



Insect assemblages and their preference for *Lupinus albus* and *L. luteus*

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Abstract

While lupin has undergone extensive research to ascertain its suitability for growth as forage or grain legume crop, the present trend is for research to be centered on its applicability in the seed protein and oil industry. Study of the literature showed that no intensive study of the lupin insect fauna had been carried out in Bulgaria. The purpose of this study was to identify the insect assemblages associated with *Lupinus albus* and *L. luteus*, as well as the insect preference for them. Thrips sampling was made by the tapping-method, aphids were directly counted on the plants and the composition and population density of other species were recorded by sweepings. Insect fauna was studied for the first time in Bulgaria. The fauna was represented on *L. albus* by 64 species, belonging to eight orders, 28 families and 57 genera, including 23 beetles, 25 hemipteras, five thrips, three butterflies, three bees, one leaf aphid, two grasshoppers, one leafminer and one green lacewing. *L. luteus* had similar species composition but was less preferred by insects. The use of lupin cultivars with shorter and intense reproductive periods, with a lower content of crude protein and phosphorus, would give an environmentally friendly protection against insect pests, which would be suitable for an organic production system.

Keywords

chemical • insect species • lupin • morphological • phenological traits

Introduction

Lupin is not a traditional crop in Bulgaria, but it has long been used as a green manure source (Angelova 2001). A study of the literature showed almost no intensive study of the lupin insect fauna in Europe. Similar studies have been conducted in the past century (Kurlovich, 1995; Bublyk *et al.*, 1999). In addition, it appears that research into the lupin insect fauna has concentrated on individual pest species rather than on the overall fauna (Shawna, 2014).

In the available scientific literature, especially Bulgarian literature, even now, there is insufficient information concerning lupin insect fauna and species composition. Nevertheless, aphids are classified as very important lupin pests, and they are vectors of plant viruses.

At the beginning, the insects attack the apical buds, and afterwards, they occupy the whole plants. Aphid damage is caused by the withdrawal of sap from leaves, stems and pods; delayed plant growth; as well as reduced palatability and quality of the foliage (Golubev and Kurlovich, 2006). Aphid feeding may reduce yield in lupin seed up to 100% (Kordan *et al.*, 2008). While aphid distribution within susceptible cultivars is usually patchy, they can cause serious damage by colonising stems, leaves and buds (The Connecticut

Agricultural Experiment Station, 2007). Other insects that showed great pest potential were *Sitona* weevils (Ströcker *et al.*, 2011), *Otiorynchus* weevils (Hurej *et al.*, 2013), thrips and plant bugs (Mound, 2005).

Additionally, some authors have found that there are qualitative and quantitative differences in insect fauna among lupin species. *Sitona* weevils strongly reduced the grain yield in *L. angustifolius* (up to 40%) in Germany, and their population density was higher than in *L. luteus* (Ströcker *et al.*, 2011). Shawna (2014) reported that aphids were especially common in white lupin and were recorded in high density at the stages of budding and early pod. Direct damage by them reduces seed yield and causes flower and pod abscission. Aphids are vectors of plant viruses that can reduce lupin productivity.

The control of major harmful insects in lupin is difficult and is primarily performed through chemical insecticides. Pest control systems may include an ecological approach that is appropriate for conditions of organic production. According to Haruta *et al.* (2001), the interaction between the plant and the insect is a dynamic system, which constantly varies and changes. In order to respond to and decrease the attack of insects, plants show different protective systems, such as physical, morphological and

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chemical barriers, related to lower contents of phosphorus, alkaloids and others (Ricklefs, 2008; Philippi *et al.*, 2015). Many studies indicate that different lupin varieties contain different contents and compositions of alkaloids influencing the probing and feeding behaviours of aphids. There is a positive correlation between alkaloid content and aphid tolerance (Adhikari *et al.*, 2012; Philippi *et al.*, 2015).

Development of resistant or tolerant cultivars could largely address the problem of pest management. Concerning the modern organic farming, the use of tolerant (resistant) varieties against lupin pest insects will support and improve the biodiversity of insects, as well as providing environmental and landscape protection.

In Bulgaria, one of the pests reported to cause great damage to the seeds of lupin was a pyralid moth *Etiella zinckenella* (Grygorov, 1972). Subchev and Tóth (2006) found that the damage from the pyralid moth is huge, especially when the lupin is grown near other grain legumes or if there is a breach of the crop-rotation scheme. However, other studies concerning lupin insect abundance, species composition, especially insect preference for lupin species, and other features are not yet reported in our country.

The purpose of this study was to identify the insect assemblages associated with *L. albus* and *L. luteus*, including insect abundance and species composition, as well as the insect preference for them.

Materials and methods

The aim of this study was to investigate the insect assemblages associated with *L. albus* and *L. luteus*, including insect abundance and species composition, as well as insect preference for them. The study was carried out during the period 2012–2014 in the field of the Institute of Forage Crops, Pleven, Bulgaria, with two lupin species - *L. albus* L., white, Ukrainian variety Garant; and *L. luteus* L., yellow, Ukrainian variety Chernilovets. The experiment was designed by the long plot method: 10 m² plots were sown at a rate of 50 germinating seeds/m², with three replications, and availability of balanced soil nutrients. No pesticides were applied. Sweepings recorded the composition of species and insect density. The net used was a standard sweep net with a mouth diameter of 30 cm and a 60 cm long net of very fine mesh Terylene. Sweep netting (15 sweeps in each plot) was carried out weekly during the seedling stage. It continued until the aboveground biomass has dried. Aphids were directly counted on the plants. Thrips were sampled by tapping. A soil excavation method was used too. Direct observation of plants of both species was carried out on six occasions beginning from their early development in March. Complete plant removal was carried out in May. Twenty randomly selected

plants from each lupin species were severed at the base and placed into plastic bags. After the insects were killed in the laboratory using formaldehyde, those remaining on the plants were washed off in a tray with 70% alcohol for identification.

Species identification of thrips was made using the following: an insect collection provided by Prof. Kiran Donchev; bug species collection provided by Nikolay Simov (Ph.D.); Coleoptera species collection provided by Assoc. Prof. Romyana Koleva; Diptera collection provided by Prof. Venelin Beshovski; Cicadellidae collection provided by Assoc. Prof. Venelin Pelov.

The classification of insect species was made as follows: * – dominant species with 15% representation; ** – sub-dominant (from 5% to 15%); *** – secondary (1%–5%); **** – tertiary (less than 1%) (Boychev, 1975).

According to the classification of the seed size, they comprised three groups: 1) species with small-sized grain and weight of 1,000 seeds <150 g; 2) species with medium size of seed and 1,000-seed weight in the range of 150–250 g; 3) species with large size of seed and 1,000-seed weight >250 g.

The morphological characteristic of lupin seeds was determined according to the Protocol for distinctness, uniformity and stability tests *Pisum sativum* (UPOV, 2010). The damaged seed ratings were determined after harvesting. Bulk samples that contained nearly 2,000 seeds were taken for *L. albus* and *L. luteus*. The reviewed seeds were grouped into two kinds: healthy seeds and seeds damaged by true bugs.

The chemical composition of aboveground dry mass, harvested in the flowering stage, was determined as follows: crude protein (CP) was determined by the method of Kjeldahl, crude fibre (CF) by the method of Weende, phosphorus (P) was determined colourimetrically using the hydroquinone method and calcium (Ca) was identified complexometrically. The obtained data were statistically processed using multiple regression analysis with the software Statgraphics Plus for Windows Ver. 2.1 (1995) and Tukey's test at 5% probability ($P \leq 0.05$).

Results

Weather conditions during the 3 yr of the study affected the numbers and development of the insects. Considerably higher air temperature in June 2012, combined with uniform distribution of moderate rainfall, in comparison with 2013 and 2014 (Table 1) had the most favourable impact on the development of lupin insects and led to a considerable increase in the number of insects during the flowering and pod development phases (June). The insect number/100 sweepings during the vegetation period of 2012 reached 479.0 in *L. albus* and 192.0 in *L. luteus*. The insect number

Table 1. Meteorological characteristics in the Pleven region

Month	Ten-day periods	Temperature, °C			Rainfall, mm			Relative humidity, %		
		2012	2013	2014	2012	2013	2014	2012	2013	2014
April	1-10	12.2	10.5	11.9	14.2	33.1	37.6	57	76	71
	11-20	14.3	12.5	9.9	22.1	17.6	69.9	66	65	82
	21-30	17.8	19.5	14.9	10.0	0	32.3	54	52	76
	Average	14.8	14.2	12.2	46.3	50.7	139.8	59	65	76.3
May	1-10	20.3	20.7	14.7	6.0	3.8	23.7	57	55	72
	11-20	15.7	20.0	15.9	28.9	33.3	16.9	76	57	68
	21-31	16.3	18.2	19.4	50.3	26.6	42.4	79	67	70
	Average	17.4	19.6	16.7	85.2	63.7	83	71	60	70.0
June	1-10	22.5	18.9	19.4	13.1	5.9	19.6	63	63	70
	11-20	24.7	23.3	20.8	1.4	49.9	15.5	56	68	72
	21-30	25.0	21.8	21.7	25.8	55.8	19.2	56	66	60
	Average	24.1	21.3	20.6	40.3	111.6	54.3	58	66	67

decreased to 13.4% and 41.3% in *L. albus* and to 9.4% and 49.0% in *L. luteus* during the vegetation periods in 2013 and 2014, respectively.

Fauna on *L. albus*

Insect fauna on lupin was studied for the first time in Bulgaria, as before this, only *E. zinckenella* was known to be a lupin pest in Bulgaria. In 2012, 2013 and 2014, insect captures totaled 479, 415 and 281 specimens, respectively. The species composition in *L. albus* was represented by 64 species, belonging to eight orders, 28 families and 57 genera, including 23 beetles, 25 hemipteras, five thrips, three butterflies, three bees, one leaf aphid, two grasshoppers, one leafminer and one green lacewing (Table 2).

According to their ecological roles, it was found that 50 species were herbivorous, 11 were predators and three were pollinating insects. The species harmful to lupin belonged to six orders: Coleoptera, suborder Polyphaga; Hemiptera with three suborders: Cicadomorpha, Heteroptera and Sternorrhyncha; Thysanoptera, suborder Terebrantia; Lepidoptera; Orthoptera, suborder Caelifera; and Diptera, suborder Brachycera. Orders Hemiptera (25 species) and Coleoptera (23 species) were the most numerous and most diverse. The beneficial insects were represented by five orders - Coleoptera, Hemiptera, Hymenoptera, Neuroptera and Thysanoptera.

Nine families, 18 genera and 23 species represented order Coleoptera, suborder Polyphaga; 19 of them were herbivorous and five were predatory species. An important group of lupin pests was the weevils, belonging to family Curculionidae, which was the most numerous and diversified. They represented 50.0% of the total Coleoptera order.

Sitona weevils were found to be one of the most frequent pests

in Bulgarian lupin crops. In the present research, the dominant species were *Sitona lineatus* (27.1%) and *S. macularius* (16.8%), whose larvae attack the root nodules, while adults feed on the growing plant tips. The Cerambycidae family was represented by a single sub-dominant species *Callimoxys gracilis* (13.5%). In the flowering lupin stage, the high-representation predators were the soldier beetle *Rhagonycha fulva* (13.8%) and the softwinged flower beetle *Clanoptilus elegans* (6.6%). Other predators, such as ladybirds *Coccinella septempunctata* and *Hippodamia variegata*, were found in lupin fields, but their numbers were low.

Order Hemiptera, suborder Cicadomorpha, was represented by 12 phytophagous species from four families. Among them, *Empoasca pteridis* (38.9%), *Psammotettix striatus* (20.0%) and *Reptalus panzeri* (15.9%) had the highest density. They are potential lupin pests and stable inhabitants of many agroecosystems. These species were dominant every year, and they were found during all vegetation periods. The maximal number of *E. pteridis* was observed in the stages of pod development and maturity in bottom pods. *P. striatus* and *R. panzeri* were dominant in the total cicada collection, prevailing also in the later stages of the reproductive period. The sub-dominant species were *Allygidius (Allygidius) atomarius* (6.8%) and *Latematium (Latematium) cypricum* (7.6%).

Order Hemiptera, suborder Heteroptera included five families, 12 genera and 13 species, four of which were predators. In our study, the Miridae family, represented by seven species, was the most abundant bug family. The dominant harmful species was *Adelphocoris lineolatus* (21.9%), followed by sub-dominant species *Lygus pratensis* (13.3%), *L. rugulipennis* (9.5%) and *Piezodorus lituratus* (6.7%). Plant bugs appeared in the crop at the end of the flowering

Table 2. List of species and average number of insects/100 sweepings during the complete growing season

Taxa	<i>Lupinus albus</i>				<i>Lupinus luteus</i>			
	2012	2013	2014	Average	2012	2013	2014	Average
Coleoptera (Suborder: Polyphaga)								
Family: Cantharidae								
<i>Cantharis (Cantharis) fusca</i> Linnaeus, 1758 ^{2*****1}	1	1	0	0.7	0	0	0	0.0
<i>Rhagonycha fulva</i> (Scopoli, 1763) ^{**1}	17	20	13	16.7	0	1	1	0.7
Family: Cerambycidae								
<i>Callimoxys gracilis</i> (Brullé, 1832) ^{**}	16	22	11	16.3	5	4	3	4.0
Family: Cetoniidae								
<i>Tropinota hirta</i> (Poda, 1761) ^{***}	2	3	2	2.3	0	1	0	0.3
Family: Chrysomelidae								
<i>Bruchus pisorum</i> (Linnaeus, 1758) ^{****}	1	1	0	0.7	0	1	0	0.3
<i>Oulema melanopus</i> (Linnaeus, 1758) ^{****}	1	0	0	0.3	0	1	0	0.3
<i>Phyllotreta atra</i> (Fabricius, 1775) ^{***}	2	3	1	2.0	1	0	0	0.3
<i>Phyllotreta cruciferae</i> (Goeze, 1777) ^{****}	0	1	1	0.7	1	0	0	0.3
<i>Phyllotreta nigripes</i> (Fabricius, 1775) ^{****}	0	2	0	0.7	0	1	0	0.3
<i>Spermophagus sericeus</i> (Geoffroy, 1785) ^{***}	4	3	4	3.7	2	5	0	2.3
Family: Coccinellidae								
<i>Coccinella septempunctata</i> Linnaeus, 1758 ^{****1}	5	4	2	3.7	0	1	1	0.7
<i>Hippodamia variegata</i> (Goeze, 1777) ^{****1}	1	1	0	0.7	0	0	0	0.0
Family: Curculionidae								
<i>Otiorhynchus (Cryphiphorus) ligustici</i> (Linnaeus, 1758) ^{***}	4	3	1	2.7	2	0	1	1.0
<i>Sitona macularius</i> (Marsham, 1802) [*]	21	17	23	20.3	10	3	12	8.3
<i>Sitona lineatus</i> (Linnaeus, 1758) [*]	32	24	42	32.7	12	7	15	11.3
<i>Tanymecus (Episomecus) dilaticollis</i> (Gyllenhal, 1834) ^{****}	1	2	0	1.0	1	3	0	1.3
<i>Tychius flavus</i> (Becker, 1984) ^{****}	0	1	0	0.3	1	0	0	0.3
<i>Tychius (Tychius) lineatulus</i> (Stephens, 1831) ^{***}	4	2	3	3.0	0	0	1	0.3
<i>Tychius quinquepunctatus</i> (Linnaeus, 1758) ^{****}	0	1	0	0.3	1	0	0	0.3
Family: Elateridae								
<i>Agriotes (Agriotes) ustulatus</i> (Schaller, 1783) ^{****}	0	1	0	0.3	0	0	0	0.0
<i>Selatosomus aeneus</i> (Linnaeus, 1758) ^{***}	3	4	2	3.0	0	1	0	0.3
Family: Malachiidae								
<i>Clanoptilus (Clanoptilus) elegans</i> (Olivier, 1790) ^{**1}	9	12	3	8.0	1	4	0	1.7
Family: Tenebrionidae								
<i>Podonta nigrita</i> (Fabricius, 1794) ^{****}	0	1	1	0.7	0	0	0	0.0
Diptera								
Family: Agromyzidae								
<i>Liriomyza</i> spp.	6	4	11	7.0	9	1	6	5.3
Indeterminate species	14	12	12	12.7	10	6	9	8.3
Hemiptera (Suborder: Cicadomorpha)								
Family: Aphrophoridae								
<i>Philaenus spumarius</i> (Linnaeus, 1758) ^{****}	1	0	1	0.7	0	1	0	0.3
Family: Cicadellidae								
<i>Allygidius atomarius</i> (Fabricius, 1794) ^{**}	8	6	4	6.0	8	7	4	6.3
<i>Anaceratagallia ribauti</i> (Ossiannilsson, 1938) ^{***}	3	5	1	3.0	0	1	0	0.3
<i>Austroagallia sinuata</i> (Mulsant & Rey, 1855) ^{***}	2	3	1	2.0	1	3	1	1.7
<i>Empoasca pteridis</i> (Dahlbom, 1850) [*]	40	33	30	34.3	10	8	9	9.0
<i>Eupteryx atropunctata</i> (Goeze, 1778) ^{***}	2	1	0	1.0	1	0	0	0.3

The table is continued on the next page.

Taxa	<i>Lupinus albus</i>				<i>Lupinus luteus</i>			
	2012	2013	2014	Average	2012	2013	2014	Average
<i>Neoliturus fenestratus</i> (Herrich-Schäffer, 1834)****	0	1	0	0.3	0	0	0	0.0
<i>Psammotettix striatus</i> (Linnaeus, 1758)*	23	16	14	17.7	8	12	7	9.0
<i>Zygina flammigera</i> (Fourcroy, 1785)	0	0	0	0.0	0	2	0	0.7
Family: Cixiidae	4	3	1	2.7	1	0	0	0.3
<i>Hyalesthes obsoletus</i> (Signoret, 1865)***								
<i>Reptalus panzeri</i> (Low, 1883)*	16	7	19	14.0	2	0	3	1.7
Family: Issidae	7	8	5	6.7	5	4	3	4.0
<i>Latematium (Latematium) cypricum</i> (Dlabola, 1982)**								
Hemiptera (Suborder: Heteroptera)								
Family: Alydidae								
<i>Camptopus lateralis</i> (Germar, 1817)	0	1	0	0.3	0	0	0	0.0
Family: Lygaeidae								
<i>Geocoris (Piocoris) erythrocephalus</i> (Lepelletier & Serville, 1825) ¹	1	0	1	0.7	0	0	2	0.7
<i>Nysius helveticus</i> (Herrich-Schäffer, 1850)	1	0	0	0.3	1	1	0	0.7
Family: Miridae	8	10	5	7.7	7	5	0	4.0
<i>Adelphocoris lineolatus</i> (Goeze, 1778)*								
<i>Campylomma verbasci</i> (Meyer-Dür, 1843)* ¹	14	13	1	9.3	2	1	0	1.0
<i>Deraeocoris (Camptobrochis) serenus</i> (Douglas & Scott, 1868) ¹	1	1	2	1.3	1	0	0	0.3
<i>Lygus pratensis</i> (Linnaeus, 1758)**	5	6	3	4.7	2	1	2	1.7
<i>Lygus rugulipennis</i> (Poppius, 1911)**	4	4	2	3.3	1	3	0	1.3
<i>Platycranus</i> sp.	1	0	4	1.7	0	0	2	0.7
<i>Trigonotylus caelestialium</i> (Kirkaldy, 1902)	1	0	0	0.3	0	1	0	0.3
Family: Nabidae	3	4	1	2.7	1	0	0	0.3
<i>Nabis</i> sp.** ¹								
Family: Pentatomidae	0	0	1	0.3	0	1	0	0.3
<i>Eurydema (Eurydema) ornate</i> (Linnaeus, 1758)								
<i>Piezodorus lituratus</i> (Fabricius, 1794)**	2	3	2	2.3	0	1	1	0.7
Hemiptera (Suborder: Sternorrhyncha)								
Family: Aphididae								
<i>Acyrtosiphon pisum</i> (Harris, 1776)*	132	105	14	83.7	62	64	8	44.7
Hymenoptera (Suborder: Apocrita)								
Family: Apidae								
<i>Andrena dorsata</i> (Kirby, 1802)	1	3	0	1.3	2	1	0	1.0
<i>Bombus sylvarum</i> (Linnaeus, 1761)	6	2	1	3.0	3	0	0	1.0
<i>Halictus maroccanus</i> (Bluethgen, 1934)	2	1	0	1.0	0	0	0	0.0
Indeterminate species	9	6	4	6.3	4	0	1	1.7
Thysanoptera (Suborder: Terebrantia)								
Family: Phlaeothripidae								
<i>Haplothrips tritici</i> (Kurdjumov, 1912)	1	2	1	1.3	0	0	0	0.0
Family: Thripidae	0	1	0	0.3	1	1	0	0.7
<i>Stenothrips graminum</i> (Uzel, 1895)								
<i>Thrips atratus</i> (Haliday, 1836)	8	6	5	6.3	2	0	0	0.7
<i>Thrips tabaci</i> (Lindeman, 1889)	5	4	3	4.0	3	4	0	2.3
Family: Aeolothripidae								
<i>Aeolothrips intermedius</i> (Bagnall, 1934) ¹	1	4	4	3.0	2	6	1	3.0

The table is continued on the next page.

Taxa	<i>Lupinus albus</i>				<i>Lupinus luteus</i>			
	2012	2013	2014	Average	2012	2013	2014	Average
Lepidoptera								
Family: Noctuidae								
<i>Helicoverpa armigera</i> (Hübner, 1808)	2	1	4	2.3	0	2	3	1.7
<i>Mamestra brassicae</i> (Linnaeus, 1758)	4	2	3	3.0	1	0	0	0.3
<i>Etiella zinckenella</i> (Treitschke, 1832)	3	3	4	3.3	1	1	0	0.7
Neuroptera (Suborder: Hemerobiiformia)								
Family: Chrysopidae								
<i>Chrysoperla carnea</i> (Stephens, 1836) ¹	5	2	2	3.0	2	1	0	1.0
Orthoptera								
Family: Acrididae								
<i>Dociostaurus maroccanus</i> (Thunberg, 1815) maroccanus	4	2	3	3.0	2	1	0	1.0
Family: Tettigoniidae								
<i>Tettigonia viridissima</i> (Linnaeus, 1758)	5	1	3	3.0	0	1	2	1.0
Total	479	415	281	391.7	193	174	98	155.0

*Dominant species with 15% representation; **sub-dominant (from 5% to 15%); ***secondary (1%–5.5%); ****tertiary (<1%); ¹useful species; ² name of author's species.

stage, and a higher density was found in the reproductive stages of pod development and seed filling. Predatory bugs represented 40.0% of the total fauna of Heteroptera as the dominant species was *Campylomma verbasci* (26.7%), with sub-dominant *Nabis* sp. (7.6%). A probably regulatory role as a predator was exhibited by *C. verbasci*.

The sole representative of suborder Sternorrhyncha (Hemiptera) was *Acyrtosiphon pisum*. Aphids migrated from multiannual legumes, mainly from alfalfa crop, to lupin in the budding stage (second half of May). Their number increased proportional to the occurrence of flowering and reached the maximum values at the beginning of pod development. During seed filling, the aphid density decreased and when the seed approached physiological maturity, they migrated onto the other legumes. Aphids formed small colonies on lupins in the observed fields and did not exceed the economic threshold level of aphid populations (500 individuals/100 sweepings) in the period 2012–2014.

Hymenoptera species had a low density and were presented mainly by pollinating insects. The most common visitors were *Bombus sylvarum*, *Halictus maroccanus* and *Andrena dorsata* in the flowering stage.

Order Thysanoptera was represented by four phytophagous species and one predator, from three families. *Thrips atratus* (42.2%), *T. tabaci* (26.7%) and *Aeolothrips intermedius* (20.0%; predator) were the most widespread and were abundant on leaves, flowers and plant berries.

Insect species composition from order Diptera was represented by *Liriomyza* spp. (35.5%). Other species were indeterminate (64.3%).

Order Lepidoptera was represented by three phytophagous

species from family Noctuidae, which had low numbers in the years of study.

Order Neuroptera was represented by one species, namely *Chrysoperla carnea*, from family Chrysopidae. It had low density and was not an important potential predator.

Order Orthoptera was represented by two phytophagous species from Acrididae and Tettigoniidae families. Their numbers were low and did not pose a threat of damage to the lupin field.

Fauna on *L. luteus*

In 2012, 2013 and 2014, the captures totaled 192, 174 and 98 specimens, respectively. The insect assemblage in *L. luteus* was represented by 56 species, belonging to eight orders and 25 families. Among the identified species, 45 species were herbivorous, nine were predators and two were pollinating insects. The harmful species belonged to six orders: Coleoptera, suborder Polyphaga; Hemiptera with three suborders: Cicadomorpha, Heteroptera and Sternorrhyncha; Thysanoptera, suborder Terebrantia; Lepidoptera; Orthoptera, suborder Caelifera; and Diptera, suborder Brachycera. The most diverse were Hemiptera (24 species) and order Coleoptera (19 species). Suborder Cicadomorpha was represented by 11 species and four families. The predominant species during the vegetation period were the species from the family Cicadellidae. *E. pteridis* (26.7%), *P. striatus* (26.7%) and *A. atomarius* (18.8%) also had a relatively high representation.

Order Hemiptera, suborder Heteroptera included four families, 11 genera and 12 species, four among which were predators. The most species-rich family was Miridae, represented by

seven species. Relatively high representation was shown by *A. lineolatus* (33.3%), followed by *L. pratensis* and *L. rugulipennis*.

Seven families and 19 species represented suborder Polyphaga of order Coleoptera. Sixteen of them were herbivorous and three were predators. An important group of lupin pests were the weevils (family Curculionidae), which were the most numerous (66.3% of the total Coleoptera) and a diversified group among Coleoptera. The dominant species were *S. lineatus* (32.7%) and *S. macularius* (24.0%).

Four phytophagous species and one predator represented the assemblage of order Thysanoptera.

Order Hymenoptera included two species from one family; order Diptera showed species from *Liriomyza* genus; order Lepidoptera was represented by three species and one family; order Neuroptera had one species; and order Orthoptera included two species and two families.

In general, the results of our study showed that *L. luteus* had considerably lower insect density than *L. albus*. The insect preference for *L. albus* than for *L. luteus* was due to a number of phenological, morphological and chemical factors (described later).

Phenological plant development

The analysis of results on a comparative study of the two species of lupin showed substantial differences in the quantitative composition of the insect fauna. The species composition in white lupin was represented by 64 species, which accounted for 71.7% of the total insect number, while the yellow lupin was represented by 56 species, which accounted for only 28.3% of the total insect density. One of the main reasons for the considerable disparities in the insect density in both lupin species was related to a discrepancy between the phenological development of the plant host and life cycle of the species. *L. albus* was characterised by a significantly longer growing season (102 d) compared with *L. luteus* (90 d) (Table 3). In addition, *L. albus* had a significantly shorter vegetative growth (the period of germination–flowering was 38 d) but considerably longer reproductive period (64 d). The opposite tendency was observed in *L. luteus*, as the vegetative period (49 d) was longer than the reproductive period (40 d). The dominant harmful species, namely, cicadas, bugs and pea

aphids, predominated mainly in the reproductive period of *L. albus*. The feeding and oviposition of pests were favoured by a synchronism between the harmful insect activity and the long term after the flowering period. *L. luteus* was outlined as the less-preferred species due to its shorter and intense reproductive period.

Chemical composition of the above-ground mass

The probable reason for the considerably higher insect numbers, in particular, sucking insects (pea aphid, thrips and true bugs) and *Sitona* weevil, in *L. albus* may be associated with differences in the chemical composition of the above-ground mass. The results showed that the white lupin had a significantly higher CP content and lower content of CF (Table 4). Phosphorus content in 2012 and the average content for 2012–2014 were significantly higher in *L. albus*. A marked trend concerning the macro element calcium was not observed.

The results of the regression analysis (analysis of variance [ANOVA]) of the insect density in regard to the chemical traits showed a significant difference (Table 5).

Model 1 was obtained, which determined the complexity character of the density variation according to the investigated plant characteristics.

The equation of the obtained regression was as follows:

$$Y = 739.62 + 0.643 \cdot X_1 - 4.400 \cdot X_2 + 0.757 \cdot X_3 + 680.141 \cdot X_4 \quad (1)$$

where Y is insect density, X_1 is CP, X_2 denotes CF; X_3 stands for calcium, and X_4 represents phosphorus.

The phosphorus content of plant had the highest significant influence on insect density (680.1), while the CF content had a significant negative influence (−4.4) (Table 6). CP and calcium had little impact.

Morphological characteristics of seeds

Preferences of sucking pests could be related to some differences in the generative organs. In the present study, certain dependencies in physical and morphological characteristics of seeds in relation to the degree of damage from plant bugs were found (Tables 7 and 8).

White lupin, which had a significantly higher proportion

Table 3. Duration of vegetative, reproductive and growing seasons in *Lupinus albus* and *L. luteus*, in terms of number of days

Lupin species	2012			2013			2014			2012–2014		
	VP	RP	GS	VP	RP	GS	VP	RP	GS	VP	RP	GS
<i>Lupinus albus</i>	36a	64b	100b	34a	69b	103b	44a	60b	104b	38a	64b	102b
<i>Lupinus luteus</i>	50b	39a	89a	48b	39a	87a	50a	43a	93a	49b	40a	90a
LSD _{0.05%}	9.621	9.034	9.306	6.164	7.057	13.186	6.205	4.110	2.246	5.172	1.936	7.216

Means in each column followed by the same letters are not significantly different ($P > 0.05$).

VP = vegetative period; RP = reproductive period; GS = growing season; LSD = least significant difference.

Table 4. Chemical composition of above-ground dry mass in *Lupinus albus* and *L. luteus* (g/kg dry matter)

Lupin species	Year	Dry matter	Crude protein	Crude fibre	Calcium	Phosphorus
<i>Lupinus albus</i>	2012	91.4a	222.4b	165.5b	1.063a	0.448b
<i>Lupinus luteus</i>		91.7a	136.8a	184.0a	1.097b	0.246a
LSD _{0.05%}		3.967	11.545	15.920	0.033	0.202
<i>Lupinus albus</i>	2013	92.8a	186.0b	152.4a	1.710b	0.277a
<i>Lupinus luteus</i>		91.8a	130.3a	173.0b	1.199a	0.314a
LSD _{0.05%}		5.510	26.415	5.510	0.388	0.084
<i>Lupinus albus</i>	2014	88.4a	108.7b	176.2a	1.114a	0.355a
<i>Lupinus luteus</i>		88.0a	89.5a	193.1b	1.220a	0.309a
LSD _{0.05%}		5.614	6.803	8.659	0.216	0.056
<i>Lupinus albus</i>		90.9a	172.4 b	170.3a	1.30a	0.36b
<i>Lupinus luteus</i>	2012–2014	90.5a	118.9 a	177.7b	1.17a	0.29a
LSD _{0.05%}		4.868	13.478	5.680	0.205	0.049

Means in each column followed by the same letters are not significantly different ($P > 0.05$).
LSD = least significant difference.

Table 5. Regression coefficients of the insect density in regard to the chemical composition of above-ground dry mass

Trait	Coefficients	Standard error	t-statistic	P-value	Lower 95%	Upper 95%
CP	0.643	0.072	8.984	0.071	-0.266	1.552
CF	-4.400	0.300	-14.688	0.043	-8.207	-0.594
Ca	0.757	11.936	0.063	0.960	-150.907	152.421
P	680.141	24.393	27.882	0.023	370.194	990.087

CP = crude protein; CF= crude fibre.

Table 6. Regression analysis (ANOVA) of the insect density in regard to the chemical traits

Dispersion	df	SS	MS	F-ratio	P-value
Model	4	100,976.0	25,244.1	1,654.00	0.0182
Residual	1	15.2624	15.2624		
Total (Corr.)	5	100,992.0			

ANOVA = analysis of variance; SS = sum of squares; MS = mean of squares; Corr. = corrections.

Table 7. Morphological characteristics of lupin species (UPOV), average 2012–2014

Lupin species	Weight of 1,000 seeds, g	Seed shape	Seed colour	Seed surface	Hilum colour
<i>Lupinus albus</i>	246.51	Rounded seeds, bilaterally flattened	White	Smooth with slight recess on both seed sides	Light
<i>Lupinus luteus</i>	81.37	Rounded seeds, bilaterally flattened	White with dark brown spots on entire surface	Slightly wrinkled surface of seeds	Light

UPOV = The International Union for the Protection of New Varieties of Plants.

Table 8. Proportion of damaged seeds by harmful bugs in white and yellow lupin, in percentages

Lupin species	2012	2013	2014	2012–2014
<i>Lupinus albus</i>	17.3b	20.5b	6.4a	14.7b
<i>Lupinus luteus</i>	6.3a	6.2a	0.7a	4.4a
LSD _{0.05%}	7.910	8.953	6.396	7.579

Means in each column followed by the same letters are not significantly different ($P > 0.05$).
LSD = least significant difference.

of damaged seeds (average: 14.7% by plant bugs), was characterised by medium-to-large-sized grains (246.51 g), white colour and smooth surface with a slight recess on both seed sides. Yellow lupin had small seeds (81.37 g), white colour with dark brown spots and a slightly wrinkled surface of seeds. Regarding the form of seeds and hilum colour, differences between the two species were not observed.

Discussion

Until now, only a few studies concerning insect fauna on lupin have been reported. A study of the literature showed that almost no intensive study of the lupin insect fauna had been carried out in Europe. Insect fauna on lupin was studied for the first time in Bulgaria. In the country, *E. zinckenella* was reported as a single seed pest.

L. albus and *L. luteus*

E. pteridis, *P. striatus* and *R. panzeri* (Hemiptera, Cicadomorpha) were the dominant and potential lupin pests in every year of the study.

E. pteridis is a polyphagous insect pest and was announced as one of the main insect pests on a variety of cultivated crop plants including alfalfa, clover, soybean, vetch, sugarbeet, potatoes, vegetables and trees (Bunker *et al.*, 2007; Flory *et al.*, 2008; Nikolova and Georgieva, 2011). Apart from the direct damage due to *E. pteridis*, it is a vector for viral disease (Mazzoni, 2006). The feeding by cicada caused distortion of physiological processes in plants, in addition to suppressing the photosynthesis and stomatal conductance (Mattingly, 2008). Zhao *et al.* (2010 a, b) reported that on feeding by *E. pteridis*, plant growth slowed, and the result was a reduction of seed yield.

P. striatus and *R. panzeri* are widely distributed in Europe and Asia, and they are also a vector of viral disease (Li-qin *et al.*, 2010 a, b; Zhao *et al.*, 2010 a, b). Leafhopper vectors *E. pteridis*, *Cicadella aurata* and *P. striatus* were the main vectors in lupin fields (Maramorosch and Mitsuhashi, 2012). However, information concerning lupin leafhoppers is minimal.

The sole representative from the suborder Sternorrhyncha (Hemiptera) was *A. pisum*. Aphid species, particularly *A. pisum*, were reported to be one of the most important pests of white and yellow lupin (Kordan *et al.*, 2008). According to Walker *et al.* (2011), aphids can be an intermittent problem at the late vegetation, budding and flowering stages in lupins and they reduce pod set. Leaf aphids transmit viral diseases, such as bean yellow mosaic virus (BYMV) and cucumber mosaic virus (CMV), and were capable of causing an extensive loss in the lupins *L. albus*, *L. luteus* and *L. angustifolius* (The Connecticut Agricultural Experiment Station, 2007). In our study, aphids were found in lupins, but their numbers were

low and did not exceed the ETL (economic threshold level). One of the probable reasons for this is the existence of certain levels of aphid tolerance. Adhikari *et al.* (2012) reported that some genotypes of yellow lupin have medium tolerance to aphids, probably related to a lower content of alkaloids.

From order Hemiptera, suborder Heteroptera, the dominant harmful species was *A. lineolatus*, followed by the subdominant species *L. pratensis*, *L. rugulipennis* and *P. lituratus*. Listed bugs (*Adelphocoris* and *Lygus* genera) are the dominant insect pests in legume forage crops (Dimitrov, 2008; Nikolova, 2010; Petrova *et al.*, 2010), including lupins (Tanigoshi and Babcock, 1989b). Feeding by plant bugs and stink bugs is accompanied by the injection of saliva into the stems, leaves or seed pods and disruption in plant development. Penetration by the stink bug stylet can inflict physical impairment in plants. Both the mechanical injury and enzymatic harm to the plant's growing point may be responsible for the injury. Tanigoshi and Babcock (1989a) concluded that *Lygus hesperus* Knight and *L. elisus* Van Duzee were the main lupin pests damaging the above-ground biomass and seed pods on *L. albus* and, due to the damage, the yield reduced 1–43 times. Kondorosy (2001) studied the Heteroptera fauna of different fodder legumes in Hungary, including *Lupinus* sp., and found that the most frequent pests on *Lupinus* spp. were *Lygus rugulipennis* and *P. lituratus*. Lu and Gross (2010) reported that *Lygus* species were related to the drippy pod disease, which is due to the bug's feeding action on lupin plants. In addition, if the disease rapidly spreads across the field, almost all lupin plants will be infected.

From order Coleoptera, suborder Polyphaga, the *Sitona* weevils were the dominant species in the present study, every year as well as on average for the period, and the weevils comprised a considerable representation in the total population of injurious coleopterous insects. They feed on many annual and perennial legume crops, and some of them are very important insect pests (Hurej *et al.*, 2013). *Sitona* weevils were associated with the enormous loss in both crop yield and quality of some legume species, and the harm caused by them will increase in coming years (Corre-Hellou and Crozat, 2005). *Sitona* are important pests in lupins (White and Pty, 2002, Botha and Hardie, 2005, Hanavan *et al.*, 2008; Hurej *et al.*, 2013). However, just a few studies regarding *Sitona* damage on lupin plants have been published. In Portugal, in the past century, it was found that approximately 80%–100% infestation level on yellow lupin resulted in serious yield loss (Silva and De Oliveira, 1959). Ströcker *et al.* (2011) reported that the most frequent pests in German lupin crops were the specific lupin weevils *Sitona (Charagmus) gressorius* and *S. (Ch.) griseus*. The authors proposed the breeding of less-susceptible cultivars as an alternative pest management strategy. During our study, the weevils *Tychius lineellus* and *Otiiorhynchus* sp. were extremely rare

in the collected samples, while in other countries, at different ecologic conditions, they were an important group of lupin pests (Severns, 2008). *C. gracilis* (Cerambycidae) was a sub-dominant species. It was reported as an important pest in hawthorn, with the larval development being carried out in hawthorn branches (Walczak *et al.*, 2014). Adults may be attracted by the lupin smell and the inflorescence of plants. The soldier beetle *R. fulva* and the soft-wing flower beetle *C. elegans* were the sub-dominant species and had high representation in the collected material from Coleoptera order. These species feed on small insects inhabiting the inflorescence of plants or woodboring insects. Other predators such as *C. septempunctata* and *H. variegata* were found in lupin, but their numbers were low probably because aphid density was also low. Hodek and Michaud (2008) reported that *C. undecimpunctata* was an important potential predator, while Finlayson *et al.* (2010) found that among *C. trifasciata* L., *C. septempunctata*, *Harmonia axyridis* and *Propylea quatuordecimpunctata* (Coccinellidae) on lupin, *H. axyridis* was the most effective aphid predator. In addition, *P. quatuordecimpunctata* had the highest population density on *L. polyphyllus*.

Fauna of order Diptera was not fully identified. It was represented by 35.5% *Liriomyza* spp. and 64.3% indeterminate species. Bygebjerg *et al.* (2011) reported that the larvae of the Nearctic species *Chyliza leguminicola* consume the roots or stems of *L. polyphyllus* and probably its pest status in Europe will increase in the coming years. The project AR0138 (2003), which dealt with white lupins, reported that *Delia platura* was the major insect pest. The larvae consumed and damaged the insides of newly emerged seedlings when the crops were sown in autumn, and it was not feasible to foresee when the injury would be serious.

Among the bees, *B. sylvarum* (Hymenoptera; suborder: Apocrita; Family: Apidae) was more common than others, but the general bee density was very low. Bernhardt *et al.* (2008) found 271 pollinating insects in the lupin field in northwestern Ohio and southeastern Michigan, and the main types of bees were the species belonging to the genera *Bombus* and *Osmia*, along with *Apis mellifera* and *Xylocopa virginica*. In addition, in a project of the Food and Agriculture Organisation of the United Nations (2007), *Patellapis (Zonalictus)* spp. (Hymenoptera, Halictidae) were found in a *L. luteus* field.

The order *Lepidoptera* was represented by *Helicoverpa armigera*, *Mamestra brassicae* and *E. zinckenella*. These species had low numbers in the years of study. Miles *et al.* (2007) reported that damage by brown pasture looper (*Ciampa arietaria*, Geometridae) mainly has been recorded in lupin crops.

In general, insect fauna on *L. alba* and *L. luteus* had similar species composition as population density on *L. luteus* was considerably lower.

Phenological, morphological and chemical plant indicators

Contemporary insect pest management commonly includes chemical and alternative tactics allowing for more sustainable crop protection and support of the natural biodiversity. Practising multiple pest management approaches will strongly reduce the risk of adaptation to one or other practices (Russell 2013). The use of pest-resistant or tolerant crops where pest population is supported at allowable levels is one of the appropriate methods of control.

The synchrony between the life cycles of the dominant harmful species, such as cicadas, phytophagous bugs and *Sitona* weevils, and the availability of a long-term flowering period in *L. albus* favoured the feeding and oviposition of pests. *L. luteus* was outlined as the lesser-preferred lupin species probably due to the shorter and intense reproductive period. The duration of the reproductive period in *L. luteus* was, on average, 40 d, while in *L. albus*, it was an average of 64 d, and there is a statistically significant difference. The longer reproductive period favoured harmful insect feeding and reproduction, supplying nutrients for a longer period.

The development and use of lupin cultivars with short and intense reproductive periods can support an environmentally friendly protection against the herbivorous insect.

Factors such as plant morphological characteristics, nutrients and others could alter density-dependent processes in insect populations by affecting the availability of resources (Miller, 2007). Conflicting opinions on the role of morphological seed characteristic as universal indicators of resistance exist in the literature (Somta *et al.*, 2007). In the present study, yellow lupin had small seeds, white-coloured appearance with dark brown spots and slightly wrinkled surface. Seven harmful bug species of Miridae family caused considerably less damage on *L. luteus* plants, compared to that on *L. albus*. The results showed that harmful bugs caused negligible damage to *L. luteus*, which had small-sized seeds.

Mineral content of above-ground biomass was associated with the nutritional regime of plants and insect feeding and therefore can induce positive, negative or indifferent reactions in plants (Dale, 1988). Jansson and Ekbom (2002) studied the influence of mineral application on the physiology and behaviour of insects. In the present study, *L. albus* had higher CP and phosphorus contents and considerably higher insect density than *L. luteus*. According to some authors (Woods *et al.*, 2004; Vannette and Hunter, 2009), the fecundity of sucking insects was often reduced when they consumed plants with lower nutritional value, and herbivorous insects were more fecund or reproductive on plants with higher leaf contents of nitrogen and phosphorus. Ricklefs (2008) reported that plant leaves with higher concentrations of phosphorus and water had a higher degree of leaf damage. The author concluded that phosphorus was one of the important limiting factors for a wide range of insect pests. The presence

of toxic compounds, such as alkaloids and phenols, as well as some genetic factors, may be also a reason for lupin resistance to pests (Pilegaard and Gry, 2008). According to Schoonhoven *et al.* (2005), the quinolizidine alkaloids are repeatedly called lupin alkaloids, due to their general abundance in the plants of the genus *Lupinus* and their ability to act as feeding deterrents against various insects.

Insect fauna on lupin was studied for the first time in Bulgaria from 2012 to 2014 as *L. albus* was represented by 64 species; while *L. luteus* had similar insect species composition represented by 56 species. Insect pests preferred *L. luteus* to a lesser extent. The dominant harmful species in lupins were cicadas, phytophagous bugs and *Sitona* weevils. The use of lupin cultivars with shorter and intense reproductive periods, along with a lower content of CP and phosphorus in the above-ground dry mass, may give an environmentally friendly protection against insect pests.

A study of the literature showed that no intensive study of the lupin insect fauna had been carried out in Europe. The survey is of primary importance because of the following information:

1. The presence of potential pests is ascertained and some understanding of the possible importance of these potential pests is obtained.
2. Any possible differences between the insect faunas associated with the two lupin species (*L. albus* and *L. luteus*), which may indicate possible differences in levels of insect damage, is elucidated.
3. The possible reasons for spring-sown lupin in Europe remaining relatively pest free is as follows:

The build-up of insects that occurs by late summer is avoided because the crop is planted early or early-maturing varieties are used. Aphids thus appear as the most likely insects to seriously affect lupin because of their presence in the spring and their potential for rapid build-up in numbers.

The rapid growth and maturity of the pods mean that when grown for seeds, they remain exposed to insect attack for only a short period.

The harvest appears a less-suitable host for aphids when compared to the legumes, lucerne and broad beans. This reduces feeding and fecundity, thereby decreasing the likelihood of serious aphid damage.

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