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Article

Seasonal abundance of psyllid species associated with carrots and potato fields in Spain

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Abstract: Psyllids (Hemiptera: Psylloidea) can transmit the phloem restricted bacterium '*Candidatus* Liberibacter solanacearum' (Lso). In Europe, Lso causes severe losses to carrot and represents a threat to the potato industry. A rising concern of Lso transmission from carrot to potato and within potato has driven the need for monitoring populations of psyllid species that could serve as vectors on both crops, which would provide a fundamental understanding of the epidemiology of this pathogen. Different sampling methods were used to survey populations of psyllid species in commercial carrot and potato fields in Central and Eastern mainland Spain from 2015 to 2017. Two psyllid species, *Bactericera trigonica* and *Bactericera nigricornis* were found to be mainly associated with carrot and potato crops. In carrot fields the most abundant species was *B. trigonica* occurring from crop emergence to harvest, whereas in potato crops the most abundant psyllid species was *B. nigricornis*. The maximum psyllid population occurred between June and October its timing depending on the field location. Since *B. nigricornis* was found on both carrot and potato and is the only psyllid species able to feed and breed on both these crops in Europe, there is the potential risk of Lso transmission from carrot to potato.

Keywords: *Candidatus* Liberibacter solanacearum; vector-transmission; *Bactericera trigonica*; *Bactericera nigricornis*; psyllid yellows; vector abundance; Zebra chip; population dynamics.

1. Introduction

Vegetable crop production forms a major part of European Union agriculture, accounting for 13.7 % of its agricultural output [1]. Carrot (*Daucus carota* L.) and potato (*Solanum tuberosum* L.) are among the most important vegetable crops in the EU. In 2017 carrot and potato production within the EU was estimated at respectively 5.8 and 62 million tonnes [1]. In recent years, psyllids (Hemiptera: superfamily Psylloidea) have emerged as important pests that threaten carrot and potato production in different regions of the world including Europe.

In Europe various psyllid species affect carrot production with *Trioza apicalis* Foerster, 1848 in northern Europe and *Bactericera trigonica* Hodkinson, 1981, in the Mediterranean region being the predominant species. Both species cause direct damage to carrot and other Apiaceae and are vectors of the phloem restricted bacterium ´*Candidatus* Liberibacter solanacearum´ (Lso) [2–4]. In carrots, the main disease symptoms of Lso are purple or yellow leaf discoloration and root reduction [4]. The incidence of Lso infected plants depend on the growing zone, vector activity and environmental

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conditions but 90% and 40% of the growing crop have been reported affected in carrot fields in Spain and Tunisia respectively[2,5]. So far, Lso has been mainly associated with Apiaceae crops in Europe however, naturally Lso-infected potato plants have recently been reported in Spain and Finland [6,7].

Due to the absence of the main vector of potato-associated Lso haplotypes, the potato psyllid *Bactericera cockerelli* (Šulc, 1909), it is still uncertain how these potatoes became infected. Where *B. cockerelli* is present such as North and Central America and New Zealand [8] Lso affects a wide number of crops in the Solanaceae and it is especially important in potato where it causes a vegetative disorder called "zebra chip" [9,10]. Zebra chip is characterized by a striped pattern of necrosis in tubers that becomes more evident when chips are fried; as a result, potato chips processed from infected tubers are commercially unacceptable [10]. Due to this, the disease has led to the abandonment of entire potato fields and huge economic losses to the potato industry in North and Central America [9–11].

Bactericera cockerelli is an endemic pest of North and Central America and was introduced to New Zealand and Australia [10,12–14]. The geographical distribution of *B. cockerelli* does not overlap with the carrot psyllids *B. trigonica* or *T. apicalis* currently found in Europe [10,15], . Therefore, despite the contiguous cultivation of carrot and potato crops in Europe, current experimental evidence suggests that the risk of transmission of Lso from carrot to potato by *B. trigonica* or *T. apicalis* is very limited because these carrot psyllids are not able to colonize and continuously feed from the phloem of potato plants [7,16]. Despite the low risk of cross transmission of Lso from carrot to potato and absence of *B. cockerelli* for potato to potato transmission, Lso is still of concern to potato producers in Europe because other psyllid species could potentially transmit Lso to potato and within potato, especially in the Mediterranean countries. For example, *B. nigricornis* Foerster, 1848 which is closely related to *B. trigonica* has been reported on carrot and on potato crops [17–20] and has been tested positive for Lso in the field [21]. Accordingly, these potential vectors might represent a threat to European carrot and potato production. However, despite its importance, very little is known on its population dynamics. Thus, to assess the potential risks of Lso transmission, studies of the population dynamics of vectors and potential vectors of Lso in carrot and potato crops in European countries is urgently required.

Furthermore, despite the economic losses associated with carrot psyllids in Mediterranean countries, the population dynamics of these insects are poorly documented. This information is important because Lso reduction and management are primarily based on: the control of vector populations [10,22]; with the timing of insecticide application based on psyllid arrival into a crop; and psyllid population peak density [23,24]. To date, just one study has monitored psyllid populations in mainland Spain, reporting *B. trigonica*, *B. tremblayi* Wagner 1961 and *B. nigricornis* in celery and only *B. trigonica* in carrots in Tenerife [21]. The same study also reported occasional surveys i) in carrot in mainland Spain where these psyllids were found ii) in potato in mainland Spain where *B. nigricornis* and *B. trigonica* were found and iii) in potato in Tenerife where only *B. trigonica* was found. However, monitoring of psyllid populations during the complete carrot and potato growing season has not been reported in mainland Spain or other Mediterranean countries. Since the information of vector abundance is fundamental to design effective management practices to control Lso spread, we monitored the seasonal abundance of psyllids associated with carrots and potato crops in mainland Spain over three consecutive years using various sampling methods.

2. Materials and Methods

2.1. Psyllid sampling locations

Surveys were conducted three consecutive years from 2015 to 2017. In the summer of 2015 one single sampling of adult psyllids was performed by sweep net. Sampling was performed in one carrot field and one potato field, both located in Gomezserracín (Segovia, Spain). From May to October of 2016 and 2017 three different carrot fields located in Gomezserracín, Íscar (Valladolid, Spain) and Villena (Alicante, Spain) were sampled. For the potato cultivation cycle (May to September) of 2016

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one field located in Aldearrubia (Salamanca, Spain) and another field located in Gomezserracín were sampled. In 2017, only the potato field located in Aldearrubia was available for sampling. For potato and carrot in 2016 and 2017, sampling was conducted every two weeks from crop emergence to harvest by three different sampling methods: sweep net, horizontal green tile water traps and visual inspection. In the potato field located in Aldearrubia adult psyllids were also caught in 2017 by using a 12.2m suction trap. Details of field locations and cultivars grown are shown in Table 1.

Table 1. Location and information of carrot and potato fields surveyed by different sampling methods during the cultivation cycles of 2015, 2016 and 2017 in Spain.

Crop	Cultivar	Field location	Province	Latitude	Longitude	Altitude
Carrot	Bangor	Gomezserracín	Segovia	41° 17′24′′N	4° 19′32′′W	804
	Bangor	Íscar	Valladolid	41°20′05′′ N	4° 32′13′′W	750
	Soprano	Villena	Alicante	38° 38′47′′ N	0° 55′47′′W	450
Potato	Monalisa	Aldearrubia	Salamanca	49° 59′11.2′′N	5° 29′10.1′′W	812
	Monalisa	Gomezserracín	Segovia	41º 17' 20'' N	4º 19 '03'' W	805

2.2. Sweep net sampling

The carrot and potato canopy was sampled for adult psyllids using a telescopic folding sweep net (NHBS Ltd., 1-6 the Stables, Fort Road Totnes, UK). Each sample consisted of ten consecutive sweeps along a surface of 2 m² at ten different points covering a total distance of 100 m, obtaining a total of ten samples per sampling site and date. Quadrants where psyllids were sampled were not treated with insecticide. Sweep net samples were transferred to plastic ziploc® bags, then were brought to ICA-CSIC in Madrid-Spain, and frozen at -20ºC until taxonomic identification.

2.3. Horizontal green tile water traps

Horizontal green water tile traps (also called Irwin traps) [25], were selected for this study because they are neutral traps. They are similar to the canopy background color and were designed to specifically collect alate insects (mainly aphids) that land on a given row crop such as soybeans [25,26]. Traps used in this study consisted of a methacrylate container (16.5 × 16.5 × 4.5 cm) and a square ceramic tile (15.5 × 15.5 cm) (Cambridge 815 from Cambridge tile C., PO Box 15071, Cincinnati, OH 46215, USA), which was placed inside a container. The green ceramic tile has an absorbance spectrum similar to the soybean canopy but has been used to monitor sap sucking insects landing on several other row crops such as pepper, lettuce and broccoli [27–30]. The container was filled with a 50% solution of ethylene glycol in water. A second container was placed below the tile in order to avoid losing insect samples in case of heavy rains. The trap was always placed at canopy level. Insects captured were collected every two weeks and the solution in the container was changed at the same time. The trap content was filtered using a funnel, and the collected insects were preserved in 70% ethanol until taxonomic identification. Only insects from the Psylloidea superfamily were identified to species level. One trap per sampling site was used in all of the carrot and potato fields surveyed. Traps were located approximately 20 meters from the edge of the crop.

2.4. Visual inspection

In the carrot fields twenty whole plants were visually inspected for eggs and immatures at each sampling site at two-week intervals, from seedling to harvest. Plants were randomly selected by walking in a zig-zag pattern in a diagonal across the field covering a distance of 100 m. A 0 to 4 scale was used to rate immature and egg density: 0 = 0; 1= 1-4; 2=5-20; 3=21-50; 4=more than 50. Infestation of psyllids on potato fields was not scored because immatures were not detected on plants.

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2.5. Suction Trap

The daily psyllid flight activity was monitored using an insect 12.2 m high suction trap. The suction trap was located at Aldearrubia in the same potato field selected for sampling psyllids by other methods. The suction trap operated during the summer months of 2017 (15/06/2017-31/8/2017). The trap contents were removed every 24 hours at the same time daily and the insects preserved in 70% ethanol until taxonomic identification.

2.6. Psyllid identification

Adult psyllids collected by sweep net or green tile traps were identified to species level based on morphological characteristics following the taxonomic keys of Burckhardt and Freuler (2000) and Hodkinson (1981) [17,31]. Insects where morphological identification to species level was not achieved, were tested by molecular assays at SASA (Science & Advice for Scottish Agriculture, Edinburgh, United Kingdom). Psyllid DNA was extracted using a non-destructive method [32], in which the insect was pierced through the thorax and abdomen using a 0.14 steel pin and the DNA was extracted and purified using the DNeasy Blood & Tissue Kit (QUIAGEN) following the manufacturer's instructions. New pins were used for each psyllid specimen. Psyllid exoskeletons were preserved in 95% ethanol and 5% glycerol and then stored in plastic vials as voucher specimens. Two regions of psyllid DNA were amplified: Cytochrome oxidase I (COI) region using primers LCO1490 and HCO2198 according to [33] and the internal transcribed region 2 (ITS2) using primers CAS5p8sFcm and CAS28sB1d according to [34]. Successful amplifications of gene regions were verified via electrophoresis on a 1% agarose gel and visualized using an UV illuminator. PCR products were then cleaned using EXO-SAP treatment and ethanol precipitation. For the sequencing reaction "BigDye™ Terminator v3.1 Cycle Sequencing Kit" (NimaGen BV) was used and products were sequenced via Sanger capillary sequencing on a "3500 Genetic Analyser" (Applied Biosystems). Both DNA strands of the target gene regions were sequenced using forward and reverse primers seperately. Each contig was aligned to create a consensus using a CLUSTAL-W algorithm on "Geneious 10" software. Sequences were then identified using BLAST against GenBank, BOLD and SASA's psyllid DNA barcoding database to determine the closest match. Sequences with 98-100% identity scores to species in the SASA psyllid database were deemed the same species.

3. Results

3.1. Sweep net sampling

Bactericera trigonica was the most abundant species found in all the carrot fields surveyed across years and locations, followed by *B. nigricornis* (Table 2 and Table 3). In contrast, *B. nigricornis* was the predominant species in the potato fields surveyed from 2015 to 2017 (Table 2 and Table 3), except for one potato field surveyed in 2016 located in Gomezserraín where *B. trigonica* was the most abundant species (Table 3). For carrot and potato some other psyllid species were occasionally observed and although not included in the analysis, for the 2016 they are provided in Table S1.

Table 2. Occasional survey of adult psyllids associated to carrot and potato fields in Gomezserracín (Segovia) Spain conducted in July of 2015.

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Crop	Location	Sampling method	Psyllid species	Date of sampling	Total number of insects
Carrot	Gomezserracín	Sweep net	*B. trigonica*	02/07/2015	20
			B. nigricornis	02/07/2015	1
Potato	Gomezserracín	Sweep net	*B. nigricornis*	02/07/2015	5
			B. tremblayi	02/07/2015	3

In the carrot fields surveyed, psyllid populations followed similar trends between the years 2016 and 2017 (Figure 1). Psyllid population peaks varied depending on where the carrots were grown. In the carrot field located in Gomezserracín, the first psyllids appeared in late April (2016) or late May (2017) and maintained low populations until late June (2016) or early July (2017) when they showed a gradual increase reaching a maximum population peak in early August 2016 (mean number of psyllids per sweep 6.6 ± 1.32) or late July 2017 (mean number of psyllids per sweep 19.3 ± 6.30) (Figure 1a). In the carrot field located in Íscar, the first psyllids appeared in late May (2016 and 2017) and then increased gradually to a maximum population peak in October (early October 2016, mean number of psyllids per sweep 33.6 ± 2.67 and late October 2017, mean number of psyllids per sweep 13.0 ± 4.91) (Figure 1b). For the carrot field located in Villena, the first psyllids arrived at the crop in mid-May and then population density varied throughout the cultivation cycle to reach the maximum population peak in late August (late August 2016, mean number of psyllids per sweep 1.7± 0.24 and late August 2017, mean number of psyllids per sweep 6.7 ± 0.56) (Figure 1c).

Table 3. Percentage of psyllid species found by sweep net sampling in the carrot and potato fields surveyed in 2016 and 2017.

Crop	Location	Year					
		2016			2017		
		% *B. trigonica*	% *B. nigricornis*	Total number of psyllids	% B. *trigonica*	% B. *nigricornis*	Total number of psyllids
Carrot	Gomezserracín	97	3	1763	93	7	3069
	Íscar	98	2	8349	98	2	4683
	Villena	94	6	654	92	8	4750
Potato	Aldearrubia	2	98	52	11	89	249
	Gomezserracín	82	18	73			

In the two potato fields surveyed, maximum population peaks also varied depending on the field location. In the field located in Aldearrubia, the first psyllids were detected in late May 2016 and early June 2017 (Figure 1d) with maximum population peaks in early July 2016 (mean number of psyllids per sweep 0.1 ± 0.05) and in mid-June of 2017 (mean number of psyllids per sweep 3.56 ± 0.33) (Figure 1d). In the potato field located in Gomezserracín the first psyllids were not observed until late July. The maximum population peak was observed in September when the crop was harvested (mean number of psyllids per sweep 0.4 ± 0.11) (Figure 1e).

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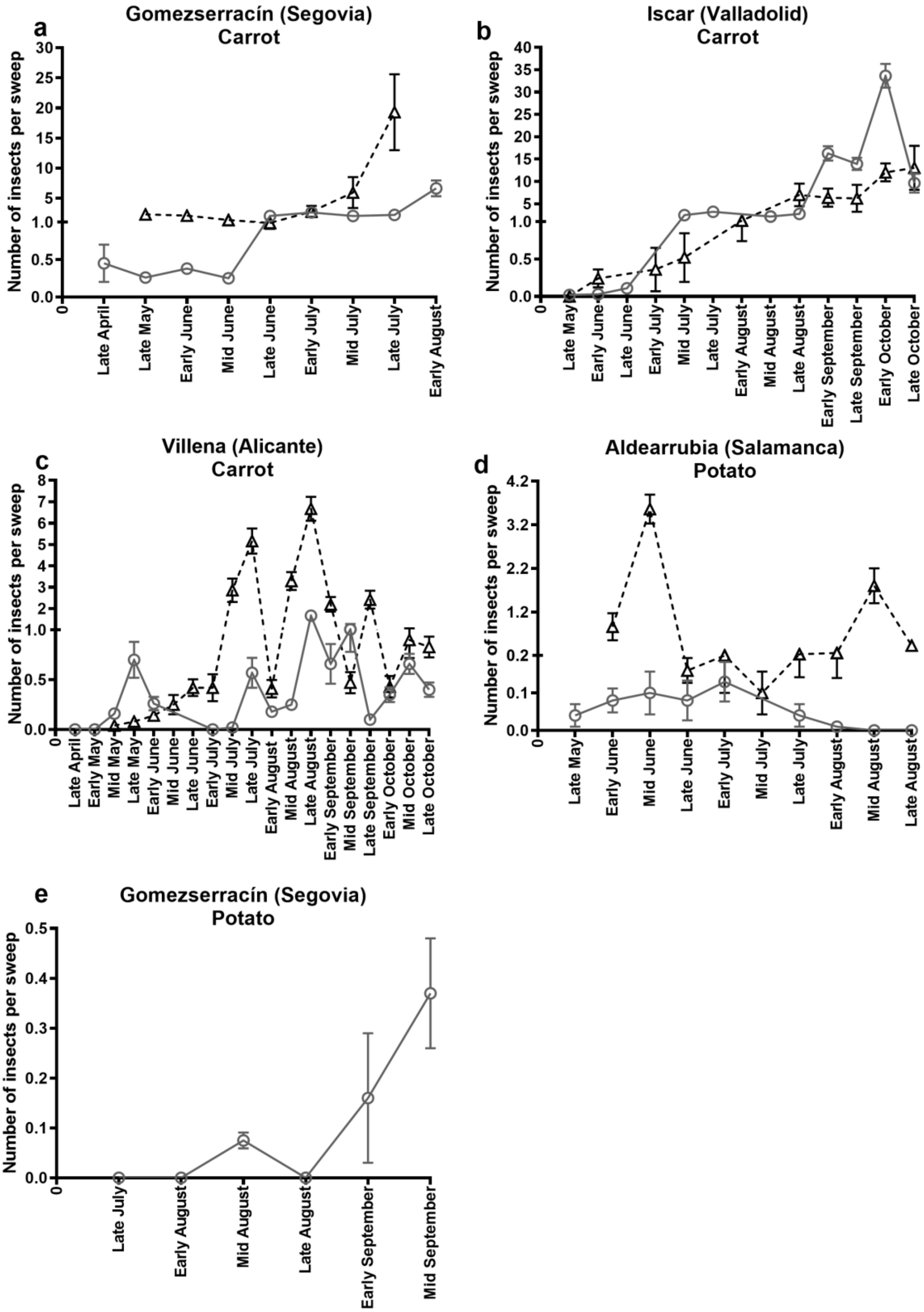

Figure 1. Population density of psyllids (*B. trigonica* + *B. nigricornis*) collected by sweep net in 2016 (Gray circles and solid line) or 2017 (Black triangles and dashed line). Samples were collected in carrot fields, (a) Gomezserracín, (b) Íscar, (c) Villena or in potato fields, (d) Albearrubia and (e) Gomezserracín. Y-axis values represent the number of insects per sweep and X-axis represents collecting dates.

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3.2. Horizontal green tile water trap sampling

Green tile traps showed *B. trigonica* to be the most dominant psyllid species in all carrot fields sampled (Table 4). *Bactericera. nigricornis* was also recorded in traps located in all carrot fields, however they were found at very low numbers (Table 4). In Gomezserracín the first psyllid adults were found in early May and remained present until the last sampling date in early August in the surveys conducted in 2016 and 2017 (Figure 2a). The maximum population peaks were observed in mid-June 2016 (total number of psyllids per trap =145) and late May in 2017 (total number of psyllids per trap =32). In the field located in Íscar, adult psyllids were consistently observed since the first samplings in May 2016 and 2017 (Figure 2b). In this field, psyllids showed its maximum population peak in early August 2016 (total number of psyllids per trap =54) and in mid-August 2017 (total number of psyllids per trap =441). Finally, the green tile trap located in the carrot field in Villena showed the presence of psyllids from late April 2016 with a maximum population peak in early September (total number of psyllids per trap =87) (Figure 2c). This contrasted with captures observed for 2017, when psyllids were absent at the first sampling dates in mid-May and were not caught until early June (total number of psyllids per trap =5). Generally, the numbers of psyllids collected in Villena for the carrot cultivation cycle of 2017 were very low.

Table 4. Percentage of psyllid species found by horizontal green tile water traps sampling in the carrot and potato fields surveyed in 2016 and 2017.

Crop	**Location**	**Year**					
		2016			2017		
		% *B. trigonica*	% *B. nigricornis*	Total number of psyllids	% *B. trigonica*	% *B. nigricornis*	Total number of psyllids
Carrot	Gomezserracín	97	3	534	74	26	121
	Íscar	94	6	365	99	1	1027
	Villena	99	1	266	75	15	8
Potato	Aldearrubia	0	100	30	10	90	21
	Gomezserracín	29	61	64			

For all potato fields, the green tile traps showed *B. nigricornis* to be the dominant species landing in the years 2016 and 2017 (Table 4). *Bactericera trigonica* was also trapped but in low numbers and not consistently observed in all surveys. It was observed in Gomezserracín in 2016 as well as in Aldearrubia in 2017, but not in 2016 in Aldearrubia (Table 4). In the potato field located in Aldearrubia, psyllids were consistently observed from May to August, showing the maximum peak in mid-June 2016 (total number of psyllids per trap =7) and in early June in the year 2017(total number of psyllids per trap =6) (Figure 2d). In the field located in Gomezserracín, psyllids were observed from the first sampling date in early June to early September. The maximum population peak occurred in early June (total number of psyllids per trap =39), and then the population decreased significantly (Figure 2e). The list of other psyllid species (*B. tremblayi*, *Trioza urticae* Linné, 1758 and *T. remota* Foerster, 1848) occasionally caught in potato is provided in supplementary material S1.

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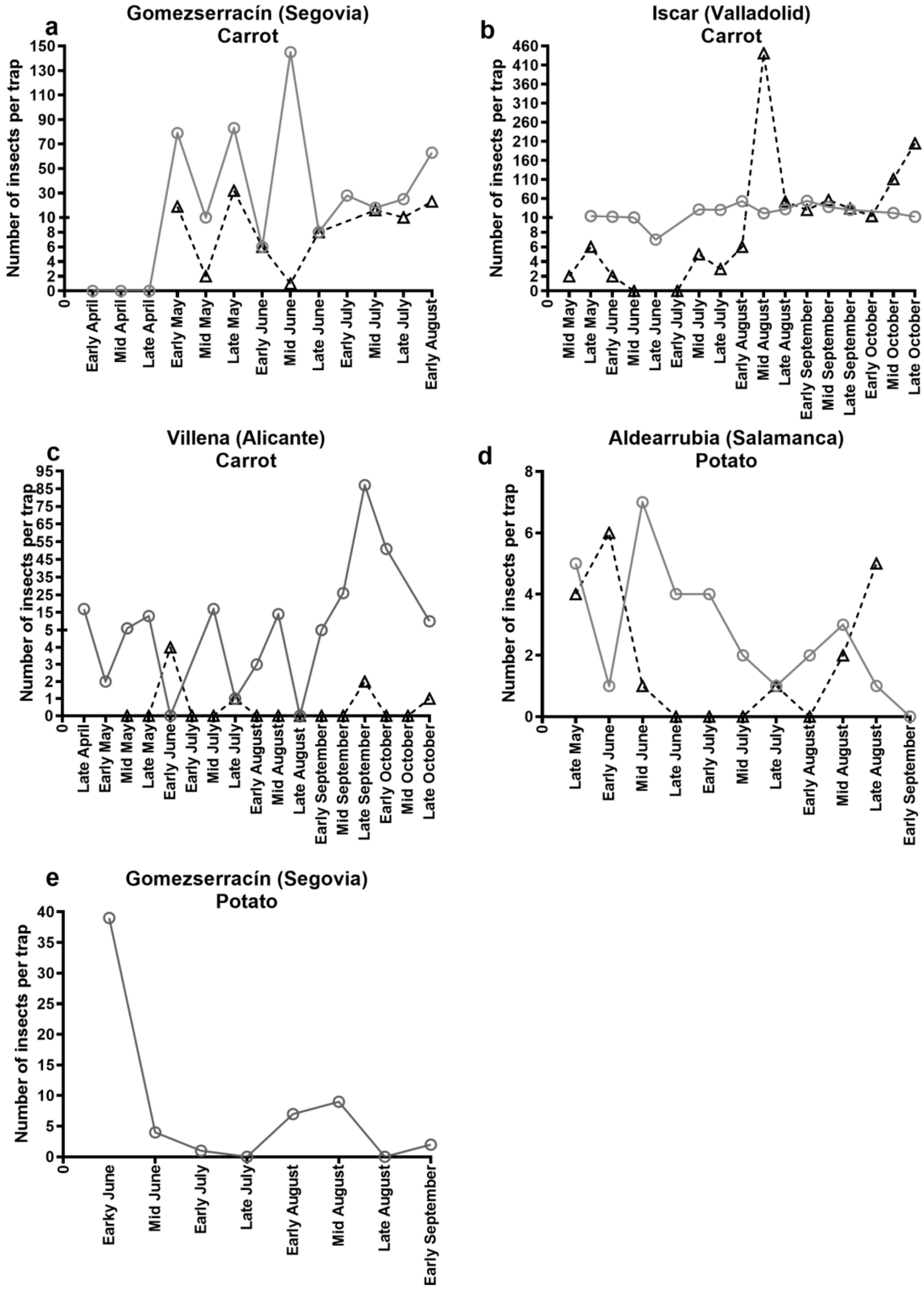

Figure 2. Population density of psyllids (*B. trigonica* + *B. nigricornis*) collected by horizontal green water tile traps in 2016 (Gray circles and solid line) or 2017 (Black, triangles and dashed line). Samples were collected in carrot fields, (a) Gomezserracín, (b) Íscar, (c) and (d) Villena or in potato fields, (e) Aldearrubia and (f) Gomezserracín.

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3.3. Visual inspection

The number of eggs and immatures sampled in carrot fields are shown in Figure 3. Overall egg and immature densities increased gradually and reached their highest densities some days earlier than the maximum population peak observed for adults: early August in Gomezserracín, early September in Íscar and mid to late September in Villena. In potato, due to the very low numbers of psyllids present in both fields, visual inspection was not performed. Despite visual identification to species level in eggs was not possible, only two psyllid species were associated to both crops, thus eggs sampled likely represent the oviposition of the two species studied.

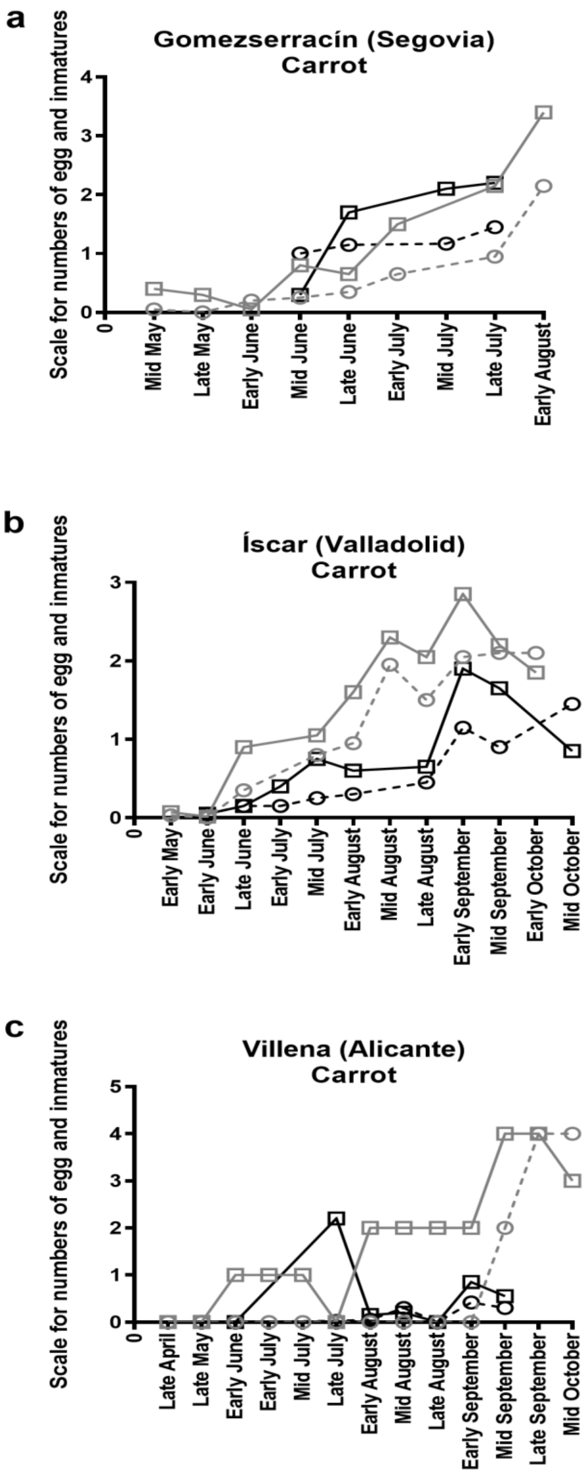

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Figure 3. Number of Eggs (Squares and solid line) or immatures (circles and dashed line) found in 2016 (Gray) and 2017 (Black) in commercial carrot fields in (a) Gomezserracín, (b) Íscar and (c) Villena. Y-axis represents scale values. Scale for eggs and nymphs used was: 0=0, 1=1-4, 2=5-20, 3=21-50, 4=more than 50.

3.4. Suction Trap

The most abundant psyllid species caught in the suction trap located in Aldearrubia in 2017 were *Blastopsylla occidentalis* Taylor, 1985 followed by *Ctenarytaina spatulata* Taylor, 1997. Just three individuals of *B. trigonica* were caught and *B. nigricornis* was not found (Table 5). According to the data collected, the psyllid species present at the crop level differed with those captured at 12.2 meters high, which likely were migrating species not capable of colonizing potato.

Table 5. Psyllid species collected by the suction trap in the potato field located in Aldearrubia Salamanca

Genus	Species	Authorship	Number	Date Collected
Arytainilla	*gredi*	(Ramirez Gomez, 1956)	1	16/06/2017
Bactericera	*trigonica*	Hodkinson, 1981	1	16/06/2017
			1	18/08/2017
			1	30/08/2017
Blastopsylla	*occidentalis*	Taylor, 1985	1	16/06/2017
			1	22/06/2017
			1	05/07/2017
			4	10/07/2017
			1	17/08/2017
			1	23/08/2017
			3	25/08/2017
Cacopsylla	*melanoneura*	(Foerster, 1848)	2	16/06/2017
			1	17/06/2017
Ctenarytaina	*spatulata*	Taylor, 1997	1	24/06/2017
			1	10/07/2017
			1	12/07/2017
			1	16/07/2017
Livia	*junci*	(Schrank, 1789)	1	16/06/2017
Trioza	*galii*	Foerster, 1848	1	16/06/2017
			1	17/06/2017

4. Discussion

Previous studies have shown that only the psyllid species that are able to colonize a given crop can efficiently transmit Lso [7,16,35]. Therefore, knowledge of the population dynamics of psyllids in European carrot and potato crops is important to understand Lso spread in the field and to assess the potential risk of Lso introduction to potato crops.

In this work, by using different sampling techniques such as sweep net, tile water traps and visual inspection we clearly showed that *B. trigonica* and *B. nigricornis* adults were consistently associated with both carrot and potato crops. However, *B. trigonica* was the most dominant species

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found in all carrot crops and *B. nigricornis* was the dominant species in potato. These results are consistent with previous work that found *B. trigonica* as the predominant species in celery and carrot crops in mainland Spain and Tenerife [21]. However, Teresani et al. (2015) [21] did not report *B. nigricornis* on carrots, except for a field located in La Rioja in an occasional survey using sticky traps (38 psyllids from one sampling) in the year 2012, and reported very low populations in celery (2 psyllids in total from 3 samplings) and potato (2 psyllids from one sampling). Our results are also consistent with field surveys in carrot crops conducted in Tunisia were *B. trigonica* and *B. nigricornis* were the only two psyllid species found [20]. On the other hand, psyllid species surveyed by the 12.2 m suction trap on potato differed from those obtained by the other sampling methods used. Psyllids caught by the suction trap were mostly migrating species not associated with carrot or potato crops. This is consistent with a recent study from Germany where no carrot psyllids were caught by using a similar suction trap in a carrot field [36]. This suggests that sampling methods performed at the crop canopy level give a more accurate estimate of the species present on carrot and potato crops than suction traps.

The psyllid population peaks from sweep nets observed in carrot occurred from June to October and although the timing of these peaks varied according to location it was consistent at each location in 2016 and 2017. This data slightly differs from that reported by Teresani et al (2015) [21], who observed the most important psyllid population peaks from April to August on celery crops in Villena and in July for carrot crops in Tenerife (Canary Islands). The dominant species found in our work, differed with those species collected in carrot fields in Valais, Switzerland, that were *Trioza apicalis*, *B. nigricornis* and *T. anthrisci* Burckhardt, 1986 [17]. In their survey, *B. nigricornis* was the most abundant species in two of the three carrot fields sampled; whereas *B. trigonica* was found in very low numbers. The very different environmental conditions between the two regions under study (Spain and Switzerland) might explain differences in the psyllid fauna composition. The current distribution of *B. trigonica* in the Mediterranean region suggests that the climatic conditions of this zone might favour this species over other species such as *B. nigricornis*, *T. apicalis* or *T. anthrisci*. To date, the latter two species commonly associated with Apiaceae plants in cooler and humid regions in Northern and Central Europe [37,38], have not been reported in Spain.

Adults of *B. trigonica* were detected at the first sampling dates in carrot fields. However, it is not known if these were adults that migrated from other zones or were already present nearby. For this species, migration behavior has not been studied and it is uncertain if adults overwinter on plant species different to those commonly reported as hosts. However, in Central Europe Burckhardt and Freuler (2000) [17], suggested that *B. trigonica* can overwinter as adult in conifers or evergreen shrubs. *Bactericera trigonica* may also migrate from carrots or celery crops that are widely present, because of the favorable climatic conditions the hole year-round in the regions where the surveys were conducted. In contrast to *B. trigonica*, *B. nigricornis* was absent in the first sampling dates suggesting that the migration from other hosts or fields occurs later than *B. trigonica*. Although *B. nigricornis* is present in many countries [39] little is known about its biology and dispersal ability, but it has been mentioned as a conifer overwintering species [40]. Furthermore, *B. nigricornis* has been reported as a polyphagous species able to feed from wild species belonging to the Amarantaceae Boraginaceae, Brassicaceae, Liliaceae, Papaveraceae and Solanaceae [31]. Thus, further research on the life cycle of this species is needed to identify its overwintering hosts or migration habits from wild host species under Mediterranean conditions.

Although eggs and immatures of *B. nigricornis* were not observed during visual inspections, previous reports have confirmed that this psyllid species is able to breed on potato [31] and has been reported causing severe yield losses in Iran [19]. Interestingly, *B. nigricornis* has not caused economic damage to potato crops in Spain, perhaps due to its low population density on these crops. Nevertheless, monitoring its populations is highly recommended as a preventive measure to avoid potential outbreaks.

The high numbers of *B. trigonica* detected in the potato field located in Gomezserracín in 2016 were unexpected as previous studies have suggested that this species is only restricted to Apiaceae plants and does not breed on potato [16,18]. However, *B. trigonica* arrived late to that potato crop and no immature stages were observed. Since the arrival dates of *B. trigonica* on the potato field coincides with the harvesting of carrot fields in the same region we suggest that the high population density of *B. trigonica* recorded on that potato field is due to the active movement of *B. trigonica* into potato when carrots were no longer available. Despite not being able to colonize potato, adults of *B. trigonica* might be able to survive on "Food plants" [41] for a short time as has been reported for related psyllid species such as *B. cockerelli* [35].

Transmission of vector- borne pathogens is affected by several factors with vector activity (the abundance of insects landing on the crop) and vector propensity especially important [26]. High populations of *B. trigonica* in carrots reported here and evidence of high Lso vector transmission ability of *B. trigonica* from previous works [16,42,43], could explain the high Lso incidence reported in carrot fields in Spain [2,5]. However, for potato, the low number of *B. trigonica* found on the crop and previous experimental evidence suggests that the risk of Lso transmission from carrot to potato by this psyllid species is negligible [16].

On the other hand, since *B. nigricornis* was consistently found on both crops and to date is the only known European psyllid able to breed on potato crop, there is a potential risk of Lso transmission from carrot to potato. Although the vector efficiency of *B. nigricornis* has not been assessed, previous field works have shown that it can become naturally infected with Lso [21]. Preliminary work being conducted at ICA-CSIC suggests that infected *B. nigricornis* is able to transmit Lso to carrot and potato and cause typical symptoms of disease (Ontiveros et al, in preparation). However, even if Lso transmission is possible by *B. nigricornis*, the frequency and significance of this transmission remains uncertain for field conditions since the observed population numbers for this species were low. Thus, further research aimed at understanding Lso primary transmission from carrot to potato and secondary transmission from potato to potato plants by *B. nigricornis* under field conditions in Europe is needed.

5. Conclusions

The psyllid *B. trigonica* was the most abundant species associated to carrot whereas *B. nigricornis* was the most abundant associated to potato. Overall, our research in Spain contributes significantly to understanding population dynamics of psyllids in carrot and potato crops which is required for Lso pest risk analysis by National and Regional Plant Protection Organizations.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: List of psyllid species occasionally found in the carrot and potato fields surveyed in 2016.

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References

1. 1. Eurostat Agricultural production - crops - Statistics Explained Available online: https://ec.europa.eu/eurostat/statistics-explained/index.php/Agricultural_production_-_crops#Vegetables (accessed on Jun 27, 2019).
2. 2. Alfaro-Fernández, A.; Cebrián, M.C.; Villaescusa, F.J.; de Mendoza, A.H.; Ferrándiz, J.C.; Sanjuán, S.; Font, M.I. First Report of "*Candidatus* Liberibacter solanacearum" in Carrot in Mainland Spain. *Plant Dis.* **2012**, *96*, 582.
3. 3. Alfaro-Fernández, A.; Siverio, F.; Cebrián, M.C.; Villaescusa, F.J.; Font, M.I. "*Candidatus* Liberibacter solanacearum" Associated with *Bactericera trigonica*-Affected Carrots in the Canary Islands. *Plant Dis.* **2012**, *96*, 581.
4. 4. Munyaneza, J.E.; Fisher, T.W.; Sengoda, V.G.; Garczynski, S.F.; Nissinen, A.; Lemmetty, A. First Report of "*Candidatus* Liberibacter solanacearum" Associated with Psyllid-Affected Carrots in Europe. *Plant Dis.* **2010**, *94*, 639.
5. 5. Ben Othmen, S.; Morán, F.E.; Navarro, I.; Barbé, S.; Martínez, C.; Marco-Noales, E.; Chermiti, B.; López, M.M. '*Candidatus* Liberibacter solanacearum' haplotypes D and E in carrot plants and seeds in Tunisia. *J. Plant Pathol.* **2018**, *100*, 197–207.
6. 6. Palomo, J.; Bertolini, E.; Martin-Robles, M.; Teresani, G.; Lopez, M.; Cambra, M. Detección en Patata en España de un haplotipo de "*Candidatus* Liberibacter solanacearum" no descrito en solanáceas.; Lleida España, 2014; p. 125.
7. 7. Haapalainen, M.; Latvala, S.; Rastas, M.; Wang, J.; Hannukkala, A.; Pirhonen, M.; Nissinen, A.I. Carrot Pathogen '*Candidatus* Liberibacter solanacearum' Haplotype C Detected in Symptomless Potato Plants in Finland. *Potato Res.* **2018**, *61*, 31–50.
8. 8. Liefting, L.W.; Sutherland, P.W.; Ward, L.I.; Paice, K.L.; Weir, B.S.; Clover, G.R.G. A New "*Candidatus* Liberibacter" Species Associated with Diseases of Solanaceous Crops. *Plant Dis.* **2009**, *93*, 208–214.
9. 9. Munyaneza, J.E.; Crosslin, J.M.; Upton, J.E. Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with "zebra chip," a new potato disease in southwestern United States and Mexico. *J. Econ. Entomol.* **2007**, *100*, 656–663.
10. 10. Munyaneza, J. Zebra Chip Disease of Potato: Biology, Epidemiology, and Management. *Am. J. Potato Res.* **2012**, *89*, 329–350.
11. 11. Munyaneza, J.E.; Buchman, J.L.; Upton, J.E.; Goolsby, J.A.; Crosslin, J.M.; Bester, G.; Miles, G.P.; Sengoda, V.G. Impact of Different Potato Psyllid Populations on Zebra Chip Disease Incidence, Severity, and Potato Yield. *Subtrop. Plant Sci.* **2008**, *60*, 27–37.
12. 12. Hansen, A.K.; Trumble, J.T.; Stouthamer, R.; Paine, T.D. A New Huanglongbing Species, "*Candidatus* Liberibacter psyllaurous," Found To Infect Tomato and Potato, Is Vectored by the Psyllid Bactericera cockerelli (Sulc). *Appl. Environ. Microbiol.* **2008**, *74*, 5862–5865.
13. 13. Teulon, D. a. J.; Workman, P.J.; Thomas, K.L.; Nielsen, M.C. *Bactericera cockerelli*: incursion, dispersal and current distribution on vegetable crops in New Zealand. *N. Z. Plant Prot.* **2009**, *62*, 136–144.
14. 14. IPPC Detection of *Bactericera cockerelli* (Tomato-potato psyllid) in Western Australia Available online: https://www.ippc.int/en/countries/australia/pestreports/2017/02/detection-of-bactericera-cockerelli-tomato-potato-psyllid-in-western-australia/ (accessed on Jun 25, 2019).
15. 15. Ouvrard, D. The World Psylloidea Database Available online: https://www.hemiptera-databases.org/psyllist/?db=psylles&lang=en&card=taxon&rank=species&id=1337 (accessed on Jun 1, 2019).
16. 16. Antolinez, C.A.; Fereres, A.; Moreno, A. Risk assessment of '*Candidatus* Liberibacter solanacearum' transmission by the psyllids *Bactericera trigonica* and *B. tremblayi* from Apiaceae crops to potato. *Sci. Rep.* **2017**, *7*, 45534.
17. 17. Burckhardt, D.; Freuler, J. Jumping plant-lice (Hemiptera, Psylloidea) from sticky traps in carrot fields in Valais, Switzerland. *Mitteilungen Schweiz. Entomol. Ges.* **2000**, *73*, 191–209.
18. 18. Hodkinson, I.D. Life cycle variation and adaptation in jumping plant lice (Insecta: Hemiptera: Psylloidea): a global synthesis. *J. Nat. Hist.* **2009**, *43*, 65–179.
19. 19. Fathi, S.A.A. Population density and life-history parameters of the psyllid *Bactericera nigricornis* (Forster) on four commercial cultivars of potato. *Crop Prot.* **2011**, *30*, 844–848.

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Peer-reviewed version available at *Insects* **2019**, *10*, 287; doi:10.3390/insects10090287

20. 20. Othmen, S.B.; Abbes, K.; Imem, M.E.; Ouvrard, D.; Rapisarda, C.; Chermiti, B. *Bactericera trigonica* and *B. nigricornis* (Hemiptera: Psylloidea) in Tunisia as potential vectors of '*Candidatus* Liberibacter solanacearum' on Apiaceae. *Orient. Insects* **2018**, *0*, 1–13.
21. 21. Teresani, G.; Hernández, E.; Bertolini, E.; Siverio, F.; Marroquín, C.; Molina, J.; Mendoza, A.H. de; Cambra, M. Search for potential vectors of '*Candidatus* Liberibacter solanacearum': population dynamics in host crops. *Span. J. Agric. Res.* **2015**, *13*, 1002.
22. 22. Gharalari, A.H.; Nansen, C.; Lawson, D.S.; Gilley, J.; Munyaneza, J.E.; Vaughn, K. Knockdown mortality, repellency, and residual effects of insecticides for control of adult *Bactericera cockerelli* (Hemiptera: Psyllidae). *J. Econ. Entomol.* **2009**, *102*, 1032–1038.
23. 23. Tsai, J.H.; Wang, J.-J.; Liu, Y.-H. SEASONAL ABUNDANCE OF THE ASIAN CITRUS PSYLLID, DIAPHORINA CITRI (HOMOPTERA: PSYLLIDAE) IN SOUTHERN FLORIDA. *Fla. Entomol.* **2002**, *85*, 446–451.
24. 24. Munyaneza, J.E.; Crosslin, J.M.; Buchman, J.L. Seasonal Occurrence and Abundance of the Potato Psyllid, *Bactericera cockerelli*, in South Central Washington. *Am. J. Potato Res.* **2009**, *86*, 513.
25. 25. Irwin, M.E. Sampling Aphids in Soybean Fields. In *Sampling Methods in Soybean Entomology*; Kogan, M., Herzog, D.C., Eds.; Springer Series in Experimental Entomology; Springer New York: New York, NY, 1980; pp. 239–259 ISBN 978-1-4612-9998-1.
26. 26. Irwin, M.E. *Vector Intensity: A product of Propensity and Activity. In: McLean GD, Garret RG, Ruesink WG (Eds) Plant virus epidemics: monitoring, modeling and predicting outbreaks*; Academic Press: London, 1986;
27. 27. Summers, E.G.; Newton, A.S.; Kirk, M.; Temple, S.R. Transmission of Beet Yellows and Beet Mosaic Viruses by Noncolonizing Aphid Vectors. *J. Econ. Entomol.* **1990**, *83*, 2448–2451.
28. 28. Kirk, M.; Temple, S.R.; Summers, C.G.; Wilson, L.T. Transmission Efficiencies of Field-Collected Aphid (Homoptera: Aphididae) Vectors of Beet Yellows Virus. *J. Econ. Entomol.* **1991**, *84*, 638–643.
29. 29. Perez, P.; Duque, M.; Collar, J.L.; Avilla, C.; Fereres, A. Activity of alatae aphids landing on open-field pepper crops in Spain / Aktivität und Vorkommen geflügelter Blattläuse auf Freilandpaprika in Spanien. *Z. Für Pflanzenkrankh. Pflanzenschutz J. Plant Dis. Prot.* **2003**, *110*, 195–202.
30. 30. Nebreda, M.; Moreno, A.; Pérez, N.; Palacios, I.; Seco-Fernández, V.; Fereres, A. Activity of aphids associated with lettuce and broccoli in Spain and their efficiency as vectors of Lettuce mosaic virus. *Virus Res.* **2004**, *100*, 83–88.
31. 31. Hodkinson, I.D. Status and taxonomy of the *Trioza* (*Bactericera) nigricornis* Förster complex (Hemiptera: Triozidae). *Bull. Entomol. Res.* **1981**, *71*, 671–679.
32. 32. Sjölund, M.; Kenyon, D.; Highet, F.; Ouvrard, D. Developing an RT-PCR assay for the identification of psyllid species. *Proc. Crop Prot. North. Br.* **2016**.
33. 33. Vossenberg, B.T.L.H. van de; Westenberg, M.; Bonants, P.J.M. DNA barcoding as an identification tool for selected EU-regulated plant pests: an international collaborative test performance study among 14 laboratories. *EPPO Bull.* **2013**, *43*, 216–228.
34. 34. Peccoud, J.; Labonne, G.; Sauvion, N. Molecular test to assign individuals within the *Cacopsylla pruni* complex. *PloS One* **2013**, *8*, e72454.
35. 35. Munyaneza, J.E.; Mustafa, T.; Fisher, T.W.; Sengoda, V.G.; Horton, D.R. Assessing the Likelihood of Transmission of *Candidatus* Liberibacter solanacearum to Carrot by Potato Psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae). *PloS One* **2016**, *11*, e0161016.
36. 36. Sjolund, M.J.; Arnsdorf, Y.M.; Carnegie, M.; Fornefeld, E.; Will, T. '*Candidatus* Liberibacter solanacearum' detected in *Trioza urticae* using suction trap-based monitoring of psyllids in Germany. *J. Plant Dis. Prot.* **2019**, *126*, 89–92.
37. 37. Burckhardt, D. Taxonomy and host plant relationships of the *Trioza apicalis* Forster complex (Hemiptera, Homoptera: Triozidae). *Entomol. Scand.* **1985**, *16*, 415–432.
38. 38. Hodkinson, I. The Psylloidea (Homoptera) of Fennoscandia and Denmark. Volume 26—Fauna Entomologica Scandinavica. *Ann. Entomol. Soc. Am.* **1994**, *87*, 401–402.
39. 39. Ouvrard, D. The World Psylloidea Database Available online: https://www.hemiptera-databases.org/psyllist/?db=psylles&lang=en&card=taxon&rank=species&id=1332 (accessed on Jun 20, 2019).
40. 40. Cermak, V.; Lauterer, P.; Maini, S. Overwintering of psyllids in South Moravia (Czech Republic) with respect to the vectors of the apple proliferation cluster phytoplasmas.; 2008.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 8 July 2019 doi:10.20944/preprints201907.0094.v1

Peer-reviewed version available at *Insects* **2019**, *10*, 287; doi:10.3390/insects10090287

41. 41. Burckhardt, D.; Ouvrard, D.; Queiroz, D.; Percy, D. Psyllid Host-Plants (Hemiptera: Psylloidea): Resolving a Semantic Problem. *Fla. Entomol.* **2014**, *97*, 242–246.
42. 42. Antolinez, C.A.; Moreno, A.; Appezzato-da-Gloria, B.; Fereres, A. Characterization of the electrical penetration graphs of the psyllid *Bactericera trigonica* on carrots. *Entomol. Exp. Appl.* **2017**, *163*, 127–139.
43. 43. Antolínez, C.A.; Fereres, A.; Moreno, A. Sex-specific probing behaviour of the carrot psyllid *Bactericera trigonica* and its implication in the transmission of '*Candidatus* Liberibacter solanacearum.' *Eur. J. Plant Pathol.* **2017**, *147*, 627–637.