Journal of Insect Science (*2016*) 16(1): 52; 1–7
doi: 10.1093/jisesa/iew033
Research

Relationships Between Aphids (Insecta: Homoptera: Aphididae) and Slugs (Gastropoda: Stylommatophora: Agriolimacidae) Pests of Legumes (Fabaceae: *Lupinus*)

Jan Kozłowski,[1] Przemysław Strażyński,[2] Monika Jaskulska,[1] and Maria Kozłowska[3,4]

[1]Department of Zoology, [2]Department of Entomology, Institute of Plant Protection – National Research Institute, Władysława Węgorka 20, 60–318 Poznań, Poland, [3]Department of Mathematical and Statistical Methods, Poznań University of Life Sciences, Wojska Polskiego 28, 60–637 Poznań, Poland, and [4]Corresponding author, e-mail: markoz@up.poznan.pl

Subject Editor: Xinzhi Ni

Received 17 January 2016; Accepted 11 April 2016

Abstract

Lupin plants are frequently damaged by various herbivorous invertebrates. Significant among these are slugs and aphids, which sometimes attack the same plants. Relationships between aphids, slugs and food plant are very interesting. Grazing by these pests on young plants can lead to significant yield losses. There is evidence that the alkaloids present in some lupin plants may reduce grazing by slugs, aphids and other invertebrates. In laboratory study was analyzed the relationships between aphid *Aphis craccivora* and slug *Deroceras reticulatum* pests of legumes *Lupinus angustifolius*. It was found that the presence of aphids significantly reduced slug grazing on the plants. The lupin cultivars with high alkaloid content were found to be less heavily damaged by *D. reticulatum*, and the development of *A. craccivora* was found to be inhibited on such plants.

Key words: Aphis craccivora, Deroceras reticulatum, Lupinus angustifolius cultivar, damage

Introduction

Herbivorous land snails and slugs (Gastropoda: Pulmonata: Stylommatophora) and aphids (Aphidoidea) cause damage to certain crop plants. The world's widespread slug is *Deroceras reticulatum* (O.F. Müller, 1774) (Agriolimacidae), which occurs in temperate regions in Europe, Asia, North and South America, Australia, and New Zealand (Godan 1979; Wiktor 1983; South 1992; Speiser et. al. 2001; Kozłowski and Kozłowska 2002; Dedov and Mitev 2011). It is a very significant pest to winter wheat, winter rape, vegetables, orchard and ornamental plants, and many species of legumes, including lupins (Frank 1998; Glen and Moens 2002; Moens and Glen 2002; Port and Ester 2002; Byers 2002; Kozłowski and Kozłowska 2002; Brooks et al. 2003). It causes damage to plants during the whole of the vegetative season, posing the greatest danger at the stage of first leaf development. The aphid *Aphis craccivora* (Koch, 1854), is a species whose development is almost entirely anholocyclic and takes place on a single type of plant. It is widespread throughout the world, occurring chiefly in warm and tropical regions (Blackman and Eastop 2006; Soffan and Aldawood 2014). It grazes on plants from 80 different families, chiefly Asteraceae and Fabaceae (Szelęgiewicz 1968; Holman 2009), and carries plant viruses (Jones and Proudlove 1991; Berlandier et al. 1997). Populations of *A. craccivora* can grow rapidly (Talati and Butani 1980; Soffan and Aldawood 2014) and can cause significant damage to legume crops, particularly beans, peas, alfalfa and lupin (Holtkamp and Bishop 1983; Kamphuis et al. 2012; Strażyński et al. 2013).

Cultivations of lupin are more and more frequently being damaged by slugs, especially *D. reticulatum* (unpublished results of own studies). Sometimes the same plants are also inhabited by *A. craccivora* or other aphid species. The most commonly recommended method of protecting plants from slugs and aphids is the use of chemicals. However, in view of the unfavorable impact of these on the environment and their unsatisfactory effectiveness, there are many problems associated with chemical use. One potential method of reducing the damage done to various crop plants by these pests is the use of cultivars that are tolerant to their grazing, or of secondary plant metabolites contained in them, such as terpenoids, glycosides, alkaloids, saponins, tannins, polysaccharides, and others (Kloos and McCullough 1982; Webbe and Lambert 1983; Stahl 1988; Adewunmi and Monache 1989; Airey et al. 1989). In plants of the genus *Lupinus* such effects are exhibited by alkaloids. These are present both in the phloemic fluid and in the extraphloemic tissues of those plants. Quinolizidine alkaloids have been found to have antifeedant or deterrent properties against slugs (Wink 1984; Carey and Wink 1994; Aguiar and Wink 1999, 2005; Chevalier et al. 2000) and against other herbivorous organisms such as nematodes, caterpillars, beetles, locusts, rabbits, cows, and aphids (Wink et al. 1982; Wink 1998; Zehnder et al. 2001). There are examples to show that slugs and aphids graze on many plants of Leguminosae, and the

levels of alkaloids, particularly lupanine, cysteine, sparteine, angustifoline, and dehydrolupanine, have a significant effect on the intensity of grazing by slugs and on the penetration of tissues by aphids (Aguiar and Wink 1999; Zehnder et al. 2001; Philippi et al. 2012).

Main goal of experimental studies was the verification of effect of presence *A. craccivora* on grazing of plants by *D. reticulatum*. We also studied susceptibility of two chemotypes *L. angustifolius* on damage caused by the slug and how the aphid colonizes the plants.

Materials and methods

The Preparation of Experimental Material—Slugs, Aphids, and Plants

Slugs of the species *D. reticulatum* were collected in spring 2013 and 2014 from a garden in Poznań, Poland. The collected specimens were kept in plastic containers measuring 26 × 26 × 14 cm, one-fifth filled with soil, which were placed in a climate chamber at air temperature 16°C with a photoperiod of 12:12 (L:D)h cycle. The slugs' diet consisted of cabbage leaves, potato tubers, carrot roots, and wheat bran with added calcium carbonate. New food was given twice a week. Before the start of each experiment the slugs were starved for 24 hours, and weighed. The average weights of the slugs were 0.453 g in the first year and 0.445 g in the second year of the study.

Aphids were collected several days before the start of the experiments. *A. craccivora* was taken from a field plantation of alfalfa (Plato cultivar). The aphids were then kept at a temperature of 23°C, on plants of narrow-leafed lupin (Graf cultivar). New narrow-leafed lupin seedlings were provided continuously to replace old ones for the maintenance and continuous growth of aphid cultures.

Plants of narrow-leafed lupin [*Lupinus angustifolius* (L.)] representing the cultivars Karo and Graf and pea [*Pisum sativum* (L.) cultivar Telefon] were grown in garden boxes at a greenhouse of the Institute of Plant Protection - National Research Institute in Poznań, Poland. The seeds were obtained from breeders at Experimental Stations for Variety Evaluation.

Experimental Methods

To study the relationships between aphids and slugs and plant, in particular for comparison of effects of two chemotypes *L. angustifolius* on development *A. craccivora* and plant damage caused by *D. reticulatum* the research of dry mass alkaloid content in studied plants was carried out. Determine the content of alkaloids in plants of both cultivars lupine were performed using a gas chromatograph coupled with mass detector (GC and MS). The studies were carried out by the Chemical Laboratory of Research Centre for Cultivar Testing in Słupia Wielka, Poland.

Followed, studies were made of the development of aphids (in the absence of slugs). Prior to testing, plants at the stage of 3–4 true leaves were planted in a 5 cm layer of soil, in plastic containers (33 × 22 × 15.5 cm), covered over with gauze. In each container were three plants of the studied cultivars of lupin or pea. The development of aphids was observed using a modified method earlier described by Soffan and Aldawood (2014). On each of the plants there were placed three aphids at stage L2 in the first year, and five aphids at stage L3 in the second year of the study. Aphids were able to develop unhindered in the conditions close to natural as possible. The number of aphids (all morphs) was recorded every 2 d until the end of the experiment (17 d). The experiment was carried out in a climate chamber at a temperature of 17°C in the first year and 21°C in the second year, with RH 70 ± 3% and a photoperiod of 12:12 (L:D)h cycle. The use of different parameters in the two years had the objective of enabling generalization of the results, based on the differences in the numbers and developmental stage of the aphids, and in the temperature. The use of a higher temperature in the second year of the study was to create better conditions for the development of aphids. Tests of the development of aphids in the absence of slugs were performed in three repetitions. The intrinsic rate of increase r is a basic parameter which we calculated for aphids (all morphs) as the rate of increase under specified conditions of our research using the formula described by Birch (1948: 16). In the studies of slug damage to plants, we applied containers which were prepared in the same way as it was described in second sentence this section. Next two slugs were placed in each container 5 d after the introduction of the aphids. For a period of more than 2 wk, observations were made every 2 d of the amount of plant damage caused by the slugs, using a five-point scale (0 for no damage, 25, 50, 75, and 100% of damaged plant surface area). The measurement was taken to be the average for the three plants in a container. For all combinations for which the amount of plant damage was assessed (the Karo, Graf and Telefon cultivars, with and without aphids), nine repetitions were performed. The pea plants in the second year were used as an excluded control. These plants are an attractive food for slugs, and were used for the purpose of comparing the level of damage suffered by them with that recorded for the cultivars of narrow-leafed lupin. The tests of slug damage to plants were two-factor experiments of type 2 × 2 with an excluded control, performed for 2 yr with variable parameters, in a completely random design with a constant number of replications equal to 9.

Statistical Analysis

The main results obtained from the observations of slug damage to plants were subjected to statistical analysis. To obtain generalized conclusions, a synthesis was made of the results from both study years. Mixed model of observations for synthesis was derived and has the following form:

$$y_{kijl} = \mu + \gamma_k + \rho_i + (\gamma\rho)_{ki} + \tau_j + (\gamma\tau)_{kj} + (\rho\tau)_{ij} + (\gamma\rho\tau)_{kij} + \varepsilon_{kijl},$$

where μ is a common parameter and γ_k is the random effects of year ($k = 1, 2$), ρ_i and τ_j are fixed effects of factor A (cultivars of lupin, $i = 1, 2$) and factor B (with or without aphids, $j = 1, 2$), respectively and $(\rho\tau)_{ij}$ denotes effect of interaction factor A with factor B. Symbols $(\gamma\rho)_{ki}$, $(\gamma\tau)_{kj}$ *and* $(\gamma\rho\tau)_{kij}$ denote interactions years with factor A, years with factor B and years with factor A and with factor B, respectively. Here ε_{kijl} is error representing uncontrolled variability of the experimental units ($l = 1, 2, \ldots, 9$). For detailed comparisons, Fisher's procedure with the corresponding denominator for synthesis was used with a significance level of $\alpha = 0.05$. Analysis of variance (ANOVA) was performed for each year separately too, due to the use of different parameters relating to the aphids and the temperature.

Two methods of exploratory data analysis were also used, in order to search effectively for similarities in the levels of slug damage to the studied plants, including the excluded controls. Cluster analysis and object and feature grouping were applied to standardized data, the results being presented graphically.

Results

In the study of the content of alkaloids in plants of both cultivars lupine it was found that plants of narrow-leafed lupin representing

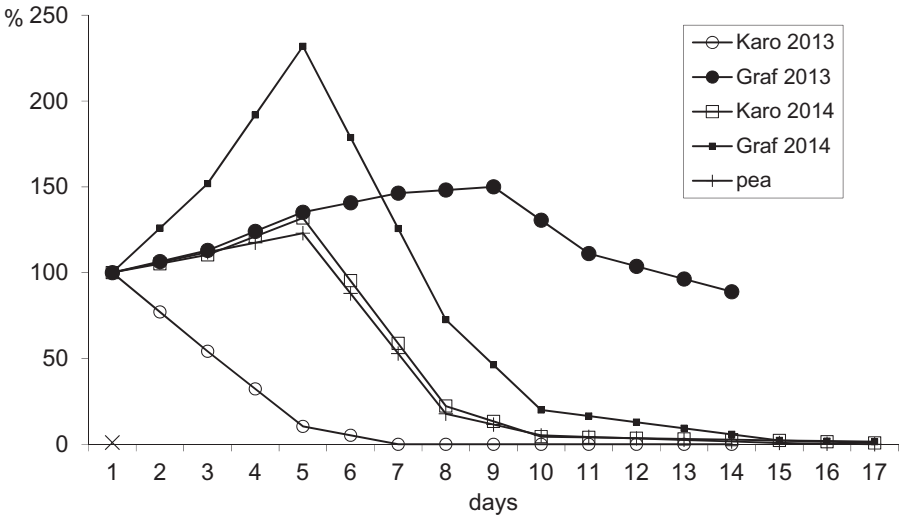

Fig. 1. Rate of development of *A. craccivora* on plants of *L. angustifolius* cultivars Karo (high alkaloid content) and Graf (low alkaloid content) and *P. sativum* cultivar Telefon, in the 2 yr of the study.

the cultivars Karo and Graf contain 0.726 and 0.002% dry mass alkaloid, respectively.

In the study of aphid development, in the first study year, no permanent colonization by the aphid *A. craccivora* was observed on the Karo cultivar of narrow-leafed lupin. Five days after the introduction of the aphids, only isolated specimens of the aphid remained on the plants (<10%; Fig. 1). The intrinsic rate of increase under given conditions does not exceed zero. This implies that plants of the Karo cultivar were not an attractive food to *A. craccivora*. The aphids developed to a greater degree on the Graf cultivar of lupin. From the first to the ninth day after aphids were placed on plants of that cultivar, their population was found to increase by ~50% and over the following days it fell to below its initial size. Although the parameters of the rate of increase were the greatest on the fifth day of observation. The difference quotient $\Delta N/\Delta t$ was equal 0.53 and the intrinsic rate of increase $r = 0.075$. In the second study year the observations, the population of aphids on plants of the Karo cultivar of narrow-leafed lupin had increased by ~30% by the fifth day and $r = 0.069$ (Fig. 1). On subsequent days there was a marked decrease in their population, and from day 10 onward only isolated specimens were present on a small number of plants. Like in the first study year, aphids developed better on plants of the Graf cultivar, on which their population more than doubled (reaching ~230% on the fifth day) and was significantly higher than on the plants of the Karo cultivar. After 5 d their population was observed to have more than doubled on almost all plants and the difference quotient $\Delta N/\Delta t$ was equal 4.94 and the intrinsic rate of increase $r = 0.210$, although after eight days the number of aphids had fallen to ~30% below the initial value, and on subsequent days it continued to decrease. On pea plants, *A. craccivora* aphids developed in a similar manner as on lupin plants of the Karo cultivar.

In the first study year of damage caused to plants by slugs of *D. reticulatum*, carried out on the Karo and Graf cultivars of narrow-leafed lupin, with and without the introduction of *A. craccivora* aphids, no interaction was detected between the studied factors (Table 1). Comparisons of the experimental combinations indicate that from the first to the final day of slug grazing the greatest amount of damage was done to plants of the Graf cultivar without aphids. From days 5 to 17 the Graf plants with aphids suffered significantly more slug damage than the plants of the Karo cultivar with aphids. Comparisons of the principal effects of the studied factors show that the lupin plants with aphids suffered less damage than those without aphids. Statistically significant differences were detected after days 1, 9, 13, 15, and 17 of slug grazing. Moreover, on all days of observation, plants of the Graf cultivar suffered significantly more slug damage than those of the Karo cultivar.

In the second study year of damage caused to plants by the slug, a comparison was again made between the levels of damage caused by *D. reticulatum* to plants of the Karo and Graf cultivars of narrow-leafed lupin with and without *A. craccivora* aphids (Table 2). As in the previous year, no interaction was found. Comparison of the experimental combinations showed that from the first to the final day of slug grazing, the damage to plants of the Graf cultivar was significantly greater than the damage to plants of the Karo cultivar, both on plants with aphids and on those without. Moreover, from days 1 to 9, the plants of the Graf cultivar without aphids suffered a significantly greater amount of damage than plants of the same cultivar with aphids, although from day 11 onwards the damage to the two groups of plants was similar. Comparison of the principal effects of the studied factors showed that the lupin plants with aphids suffered less damage than those without aphids. Statistically significant differences were recorded on days 1, 3, 5, 7, and 13. Moreover, during the entire period of slug grazing, plants of the Graf cultivar were significantly more heavily damaged than those of the Karo cultivar.

The excluded control consisted of plants of the Telefon cultivar of pea, with and without the introduction of *A. craccivora* aphids. The differences in the levels of damage suffered by the pea plants with and without aphids were insignificant (Table 2). During the whole period of observations, the damage to the pea plants was greater than the damage to the lupin plants of the Karo cultivar.

Analysis of the results from both study years, based on the derived mixed model (random testing conditions in the years) showed an absence of interaction on particular days of observation, except for the fifth day (Table 3). Based on pairwise comparison of combinations, it can be concluded that after days 5, 7, 9, and 13 of slug grazing the damage done to lupin plants of the Graf cultivar without aphids was significantly greater than the damage done to

Table 1. Damage done by *D. reticulatum* to cultivars of narrow-leafed lupin with and without aphids, *P*-values from ANOVA (fixed model) and results of Fisher's test at significance level $\alpha = 0.05$ (first study year)

Plant cultivar	Aphid species	Days of slug grazing								
		1	3	5	7	9	11	13	15	17
A	B									
Karo	*A. craccivora*	9.7a	22.2a	29.2a	37.5a	43.1a	52.8a	56.9a	59.7a	65.3a
Karo	none	11.1a	23.6a	31.9a	45.8a	54.2a	69.4ab	79.2b	79.2b	83.3b
Graf	*A. craccivora*	11.1a	34.7ab	59.7b	70.8b	75.0b	84.7bc	90.3bc	93.1bc	94.4bc
Graf	none	25.0b	48.6b	76.4b	83.3b	90.3b	94.4c	100.0c	100.0c	100.0c
A × B	F(AB) (*P*-value)	3.7 (0.063)	0.8 (0.364)	1.3 (0.270)	0.1 (0.770)	0.1 (0.774)	0.2 (0.625)	1.2 (0.280)	1.3 (0.255)	1.6 (0.209)
	A. craccivora	10.4a	28.5a	44.4a	54.2a	59.0a	68.8a	73.6a	76.4a	79.9a
	none	18.1b	36.1a	54.2a	64.6a	72.2b	81.9a	89.6b	89.6b	91.7b
B	F(B) (*P*-value)	5.6 (0.025)	1.3 (0.269)	2.5 (0.126)	2.2 (0.150)	3.4 (0.077)	3.5 (0.070)	7.9 (0.008)	6.0 (0.020)	5.9 (0.021)
Karo		10.4a	22.9a	30.6a	41.7a	48.6a	61.1a	68.1a	60.4a	74.3a
Graf		18.1b	41.7b	68.1b	77.1b	82.6b	89.6b	95.1b	96.5b	97.2b
A	F(A) (*P*-value)	5.6 (0.025)	7.6 (0.009)	36.7 (<0.001)	25.1 (<0.001)	22.2 (<0.001)	16.3 (<0.001)	22.6 (<0.001)	25.1 (<0.001)	22.1 (<0.001)

Karo, Graf, cultivars of narrow-leafed lupin.

Column values marked with the same letter do not differ significantly

plants of the Karo cultivar both with and without aphids. Moreover, after days 5, 7, and 13 there was significantly greater damage to the plants of the Graf cultivar than to those of the Karo cultivar. During the entire period of slug grazing, the plants with aphids were less damaged. However, the differences were not shown to be statistically significant for most days of observation, because for the adopted mixed model the sources of variation and their respective interactions with the random factor have only one degree of freedom (type 2 × 2 experiment over 2 yr). Significant differences were shown only for days 3 and 5 of slug grazing. Similarly, only for the results from days 5 and 15 was it shown that lupin plants of the Graf cultivar suffered significantly more slug damage than those of the Karo cultivar.

The amounts of damage to the plants of both lupin cultivars increased on successive days of observation, and on each day were greater on the plants without aphids (Fig. 2). The difference between the levels of damage done by *D. reticulatum* to plants with and without *A. craccivora* aphids decreased on successive days of observation, for both the Karo and Graf cultivars of lupin.

The progressive damage to the studied experimental combinations and the plants used as excluded controls was compared using exploratory techniques. Cluster analysis was used to produce a dendrogram (Fig. 3), taking a Euclidean distance of 3, which produced three clusters. The first cluster contains the Telefon cultivar of pea with and without *A. craccivora* aphids, and the Graf cultivar of lupin with aphids. The second cluster consists of the Graf cultivar of lupin without aphids. The third cluster contains the Karo cultivar of lupin with and without aphids. This division reflects the high degree of similarity in the progressive damage caused by *D. reticulatum* to plants listed as belonging to the same cluster. It should be noted that only for the Graf cultivar of lupin was there a difference in the progressive damage with and without the application of aphids, these two combinations appearing in different clusters.

To determine which variables affected the grouping of the studied combinations, object and feature grouping analysis was carried out. The results are shown in Figure 4. A similar consistency in progressive damage on successive days was found for lupin plants of the Karo cultivar with and without aphids. The Telefon cultivar of pea shows a smaller degree of similarity, and the least similarity is recorded for the Graf cultivar of lupin, as evidenced by the significant differences in the results obtained on days 1, 3, and 5.

Discussion

The results obtained in this study indicate that the presence of *A. craccivora* on plants of both cultivars of lupin reduced grazing activity and consequently the amount of damage caused by *D. reticulatum*, in particular during the period of growth in the aphid population. It is known that certain aphids (*Aphis cytisorum, Acyrthosiphon spartii*) are able to accumulate alkaloids which are toxic both to the aphids themselves and to their predators (Wink et al. 1982; Wink 1984). In the case of slugs, the reduction in their grazing on plants colonized by aphids can probably be explained by the deterioration in food quality as a result of grazing on aphids.

As expected, both the colonization of the studied lupins by aphids and the amount of slug damage were significantly affected by the properties of the particular plant cultivars. Two cultivars of *L. angustifolius* were used in the study, having respectively high and low alkaloid content. The high-alkaloid Karo cultivar (0.726% dry mass) was less heavily colonized by aphids and significantly less damaged by *D. reticulatum* than the low-alkaloid Graf cultivar (0.002% dry mass). This suggests that the alkaloids had an antifeedant effect on the slugs, reducing the level of their grazing on the plants. Similar results were obtained by Chevalier et al. (2000) in a study of bitter cultivars of *Lupinus albus* (L.) (with a seed alkaloid content of 1.5% dry mass), which were rejected by the snail *Helix aspersa* (Müller) after several days of grazing on the plants. Wink (1983) found that an increase in the content of alkaloids in the leaves of lupin can occur within just 2–4 h of their being damaged, and that the leaves quickly become repellent to herbivores.

It was also found that the high-alkaloid Karo cultivar was less heavily colonized by *A. craccivora* than the low-alkaloid Graf cultivar. It should be noted that a large influence on the colonization and development of aphids on the plants, not connected with the properties of the particular lupin cultivars, came from the conditions of the experiment (17 or 21°C, RH 70 ± 3%). These conditions were optimal for *D. reticulatum* (Dmitrieva 1969; South 1982), but unfavorable to *A. craccivora*, which develops better at a higher temperature and lower air humidity (in field conditions it is not able to survive periods of intense rainfall). The optimum temperature for the development of that species is 24–28.5°C at a humidity of around 65% (Mayeux 1984). When in the second year of the study the temperature was increased to 21°C, the aphids displayed better development on the low-alkaloid Graf cultivar. This development was nonetheless significantly shorter than in the case of the high-alkaloid Karo

Table 2. Damage done by *D. reticulatum* to cultivars of narrow-leafed lupin and pea plants with and without aphids, *P*-values from ANOVA (fixed model) and results of Fisher's test at significance level $\alpha = 0.05$ (second study year)

		Days of slug grazing								
Plant cultivar	Aphid species	1	3	5	7	9	11	13	15	17
Telefon	*A. craccivora*	25.9	44.4	54.6	64.8	73.1	86.1	89.8	91.7	99.1
Telefon	none	23.1	44.4	50.0	59.3	68.5	75.0	77.8	83.3	93.5
A	B									
Karo	*A. craccivora*	10.2a	19.4a	25.9a	30.6a	41.7a	50.9a	50.9a	62.0a	73.1a
Karo	none	14.8a	25.0a	29.6a	37.0a	43.5a	56.5a	65.7a	69.4a	71.3a
Graf	*A. craccivora*	20.4b	44.4b	61.1b	74.1b	82.4b	89.8b	91.7b	93.5b	100.0b
Graf	none	28.7c	54.6c	78.7c	92.6c	97.2c	98.1b	99.1b	99.1b	100.0b
A × B	F(AB) (*P*-value)	1.0 (0.313)	0.6 (0.426)	2.3 (0.135)	2.2 (0.146)	1.9 (0.175)	0.1 (0.740)	0.5 (0.482)	0.03 (0.856)	0.04 (0.840)
	A. craccivora	15.3a	31.9a	43.5a	52.3a	62.0a	70.4a	71.3a	77.8a	85.6a
	none	21.8b	39.8b	54.2b	64.8b	70.4a	77.3a	82.4b	84.3a	86.6a
B	F(B) (*P*-value)	12.9 (0.001)	7.5 (0.009)	5.5 (0.025)	9.5 (0.004)	3.2 (0.083)	2.8 (0.104)	4.5 (0.040)	1.6 (0.210)	0.04 (0.840)
Karo		12.5a	22.2a	27.8a	33.8a	42.6a	53.7a	58.3a	65.7a	72.2a
Graf		24.5b	49.5b	69.9b	83.3b	89.8b	93.9b	95.4b	96.3b	100.0b
A	F(A) (*P*-value)	44.3 (<0.001)	90.4 (<0.001)	86.3 (<0.001)	149.7 (<0.001)	102.3 (<0.001)	94.0 (<0.001)	50.5 (<0.001)	36.3 (<0.001)	37.3 (<0.001)

Karo, Graf, cultivars of narrow-leafed lupin; Telefon – pea (excluded control).
Column values marked with the same letter do not differ significantly.

Table 3. Damage done by *D. reticulatum* to cultivars of narrow-leafed lupin with and without aphids, *P*-values from ANOVA (mixed model) and results of Fisher's test at significance level $\alpha = 0.05$ (synthesis of two years of study)

Plant cultivar	Aphid species	Days of slug grazing								
		1	3	5	7	9	11	13	15	17
A	B									
Karo*	*A. craccivora*	9.9a	20.8a	27.5a	34.0a	42.4a	51.9a	53.9a	60.9a	69.2a
Karo	none	13.0a	24.3a	30.8b	41.4ab	48.8a	63.0ab	72.5b	74.3a	77.3a
Graf*	*A. craccivora*	15.7a	39.6a	60.4c	72.5bc	78.7ab	87.3ab	91.0c	93.3a	97.2a
Graf	none	26.9a	51.6a	77.5d	88.0c	93.8b	96.3b	99.5c	99.5a	100.0a
A x B	F(AB) (*P*-value)	3.4 (0.317)	330.1 (0.274)	21702.5 (0.004)	4.2 (0.288)	3.8 (0.302)	0.2 (0.742)	15.3 (0.160)	1.8 (0.406)	0.6 (0.594)
	A. craccivora	12.8a	30.2a	44.0a	53.3a	60.5a	69.6a	72.5a	77.1a	83.2a
	none	19.9a	38.0b	54.2b	64.7a	71.3a	79.6a	86.0a	86.9a	88.7a
B	F(B) *P*-value	148.8 (0.052)	4510.1 (0.009)	478.8 (0.029)	121.2 (0.058)	19.6 (0.141)	10.4 (0.192)	31.1 (0.113)	8.6 (0.209)	0.7 (0.550)
Karo		11.5a	22.6a	29.2a	37.7a	45.6a	57.4a	63.2a	67.6a	73.3a
Graf		21.3a	45.6a	69.0b	80.2a	86.2a	91.8a	95.3a	96.4b	98.6a
A	F(A) *P*-value	20.0 (0.140)	28.9 (0.117)	295.7 (0.037)	36.2 (0.105)	37.9 (0.102)	33.9 (0.108)	41.5 (0.098)	275.3 (0.038)	108.8 (0.061)

Karo, Graf, cultivars of narrow-leafed lupin; days 1–17 of slug grazing.
Column values marked with the same letter do not differ significantly.

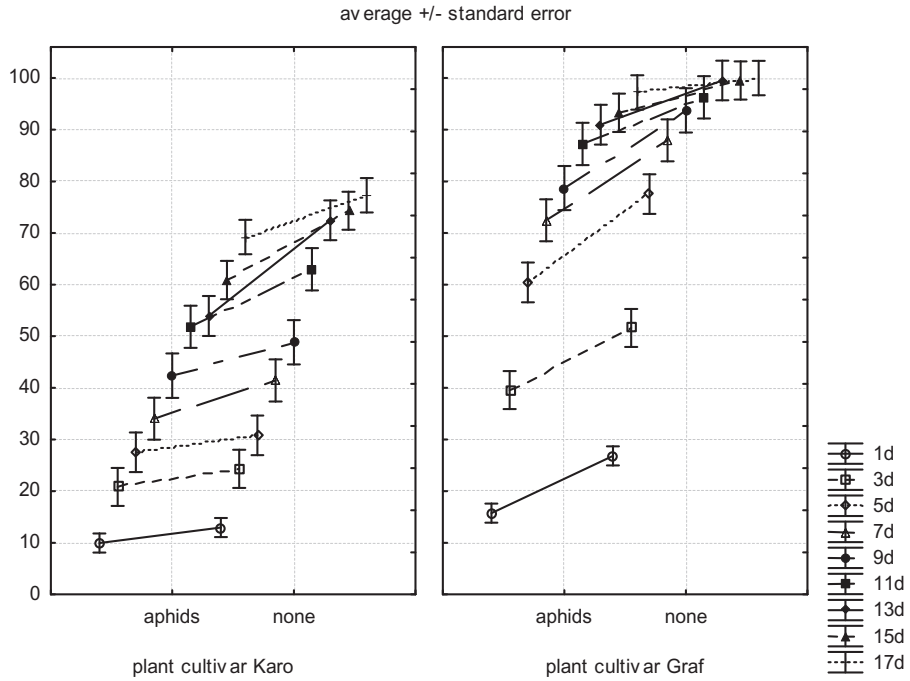

Fig. 2. Average damage (%) to plants of *L. angustifolius* cultivars Karo (high alkaloid content) and Graf (low alkaloid content) caused by the slug *D. reticulatum* on successive days of grazing, ±se (2 yr of study).

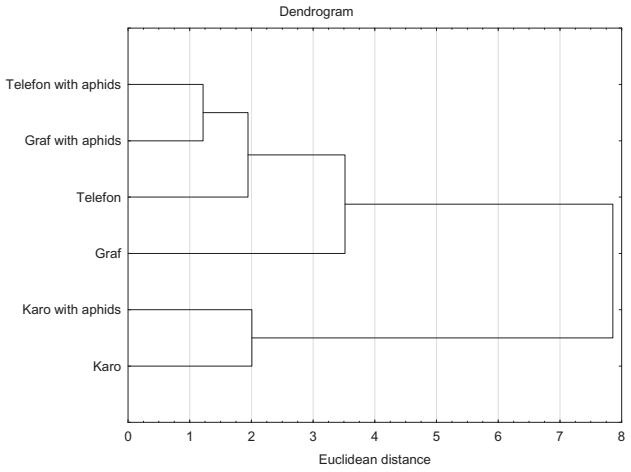

Fig. 3. Dendrogram produced by cluster analysis for the studied combinations (the Karo, Graf, and Telefon cultivars with and without aphids).

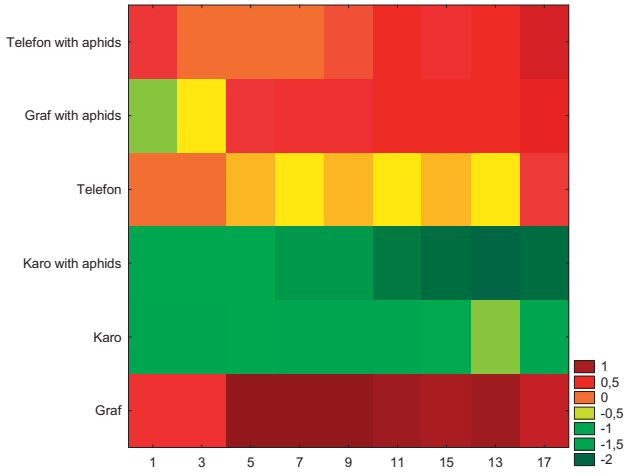

Fig. 4. Objects and feature grouping, the objects being the six studied combinations, and the features being the levels of slug damage to the plants at nine time points.

cultivar. The increase in air temperature improved the conditions for the aphids' development, but not sufficiently to maintain their population at a high level for a long period of time.

The most interesting conclusion drawn from the study is that the presence of *A. craccivora* aphids on lupin plants leads to a significant reduction in the damage caused to the plants by the slug *D. reticulatum*. It was shown that the lupin cultivar with high alkaloid content suffered less damage from the slugs and was also less heavily colonized by the aphids. Similar results concerning the antifeedant effect of high-alkaloid lupins have been obtained by other authors in relation to slugs (Wink 1984; Carey and Wink 1994; Aguiar and Wink 1999, 2005; Chevalier et al. 2000) and aphids (Zehnder et al. 2001; Philippi et al. 2012). For example, Ferguson (1994) showed, in a field experiment conducted in England, that sweet cultivars of lupin, which had a lower alkaloid content, were more often attacked by slugs, while Wink et al. (1982) found that only sweet cultivars of lupin were damaged by polyphagous aphids.

In summary, it has been found that: 1) plants of the Karo cultivar of narrow-leafed lupin were less heavily damaged by *D. reticulatum* and less heavily colonized by *A. craccivora* than plants of the Graf cultivar, the probable reason being the high alkaloid content in plants of the former cultivar, which had a deterrent effect on aphids and slugs; and 2) the presence of *A. craccivora* aphids on narrow-leafed lupin plants caused a reduction in grazing by *D. reticulatum* and in the damage to the plants caused by that slug.

References Cited

Adewunmi, C. O. and F. D. Monache. 1989. Molluscicidal activity of some coumarins. Fitoterapia 60: 79–82.

Aguiar, R. and M. Wink. 1999. Mollusc-deterrent activity of lupin alkaloids, pp. 97–98. *In* Proceedings, 9th Intern Lupin Conference Klink/Mültriz, 20–24 June, International Lupin Association. Canterbury, New Zealand.

Aguiar, R., and M. Wink. 2005. How do slugs cope with toxic alkaloids? Chemoecology 15: 167–177.

Airey, W. J., I.F. Henderson, J. A. Pickett, G. C. Scott, J. W. Stephenson and C. M. Woodcock. 1989. Novel chemical approaches to mollusc control, pp. 301–307. *In* I. F. Henderson (ed.), Slugs and snails in world agriculture. Monograph 41, British Crop Protection Council, Thornton Heath.

Berlandier, F. A., D. J. Thackray, R.A.C. Jones, L. J. Latham, and L. Cartwright. 1997. Determining the relative roles of different aphid species as vectors of cucumber mosaic and bean yellow mosaic viruses in lupins. Ann. Appl. Biol. 131: 297–314.

Birch, L. C. 1948. The intrinsic rate of natural increase of an insect population. J. Anim. Ecol. 17: 15–26.

Blackman, R. L., and V. F. Eastop. 2006. Aphids on the world's herbaceous plants and shrubs, pp. 1025–1439, vol. 2. *In*: John Wiley and Sons with the Natural History Museum (eds), The Aphids London.

Brooks, A. S., M. J. Crook, A. Wilcox and R. Cook. 2003. A laboratory evaluation of the palatability of legumes to the field slug *Deroceras reticulatum* Müller. Pest Manag. Sci. 59: 245–251.

Byers, R. A. 2002. Agriolimacidae and Arionidae as pests in lucerne and other legumes in forage systems of north-eastern North America, pp. 325–335. *In*: G. M. Barker (ed.), Molluscs as crop pests. Landcare Res Ham New Zealand, CABI Publish.

Carey, D. B. and M. Wink. 1994. Elevational variation of quinolizidine alkaloid contents in a lupine (*Lupinus argenteus*) of the Rocky Mountains. J. Chem. Ecol. 20: 849–857.

Chevalier, L., C. Desbuquois, J. Papineau and M. Charrier. 2000. Influence of the quinolizidine alkaloid content of *Lupinus albus* (Fabaceae) on the feeding choice of *Helix aspersa* (Gastropoda: Pulmonata). J. Moll. Stud. 66: 61–68.

Dedov I. K. and T. Mitev. 2011. Mollusks fauna (Mollusca: Gastropoda: Bivalvia) of mountain Osogovo. Acta Zool. Bulg. 63: 37–46.

Dmitrieva, E. F. 1969. Population dynamics, growth, feeding and reproduction of field slug (*Deroceras reticulatum*) in Leningrad Oblast. Zool. Zh. 48: 802–810.

Ferguson, A. W. 1994. Pests and plant injury on lupins in the south of England. Crop Prot. 13: 201–210.

Frank, T. 1998. Slug damage and numbers of the slug pests, *Arion lusitanicus* and *Deroceras reticulatum* in oilseed rape grown beside sown wildflower strips. Agric. Ecos. Env. 67: 67–78.

Glen, D. M. and R. Moens. 2002. Agriolimacidae, Arionidae and Milacidae as pests in West European cereals, pp. 271–300. *In* G. M. Barker (ed.), Molluscs as crop pests. Landcare Res Ham New Zealand, CABI Publish.

Godan, D. 1979. Schadschnecken und ihre Bekämpfung, p. 467. Ulmer: Stuttgart.

Holman, J. 2009. Host plant catalog of aphids: palearctic region, p. 1216. Springer Science and Business Media BV.

Holtkamp, R. H., and A. L. Bishop. 1983. Lucerne aphids, p. 6. Department of Agricultural, New South Wales, Agfacts P2 AE 4.

Jones, R.A.C., and W. Proudlove. 1991. Further studies on cucumber mosaic virus infection of narrow-leafed lupin (*Lupinus angustifolius*): seed borne infection, aphid transmission, spread and effects on grain yield. Ann. Appl. Biol. 118: 319–329.

Kamphuis, L. G., L. Gao, and K. B. Singh. 2012. Identification and characterization of resistance to cowpea aphid (*Aphis craccivora* Koch) in Medicago truncatula. BMC Plant Biol. 12 (http://www.biomedcentral.com).

Kloos, H., and F. S. McCullough. 1982. Plant molluscicides. Planta Med. 46: 195–209.

Kozłowski, J., and M. Kozłowska. 2002. Assessment of plant damages and intensity of *Deroceras reticulatum* (Müller) occurrence in winter oilseed rape and winter wheat. J. Plant Prot. Res. 42: 229–237.

Mayeux, A. 1984. The groundnut aphid. Biology and control. Oleagineux 39: 425–434.

Moens, R., and D. M. Glen. 2002. Agriolimacidae, Arionidae and Milacidae as pests in West European oilseed rape, pp. 301–314. *In* G. M. Barker (ed.), Molluscs as Crop Pest. Landcare Res Ham New Zealand, CABI Publish.

Philippi, J., E. Schliephake, and F. Ordon. 2012. Penetration behavior of different aphid species on *Lupinus angustifolius* L. genotypes, p 46. Nachwuchswissenschaftlerforum/Young Scientists Meeting, Berichte aus dem Julius Kühn-Institut 167.

Port, R., and A. Ester. 2002. Gastropods as pests in vegetables and ornamental crops in Western Europe, pp. 337–352. *In* G. M. Barker (ed.), Molluscs as Crop Pests. Landcare Res Ham New Zealand, CABI Publish.

Soffan, A., and A. S. Aldawood. 2014. Biology and demographic growth parameters of cowpea aphid (*Aphis craccivora*) on faba bean (*Vicia faba*) cultivars. J. Insect Sci. 14: 120 (www.isectscience.org/14.120).

South, A. 1982. A comparison of the life cycles of *Deroceras reticulatum* (Müller), and *Arion intermedius* (Normand) (Pulmonata: Stylommatophora) at different temperatures under laboratory conditions. J. Moll. Stud. 48: 233–244.

South, A. 1992. Terrestrial slugs: biology, ecology and control. Chapman and Hall, London.

Speiser, B., J. G. Zaller, and A. Neudecker. 2001. Size–specific susceptibility of the pest slugs *Deroceras reticulatum* and *Arion lusitanicus* to the nematode biocontrol agent *Phasmarhabditis hermaphrodita*. Biocontrol 46: 311–320.

Stahl, E. 1988. Pflanzen und Schnecken. Jenaische Zeitschrift 22: 557–684.

Strażyński, P., A. Bandyk, W. Bieniewicz, and A. Bartkowski. 2013. Ocena podatności wybranych odmian łubinu żółtego i wąskolistnego na zasiedlanie przez mszyce [Evaluation of selected varieties of yellow lupine and narrow-leaf lupine for colonization by aphids]. Prog. Plant Prot./Post. Ochr. Roślin 53: 713–716.

Szelęgiewicz, H. 1968. Mszyce–Aphidoidea, p. 316. Catalogus faunae Poloniae XXI(4), PWN Warszawa.

Talati, G. M., and P. G. Butani. 1980. Reproduction and population dynamics of groundnut aphid. Gujarat Agric. Univ. Res. J. 5: 54–56.

Webbe, G., and J. D. H. Lambert. 1983. Plants that kill snails and prospects for disease control. Nature 302: 754.

Wiktor, A. 1983. The slugs of Bulgaria (Arionidae, Limacidae, Agriolimacidae – Gastropoda, Stylommatophora). Annal. Zool. (Polska Akademia Nauk) 37: 71–206.

Wink, M. 1983. Inhibition of seed germination by quinolizidine alkaloids. Aspects of allelopathy in *Lupinus albus* L. *Planta* 158: 365–368.

Wink, M. 1984. Chemical defense of lupins. Mollusc-repellent properties of quinolizidine alkaloids. Z. Naturforschung 39c: 553–558.

Wink, M. 1998. Chemical ecology of alkaloids, pp. 265–300. *In* M. F. Roberts, and M, Wink (eds.), Alkaloids: biochemistry, ecology and medical applications. Plenum Press, New York.

Wink, M., T. Hartmann, L. Witte, and J. Rheinheimer. 1982. Interrelationship between quinolizidine alkaloid producing legumes and infesting insects: exploitation of the alkaloid-containing phloem sap of *Cytisus scoparius* by the broom aphid *Aphis cytisorum*. Z. Naturforsch. 37c: 1081–1086.

Zehnder, G. W., A. J. Nichols, O. R. Edwards, and T. J. Ridsdill-Smith. 2001. Electronically monitored cowpea aphid feeding behavior on resistant and susceptible lupins. Ent. Exp. Appl. 98: 259–269.