



# Article Survival and Feeding Behavior of *Diaphorina citri* (Hemiptera: Liviidae) Adults on Common Cover Crops in Citrus

Justin George <sup>1,2,\*</sup>, Ramdas Kanissery <sup>3</sup>, Mahesh Bashyal <sup>3</sup>, Blessy Tamayo <sup>2</sup> and Lukasz L. Stelinski <sup>2</sup>

- <sup>1</sup> USDA-ARS, Southern Insect Management Research Unit, 141 Experiment Station Rd., Stoneville, MS 38776, USA
- <sup>2</sup> Department of Entomology and Nematology, Citrus Research and Education Center, University of Florida, 700 Experiment Station Rd., Lake Alfred, FL 33850, USA
- <sup>3</sup> Department of Horticultural Sciences, Southwest Florida Research and Education Center,
- University of Florida, 2685 SR 29 North Immokalee, Immokalee, FL 34142, USA
- Correspondence: justin.george@usda.gov

**Abstract:** Asian citrus psyllid, *Diaphorina citri*, transmits *Candidatus* Liberibacter asiaticus (CLas), the bacterial pathogen responsible for citrus greening disease. To explore the possibility that cover crops in citrus groves may serve as refuges for this pathogen vector during unfavorable host conditions, psyllid feeding was investigated on six common cover crop species and citrus using electrical penetration graph (EPG) recordings and behavioral bioassays. EPG recordings showed that the proportion of time spent by *D. citri* feeding on xylem was similar or higher on all tested cover crops (17%–32%) compared to the positive control (12%), the preferred host, *Citrus macrophylla*. Very little to no phloem feeding was observed on cover crops by the adults. In the choice assays, more *D. citri* adults settled on buckwheat (*Fagopyrum esculentum*) and cowpea (*Vigna unguiculata*) than on the host, *C. macrophylla*, 24 h after release. No-choice assays showed that the citrus cover crop species evaluated extend the survival of *D.* citri up to 8 d because of xylem feeding. Our results indicate that some cover crop species may be less suitable refuge sites for *D. citri* than others, but none served as breeding sites or supported more than 8 d of survival.

**Keywords:** Asian citrus psyllids; *Diaphorina citri*; cover crops; electrical penetration graph; xylem feeding; phloem feeding

# 1. Introduction

Cover crops have long been used in citrus for weed management and are especially important for organic citrus production, where agrochemical use is discouraged [1]. Besides weed suppression, additional benefits from planting cover crops include increased microbial diversity and reduced weed seed vigor from unremoved residues [2], increased soil C and N content, as well as suppressed activity from parasitic nematodes [3], and soil pathogens via allelochemicals [4]. The additional abundance and diversity from ground cover vegetation are positively correlated with the presence of insects [5], which can benefit or, in some cases, encumber pest management. While many previous studies have linked cover crops to increases in arthropod predator abundance and diversity [6,7], studies have also suggested that pest species can benefit from cover crops [8,9].

Suppression of Asian citrus psyllid, *Diaphorina citri* Kuwayama, populations is an important component of managing the currently incurable citrus greening disease [10]. A feeding study conducted by George et al. [11] suggested that when host conditions are unfavorable, adult *D. citri* can use weed species commonly found in Florida citrus groves as a short-term refuge. This highlights the importance of choosing cover crop species that both outcompete weed species in citrus but that do not themselves act as suitable refuges for *D. citri* in Florida citrus groves. Selecting a cover crop that both attracts predators, pollinators, and herbivore natural enemies while simultaneously repelling or outcompeting damaging



Citation: George, J.; Kanissery, R.; Bashyal, M.; Tamayo, B.; Stelinski, L.L. Survival and Feeding Behavior of *Diaphorina citri* (Hemiptera: Liviidae) Adults on Common Cover Crops in Citrus. *Agriculture* **2022**, *12*, 2175. https://doi.org/10.3390/ agriculture121222175

Academic Editor: Wenqing Zhang

Received: 31 October 2022 Accepted: 9 December 2022 Published: 19 December 2022

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). herbivores and weeds is challenging and unlikely possible without some compromise [12]. As a starting point, an evaluation of cover crop suitability as hosts to key pests of a particular cultivated crop could allow growers to make more informed choices that benefit production and yield.

Commonly used cover crop species in Florida citrus production include buckwheat Fagopyrum esculentum, (Polygonacea), daikon radish, Raphanus sativus var. longipinnatus, (Brassicaceae), and numerous legumes (Fabaceae) such as cowpea (Vigna unguiculata), clover (Trifolium incarnatum), perennial peanut (Arachis glabrata), hairy vetch (Vicia villosa), or a selected mixture of these plants. Among these six species, buckwheat has been demonstrated to increase the diversity of beneficial parasitoids, predators, and pollinators in various agriculture settings, and numerous studies conducted in vineyards with buckwheat as ground covers showed enhanced biological control of various arthropod pests [13–15]. Certain cultivars of cowpea, such as 'Mississippi Silver' and 'Iron Clay', exhibit resistance to the root-knot nematode, *Meloidogyne incognita*, while other species may increase nematode populations [16]. Cowpea also exhibits resistance to arthropods, such as fall armyworms, Spodoptera frugiperda (J.E. Smith) [17]. Moreover, cowpea plants can attract parasitoid wasps and other beneficial insects following damage by stink bugs, leaf-footed bugs, and aphids [18]. Manandhar and Wright [19] reported an increase in parasitization of *Helicoverpa zea* eggs by *Trichogramma* spp. from an established cover crop mixture of cowpeas and sunn hemp (Crotalaria juncea) between rows of sweetcorn. The same study also revealed an increase in predator populations (*Orius* spp.), which is likely a consequence of additional nectar and pollen provided by the cover crops. When used as cover crop monocultures, clover and buckwheat exhibited repellent activities against silverleaf whitefly, Bemisia argentifolii, in zucchini production fields [20]. Clover enhanced biological control of grape leafhopper (*Erythroneura* spp.) by *Anagrus* wasps in commercial vineyards [21]. A radish-clover mixture was reported to increase beneficial insects such as big-eyed bugs (Geocoris punctipes) and lady beetles (Coccinellidae) in Southeastern United States (U.S.) while also being preferred by pest insects such as the tarnished plant bug (Lygus lineolaris) [22]. Coincidentally, Bugg and Ellis [23] reported that hairy vetch (Vicia villosa) harbored exceptionally high densities of aphids (Acyrthosiphon pisum), while both hairy vetch and buckwheat harbored high densities of the insidious flower bug (Orius *insidiosus*) and the tarnished plant bug (*Lygus lineolaris*). A related investigation suggested that interspersion of ryegrass, *Lolium multiflorum* (Poaceae) with hairy vetch and clover reduced both aphid and coccinellid densities; however, this suppressive effect was not consistent over time [24].

Out of these six species listed above, cowpea, buckwheat, and clover have been evaluated as cover crops, most often including their interactions with beneficial/pest insect populations. In contrast, daikon radish and perennial peanuts have received little attention previously with respect to their interactions with commonly occurring pests in tree fruit or citrus in particular. With a growing interest in introducing cover crops into citriculture as a sustainable weed management practice, there is a need to investigate their compatibility with key pests of cultivated citrus. Therefore, our objectives were to determine if any of the six potential cover crop species could serve as potential hosts or feeding resources for *D. citri* as compared to a true host, *Citrus macrophylla* (Rutaceae), serving as a positive control. Cryptic xylem- and phloem-feeding behaviors of *D. citri* were compared between cover crops and hosts using electrical penetration graph recordings. Furthermore, choice and no-choice assays were performed to investigate plant acceptance by *D. citri* as well as the duration of survival.

#### 2. Materials and Methods

#### 2.1. Plants

Cover crop plant species evaluated in electrical penetration graph (EPG) recordings and behavioral assays were buckwheat, cowpea, clover, daikon radish, perennial peanut (not shown), and hairy vetch (Figure 1). Seeds were procured from the Hancock seed company (Dade City, FL, USA) and planted in 1-liter plastic pots filled with a 2:1 potting mix: sand mixture (Pro-Mix BX Mycorrhizae growing medium, Quakertown, PA, USA). The test plants were raised in a temperature-controlled greenhouse under natural sunlight conditions. *C. macrophylla*, a known host of *D. citri*, was used as a positive control and was grown under identical conditions. Young leaves (soft, immature, and fully expanded) of *C. macrophylla* and cover crop plants were used in the EPG recordings. Plants of the same approximate age (5 weeks) and size were used in the choice and no-choice experiments. Plants were watered every other day with tap water during the duration of the experiment.



**Figure 1.** Feeding activities of *Diaphorina citri* on various cover crops. (**A**) Cowpea (*Vigna unguiculata*); (**B**) buckwheat (*Fagopyrum esculentum*); (**C**) hairy vetch (*Vicia villosa*); (**D**) daikon radish (*Raphanus sativus* var. *longipinnatus*); (**E**) clover (*Trifolium incarnatum*); (**F**) *Citrus macrophylla*.

#### 2.2. Insects

Adult *D. citri* used in the experiments were acquired from a *D. citri* colony established in 2000 at the USDA-ARS U.S. Horticultural Research Laboratory, Fort Pierce, FL. This colony was maintained on *C. macrophylla*, as described by Skelley and Hoy in [25]. To confirm the absence of CLas, the colony was tested quarterly by qPCR [26]. All the adults used for the EPG recordings were 6–8 days old. Previous research has shown no differences in the EPG waveforms produced by male and female *D. citri* [27,28]. Therefore, the sex of the psyllids used was not considered a factor in the data analysis.

# 2.3. Feeding Behavior of D. citri Adults on Cover Crop Plants versus Citrus Using an Electrical Penetration Graph (EPG)

The feeding behavior of *D. citri* adults was quantified, as described by George et al., in [11]. In brief, young, fully expanded leaves of cover crop species and *C. macrophylla* were used for EPG recordings. A DC-monitor was used to perform the EPG recordings (GIGA-8 model, EPG-Systems, Wageningen, The Netherlands) adjusted to a 50x gain [29]. To digitize the analog signal, a DI-710 board was used and displayed using the Windaq Lite ver. 2.40 software (Dataq Instruments Inc. Akron, OH, USA) on a desktop computer (Dell, Austin,

TX, USA). Recordings were performed for 18 h under continuous photophase, and the EPG system was set up inside a Faraday cage housed in an environmentally regulated room. The temperature was set to 26 °C with 60–65% RH. Prior to the start of the experiment, psyllid adults were aspirated into glass vials and starved for 2 h.

Psyllid adults were attached to a 25  $\mu$ m gold wire (Sigmund Cohn Corp., Mt. Vernon, New York, NY, USA) by a droplet of silver conducting paint (Ladd Research Industries, Burlington, VT, USA) applied to the pronotum [28,29]. The gold wire lead was attached to a copper electrode (3 cm × 1 mm diameter) connected to the EPG probe. A reference copper electrode (10 cm × 2 mm) was inserted into the soil medium near the base of each plant to complete the electrical circuit. Psyllid adult feeding was restricted on the abaxial surface of fully expanded leaves (the preferred feeding site on leaves) of cover crop plants or citrus [28,30,31]. The feeding behaviors of individual *D. citri* adults were monitored on cowpea (n = 12), daikon radish (n = 12), hairy vetch (n = 12), buckwheat (n = 11), perennial peanut (n = 10), clover (n = 6), and *C. macrophylla* (n = 17) for a continuous period of 18 h.

EPG waveform characterization was performed by visually identifying and annotating waveforms based on previous histological studies [28,30]. Waveform annotations were conducted using the Windows Dataq waveform browser (Dataq Instruments Inc., Akron, OH, USA). The waveforms were visually inspected for frequency patterns and annotated as non-probing (Np), mesophyll intercellular pathway (C), phloem penetration (D), phloem salivation (E1), phloem ingestion (E2), or xylem ingestion (G) phases. JMP (v. 10, SAS Inc, Cary, NC, USA) was used for performing the statistical analysis. The mean numbers of feeding bouts, duration, and total duration of different feeding activities were analyzed by analysis of variance (ANOVA) and mean comparisons using Tukey's HSD using log-transformed data. The Kruskal–Wallis test, followed by Dunn's test, was performed for nonparametric pairwise comparison using JMP (v. 10, SAS Inc., Cary, NC, USA) if normal distribution criteria were not met. Treatments that have no letters in common were significantly different ( $\alpha = 0.05$ ).

#### 2.4. Behavioral Choice of D. citri to Various Citrus Cover Crop Species and Citrus

The host preference and feeding choice of *D. citri* adults to various cover crop plants and citrus were investigated in a choice assay. One-liter pots containing either buckwheat, cowpea, clover, daikon radish, perennial peanut, hairy vetch, or *Citrus macrophylla* were used in the choice assay (Figure 1). The plants were five weeks old. Six cover crop plants and one citrus plant were arranged in a circular pattern in a completely randomized design inside screened cages ( $60 \times 60 \times 60$  cm, BioQuip, San Diego, CA, USA) and placed under grow lights on a bench in a walk-in growth chamber ( $26 \degree C$ , 65% RH, 14: 10 L/D). Each cage received 100 adult *D. citri* adults. The experiment was replicated six times. *D. citri* were allowed to settle and feed on host plants of their choice, and their host preferences were recorded at 4, 24, and 48 h after release. The numbers of *D. citri* alighting on each plant species were analyzed using the Kruskal–Wallis test followed by Dunn's test for nonparametric pairwise comparison ( $\alpha = 0.05$ ).

#### 2.5. Survival of D. citri Adults on Citrus Cover Crop Species in No-Choice Assays

To measure the survival of psyllids on various cover crop plants versus the true citrus host, no-choice assays were conducted using potted plants within screened cages. Individual plants were placed into mesh cages, after which twenty-five *D. citri* adults were introduced. The adults were allowed to feed on the plants, and the number of surviving psyllid adults was counted 2, 4, 6, 8, and 10 d after their release on the plant treatments. A negative control was included, in which twenty-five adults were released into cages containing only moistened cotton rolls as a water source. This quantified the survival of psyllids under starvation conditions. All treatments tested in choice and no-choice assays were identical to those tested in EPG recordings, and each treatment was replicated six times. To assess the influence of these cover crops on *D. citri* survival, log-transformed data were analyzed by ANOVA using JMP (v. 10, SAS Inc., Cary, NC, USA). Data from no-choice

assays were subjected to the Kaplan–Meier test to determine the survival probability of *D. citri* on days 2, 8, and 10. The Kaplan–Meier test was implemented in the R programming language (v 4.2.0) using the 'ggfortify' package.

### 3. Results

# 3.1. Feeding Behavior of D. citri Adults on Cover Crop Plants versus Citrus Using Electrical Penetration Graph (EPG)

All *D. citri* adults that were recorded engaged in xylem and/or phloem feeding activities on cover crop plants and citrus. Sixty percent of *D. citri* adults engaged in phloem-feeding activities on *C. macrophylla* plants, whereas no phloem penetration activities that could result in phloem ingestion occurred on any of the cover crop species. The feeding waveforms produced on citrus were similar to those reported in previous EPG studies with *D. citri* [28,30,31].

No differences were observed in the frequency, mean duration, or total duration (frequency x mean duration) of non-probing (Np) activities between the cover crop plant species and citrus. *D. citri* adults spent 58 to 71% of their total feeding time in non-probing activities on the various cover crop plants and citrus (Figure 2). No statistical differences were observed in the frequency of feeding bouts or total duration of intercellular stylet penetration activities between the various cover crop plants tested versus citrus. The mean duration of intercellular stylet penetration (waveform C) activities was significantly longer on clover ( $12.9 \pm 1.7 \text{ min}$ ) than on the other cover crop plants, such as buckwheat ( $6.4 \pm 1.2 \text{ min}$ ) and cowpea ( $5.3 \pm 1.1 \text{ min}$ ) ( $F_{6,74} = 4.13$ ; p = 0.0012, n = 6) (Figure 3). During the 18 h recordings, 9 to 20% of the total time was spent on intercellular stylet penetration activities (Figure 2).



**Figure 2.** Percent duration of various feeding activities performed by *Diaphorina citri* adults on various cover crop species as determined by waveforms (C, D, E1, E2, G, and Np) recorded over an 18 h period by electrical penetration graph recordings. (**A**) Buckwheat, n = 11; (**B**) cowpea, n = 13; (**C**) clover, n = 6; (**D**) radish, n = 12; (**E**) peanut, n = 10; (**F**) vetch, n = 12; (**G**) *Citrus macrophylla*, n = 17.

The proportion of overall time that *D. citri* spent in xylem feeding activities was similar between the various cover crop species (17 to 33%); however, this duration was significantly higher than the proportion of overall time spent xylem feeding (9%) on *C. macrophylla* (Figures 2 and 4). There were no differences observed in the number of xylem feeding bouts on the various cover crop species versus citrus (p = 0.2). The mean duration of feeding on the xylem was significantly longer on cowpea (65.5 ± 12.3 min) than *C. macrophylla* (27.1 ± 11.3) ( $\chi^2 = 20.93$ , df = 6, p < 0.05). In addition, the total duration of xylem feeding



activities performed by *D. citri* during the 18 h period was significantly higher on cowpea  $(352 \pm 65.2 \text{ min})$  than on *C. macrophylla*  $(95.4 \pm 29.2)$  ( $\chi^2 = 14.71$ , df = 6, *p* < 0.05) (Figure 4).

**Figure 3.** Mean duration ( $\pm$  SE) of stylet penetration (Waveform C) activities performed by *Diaphorina citri* adults on various cover crop species and citrus. Log-transformed data were analyzed by ANOVA followed by Tukey's HSD. Treatments that have no letters in common were significantly different ( $\alpha = 0.05$ ).



**Figure 4.** Total duration of xylem feeding activities (Waveform G) performed by *Diaphorina citri* adults on various cover crop species and citrus. Data were analyzed by the Kruskal-Wallis test followed by Dunn's test for nonparametric pairwise comparison. Treatments that had no letters in common were significantly different ( $\alpha = 0.05$ ).

There were a few phloem penetration (waveform D) and salivation (waveform E1) attempts observed on certain cover crop plant species such as clover and daikon radish, but none of them resulted in any phloem ingestion (waveform E2) activities (Figure 2). The number of phloem-feeding bouts ( $\chi^2 = 42.22$ , df = 6, p < 0.05) as well as their total duration ( $\chi^2 = 34.79$ , df = 6, p < 0.05) were significantly higher on *C. macrophylla* than on any of the cover crop species. Adults spent 11% of their time in phloem ingestion on citrus plants (Figure 2G), whereas no phloem ingestion (<1%) was observed on any cover crop species (Figure 2A–F).

## 3.2. Behavioral Choice of D. citri to Various Citrus Cover Crop Species and Citrus

Significantly more *D. citri* adults were found on buckwheat than on citrus or any other cover crop plant species at 4 h after psyllid release ( $\chi^2 = 27.85$ , df = 6, p < 0.05) (Figure 5A). Adult *D. citri* were found in the feeding position on these plants at 4 and 24 h after release, shown in Figure 1A–F. After 24 h, significantly more *D. citri* were observed on buckwheat and cowpea than on citrus or any of the other cover crop plant species ( $\chi^2 = 33.10$ , df = 6, p < 0.05) (Figure 5B). *D. citri* adults were observed successfully feeding on both cowpea and buckwheat. This observation was congruent with the EPG recordings indicating that *D. citri* were found alighting on these cover crops and were able to feed on the xylem. At 48 h post psyllid release, there was no significant difference observed between the number of insects that chose buckwheat, cowpea, and citrus; however, significantly fewer psyllids were observed on peanut, hairy vetch, and clover. ( $\chi^2 = 33.74$ , df = 6, p < 0.05) (Figure 5C). The number of *D. citri* number found colonizing citrus gradually increased over time after insect release (Figure 5A–C).

## 3.3. Survival of D. citri Adults on Citrus Cover Crop Species in No-Choice Assays

A significantly higher number of adults were found alive on cover crops or *C. macrophylla* than in the no plant (starvation) negative control (p < 0.0001, n = 5) during each day of the experiment. There was no significant difference between the survival of *D. citri* on any of the cover crop plants versus on citrus 2 d after release (p = 0.13, n = 5) (Table 1). However, on day 4, *D. citri* survival was significantly higher on the radish than on hairy vetch and peanut (Table 1). By 6–10 d post-release, psyllid survival was greater on the true citrus host than on any of the cover crop species tested (Table 1). No differences were observed in the survival of *D. citri* on the various cover crops at 8 days after release; however, survival on these non-hosts did not extend beyond day 10. Survival of *D. citri* was negligible by 6 d after release on the negative control (access to moist cotton) and did not extend beyond 6 days; all of the cover crops had higher psyllid survival than the negative control (Table 1).

**Table 1.** Mean ( $\pm$  SEM) survival of *Diaphorina citri* adults on various cover crop species and citrus after the release on plants within cages in no-choice experiments. Twenty-five *D. citri* adults were introduced at the start of the experiment. Log-transformed data was analyzed by ANOVA followed by Tukey's HSD. Treatments that have no letters in common within the same row were significantly different ( $\alpha = 0.05$ ).

	Plant Type									
Time	Radish	Peanut	Buckwheat	Vetch	Citrus	Cowpea	Clover	Negative Control	F-Ratio	<i>p</i> -Value
Day 2	$23\pm1~^{a}$	$22\pm1~^{a}$	$24\pm1~^{a}$	$23\pm1~^{a}$	$21\pm1~^{a}$	$22\pm1~^{a}$	$22\pm1~^{a}$	$16\pm1~^{b}$	19.78	< 0.0001
Day 4	$21\pm1~^{a}$	$15\pm2^{b}$	$17\pm2~^{ab}$	$15\pm1$ <sup>b</sup>	$20\pm1~^{ab}$	$16\pm1$ <sup>b</sup>	$18\pm1~^{\mathrm{ab}}$	$10\pm1~^{\rm c}$	9.1	< 0.0001
Day 6	$12\pm1$ <sup>b</sup>	$11\pm1~^{ m bc}$	$12\pm2^{b}$	$11\pm1~^{ m bc}$	$19\pm1~^{a}$	$5\pm2$ <sup>cd</sup>	$13\pm1~^{\mathrm{ab}}$	$2\pm1$ <sup>d</sup>	13.4	< 0.0001
Day 8	$10\pm2^{\ b}$	$9\pm1$ <sup>b</sup>	$8\pm2^{b}$	$6\pm1^{b}$	$18\pm1~^{\rm a}$	$5\pm1^{ m bc}$	$10\pm1$ <sup>b</sup>	$0\pm0^{ m b}$	19.1	< 0.0001
Day 10	$0\pm0^{ m b}$	$1\pm1$ <sup>b</sup>	$0\pm0$ <sup>b</sup>	$0\pm0^{b}$	$15\pm1~^{a}$	$0\pm0$ <sup>b</sup>	$0\pm0^{b}$	$0\pm0^{\ b}$	344.3	< 0.0001



**Figure 5.** Mean ( $\pm$  SEM) number of *Diaphorina citri* adults observed alighting on various cover crop species and citrus plants (**A**) 4, (**B**) 24, and (**C**) 48 h after release in choice experiments. Data were analyzed by the Kruskal–Wallis test followed by Dunn's test for nonparametric pairwise comparison. Treatments that have no letters in common were significantly different ( $\alpha = 0.05$ ).

Kaplan–Meier plots were employed to assess the survival of *D. citri* adults on citrus and the different cover crops (Figure 6). In general, the survival of *D. citri* was higher on *C. macrophylla* (Cmac) than the cover crop species evaluated (Figure 6). Although *D. citri* survived on the cover crop plants initially, their survival probability declined rapidly over time. Survival probability on the negative control was lower than on cover crops or citrus (Figure 6).



**Figure 6.** Kaplan–Meier plots showing the survival probabilities of *Diaphorina citri* on days 2, 8, and 10 on different cover crops, citrus, or a negative control under no-choice conditions. *D. citri* showed a higher survival rate on citrus plants by day 6 than the cover crop plants evaluated.

### 4. Discussion

We examined the feeding behavior of *D. citri* on selected cover crop species commonly grown in citrus groves for the purpose of weed management to determine if these non-host plants used for cultural control could act as alternate feeding resources for this key pest. Although a recent report has indicated that adult psyllids can take short-term refuge in weed species commonly found in citrus groves when conditions on the host are unfavorable, such as directly after insecticide application, interactions between citrus pests and cover crops commonly have not been previously investigated.

Our EPG recordings showed that *D. citri* adults engaged in xylem-feeding activities after colonizing each of the cover crop species investigated; however, none of them engaged in phloem-feeding activities (Figure 2). There were attempts made by *D. citri* to access phloem tissues when probing leaves on cover crop plants (waveforms D and E1), which were unsuccessful and did not result in phloem ingestion (waveform E2) (Figure 2). Previous research has indicated that xylem feeding is a common behavior among psyllids, including *D. citri*, which could be associated with general osmotic regulation [32,33]. The

much greater percentage of the total duration spent on xylem feeding activities by *D. citri* on cover crop plants than their preferred host, *C. macrophylla*, was likely a consequence of the ability to eventually feed on phloem on the latter (Figure 4). Phloem is more nutrient-rich than xylem sap and therefore is vital for the development of *D. citri* nymphs and eggs [11,32].

*D. citri* adults readily fed on the xylem of buckwheat and cowpea and also exhibited a preference for these two cover crop species as compared with the other species investigated during the initial 24 h post-release in the choice behavioral assays (Figure 5). Interestingly, the buckwheat flowers attract hoverflies (*Allograpta obliqua*), which are natural enemies of *D. citri* [34]. As *D. citri* may also prefer buckwheat for xylem feeding, this cover crop may encourage biological control of *D. citri* as an ecosystem service in citrus groves. However, the prospects of utilizing cover crops, such as buckwheat, as refuges for natural enemies, trap crops or similar strategies to regulate populations of *D. citri* in citrus will require a detailed investigation.

The survival of *D. citri* on the various cover crop plants evaluated in a no-choice setting was extended to 8 days as compared with a 6-day lifespan observed under starvation conditions where only moist cotton was provided. In contrast, the majority of released psyllids were still thriving at 10 d post-release on the known host, *C. macrophylla* (Table 1). Kaplan–Meier survival plots also showed a higher survival rate of *D. citri* on citrus plants than the cover crop species evaluated here (Figure 6). This demonstrates that while *D. citri* adults can briefly colonize these cover crop species to obtain refuge and water, the phloem content and/or chemistry of secondary metabolites characterizing these species does not support their long-term survival. For example, Brassicaceae species possess insect-feeding deterrent compounds such as glucosinolates and saponins [35]. Similarly, secondary metabolites, such as flavones, are a known plant defense mechanism in white clover (*Trifolium repens* L.) [36]. Furthermore, no egg-laying was observed on any of these plants during either choice or no-choice experiments.

Cover crops have been reported to improve soil properties in Florida citrus groves by the addition of soil organic matter, improved infiltration, moisture retention, and addition of soil N [37,38]. In addition, they aid in weed suppression, increase soil microbial diversity [2], and benefit ecosystem services such as the biological control of insect pests and pollination [23,39]. The selection of optimal cover crop species or mixtures of species to achieve both weed and pest management would benefit citrus management in Florida, where citrus greening has decimated yields and more than doubled production costs [40]. More importantly, evaluating the feeding behavior of *D. citri* on these selected cover crops is essential to determine whether this phytopathogen vector is able to take refuge and feed on these plants when conditions are inhospitable on their preferred citrus host. This is relevant in the context of managing the vector of the causal pathogen of the currently incurable citrus greening disease. Our findings indicate that D. citri can utilize these cover crop species as alternate hosts or refuges for several days, which may promote population survival following insecticide treatment of the citrus crop or during dispersal. However, these cover crop species do not support the long-term survival or population growth of D. citri.

### 5. Conclusions

Our results indicate that *D. citri* can survive up to 8 d on certain cover crop species by feeding on xylem, which could provide a short-term refuge for psyllids when access to host plants is restricted. However, our results showed that adult *D. citri* could not perform phloem feeding on all cover crop species evaluated and, therefore, could not obtain the required nutrition from these species. Furthermore, *D. citri* did not appear to lay eggs on these plants. Radish, peanut, and vetch were not attractive to psyllids in choice tests, while cowpea was marginally suitable as a non-host under no-choice conditions. Therefore, radish, peanut, vetch, and cowpea may be the most useful candidates for use as cover crops in citrus groves where *D. citri* and citrus greening disease are prevalent.

Author Contributions: Conceptualization and experiment design, J.G., R.K. and L.L.S.; validation, J.G., R.K. and B.T.; data analysis, J.G., R.K., B.T. and M.B.; provided materials, R.K., M.B. and L.L.S.; writing—original draft, J.G., R.K., and M.B.; writing—review and editing, L.L.S., J.G. and R.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research project received partial funding from the USDA-APHIS Huanglongbing Multi-Agency Coordination (HLB-MAC), grant # AP19PPQS&T00C073.

Institutional Review Board Statement: Not applicable.

**Data Availability Statement:** All the data are reported in the manuscript. Additional details are available from the corresponding author upon request.

Acknowledgments: Special thanks to Anna Sara Hill (USDA-ARS, Ft. Pierce, FL) for insect rearing and Robert Reifer for help with the planting of cover crops and maintenance. USDA is an equal opportunity provider and employer. The findings and conclusions in this publication are those of the author(s) and should not be construed to represent any official USDA or U.S. Government determination or policy. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Conflicts of Interest: The authors declare no competing interest.

### References

- 1. Linares, J.; Scholberg, J.; Boote, K.; Chase, C.A.; Ferguson, J.J.; McSorley, R. Use of the cover crop weed index to evaluate weed suppression by cover crops in organic citrus orchards. *HortScience* **2008**, *43*, 27–34. [CrossRef]
- 2. Ngouajio, M.; McGiffen, M.E. Going organic changes weed population dynamics. HortTechnology 2002, 12, 590–596. [CrossRef]
- 3. Wang, K.H.; McSorley, R.; Marshall, A.; Gallaher, R.N. Influence of organic *Crotalaria juncea* hay and ammonium nitrate fertilizers on soil nematode communities. *Appl. Soil Ecol.* **2006**, *31*, 186–198. [CrossRef]
- Bailey, K.L.; Lazarovits, G. Suppressing soil-borne diseases with residue management and organic amendments. *Soil Tillage Res.* 2003, 72, 169–180. [CrossRef]
- 5. Letourneau, D.K.; Armbrecht, I.; Rivera, B.S.; Lerma, J.M.; Carmona, E.J.; Daza, M.C.; Trujillo, A.R. Does plant diversity benefit agroecosystems? A synthetic review. *Ecol. Appl.* **2011**, *21*, 9–21. [CrossRef] [PubMed]
- Sáenz-Romo, M.G.; Veas-Bernal, A.; Martínez-García, H.; Ibáñez-Pascual, S.; Martínez-Villar, E.; Campos-Herrera, R.; Pérez-Moreno, I. Effects of ground cover management on insect predators and pests in a mediterranean vineyard. *Insects* 2019, 10, 421. [CrossRef]
- Aguilar-Fenollosa, E.; Ibáñez-Gual, M.V.; Pascual-Ruiz, S.; Hurtado, M.; Jacas, J.A. Effect of ground-cover management on spider mites and their phytoseiid natural enemies in clementine mandarin orchards (I): Bottom-up regulation mechanisms. *Biol. Control.* 2011, 59, 158–170. [CrossRef]
- 8. Froud, K.; Stevens, P.; Steven, D. Survey of alternative host plants for Kellys citrus thrips (*Pezothrips kellyanus*) in citrus growing regions. *NZ Plant Prot.* 2001, 54, 15–20. [CrossRef]
- Karp, D.S.; Chaplin-Kramer, R.; Meehan, T.D.; Martin, E.A.; DeClerck, F.; Grab, H.; Gratton, C.; Hunt, L.; Larsen, A.; Martínez-Salinas, A.; et al. Crop pests and predators exhibit inconsistent responses to surrounding landscape composition. *Proc. Natl. Acad. Sci. USA* 2018, 115, 863–870. [CrossRef] [PubMed]
- Stansly, P.A.; Arevalo, H.A.; Qureshi, J.A.; Jones, M.M.; Hendricks, K.; Roberts, P.D.; Roka, F.M. Vector control and foliar nutrition to maintain economic sustainability of bearing citrus in Florida groves affected by huanglongbing. *Pest. Manag. Sci.* 2014, 70, 415–425. [CrossRef]
- George, J.; Kanissery, R.; Ammar, E.D.; Cabral, I.; Markle, L.T.; Patt, J.M.; Stelinski, L.L. Feeding behavior of Asian citrus psyllid (*Diaphorina citri* (Hemiptera: Liviidae)) nymphs and adults on common weeds occurring in cultivated citrus described using electrical penetration graph recordings. *Insects* 2020, 11, 48. [CrossRef]
- 12. Martinez, L.; Soti, P.; Kaur, J.; Racelis, A.; Kariyat, R.R. Impact of cover crops on insect community dynamics in organic farming. *Agriculture* **2020**, *10*, 209. [CrossRef]
- 13. Campbell, J.W.; Irvin, A.; Irvin, H.; Stanley-Stahr, C.; Ellis, J.D. Insect visitors to flowering buckwheat, *Fagopyrum esculentum* (Polygonales: Polygonaceae), in north-central Florida. *Fla. Entomol.* **2016**, *99*, 264–268. [CrossRef]
- 14. Nicholls, C.I.; Parrella, M.; Altieri, M.A. The effects of a vegetational corridor on the abundance and dispersal of insect biodiversity within a northern California organic vineyard. *Landscape Ecol.* **2001**, *16*, 133–146. [CrossRef]
- Irvin, N.A.; Hagler, J.R.; Hoddle, M.S. Measuring natural enemy dispersal from cover crops in a California vineyard. *Biol. Control* 2018, 126, 15–25. [CrossRef]
- 16. Gill, H.K.; McSorley, R. Cover crops for managing root-knot nematodes. ENY063/IN892. EDIS 2011, 7.
- 17. Meagher, R.L., Jr.; Nagoshi, R.N.; Stuhl, C.; Mitchell, E.R. Larval development of fall armyworm (Lepidoptera: Noctuidae) on different cover crop plants. *Fla. Entomol.* **2004**, *87*, 454–460. [CrossRef]

- SARE Outreach. Cover Cropping for Pollinators and Beneficial Insects. Available online: https://www.sare.org/Learning-Center/Bulletins/Cover-Cropping-for-Pollinators-and-Beneficial-Insects/Text-Version (accessed on 30 March 2020).
- 19. Manandhar, R.; Wright, M.G. Effects of interplanting flowering plants on the biological control of corn earworm (Lepidoptera: Noctuidae) and thrips (Thysanoptera: Thripidae) in sweet corn. *J. Econ. Entomol.* **2016**, *109*, 113–119. [CrossRef]
- 20. Manandhar, R.; Hooks, C.R.; Wright, M.G. Influence of cover crop and intercrop systems on *Bemisia argentifolii* (Hemiptera: Aleyrodidae) infestation and associated squash silverleaf disorder in zucchini. *Environ. Entomol.* **2009**, *38*, 442–449. [CrossRef]
- English-Loeb, G.; Rhainds, M.; Martinson, T.; Ugine, T. Influence of flowering cover crops on *Anagrus* parasitoids (Hymenoptera: Mymaridae) and *Erythroneura* leafhoppers (Homoptera: Cicadellidae) in New York vineyards. *Agric. Forest Entomol.* 2003, 5, 173–181. [CrossRef]
- 22. Akins, J. Cover Crop Effects on Insect Dynamics in Cropping Systems of the Southeastern US 2020. Master's Thesis, Auburn University, Auburn, AL, USA, 12 December 2020.
- 23. Bugg, R.L.; Ellis, R.T. Insects associated with cover crops in Massachusetts. Biol. Agric. Hortic. 1990, 7, 47-68. [CrossRef]
- 24. Bugg, R.L.; Phatak, S.C.; Dutcher, J.D. Insects associated with cool-season cover crops in southern Georgia: Implications for pest control in truck-farm and pecan agroecosystems. *Biol. Agric. Hortic.* **1990**, *7*, 17–45. [CrossRef]
- Skelley, L.H.; Hoy, M.A. A synchronous rearing method for the Asian citrus psyllid and its parasitoids in quarantine. *Biol. Control.* 2004, 29, 14–23. [CrossRef]
- Li, W.B.; Hartung, J.S.; Levy, L. Quantitative real-time PCR for detection and identification of *Candidatus* Liberibacter species associated with citrus Huanglongbing. J. Microbiol. Methods 2006, 66, 104–115. [CrossRef] [PubMed]
- Luo, X.; Yen, A.L.; Powell, K.S.; Wu, F.; Wang, Y.; Xeng, L.; Yang, Y.; Cen, Y. Feeding behavior of *Diaphorina citri* (Hemiptera: Liviidae) and its acquisition of *'Candidatus* Liberibacter asiaticus', on huanglongbing-infected *Citrus reticulata* leaves of several maturity stages. *Fla. Entomol.* 2015, *98*, 186–192. [CrossRef]
- George, J.; Ammar, E.-D.; Hall, D.G.; Lapointe, S.L. Sclerenchymatous ring as a barrier to phloem feeding by Asian citrus psyllid: Evidence from electrical penetration graph and visualization of stylet pathways. *PLoS ONE* 2017, *12*, e0173520. [CrossRef] [PubMed]
- 29. Tjallingii, W. Electronic recording of penetration behaviour by aphids. Entomol. Exp. Appl. 1978, 24, 721–730. [CrossRef]
- 30. Bonani, J.P.; Fereres, A.; Garzo, E.; Miranda, M.P.; Appezzato-Da-Gloria, B.; Lopes, J.R.S. Characterization of electrical penetration graphs of the Asian citrus psyllid, *Diaphorina citri*, in sweet orange seedlings. *Entomol. Exp. Appl.* **2010**, *134*, 35–49. [CrossRef]
- 31. George, J.; Ammar, E.-D.; Hall, D.G.; Lapointe, S.L. Prolonged phloem feeding activities by *Diaphorina citri* nymphs may explain their greater acquisition of citrus greening pathogen. *Sci. Rep.* **2018**, *8*, 10352. [CrossRef]
- 32. Ebert, T.A.; Backus, E.A.; Shugart, H.J.; Rogers, M.E. Behavioral plasticity in probing by *Diaphorina citri* (Hemiptera, Liviidae): Ingestion from phloem versus xylem is influenced by leaf age and surface. *J. Insect Behav.* **2018**, *31*, 119–137. [CrossRef]
- Pompon, J.; Quiring, D.; Goyer, C.; Giordanengo, P.; Pelletier, Y. A phloem-sap feeder mixes phloem and xylem sap to regulate osmotic potential. J. Insect Physiol. 2011, 57, 1317–1322. [CrossRef] [PubMed]
- 34. Irvin, N.A.; Pierce, C.; Hoddle, M.S. Evaluating the potential of flowering plants for enhancing predatory hoverflies (Syrphidae) for biological control of *Diaphorina citri* (Liviidae) in California. *Biol. Control* **2021**, *157*, 104574. [CrossRef]
- Badenes-Perez, F.R.; Gershenzon, J.; Heckel, D.G. Insect attraction versus plant defense: Young leaves high in glucosinolates stimulate oviposition by a specialist herbivore despite poor larval survival due to high saponin content. *PLoS ONE* 2014, *9*, e95766. [CrossRef]
- Carlsen, S.C.; Fomsgaard, I.S. Biologically active secondary metabolites in white clover (*Trifolium repens* L.)—A review focusing on contents in the plant, plant–pest interactions and transformation. *Chemoecology* 2008, 18, 129–170. [CrossRef]
- 37. Bugg, R.L.; Waddington, C. Using cover crops to manage arthropod pests of orchards: A review. *Agric. Ecosyst. Environ.* **1994**, 50, 11–28. [CrossRef]
- Castellano-Hinojosa, A.; Martens-Habbena, W.; Smyth, A.R.; Kadyampakeni, D.M.; Strauss, S.L. Short-term effects of cover crops on soil properties and the abundance of N-cycling genes in citrus agroecosystems. *Appl. Soil Ecol.* 2022, 172, 104341. [CrossRef]
- Grafton-Cardwell, E.E.; Ouyang, Y.; Bugg, R.L. Leguminous cover crops to enhance population development of *Euseius tularensis* (Acari: Phytoseiidae) in citrus. *Biol. Control* 1999, 16, 73–80. [CrossRef]
- 40. Spreen, T.H.; Baldwin, J.P.; Futch, S.H. An economic assessment of the impact of Huanglongbing on citrus tree plantings in Florida. *HortScience* **2014**, *49*, 1052–1055. [CrossRef]