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Citrus Aphids in Algarve Region (Portugal): Species, Hosts, and Biological Control

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Abstract: Aphids affect citrus by causing leaf deformations and reducing fruit production. Additionally, aphids are a great concern due to their ability to transmit *Citrus tristeza virus* (CTV), the cause of tristeza, one of the main citrus diseases. In the last four years, citrus orchards in the south of Portugal (Algarve region) were sampled for aphid species identification and counting. *Aphis spiraecola* was the most abundant species, representing more than 80% of all identified aphids, and the damage (leaf deformation) it causes was directly proportional to its density. *A. gossypii* was the second most common species, followed by *A. aurantii* and *Macrosiphum euphorbiae*. The number of aphids in nymph stages was predominant over the adult stages (both wingless and winged) in all species. *A. citricidus*, the most efficient CTV vector, was not detected. The largest populations of *A. spiraecola* were observed in lemon and orange trees during spring (>100 individuals per shoot), with great damage observed in orange, lemon, and mandarin trees. *A. gossypii* was observed mainly in mandarin and tangor trees. There was a low activity of natural biological control agents, with the parasitism of *A. spiraecola* by *Lysiphlebus* spp. and *Binodoxys* spp. ranging from 0.3 to 1.5%. The numerical ratio ranged from 150 to 440 aphids per predator, and among these, syrphids were the most abundant, followed by lacewings and coccinellids (*Scymnus*).

Keywords: *Aphis spiraecola*; *A. gossypii*; *A. aurantii*; *A. citricidus*; infestation; kumquat; grapefruit; parasitism; shoots; tangor

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Citation: Paiva, P.E.B.; Neto, L.M.; Marques, N.T.; Duarte, B.Z.; Duarte, A.M. Citrus Aphids in Algarve Region (Portugal): Species, Hosts, and Biological Control. *Ecologies* **2024**, *5*, 101–115. https://doi.org/10.3390/ecologies5010007

Academic Editor: José Ramón Arévalo Sierra

Received: 9 January 2024
Revised: 8 February 2024
Accepted: 14 February 2024
Published: 19 February 2024

1. Introduction

Citrus is the second most economically important group of fruit crops in Portugal, after pome fruits. Average annual production over the last ten years was 374,000 t. Around 85% of this production was obtained in the Algarve, the southernmost region of Portugal, where citrus is the main crop, occupying an area of 16,000 ha [1]. Furthermore, citrus fruits have great importance in the culture and traditions of the country and the entire Mediterranean basin [2] and are an important contribution to a healthy diet due to their high vitamin C content [3].

Citrus plants are affected by aphids, which cause direct damage mainly by deformations of young leaves (Figure 1). Indirect damage resulting from virus transmission by aphids is often more impactful than direct damage. Damage depends mostly on the aphid species present in the orchard [4]. For a given aphid species, injury depends on the ecological characteristics of the agricultural system, which, in addition to regional climatic conditions, is very dependent on human activities. The plant (citrus host species and its sprouting pattern), agricultural practices (irrigation, fertilization, and pruning practices) [5], regional and local climatic conditions, and some ecological factors like the activity of aphids' natural enemies work in an integrated manner, resulting in a certain degree of damage [4]. Under favorable conditions, high aphid density may cause a reduction in fruit production by more than 50%, as previously reported for *Aphis gossypii* [6].

Figure 1. Leaf deformation (curling) caused by *Aphis spiraecola* on young shoots (**left**) that persists as shoots mature (**right**).

In Portugal, 11 aphid species were reported to affect citrus, four of them with economic impact, namely *A. gossypii* Glover, *A. spiraecola* Patch, *A. (Toxoptera) citricidus* (Kirkaldy), the brown citrus aphid, and *A. aurantii* (Boyer de Fonscolombe) (Homoptera: Aphididae) [7,8]. All four species inflict direct damage to the plant and can transmit *Citrus tristeza virus* (CTV), a pathogen that causes one of the most serious citrus diseases, the tristeza disease, which was responsible for the decline and death of tens of millions of trees worldwide [9]. Among the CTV aphid vectors, *A. citricidus* is very efficient and able to transmit the most severe strains [10]. *A. citricidus* was detected for the first time on Madeira Island in 1994 and in the north of mainland Portugal in 2003 [11,12]. According to the General Directorate of Agriculture and Veterinary of Portugal, *A. citricidus* is present in the northern and central regions of Portugal [13] and has not yet been found in the south, where the main citrus industry is established. With respect to *A. citricidus*, the European Food Safety Authority (EFSA) has published a set of guidelines to assist member states in monitoring this pest, including its distribution, host range, risk factors, and detection and identification methodologies [14].

The ability of aphid vectors to transmit CTV depends on several factors, such as the aphid population, CTV strains, the virus source host plant, and the target host plant [15]. Despite *A. gossypii* being less efficient in the transmission of CTV compared to *A. citricidus*, its predominance in Spain led to the dissemination of tristeza disease and loss (uproot was necessary) of more than 20 million trees grafted on sour orange (*Citrus aurantium*) from 1989 to 2000 [16]. Species such as *A. spiraecola* and *A. aurantii* were reported to be less efficient in transmitting CTV [17].

Tristeza disease develops when citrus species *C.* ×*sinensis* L. Osbeck (sweet orange), *C. reticulata* Blanco (mandarin), and *C.* ×*paradisi* Macfad. (grapefruit) are grafted onto sour orange, preventing further use of this rootstock [18,19]. The citrus industry in Portugal is at serious risk due to the presence of *A. citricidus*, the predominance of sour orange rootstock in many orchards, and the circulation of isolates from all known CTV haplotypes [20]. This polyphagous aphid has a preference for Rutaceae; however, it may establish dense colonies in young plants of other families, making population control difficult.

The ability of the most abundant aphids (*A. spiraecola* and *A. gossypii*) to acquire CTV from contaminated trees [21] and to transmit the virus [22] also contributes to pressure on the citrus industry. In the southern region of Portugal, where citrus orchards predominate, knowledge of the dynamics of citrus aphid populations related to the ecological conditions of the agricultural systems is important for their control. A systematic survey is needed to identify the aphid species and their preferred hosts in this region. Evaluation of aphid populations, both for control and research purposes, can be expressed by several density measures such as the number of aphids per shoot or per leaf [23], number of insects per canopy square meter [6], or density per leaf square centimeter [24]. The use of insect density classes allows a faster assessment of aphid populations [25,26]. The proportion of aphid infestation in new shoots has been the most recommended sampling methodology and the most practical tool for decision making [27].

The ability of a pest to develop into a large population depends on the intensity and effectiveness of the natural control by biotic and abiotic factors. The biological control of aphids is mainly carried out by invertebrate arthropods through the processes of predation and parasitism. However, the efficacy of this control depends on several factors: the edapho-climatic conditions [28], the agroecosystem type [29], the host plants and the predominant aphid species [30], hyperparasitism [25], the way the agroecosystem is managed [29], and the season of the year [31], among others. The aphid parasitoids belong fundamentally to the order Hymenoptera, namely to the families of the Parasitica group, and the most frequently reported genera that affect aphids in the Mediterranean region are the *Binodoxys*, *Lysiphlebus,* and *Trioxys* (Braconidae, Aphidiinae) [31,32]. Predatory arthropods with major importance in terms of control of aphid populations belong to Coleoptera: Coccinellidae, Hemiptera: Miridae, Neuroptera: Chrysopidae and Coniopterygidae, Diptera: Syrphidae, Araneae, and Mesostigmata: Phytoseiidae (predatory mites) [32].

The objective of the present research was to determine the aphid species present in the Algarve region, their respective abundance and damages, and the level of their natural biological control. An attempt was made to establish a relationship between the type of agricultural system, the time of year, the citrus species, and the incidence of the various aphid species.

2. Materials and Methods

Aphid surveys were carried out in citrus orchards distributed throughout the main citrus area of the Algarve region between Silves and Vila Real de Santo António. Aphids and citrus shoots were collected in eight commercial orchards and a citrus collection orchard, the last one located in Faro (Figure 2). Six citrus species were sampled, namely sweet orange (*Citrus* ×*sinensis*), mandarin (hybrid of *C. reticulata*), lemon (*C.* ×*limon*), tangor (*C.* ×*sinensis* × *C. reticulata*), grapefruit (*C.* ×*paradisi*), and kumquat (*C. japonica*) on three surveys carried out between March and August 2019 (Figure 2, surveys 1, 2, and 3). All commercial orchards and the citrus collection are managed conventionally, in accordance with integrated production standards (including integrated pest management), which is the mandatory minimum in the European Union for commercial orchards. In a *C.* ×*sinensis* 'Lane Late' orchard, hereafter designated as the experimental orchard, located in Faro, with half of the area managed conventionally and the other half organically, the presence of aphids on new shoots was compared by counting those that had at least one aphid and those that did not have aphids (Figure 2, survey 4). The commercial orchards belong to different citrus growers and were distributed across the Algarve citrus zone, while the citrus collection and experimental orchards belong to the Ministry of Agriculture (DRAP Algarve). In total, four surveys were carried out over the years 2019, 2021, and 2022.

Figure 2. Location of sampling points in the Algarve region (Southern Portugal). Survey 1; Survey 2 and 3; Survey 4.

2.1. Incidence of Aphid Species and Their Natural Enemies

From March to August 2019, three aphid surveys (surveys 1, 2, and 3) were carried out to study the incidence of aphid species in citrus orchards. The plant species and cultivars sampled in each survey and the number of samples for each citrus species in each survey are presented in Table 1.

Table 1. Characterization of the four aphid surveys carried out in citrus orchards of the Algarve region in spring and summer 2019 (surveys 1, 2, and 3) and between August 2021 and December 2022 (survey 4).

Survey	Location	Period	Citrus Species (Number of Samples)	Citrus Cultivars
1 Citrus commercial orchards	Algoz, Alte, Asseca, Benafim, Cacela, Conqueiros, Faro, Peral, Tavira	Spring (March–May)	*C.* ×*sinensis* (101)	Dom João, Lane Late, Newhall, Valencia Late
			C. ×*limon* (9)	Eureka
			C. reticulata (18)	Encore, Mioro
2 Citrus collection orchard	Faro	Spring (March–May)	*C. japonica* (14)	Marumi
			C. ×*sinensis* (29)	Lane Late, Navelate, Navelina, Newhall, Rohde
			C. ×*limon* (25)	Eureka
			C. ×*paradisi* (8)	Star Ruby
			C. reticulata (29)	Emperor, Fortune, Fremont, Marisol, Nova, Nules
3 Citrus collection orchard	Faro	Summer (July–August)	*C. japonica* (5)	Nagami
			C. ×*sinensis* (39)	Navelate, Newhall, Rhode, Sanguinelli
			C. ×*limon* (34)	Eureka, Lisbon
			C. ×*paradisi* (12)	Star Ruby
			C. reticulata (44)	Arrufantina, Clauselina, Clementina, Fairchild, Fortune, Hernandina
			C. reticulata × *C. sinensis* (22)	Murcott, Ortanique
4 Citrus experimental orchard	Faro	August 2021–December 2022	*C.* ×*sinensis* (26)	Lane Late

For each citrus host, four to five new shoots per tree were evaluated. The shoots were cut out, counted, placed in plastic bags, and transferred on the same day to the laboratory for further processing. To evaluate the incidence of aphids, the number of living insects of each species was counted under a stereoscopic microscope, distinguishing three developmental aphid stages: nymphs, wingless adults, and winged adults. For each species, the proportion of each aphid stage was determined in relation to the total number of aphids of that species. The taxonomic keys from Brown et al. [33] and Ilharco and Sousa-Silva [12] were used in the identification.

To evaluate the incidence of some natural control agents, the number of mummified (parasitized) aphids and predatory insects was recorded. Leaves with mummified aphids were placed in rearing boxes to obtain adult parasitoids for posterior identification [34]. The mummification rate was determined by the rate of mummified aphids in relation to the total number of aphids (alive + mummified). The numerical ratio between the total number of aphids and the total number of predatory insects obtained in each survey was calculated.

The incidence of aphids in new shoots was also evaluated during the years 2021 and 2022 in a 'Lane Late' experimental orchard managed with two different production modes—organic and conventional (survey 4). For this survey, a simplified methodology was adopted: 20 trees in each production mode were randomly selected, and the number of shoots that had at least one aphid and shoots without aphids within a circle of 0.25 m^2 was counted (survey 4). The phytosanitary treatments applied to the plots during the monitoring period were as follows:

- Conventional: copper oxychloride (30 December 2021), bromadiolone (27 January 2022), glyphosate (2 March 2022 and 19 July 2022), abamectin (14 July 2022), spirotetramat + abamectin + paraffin oil (28 September 2022);
- Organic: paraffin oil (26 August 2021 and 24 June 2022), copper oxychloride (30 December 2021), azadirachtin (26 July 2022).

2.2. Incidence of Aphid Damage

Citrus shoots obtained in the previous study (surveys 1 to 3) were further evaluated for their length, total number of leaves, and number of leaves deformed by aphids. To evaluate the aphid damage (leaf deformation), the proportion of damaged leaves in relation to the total number of leaves per shoot was calculated.

2.3. Statistical Analysis

In surveys 1, 2, and 3, statistical analysis was carried out considering each shoot as a sampling unit. Data from aphid counting do not follow the normal distribution of errors and homogeneity of variances, so generalized linear models with Poisson distribution and log link were adopted. Deviance analyses and mean comparison tests were performed by a generalized Tukey test ($p < 0.05$). The relationship between the number of *A. spiraecola* (x) and the proportion of damaged leaves (y) was analyzed by linear regression. Analyses were computed in the R program, version 3.5.3 (2019). To analyze results from survey 4, the comparison of the proportion of shoots with aphids between the two modes of production was made by 2-factorial ANOVA without replication (Excel, Data analysis module).

3. Results and Discussion

3.1. Incidence of Aphid Species

The most abundant aphid species in citrus orchards in the spring and summer of 2019 was *A. spiraecola* (Table 2). This species represented 95.56% of the individuals observed in commercial orchards and around 80% in the citrus collection orchard (82.59% in spring and 79.02% in summer) (Table 2). The second most abundant species was *A. gossypii*. In commercial orchards, *A. gossypii* represented only 4.36% of aphids, and in the citrus collection orchard, it was present in 15.38% and 20.98%, respectively, in the spring and summer of 2019. Two other aphid species were observed in small numbers, *A. aurantii* and

Macrosiphum euphorbiae (Table 2), and no records of the brown citrus aphid *A. citricidus* were found.

Table 2. Relative abundance of aphid species (%) in new shoots and total number of individuals of each species at the following stages: winged aphids (wa), wingless adults (wsa), and nymphs (nym). Data obtained from new shoots from commercial orchards and citrus collection orchards in the Algarve region, sampled in the spring and summer of 2019.

Survey	*Aphis spiraecola*				*Aphis gossypii*				*Aphis aurantii*				*Macrosiphum euphorbiae*			
	wa	**wsa**	**nym**	**%**	**wa**	**wsa**	**nym**	**%**	**wa**	**wsa**	**nym**	**%**	**wa**	**wsa**	**nym**	**%**
1 Commercial orchards (spring)	758	2994	8212	95.56	146	184	216	4.36	0	0	7	0.06	0	0	2	0.02
2 Citrus collection (spring)	178	1342	5404	82.59	41	259	990	15.38	2	41	126	2.02	0	1	0	0.01
3 Citrus collection (summer)	19	378	1561	79.02	8	117	395	20.98	0	0	0	0.00	0	0	0	0.00

Previous research in 2000 [22] already reported *A. spiraecola* as the most abundant aphid in the Algarve citrus orchards, revealing that there has been no change in the dominant aphid species in the region over the last 20 years. This aphid species invaded the Mediterranean region in the 1960s [4] and was therefore also described as the most common in citrus orchards in other countries in this region [26,35–37]. The outstanding abundance of *A. spiraecola* confirms this aphid is well adapted to the conditions of the southern Portugal region. Despite the higher abundance of *A. spiraecola* in comparison with *A. gossypii* and *A. aurantii*, some studies have pointed out that the proportion of aphid species could depend on the sampling method used [17], being distinct when counts are made in yellow sticky traps or by direct observation in citrus shoots [17,22]. Only the winged forms are collected in yellow sticky traps, so the differences obtained between the two methodologies may be related to the ability of an aphid species to produce winged forms on a specific host within a certain time. In fact, our results show that the proportion of winged adults is higher in *A. gossypii* than in *A. spiraecola* in all surveys (Table 2). In general, the greatest importance of aphids in agriculture is related to their ability to transmit viruses. Fortunately, the brown aphid *A. citricidus,* the most efficient vector in the transmission of CTV, was not found in the present surveys. However, although with lower efficiency, both *A. spiraecola* and *A. gossypii* can transmit CTV [10,21,38]. Therefore, the risk of epidemics of tristeza disease in the southern region of Portugal seems to be real, taking into consideration the presence of severe CTV strains [20]. The analysis of *A. spiraecola* and *A. gossypii,* collected in a CTV-positive tangor 'Ortanique' in the Algarve, showed that 75% and 70% of individuals, respectively, carried CTV [21]. In Spain, the natural spread of CTV by *A. spiraecola*, *A. gossypii,* and *A. aurantii* ranged from 19 to 27% [17].

3.2. Incidence of Aphid Natural Enemies

The rate of mummified aphids detected in 2019 was 1.53% in spring (Table 3, survey 2) and 0.28% in summer (Table 3, survey 3) in the same citrus collection orchard. All the inspections carried out elsewhere in the Algarve region (survey 1) showed an average rate of mummified aphids of 1.57%. The hymenopterous parasitoids that emerged from *A. spiraecola* mummies all belong to the Braconidae family: 60% belong to the genus *Lysiphlebus* and 40% belong to the genus *Binodoxys*. Parasitoids of these two genera are considered primary parasitoids of citrus aphids [30].

Table 3. Abundance of aphids (all species), both live and mummified, mummification rate, and associated predators: syrphids, chrysopids, and coccinellids. Numerical rate between aphids and predators on new shoots during spring and summer 2019. Total aphids are winged, wingless adults, and nymph forms.

Survey	Aphids		Rate	Predators			Aphids/Predator
	Total	Mummified [1]	%	Syrphids	Chrysopids	Coccinellids [2]	Numerical Rate
1 Citrus commercial orchards spring	12,519	197	1.57	71	3	1	167
2 Citrus collection orchards spring	8384	128	1.53	16	1	2	441
3 Citrus collection orchards summer	2478	7	0.28	6	6	4	155

[1]: Lysiphlebus and Binodoxys, [2]: Scymnus.

Among the predatory insects, the most abundant group belongs to the syrphids (Diptera: Syrphidae), followed by lacewings (Neuroptera: Chrysopidae) and coccinellids (Coleoptera: Coccinelidae), with the last one from the genus *Scymnus* (Table 3). All predatory insects were sampled in the larval stage, and for this reason, except for coccinellids, they were not identified at the genus level.

Many predatory insects and parasitoids have been reported as natural biological control agents of aphids in the Mediterranean basin [30,39,40]. As shown in this study, the action of these beneficial agents seems to be limited, an aspect already reported for *A. spiraecola* as one of the factors that explain the high populations of this species [6]. In a similar way, low rates of mummification in *A. aurantii, A. spiraecola,* and *A. gossypii* by *Lysiphlebus testaceipes* and *Binodoxys angelicae* have been found in Algeria, ranging from 0.76 to 2.66% [37]. Several aspects may explain the low action of the beneficial agents: the gap between the onset of aphid populations and that of their natural enemies [4] and the weed management that contributes to reducing the population of aphidophagous organisms in the orchards [40]. Data from these studies are valuable information that allow farm management to improve natural biological control.

3.3. A. spiraecola and A. gossypii Host Preference

In commercial orchards, the abundance of *A. spiraecola* winged aphids did not differ among the three citrus species sampled in spring 2019 (lemon, orange, and mandarin), but all the other developmental stages were influenced by the citrus host plant (Table 4, survey 1). The number of wingless adults of *A. spiraecola* was found to be significantly higher on orange trees, while nymphs and the total number of aphids were significantly higher on lemon trees. In this survey, *A. gossypii* was only observed on orange trees.

In citrus collection orchards, the distribution of the various aphid developmental stages was found to be dependent on the plant host or the sampling season (spring or summer) (Table 4, surveys 2 and 3). During the spring of 2019, adults of *A. spiraecola* were found to be more abundant on grapefruit, while nymphs and the total number of aphids were in a higher number on sweet orange trees (Table 4, survey 2). In the case of *A. gossypii*, excepting for winged adults, whose prevalence did not show significant differences between citrus hosts, a higher incidence of all other aphid stages was found on tangor, followed by mandarin.

Table 4. Infestation of *Aphis spiraecola* and *Aphis gossypii* on new citrus shoots in spring and summer 2019. Number of shoots evaluated (n); shoot characteristics: shoot length (sl), average number of leaves per shoot (nl). Aphid infestation—average number of individuals per shoot: winged adults (wa), wingless adults (wsa), nymphs (nym), and total number of aphids. Leaf damage: average number of leaves damaged by aphids (nlda), proportion of leaves damaged by aphids (plda).

Survey	Host		Shoot Characteristics		Aphid Infestation								Leaf Damage	
					Aphis spiraecola				*Aphis gossypii*					
		n	sl (cm)	nl	wa	wsa	nym	Total	wa	wsa	nym	Total	nlda	plda (%)
1 Citrus commercial orchards (spring)	Orange	77	7.8 a [1]	5.3 a	4.3 a	27.9 a	79.4 b	111.6 b	1.5 a	1.8 a	2.2 a	5.5 a	2.3 b	45.6 b
	Lemon	5	7.0 a	6.8 a	4.4 a	19.0 b	124.0 a	147.4 a	0.0 b	0.0 b	0.0 b	0.0 b	4.2 a	60.8 a
	Mandarin	16	8.3 a	6.7 a	3.8 a	4.2 c	11.7 c	19.7 c	0.0 b	0.0 b	0.0 b	0.0 b	1.8 b	33.9 c
	Deviance [2]		0.88	5.32	0.94	450.49	1454.8	1738.9	72.08	90.85	106.64	269.57	8.03	72.71
	p-value		0.645	0.0698	0.6220	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0180	<0.001
2 Citrus collection orchards (spring)	Kumquat	14	6.2 bc	5.0 ab	1.8 b	15.9 b	51.3 b	68.9 b	0.07 a	0.3 c	0.5 e	0.8 e	1.0 bc	16.4 d
	Orange	29	7.8 b	5.4 ab	1.8 b	15.4 b	70.1 a	87.3 a	0.00 a	0.5 c	0.8 d	1.3 d	2.3 a	46.6 a
	Lemon	25	11.4 a	6.2 a	0.7 c	3.6 c	16.0 d	20.3 d	0.04 a	0.1 c	0.2 e	0.3 e	0.5 c	6.4 e
	Grapefruit	8	10.2 a	5.4 ab	4.7 a	25.2 a	40.1 c	70.1 b	0.00 a	2.3 b	4.6 c	6.9 c	1.2 abc	22.6 c
	Mandarin	29	5.1 c	4.2 b	0.6 c	5.2 c	37.2 c	43.1 c	0.14 a	1.7 b	11.7 b	13.5 b	1.6 ab	34.6 b
	Tangor	36	4.9 c	4.8 ab	0.7 c	4.2 c	16.3 d	21.2 d	1.00 a	4.8 a	16.0 a	21.8 a	1.0 bc	21.8 c
	Deviance		111.52	11.07	82.33	604.45	1659.00	2142.20	72.19	266.40	1112.00	1398.20	40.57	1079.70
	p-value		<0.001	0.0499	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
3 Citrus collection orchards (summer)	Kumquat	5	11.2 a	8.2 a	0.2 a	28.0 a	55.8 a	84.0 a	0.00 a	0.0 b	0.0 b	0.0 b	4.8 a	44.7 a
	Orange	39	7.8 a	6.4 b	0.1 a	0.0 c	0.3 c	0.3 c	0.03 a	0.0 b	0.1 b	0.1 b	0.0 c	0.0 b
	Lemon	34	8.4 a	4.5 b	0.0 a	0.0 c	0.2 c	0.2 c	0.00 a	0.0 b	0.0 b	0.0 b	0.0 c	0.0 b
	Grapefruit	12	9.3 a	5.3 b	0.0 a	0.0 c	0 c	0.0 c	0.00 a	0.0 b	0.0 b	0.0 b	0.0 c	0.0 b
	Mandarin	44	5.6 b	5.2 b	0.3 a	5.4 b	28.5 b	34.2 b	0.14 a	2.7 a	8.9 a	11.7 a	1.3 b	19.8 a
	Tangor	22	8.1 ab	7.6 a	0.2 a	0.0 c	0.5 c	0.7 c	0.04 a	0.0 b	0.0 b	0.0 b	0.2 c	2.7 b
	Deviance		39.72	30.79	20.08	1067.5	3383.10	4369.80	10.10	296.2	965.34	1257.20	192.66	2397.60
	p-value		<0.001	<0.001	0.0012	<0.001	<0.001	<0.001	0.072	<0.001	<0.001	<0.001	<0.001	<0.001

[1]: In the same survey and column, means followed by a common letter did not differ by the generalized Tukey test ($p < 0.05$). [2]: Deviance value and *p*-value.

During the summer of 2019 (survey 3), populations of both aphid species decreased on all hosts except kumquat. As for *A. spiraecola*, winged adult populations had a low incidence on all citrus hosts with no significant differences between host species. All other developmental stages were more abundant on kumquat, followed by mandarin. Mandarin was also the host with the highest number of *A. gossypii* aphids in all stages (Table 4), except for winged adults for which no statistical differences were detected between citrus hosts.

Theoretically, small shoots with a low number of leaves could host fewer number of aphids than larger ones. Data from the present study reveal differences in the number of leaves on new shoots dependent on citrus species (Table 4); thus, eventually, a similar variation would be expected in relation to the colonization of new shoots by aphids, which was not always evident in our study. In survey 1 (Table 4), no significant differences were observed in the number of leaves per shoot between citrus hosts. Similarly, for winged adults of *A. spiraecola*, no significant differences were also observed in their mean values. Yet, for wingless adults and nymphs of *A. spiraecola* and for all stages of *A. gossypii*, significant differences in aphid colonization were identified depending on the host. In survey 2, lemon tree new shoots had significantly more leaves than mandarin, but the number of adult aphids was not significantly different, and mandarin had significantly more nymphs than lemon (Table 4). Finally, on survey 3, kumquat new shoots had the highest number of leaves and also the highest number of aphid nymphs. However, by comparison with tangor, which showed a similar number of leaves per shoot, the number of total aphids was significantly higher on kumquat (Table 4).

The present results suggest that the number of leaves per shoot is not per se a factor that explains the number of aphids in a specific citrus species or cultivar, but instead, several factors are involved. Aphids use a variety of cues to select a suitable host to reproduce and maximize the success of their progeny [41]. These cues can be visual, olfactory, gustatory, and tactile stimuli [42,43], and in a heterogeneous and dynamic environment in which the host plant's quality changes over time, the ability to interpret these cues is essential for aphid colonization. The intensity of a stimulus is essential in the aphids' choice of a host plant, an aspect that involves the flush intensity study which was not considered in the present study. It seems possible that aphids' choice and reproduction may occur on shoots with fewer leaves, in case the tree, among other factors, has abundant flushing. Several other factors may also influence aphids' choice. In cotton, antixenosis is activated after the first aphid attack through the release of plant defense elicitors [44], making the crop less susceptible to aphids. Using electropenetrography, it was shown that the feeding parameters of the brown citrus aphid were different depending on the citrus host species [45]. These results may indicate a potential fitness of aphids dependent on citrus hosts. A differential development that relies on the cultivar was already reported for *Acyrthosiphon pisum* on pea cultivars [46]. These factors contribute to explaining the differences observed in the number of aphids, at distinct stages of aphid development, on different citrus host species (Table 4).

3.4. Aphid Host Damage by A. spiraecola and A. gossypii

In citrus commercial orchards, where the number of leaves per shoot was similar for the three host tree species sampled, damage caused by aphids *A. spiraecola* and *A. gossypii* was greater on lemon trees than on orange or mandarin trees (Table 4, survey 1). On the other hand, in citrus collection orchards (Table 4, surveys 2 and 3), the number of leaves per shoot and the aphid damage were more heterogeneous among the citrus hosts. In the spring of 2019, the lemon trees showed the highest number of leaves per shoot, only different from mandarin, which had significantly fewer leaves per shoot (Table 4, survey 2), but the orange trees had a significantly higher number of leaves damaged by aphids (in absolute numbers and in relation to the number of leaves per shoot) than lemon. Concerning the summer of 2019, the number of leaves on new shoots was higher in kumquat and tangor than in other citrus species (Table 4, survey 3). The greatest damage caused by aphids was found on kumquat and mandarin, with 44.7% and 19.8%, respectively, of damaged leaves.

Despite the high number of leaves on tangor shoots, it was one of the hosts with negligible damage (Table 4). These results reinforce what was discussed in the previous section about the importance, per se, of the number of leaves per shoot on the level of infestation and in this case also on the damage level, particularly in situations where the orchard has different citrus host species/cultivars.

The level of aphid damage is due, above all, to the number of insects present in an orchard. Thus, it is expected to find more damaged leaves in orchards/regions with growing conditions favorable to aphid development. Beyond the vital climatic factors, the number of new leaves is an important issue that depends on the density of new shoots, the shoot length, the number of leaves attached, and the leaf stage. However, the present study does not show a clear relationship between the mean shoot length and the mean number of leaves per shoot (Table 4), possibly due to the orchard heterogeneity in terms of citrus species and cultivars. It is expected that a citrus orchard of the same species/cultivar will exhibit shoots with regular leaf development, although dependent on cultural management (mainly fertilization and irrigation). Orchards that have different citrus species and whose sprouting characteristics are diverse and occur at different times will also present a diverse environment throughout the year for aphid development. Knowledge of the correspondence between new shoot length and the number of developed leaves, supplemented by an estimation of shoot density, is a key factor in establishing pest sampling procedures and control decision levels [47].

Regarding the citrus collection orchard and during spring flush (Table 4, survey 2), the proportion of damaged leaves was directly related to the average total number of *A. spiraecola* per shoot (winged, wingless, and nymphs) (Figure 3). Sucking insects are dependent on new shoots; therefore, damages tend to increase during these phenological stages. The relationship between new shoot growth and the development of piercing/sucking insects is well described in several studies. The colonies of *A. citricidus* develop better during the intermediate stages of shoot development, a stage known as the "recent expansion" [25]. Cifuentes-Arenas et al. [48] described in detail the shoot development stages in orange trees and their relationship with the development of another sucking insect, the psyllid *Diaphorina citri*. Our results confirm a direct relationship between the number of aphids and the respective damage. In addition, factors such as the host tree species/cultivar and climatic conditions favorable to aphid development must also be considered. Furthermore, aphids share the same ecological niche with other citrus pests, such as the citrus leaf miner (*Phyllocnistis citrella*), which was also present in the orchard but was not evaluated in the present study. The competition for the same leaves could influence the amount of damage depending on the ecological conditions.

A major factor that influences insect pest damage is the pest management strategy. Interestingly, in the experimental orchard and during 2022 (Table 1, survey 4), there were no significant statistical differences between organic and conventional orchards in relation to the proportion of shoots with aphids (Figure 4). This result substantiates that organic pest control is, at least, equivalent to the control achieved with conventional chemical products and highlights the positive action of natural enemies in organic farming complemented with organically authorized pest control products. The management of an agroecosystem that promotes the development of parasitic and predatory insects and mites may therefore represent an important resource for pest control [35]. However, our results may be affected by the sampling methodology used, which, although not time-consuming, may be insufficient to detect small differences between the two modes of production.

Pests associated with shoot flushes depend on their abundance. Therefore, estimations of shoot abundance may be an important tool to assist pest management programs [47]. Samplings that evaluate the proportion of aphid-infested shoots (presence/absence) are practical for deciding when to control these insects [27]; however, they may be unsuitable for experimental purposes. Instead, values based on relative densities such as the number of insects per shoot or per leaf [23], infestation classes, or the number of aphids per shoot [26], have been employed in aphid population estimates or to calculate insect numbers per m^2

of canopy or per cm^2 of leaf [6,24,37], although more accurate methodologies are more time-consuming.

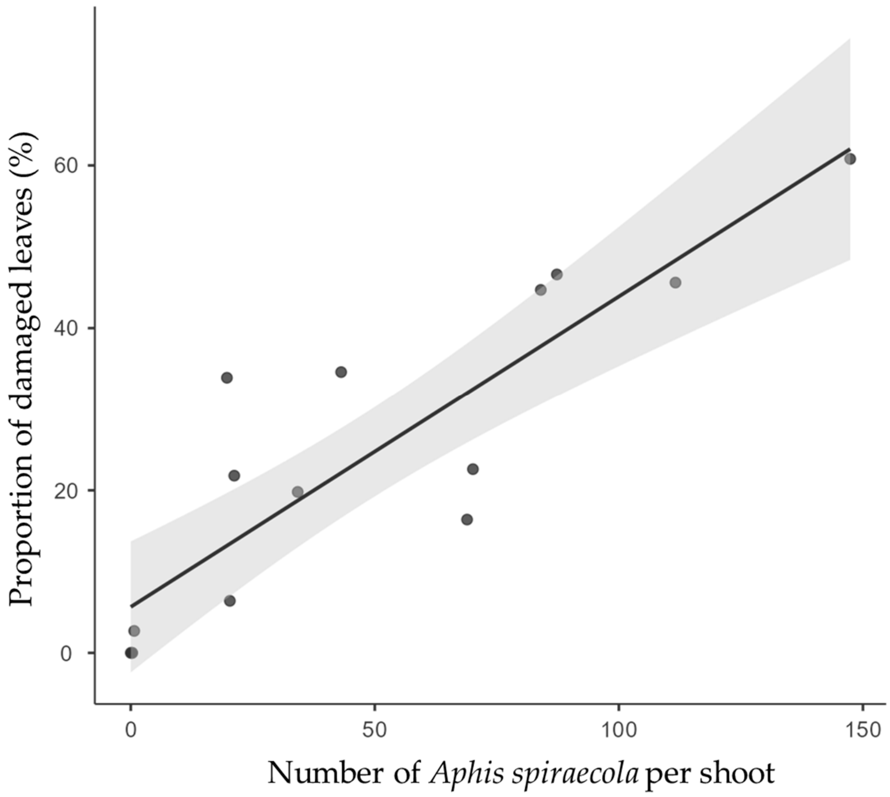

Figure 3. Relationship between the number of *Aphis spiraecola* per shoot and the proportion (%) of damaged leaves on the same shoot. These observations were carried out in the spring of 2019 on a citrus collection orchard in southern Portugal ($Y = 0.3823X + 5.6561$, $R^2 = 0.77$, ANOVA: $F = 44.07$, $p < 0.0001$). The shaded area represents the standard error of the regression.

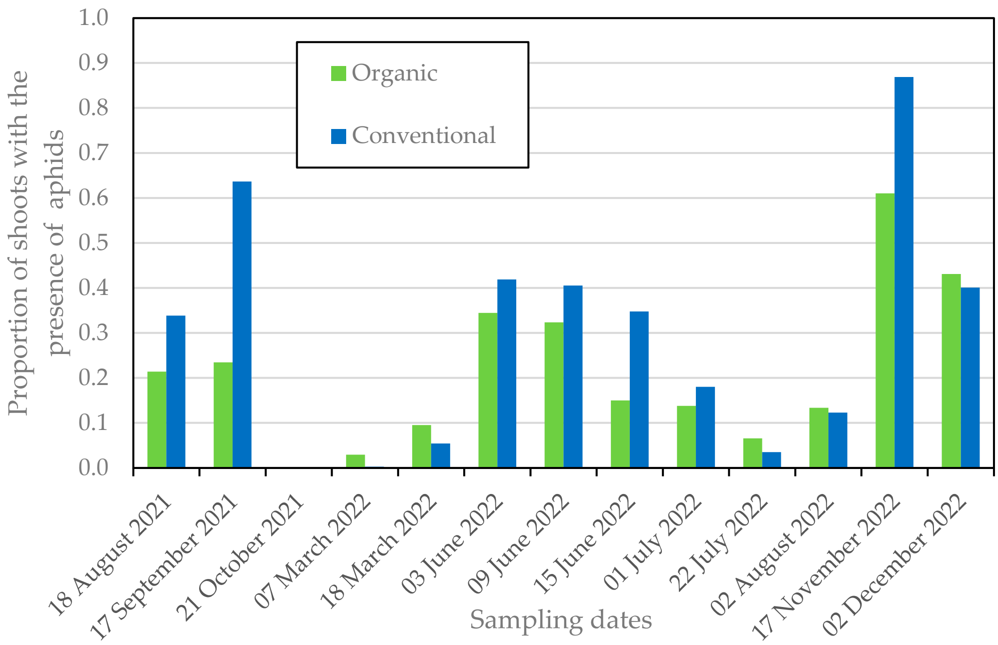

Figure 4. Proportion of shoots with aphid nymphs and adults ($F = 2.98$; $p = 0.11$) on a 'Lane Late' orange orchard managed organically (green bars) and in a conventional way (blue bars) (survey 4). Statistical analysis was performed after a data transformation of arcsin$\sqrt{p}$.

4. Concluding Remarks

Although *A. citricidus* has not yet been found in the southern region of Portugal, the presence of other aphid species with CTV transmission competencies highlights the need for continuous aphid monitoring and control. Aphids are the target of chemical control mainly in young citrus orchards, while in mandarin orchards, the control is performed regardless of the tree age. The absence of aphid control in mature orchards requires strict control by official phytosanitary authorities on all aspects related to CTV vectors and the detection of infected trees.

Unfortunately, no recent research has been carried out to evaluate the severity of CTV isolates present in Algarve orchards. The low incidence of quick decline syndromes by CTV in citrus species grafted on sour orange rootstock may be due to the low propagation of severe CTV isolates by existing aphid species, CTV genomic variability, the absence of the efficient *A. citricidus*, unfavorable climatic conditions for the development of the disease, or even other factors that have not yet been fully investigated. In the face of the presence of aphid vectors in high densities, it is important to understand the absence of abundant cases of severe CTV diseases such as tristeza in orchards with sour union rootstock.

The results from the present study indicate that organic orchard management can bring many benefits in controlling the aphid population. Therefore, increasing the natural control of aphids is a major challenge for citrus growers. Auxiliary arthropods exist naturally in orchards, although with a low ability to control aphid populations. A careful planning of orchards to provide an efficient conservation of auxiliary arthropods will lead to an increase in the natural control of aphids, even in conditions where no chemical control is implemented.

The development of new shoots usually coincides with new aphid infestations [5,49] and consequently with aphids' damage. Therefore, cultural practices, such as citrus pruning and the resulting onset of new shoots, must be used with great care and discernment, in particular when climatic conditions are favorable to the development of aphid vectors.

Planning a citrus production system—considering factors such as species/cultivars, tree density, choice of orchard location, and the intensity of agricultural practices—involves the assessment of several biotic and abiotic components of the agricultural ecosystem that can promote the development of pests, namely aphids, and may hamper the establishment of its natural enemies. A better understanding of the relationship between these components and the biology of pests is key to improving their control and preventing the spread of diseases like the one caused by CTV, which is transmitted by aphids.

Author Contributions: Conceptualization, P.E.B.P. and L.M.N.; methodology, P.E.B.P. and L.M.N.; investigation, P.E.B.P. and B.Z.D.; resources, A.M.D., L.M.N. and N.T.M.; data curation, P.E.B.P. and B.Z.D.; writing—original draft preparation, P.E.B.P.; writing—review and editing, P.E.B.P., L.M.N., N.T.M., B.Z.D. and A.M.D.; supervision, A.M.D. and L.M.N.; project administration, A.M.D.; funding acquisition, A.M.D. and P.E.B.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by the European Union, grant number LIFE18 CCA/ES/001109, Vida for Citrus. The APC was funded by National Funds through FCT—Foundation for Science and Technology—under project UIDB/05183/2020. This study also received national funds through FCT under the projects PTDC/BAA-AGR/30957/2017 and UIDB/00631/2020 CEOT BASE and from the Investment and Structural European Funds (FEEI) by Portugal 2020—Algarve's Regional Operational Programme (CRESC 2020) through project ALG-01-0145-FEDER-30957. Additional funds were from the Euphresco Project, topic 2022-A-391, "Rapid and efficient detection and identification of severe strains of Citrus tristeza virus".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to thank the Regional Directorate for Agriculture and Fisheries of the Algarve and the agronomists António Marreiros, José Carlos Tomás, Celestino Soares, Eugénia Neto, Ana Sofia Feliciano, and Sandra Germano for making available the experimental orchards where part of the samplings were carried out and for the information provided. The authors also thank all farmers who authorized sampling in their orchards. The first author is grateful to the Federal Institute of Education, Science and Technology of Triângulo Mineiro for the support.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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