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Diversity of arthropods and mollusks associated with lettuce

Diversidad de artrópodos y moluscos asociados al cultivo de lechuga

Maria Aparecida Cassilha Zawadneak[1]*, *Josélia Schuber*[1], *Osmir Lavoranti*[2], *Francine Lorena Cuquel*[3]

ABSTRACT

Despite the economic importance of lettuce, no studies have examined the diversity of species associated with this crop that could contribute to the development of an ecological management of phytophagous arthropods and mollusks. This study was aimed at determining the predominant species of arthropods and mollusks in two lettuce cultivars (curly-leaf Veronica and smooth-leaf Elisa). A total of 9,638 specimens of arthropods and mollusks was collected and identified to 27 constant species, 17 classified as accessory and two as accidental species. Seven species of aphids were identified, with Nasonovia *ribisnigri, Uroleucon sonchi*, and *Uroleucon ambrosiae* as the most frequent, as well as three species of thrips, predominantly *Caliothrips phaseoli* and *Frankliniella schultzei*. Other phytophagous arthropods and mollusks occurred at frequencies below 10%. Among natural enemies, predators of the families Syrphidae (2.74%), Coccinellidae (1.66%), Staphylinidae (0.79%) and Carabidae (0.17%) and parasitoids of the order Hymenoptera (0.93%) were observed. Of the total specimens collected, 53.87% are considered phytophagous species, mostly aphids (40.82%), followed by thrips (11.35%). The aphid *N. ribisnigri* was the main pest species, as it forms colonies in the lettuce head and can spread plant viruses. Phytophagous pests preferred smooth over curly leaves.

Key words: *Lactuca sativa*, pests, natural enemies, constancy index, biological control, biodiversity.

RESUMEN

A pesar de la importancia económica de la lechuga, no hay estudios que hayan analizado la diversidad de especies asociadas a este cultivo que podrían contribuir al manejo ecológico de los artrópodos fitófagos y moluscos. Este estudio tuvo como finalidad determinar las especies predominantes de artrópodos y moluscos en dos cultivares de lechuga (Veronica de hoja rizada y Elisa de hoja lisa). De un total de 9.638 ejemplares de artrópodos y moluscos se identificaron 27 especies comunes, 17 fueron clasificados como especies accesorias y dos especies, como accidentales. Se identificaron siete especies de áfidos, siendo Nasonovia ribisnigri, Uroleucon sonchi, Uroleucon ambrosiae*, las más frecuentes. En trips se identificaron tres especies, siendo las más frecuentes* Caliothrips phaseoli *y* Frankliniella schultzei*. Otros artrópodos fitófagos y moluscos fueron identificados en frecuencias por debajo del 10%. Entre los enemigos naturales, se identificaron depredadores de las familias Syrphidae (2,74%), Coccinellidae (1,66%), Staphylinidae (0,79%) y Carabidae (0,17%) y parasitoides del orden Hymenoptera (0,93%). De las muestras totales recolectadas, 53.87% se consideran especies de fitófagos, principalmente áfidos (40.82%), seguido por trips (11,35%). El áfido* N. ribisnigri *fue la principal especie plaga ya que forma colonias en la cabeza de lechuga y puede propagar virus en las plantas. Los fitófagos prefieren las lechugas de hojas lisas por sobre las rizadas.*

***Palabras clave**:* Lactuca sativa*, plagas, enemigos naturales, índice de constancia, control biológico, biodiversidad.*

Introduction

Lettuce is the leaf vegetable most consumed in Brazil and accounts for 50% of all domestic production and marketing of leafy greens. The two most consumed lettuce varieties are curly-leaf, representing over 50%, and iceberg, followed by smooth-leaf, mimosa, red, romaine and mini. Many insects attack this crop, impair the development of plants, affect the commercial aspect of leaves and spread several viral diseases.

[1] Departamento de Patologia Básica, Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba, Paraná, Brazil.
[2] Centro Nacional de Pesquisa de Florestas, Empresa Brasileira de Pesquisa Agropecuária, Colombo, Paraná, Brazil.
[3] Departamento de Fitotecnia e Fitossanitarismo, Universidade Federal do Paraná, Curitiba, Paraná, Brazil.
* Corresponding author: mazawa@ufpr.br

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Information on the insect species that occur in lettuce is essential for management strategies, since the correct taxonomic classification of pests allows the protection and the use of natural enemies more efficiently, and consequently decreases pesticide use. The aphids *Nasonovia ribisnigri* (Mosely), *Aulacorthum solani* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas), and *Myzus persicae* (Sulzer) are considered the most harmful to lettuce (Smith *et al*., 2008; Farsi *et al*., 2014). The thrips *Frankliniella schultzei* (Trybom), *Thrips palmi* (Karny) and *Thrips tabaci* Lindeman are reported as the main lettuce pests (Imenes *et al*., 2000; Broughton and Herron, 2009). The caterpillars *Spodoptera frugiperda* (J.E.Smith), *Trichoplusia ni* (Hübner), *Helicoverpa zea* (Boddie), *Heliothis virescens* (Fabricius) and *Agrotis ípsilon* (Hufnagel) have also been reported attacking lettuce plants (Santos *et al*., 1992; Imenes *et al*., 2000). Among other arthropods that attack lettuce are leaf-miner flies, some beetles, mole crickets, crickets, springtails, slugs and snails (Imenes *et al*., 2000; Gómez-Polo, 2014).

Insects that control pests are included in many orders, including (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae), syrphid flies (Diptera: Syrphidae), flower bugs (Hemiptera: Anthocoridae) and parasitoids (Hymenoptera). There are also predatory mites (Acari: Phytoseiidae) and spiders (Arachnida) (Smith *et al*., 2008). The identification of species with the potential to be biological control agents of pests is necessary in an integrated pest management program. This can define which measures need to be adopted to promote the conservation and reproduction of these organisms, in order to reduce the use of chemicals in pest control. Information on the fluctuation of pest and natural enemy populations is essential in an integrated pest management. Periods of highest pest infestation, distribution, and damage to plants associated with precipitation and temperature determine the suppression or growth of an insect population. This study was aimed at determining the dominant arthropod and mollusk species in two lettuce cultivars (Curly and Smooth Leaf) and to examine the influence of climatic factors on these insects.

Materials and Methods

The study was carried out in the field (25º25 ‘S, 49º08’ W, 930 m altitude); the climate is Cfb, according to the classification of Köppen. The experimental area was fallow for two years, in three cycles corresponding to the three seasons of the year (cycle I spring; II summer; III fall). The Commission of Soil Chemistry and Fertility performed fertilization and agricultural practices according to the recommended standards for lettuce. Insecticides were not applied for pest control during plant growth, weeds were removed manually and irrigation was done by rotating sprinklers in the entire field.

For each crop cycle, three randomized blocks of 33.6 x 1.20 m, spaced at 0.5 m were set up with 28 replicates each, and two lettuce cultivars (Curly Leaf Verônica and Smooth Leaf Elisa). The area of each experimental plot was 2.52 m^2 (2.10 x 1.20 m) and consisted of four rows of plants with spacing of 0.3 x 0.3 m between plants and rows, totaling 28 plants per plot, resulting in a density of 100,000 plants/ha. Combining the three crop cycles, the experimental area totaled 0.5 hectare. The two central rows were defined as useful area, totaling ten plants. A row of lettuce plants was left as border between plots.

Two sampling collections were performed in each cycle, at 24 and 44 days after transplantation. The means of the two assessments were included in the statistical analysis. Two plants were selected per plot in each sampling, covered with white polypropylene plastic bags (0.50 x 0.60 m) and then cut close to the soil. In the laboratory each lettuce plant was examined leaf by leaf, and the arthropods and mollusks present were collected and preserved in 70% ethanol. Specimens were prepared and identified.

The species collected were classified according to the Constancy index (Bodenheimer, 1955). For each species, Constancy (C) was determined with the equation: $C (\%) = (ncsp_n \times 100)/NC$, where $ncsp_n$ is the number of collections where the study species was present and NC is the total number of collections. Based on the results, the species were classified as: Constant (X) –when present in more than 50% of the collections, Accessory (Y)– when present in 25 to 50% of collections, and Accidental (Z) - when present in less than 25% of collections. The results were compared with an analysis of variance performed with the software Sisvar 4.0 after being transformed into the square root of x + 1.

The resistance to penetration of lettuce leaves was measured on ten leaves of each of the cultivars using a most consumed Texture Analyzer CT3 (Brookfield, Middleboro, USA), Kramer Shear press with blades (TA-91) and a platform to adapt the blade. Each leaf was rolled from the central vein outwards and cut at the ends, resulting in a length of 5.5 cm. The leaves were positioned transversely to the blades, which operated in the compression mode, with trigger load of 8 N, pre-test speed, test and post-test of 2.00, 1.00 and 1.00mm/s, respectively, and 70.0 mm cutting distance.

Results and Discussion

A total of 9,638 arthropod and mollusk specimens associated with lettuce were collected and identified. Of the 46 species, 27 were classified as constant, 17 as accessory and two as accidental (Tables 1, 2 and 3). Among the species considered lettuce pests, aphids (Hemiptera: Aphididae) and thrips (Thysanoptera: Thripidae) were the most frequent. Aphids were found in larger numbers, with 3,934 specimens (40.82%), followed by thrips with 1,094 specimens (11.35%) (Table 1). These results corroborate those reported by Santos *et al.* (1992), Imenes *et al.* (2000), Smith *et al.* (2008), Sæthre *et al.* (2011) and Farsi *et al.* (2014), who reported the importance of these groups as lettuce pests.

Seven species of aphids were identified associated with lettuce: *Aphis fabae* Scopoli, *A. solani, M. euphorbiae, M. persicae, N. ribisnigri, Uroleucon ambrosiae* (Thomaz), and *Uroleucon sonchi* (Linnaeus). The species *N. ribisnigi, U. ambrosiae* and *U. sonchi* were the most frequently collected (Table 1), contrary to the report of Santos *et al.* (1992) that found only *M. persicae*. Imenes *et al.* (2000), however, described the occurrence of the same species except *N. ribisnigri*. In the state São Paulo, Yuki (2000) found a higher incidence of *M. persicae*, *M. euphorbiae*, and *N. ribisnigri*, while Auad *et al.* (2002) reported the occurrence of

Table 1. Taxonomic classification, Constancy index and total number of phytophagous arthropods collected in two lettuce cultivars (*Lactuca sativa* L.) (cv. curly-leaf Verônica and cv. Smooth-leaf Elisa) Pinhais, PR, 2004/05.

Order	Family	Species	Trophic relationship[(1)]	Total	Constancy[(2)]
Coleoptera	Chrysomelidae	*Diabrotica speciosa* (Germar)	F	12	X
Diptera	Agromyzidae	*Liriomyza sativae* (Blanchard)	F	34	X
Hemiptera	Aphididae	*Aphis fabae* (Scopoli)	F	15	X
		Aulacorthum solani (Kaltenbach)	F	7	Y
		Macrosiphum euphorbiae (Thomaz)	F	45	X
		Myzus persicae (Sulzer)	F	26	X
		Nasonovia ribisnigri (Mosley)	F	3328	X
		Uroleucon ambrosiae (Thomaz)	F	200	X
		Uroleucon sonchi (Linnaeus)	F	313	X
	Cicadellidae	*Agallia* sp. (Curtis)	F	27	X
		Agalliana ensigera (Oman)	F	29	X
Lepidoptera	Noctuidae	*Spodoptera frugiperda* (J.E.Smith)	F	3	Y
		Agrotis subterranea (Fabricius)	F	6	Y
Orthoptera	Gryllidae	*Gryllus assimilis (*Fabricius)	F	4	Y
Thysanoptera	Thripidae	*Caliothrips phaseoli* (Hood)	F	707	X
		Frankliniella schultzei (Trybom)	F	383	X
	Phlaeothripidae	*Bamboosiella cingulata* (Hood)	F	4	Z

(1) F: phytophagous. (2) Constancy of species = X: Constant; Y: Accessory; Z: Accidental.

Table 2. Taxonomic classification, Constancy index and total number of natural enemies collected in lettuce (*Lactuca sativa* L.) (curly-leaf Verônica and smooth-leaf Elisa).

Order	Family	Species	Trophic relationship[1]	Total	Constancy[2]
Coleoptera	Carabidae	*Lebia concinna* (Brullé)	P	16	X
	Coccinellidae	*Coleomegilla maculata* (DeGeer)	P	14	Y
		Cycloneda pulchella (Klug)	P	7	Y
		Cycloneda sanguinea (Linnaeus)	P	17	X
		Eriopsis connexa (Germar)	P	11	Y
		Harmonia axyridis (Pallas)	P	98	X
		Hippodamia convergens Guérin-Mén.	P	13	X
	Staphylinidae	*Stenus* sp. (Latreille)	P	30	X
		Xantholinus attenuatus (Erichson)	P	19	X
		Xantholinus sp. (Serville)	P	27	X
Diptera	Syrphidae	*Toxomerus* sp. (Macquart)	P	264	X
Hymenoptera	Braconidae	*Aleiodes* sp. (Wesmael)	PS	6	X
		Cotesia sp. (Cameron)	PS	4	Y
		Opius sp. (Wesmae)	PS	9	Y
	Eulophidae	*Euplectrus* sp. (Westwood)	PS	38	Y
	Aphelinidae	n.i.	PS	6	X
	Diapriinae	n.i.	PS	7	Y
	Encyrtidae	n.i.	PS	9	X
	Eucoilidae	n.i.	PS	7	Y
	Scelionidae	n.i.	PS	7	X

[1] P: predator; PS: parasitoid; n.i.: not identified. [2] Constancy of species = X: Constant; Y: Accessory; Z: Accidental.

Table 3. Taxonomic classification, Constancy index and total number of mollusks and other arthropods collected in two lettuce cultivars (*Lactuca sativa* L.) (curly-leaf Verônica and smooth-leaf Elisa).

Order/ Suborder	Family	Species	Trophic relationship[1]	Total	Constancy[2]
		Mollusca			
Gastropoda					
Stylommatophora	Xanthonychidae	*Bradybaena similaris* (Férussac)	F	9	X
Eupulmonata	Agriolimacidae	*Deroceras laeve* (Müller)	F	8	Y
		Arthropoda			
Collembola					
Entomobryomorpha	Entomobryidae	*Entomobrya* sp. (Rondani)	(?)	37	X
		Dicranocentrus sp. (Schött)	(?)	9	Z
Poduromorpha	Hypogatruridae	*Hypogastrura* sp. (Bourlet)	(?)	3464	X
	Onychiuridae	*Orthonychiurus* sp. (Stach)	(?)	113	Y
Symphypleona	Sminthuridae	*Katianna* sp. (Börner)	(?)	32	Y
Diplopoda					
Polydesmida	Paradoxosomatidae	*Orthomorpha coarctata* (DeSauss.)	F	21	Y
		Catharosoma sp. (Silvestri)	F	11	Y

[1] F: phytophagous; P: predator; (?) various food habits. [2] Constancy of species = X: Constant; Y: Accessory; Z: Accidental.

U. ambrosiae, *M. persicae*, and *M. euphorbiae* in hydroponic lettuce. The species found in the present study were collected by Carvalho *et al.* (2002) in a survey carried out in several farms in the state of Minas Gerais. These authors characterized the species found not only in lettuce, but also in the family Asteraceae to which lettuce belongs.

Aphid species are known to attack lettuce in the field. Farsi *et al.* (2014) reported that *N. ribisnigri* is a primary pest of lettuce. In the present study, *N. ribisnigri s*pecimens, unlike other aphid species, were found scattered in the entire lettuce head, including the center and developing leaves, while other species preferred outer leaves. These observations are consistent with those reported by Yuki (2000) and Liu and Mccreight (2006). Farsi *et al.* (2014) collected a large number of *N. ribisnigri* specimens in lettuce fields in Iran, followed by the aphid species *A. solani*, *M. euphorbiae* and *M. persicae* in lower numbers. Smith *et al.* (2008) collected predominantly *N. ribisnigri* in organic lettuce in the central coast of California. Pretorius *et al.* (2010) found five aphid species associated with shadehouse-cultivated lettuce, *Acyrthosiphon lactucae* (Passerini), *Aphis craccivora* (Koch), *M. euphorbiae*, *M. persicae*, and *N. ribisnigri*. Sæthre *et al.* (2011), studying the biodiversity of aphids and their natural enemies in several plant agroecosystems in Benin, reported the aphid species *A. craccivora* Koch, *Aphis gossypii* Glover, and *M. persicae* infesting lettuce plants.

Three species of thrips, *F. schultzei*, *Caliothrips phaseoli* (Hood) and *Bamboosiella cingulata* (Hood), accounted for 35.01% (accessory species), 64.63% (constant species), and 0.37% (accidental species) of total thrips, respectively. The species *C. phaseoli* and *F. schultzei* were observed damaging lettuce plants. The market value of lettuce is reduced because feeding causes a silver discoloration on leaves due to the reaction of insect saliva with plant tissue. Stunted growth and lack of development of the internal parts of the plant are also observed. In addition to adults, thrips larvae were observed. Four specimens of the species *B. cingulata* were collected, and despite the small number and consequently being classified as an accidental, this is the second record of this species in Brazil. Its first occurrence was reported by Pinent *et al.* (2003) in Viamão State Park, RS, where a few specimens were also collected. Of the species observed, only *F. schultzei* was reported in lettuce by Imenes *et al.* (2000), while *T. tabaci* and *T. palmi* cited by Imenes *et al.* (2000) were not found in our samples. Workman *et al.* (2007) found the thrips species *T. tabaci* and *F. occidentalis* in lettuce and the latter was frequently the dominant species and occurred in lettuce year round in New Zealand.

The phytophagous species with frequencies below 10% were classified as secondary (Tables 1 and 3) but are considered pests according to the literature (Imenes *et al.*, 2000). Mollusks represented 0.94% of the total phytophagous species collected (Table 3). *Bradybaena similaris* (Férussac) and *Deroceras laeve* (Müller) were classified as constant and accessory species, respectively. These mollusks cause lesions in the leaves, compromising the quality of the plant, and contaminate them with their feces. These data confirm the report of Imenes *et al.* (2000) but contradict the results reported by Bruschi-Figueiró and Veitenheimer-Mendes (2002), who considered *B. similaris* an accidental species.

Twenty-one species of natural enemies were identified among the 516 collected specimens, belonging to the families Coccinellidae and Carabidae of the order Coleoptera, and the family Syrphidae, Diptera. The parasitoids found belong to the order Hymenoptera (Braconidae, Eulophidae, Aphelinidae, Diapriinae, Encyrtidae, Eucoilidae and Scelionidae) (Table 2).

Six species of the family Coccinellidae were identified: *Coleomegilla maculata* (DeGeer), *Eriopsis connexa* Germar, *Cycloneda pulchella* (Klug), *Cycloneda sanguinea* (Linnaeus), *Hippodamia convergens* (Guérin) and *Harmonia axyridis* (Pallas), with the last as the most abundant (Table 2). Pretorius *et al.* (2010) found five species of predators associated with shadehouse-cultivated lettuce grown in South Africa, *Scymnus levaillanti* (Mulsant), *Hippodamia variegata* (Goeze), *Exochomus flavipes* (Thunberg), *Cheilomenes luneta* (Fabricius) and *Harmonia* sp. Farsi *et al.* (2014) surveyed the natural enemies of *N. ribisnigri* in lettuce and reported the coccinellid *S. levaillanti* (Mulsant.), *Coccinella septempunctata* (L.), *Coccinella novemnotata* (Herbst) and *Coccinella undecimpunctata* (L.). The first species was the most frequently observed, collected in high densities in the months of March and April. *C. septempunctata* was first observed in February and peaked in March.

Two morphospecies of a syrphid predator of the genus *Toxomerus* were found in the three crop cycles. In Cycle II, with the reduction of the aphid population, the number of coccinelids decreased drastically. However, a growing population of syrphid larvae was observed during Cycles II and III, despite the reduced number of aphids. The population of syrphid larvae was maintained and observed feeding on *Hypogastrura* sp. (Collembola: Poduridae). Springtails can be an alternative food for natural enemies when their preferred prey is scarce, helping stabilize the population of natural enemies in the field. The feeding habits of larvae and their stable incidence during the three cycles is in agreement with the observations by Marinoni and Bonatto (2002), who described polyphagic behavior in syrphid larvae. In the surveys conducted by Marinoni and Bonatto (2002) in several localities of the state of Paraná, *Toxomerus* sp. was also the most abundant species. Smith *et al.* (2008) obtained adults from eggs, larvae and pupae collected from organically grown lettuce and identified mainly the syrphids *Toxomerus marginatus* (Say), *Platycheirus stegnus* (Say), *Sphaerophoria sulfuripes* (Thomson) and *Allograpta obliqua* (Say). *Syrphus pinator* Osten Sacken, *Toxomerus occidentalis* (Curran) and *Eupeodes volucris* Osten Sacken were less common.

Eighty-six specimens of parasitoids were collected and most belonged the superfamilies Chalcidoidea and Ichneumonoidea, followed by Cynipoidea and Platygastroidea (Table 2). Noctuidae caterpillars were parasitized by chalcid wasps of the families Eulophidae (*Euplectrus* sp.) and Braconidae (*Cotesia* sp.). Braconids of the genus *Cotesia* (Microgastrinae) are solitary or gregarious endoparasitoids of many macrolepidopterans and are used in biological control programs. The genus *Opius* (Opiinae) includes mostly parasitoids of leaf-miner flies of the genus Agromyzidae (Diptera), while *Aleiodes* (Rogadinae) parasitizes several groups of Macrolepidoptera (Noctuoidea, Geometroidea and Sphingoidea), and both are potential natural control agents of several pest lepidopterans of agroecosystems (Cirelli and Penteado-Dias, 2003). Andorno *et al.* (2014), studying aphids in lettuce in Argentina, observed the presence of mummies (visible signal of parasitism) from June and July until October.

Regarding the frequencies of phytophagous organisms sampled per cycle, in Cycle I (spring) aphids represented 94.60% of the total number of pests collected. In Cycle II, (summer) thrips accounted for 43.44% of the total of pests collected, followed by aphids with 40.34%. In Cycle III (fall) thrips predominated, with 72.09% of the total pests. The remaining pests were classified as secondary, as they were found at frequencies below 10%, but are phytophagous species recognized as pests in the literature (Figure 1).

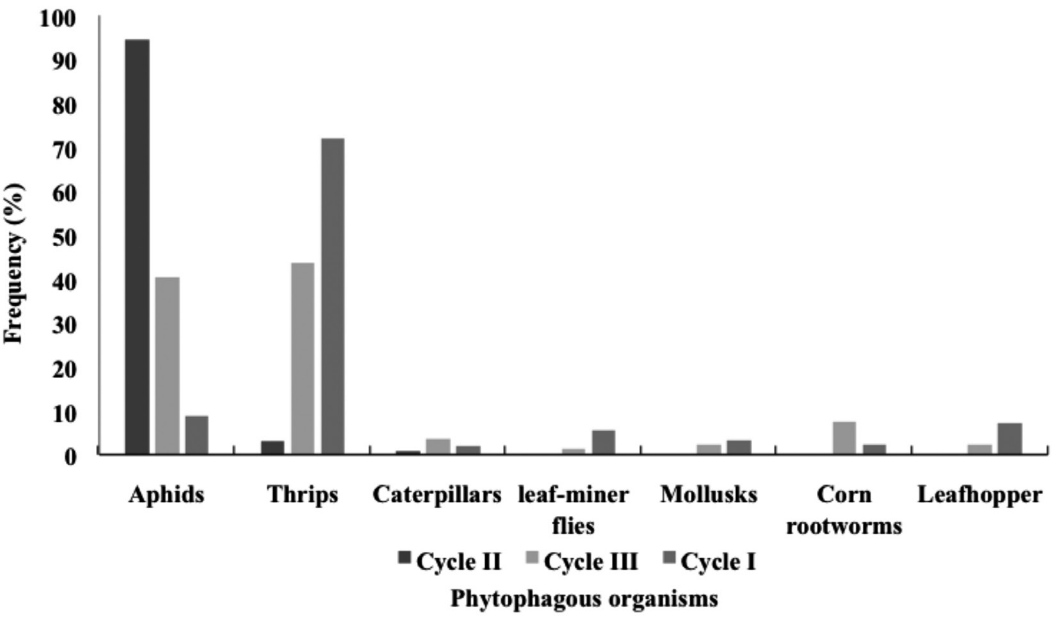

Figure 1. Frequency (%) of phytophagous organisms in lettuce (*Lactuca sativa* L.) in three crop cycles.

The analysis of variance of the number of dominant phytophagous insects (aphids and thrips) and secondary insects collected in the cultivars curly-leaf Verônica and smooth-leaf Elisa revealed significant differences (Table 4). For leaf-miner flies, caterpillars, mollusks, corn rootworms and leafhoppers, no statistically significant differences were found between means of the two lettuce cultivars. For aphids, smooth-leaf Elisa had 187% more aphids than curly-leaf Verônica. The same was observed for thrips, with 43% more individuals in smooth-leaf Elisa. The effect of the cultivars on the two dominant pests, aphids and thrips, was then examined.

A relation between aphid population and lettuce planting time and cultivar was found (Table 4); the same was observed for mollusks and leafhoppers. Aphid populations in both cultivars were higher in the spring. The population of mollusks and leafhoppers in smooth-leaf Elisa was significant in the fall, but no differences in the populations of mollusks and leafhoppers were observed for the curly-leaf variety. In the analysis of each crop cycle for each cultivar, the aphid population differed in Cycle I; it was greater in Elisa lettuce. No significant differences were found in the aphid population in the other two cycles.

The analysis of resistance to penetration demonstrated that resistance is much higher in curly leaves (207.25 ± 2.70), suggesting a preference of phytophages for smooth leaves (165.96 ± 2.48) (F = 5.191, *p* value = 0.004, CV = 10.89%) according to the Scott-Knott test. The occurrence of the two most abundant pests, *N. ribisnigri* and *C. phaseoli*, was correlated with meteorological variables. A negative and significant correlation (p<0.0001) was observed between temperature and the mean occurrence of aphids for both Verônica (r = –0.6022) and Elisa (r = –0.64161). These results indicate that under milder temperatures aphids are found in larger numbers in lettuce. These results agree with the findings reported by Auad *et al.* (2002), who concluded that temperature significantly influenced the population density of *U. ambrosiae* (r = –0.5294), for which 20 ºC is the most suitable temperature in hydroponic lettuce. A decrease in the development period in response to temperature increase is a common response in aphids. Morales and Fereres (2008) found that *N. ribisnigri* cannot fly when temperatures are under 17 ºC, concluding that the distribution of this species within the crop is carried out mainly by wingless individuals that move from leaf to leaf and on the soil.

A positive and significant correlation (p <0.0001) was found between relative air humidity and mean aphid incidence for both Verônica (r = 0.4759) and Elisa (r = 0.53287) cultivars. This might be due to high air humidity that favors the maintenance of water content in the insect body tissues, avoiding weight loss (Silveira Neto *et al.*, 1976). Since the body structure of aphids is poorly sclerotized with a high water content, an increase in temperature and decrease in relative air humidity could affect them adversely.

The populations of winged aphids vary considerably with changes in temperature (Diaz *et al.*, 2007). The optimum temperature for the development of *N ribisnigri* is between 25-27 ºC. Higher temperatures (26-28 ºC) have a greater impact on the mortality of adults and nymphs in the last instars than on nymphs in early instars (Diaz *et al.*, 2007). The populations of *N. ribisnigri* in lettuce decreased in late spring or early summer, indicating that high temperatures hinder the reproduction of *N. ribisnigri*, since no nymphs were found at or above 28 ºC (Diaz *et al.*, 2007). Morales and Fereres (2008) found that while lettuce plants are in good condition, *N.*

Table 4. Mean number of specimens and mean square error and *p*-value of aphids, thrips, leaf-miner flies, caterpillars, mollusks, corn rootworms and leafhoppers collected in the lettuce cultivars curly-leaf Verônica and smooth-leaf Elisa with organic and mineral soil amendments.

Letture cultivar	Pests						
	Aphids	Thrips	Caterpillars	Leaf-miner flies	Mollusks	Cornrootworms	Leafhopper
Curly-leaf	4.0 ± 0.25	1.8 ± 0.11	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.2 ± 0.01
Smooth-leaf	11.6 ± 0.73	2.6 ± 0.16	0.1 ± 0.01	0.2 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.2 ± 0.02
General mean	7.8	2.2	0.2	0.2	0.2	0.2	0.2
P-valor	< 0.0001	0.0361	0.3314	0.6381	0.2438	0.2911	0.0833

ribisnigri cannot fly at temperatures below 17 ºC and that the spread within the crop occurs mainly by wingless individuals from one plant to another. The minimum temperature for *N. ribisnigri* is similar to those found for syrphid flies such as *Episyrphus balteatus* (De Geer), *Melasyrphus corollae* (F.), *Melangyna viridiceps* (Macquart) and *Symnosyrphus grandicornis* (Marquart) (4 ºC). This suggests that this group of predators is able to be established and complete its development in lettuce at the same time as its prey in spring and autumn, when temperatures are low and before aphids disappear from the area (Diaz *et al*., 2007).

A positive and significant correlation ($p<0.0001$) was found between aphid populations and precipitation ($r = 0.39902$ for Veronica and $r = 0.43526$ for Elisa lettuce). Intense or constant rain has a direct mechanical action, decreasing the populations of small insects such as thrips and aphids (Silveira Neto *et al*., 1976; Leite *et al*., 2005). However, they live in parts of the plant that provide shelter and according to Liu and Mccreight (2006) the highest concentration of aphids is within the lettuce head, while in *N. ribisnigri* aphid populations were higher in the outer leaves than in other leaves. In moist conditions entomopathogenic fungi can develop, reducing the population of aphids.

An increase in thrips was observed starting in the summer (Figure 1) and continuing throughout the three cycles, contrary to the results obtained by Leite *et al.* (2005) who found no significant differences in the occurrence of *T. tabaci* in *Brassica oleracea* var. *acephala*. In the present study, significant differences were observed among the means of occurrence of thrips in the three cycles studied (Table 4); infestations increased in each cycle. One of the possible explanations for the variation among crop cycles is the positive and significant correlation ($p<0.0001$) between temperature and the average incidence of thrips, in both Verônica ($r = 0.6109$) and Elisa ($r = 0.54104$) cultivars. In Cycles II and III, thrips populations increased, coinciding with the gradual increase in temperature, from 16.4 ºC to 25.2 ºC (mean 19.8 ºC) in Cycle II and 16.8 ºC to 26.8 ºC (mean 20.6 ºC) in Cycle III. These results indicate that thrips are more active at higher temperatures, similar to most insects, as discussed by Silveira Neto *et al.* (1976). Jesus *et al.* (2010) reported that the highest incidence of *C. phaseoli* occurred from 46 to 60 days after the emergence of bean seedlings, and the population increased as plants matured, reaching the highest infestation at 60 days. However, Boiça Júnior *et al.* (2015) found that infestation by *C. phaseoli* decreased as temperature increased and that the largest *C. phaseoli* population occurred in the winter, but infestation in bean cultivars was not significantly influenced by temperature, relative humidity, or rainfall.

A negative and significant correlation ($p<0.0001$) was observed between thrips populations and precipitation ($r = -0.6670$ for Veronica and $r = -0.59813$ for Elisa). Species of insects such as thrips which have a developmental stage in the soil are strongly influenced by rainfall (Silveira Neto *et al*., 1976). The results obtained in this study are in agreement with those obtained by Leite *et al.* (2005).

Conclusions

Aphids and thrips prefer lettuce varieties with smooth instead of curly leaves. Of the total of specimens collected, 53.87% are considered phytophagous species. Aphids (Hemiptera: Aphididae) represented 40.82% of the total specimens, with *N. ribisnigri* as the most abundant. Thrips (Thysanoptera: Thripidae) of the species *F. schultzei* and *Caliothrips phaseoli* accounted for 11.35% of the total species. The natural enemies found were predators of the family Syrphidae, Coccinellidae and Carabidae and parasitoids of the order Hymenoptera; the two most frequent superfamilies were Ichneumonoidea and Chalcidoidea.

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