PROBING BEHAVIORS OF ADULT ASIAN CITRUS PSYLLID (HEMIPTERA: LIVIIDAE) ARE NOT APPRECIABLY AFFECTED BY SOIL APPLICATION OF FIELD-RATE ALDICARB TO CITRUS

R. H. Serikawa[1,2], E. A. Backus[3] and M. E. Rogers[1,*]
[1]University of Florida, Department of Entomology, Citrus Research and Education Center, 700 Experiment Station Rd, Lake Alfred, FL 33850, USA

[2]Current address: Dupont do Brasil S.A., Rua Bortolo Ferro, 500A, Bairro Boa Esperança, CEP. 13148-903, Paulínia/SP, Brazil

[3]USDA Agricultural Research Service, Crop Diseases, Pest and Genetics Research Unit, 9611 S. Riverbend Ave, Parlier, CA 93648, USA

*Corresponding author, E-mail: mrgrs@ufl.edu

Abstract

In 2005, Huanglongbing disease (HLB), also known as citrus greening, was discovered in Florida. The presumptive causal agent of this disease is the phloem-limited bacterium '*Candidatus* Liberibacter asiaticus' (Las) which is spread by the Asian citrus psyllid (*Diaphorina citri* Kuwayama). Following the discovery of HLB, insecticide use for control of the vector has increased dramatically. One such insecticide is aldicarb, a soil-applied systemic carbamate insecticide that has been used in Florida citrus since the 1970's for both control of insect and mite pests and for its potential plant growth promoting benefits. The objective of this study was to determine the effects of soil-applications of aldicarb to citrus on the feeding behavior of *D. citri*, including whether this systemic insecticide disrupts feeding behaviors responsible for Las transmission. To achieve this goal, an electrical penetration graph monitor was used to examine *D. citri* feeding behavior when given a feeding access period of 12 h on aldicarb-treated and untreated citrus plants. Results showed no reduction in *D. citri* probing behaviors between treatments, and no insects died during recordings. Unexpectedly, at the cohort level, both phloem salivation and phloem ingestion were significantly longer on aldicarb-treated compared with untreated plants, suggesting that aldicarb application may increase the likelihood of Las transmission. Although registration of aldicarb for use in the U.S. has been discontinued for the last year, it has been reregistered for use in certain crops that may include citrus in the future. Thus, there is renewed importance in understanding the effects of aldicarb and other insecticides applied to suppress insect-transmitted diseases.

Key words: *Diaphorina citri*, electrical penetration graph, feeding disruption, insect behavior, insecticide

Resumen

En 2005, la enfermedad Huanglongbing (HLB), también conocida como enverdecimiento de los cítricos, fue descubierto en Florida. El agente causal presuntivo de esta enfermedad es 'Candidatus Liberibacter asiaticus' (Las), una bacteria limitada al floema que es transmitida por el psílido asiático de los cítricos (*Diaphorina citri* Kuwayama). Tras el descubrimiento de HLB, el uso de insecticidas para el control del vector ha incrementado de manera espectacular. Uno de estos insecticidas es el aldicarb, un insecticida carbamato sistémica aplicado al suelo que se ha utilizado en los cítricos de Florida desde la década de 1970, tanto para el control de plagas de insectos y ácaros, como por sus beneficios potenciales para promover el crecimiento de las plantas. El objetivo de este estudio fue determinar los efectos de aplicaciones de aldicarb a suelo en los cítricos sobre el comportamiento de alimentación de *D. citri*, incluyendo si este insecticida sistémico interrumpe el comportamiento de alimentación responsable para la transmisión de Las. Para lograr este objetivo, se utilizó un monitor gráfico de penetración eléctrica para examinar el comportamiento de alimentación de *D. citri* cuando se les da un periodo de acceso de alimentación de 12 horas sobre plantas de cítricos tratadas con aldicarb y plantas no tratadas. Los resultados no mostraron una reducción en el comportamiento de *D. citri* para probar las plantas entre los tratamientos, y no insectos murieron durante la grabación. Inesperadamente, a nivel de cohorte, tanto la salivación y la ingestión de floema fue significativamente más largo en plantas tratadas con aldicarb en

comparación con las plantas no tratadas, lo que sugiere que la aplicación aldicarb puede aumentar la probabilidad de transmisión de Las. Aunque el registro de aldicarb para su uso en los EE.UU. ha sido descontinuado en el último año, se ha vuelto a registrar para su uso en determinados cultivos, que pueden incluir los cítricos en el futuro. Por lo tanto, hay una importancia renovada en el entender los efectos del aldicarb y otros insecticidas aplicados para suprimir las enfermedades transmitidas por insectos.

Palabras Clave: *Diaphorina citri,* gráfico eléctrica de penetración, trastornos de alimentación, comportamiento de insectos, insecticidas

Soil-applied systemic insecticides are commonly used in Florida citrus production for control of plant-feeding pests. Due to their long residual period of activity within treated plants, systemic insecticides typically provide control of target pests for longer durations of time compared to foliar insecticide sprays. One such systemic insecticide is aldicarb (Temik® 15 G, Bayer CropSciences, Research Triangle Park, N.C.). Aldicarb is a systemic carbamate insecticide that was first registered for use in Florida citrus in the 1970's. Previously aldicarb–applied as a granular formulation incorporated into the soil surrounding citrus trees–has been used to control numerous pests of citrus including mites (i.e., *Phyllocoptruta oleivora* (Ashmead), *Brevipalpus* sp., *Eutetranuychus banksi* (McGregor)), aphids (*Aphis citricola* Van der Goot), whiteflies (*Dialeurodes citri* (Ashmead)), mealybugs (*Planococcus citri* (Risso)), scale insects (brown soft scale, chaff scale) and nematodes (i.e., *Tylenchulus semipenetrans* (Cobb) and *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuumans Stekhoven) (Boling & Dean 1968; Bullock 1980; Childers et al. 1987; Stansly & Rouse 1994). In addition to controlling these potential citrus pests, aldicarb use has been correlated with increases in yield, fruit size and brix, improved external fruit quality, increases in P and Ca levels in leaf tissues and enhancement of the plant's ability to survive freezing temperatures (Knapp et al. 1982; Wheaton et al. 1985; Stansly & Rouse 1994). Furthermore, an economic analysis by Blakeley et al. (2003) reported that the use of aldicarb improved citrus grower's net returns by more than US$ 500/ac (US$ 1,235/ha) compared with using other pest control alternatives.

In 2005 citrus greening disease (aka huanglongbing) was discovered in Florida. Huanglongbing (HLB) is putatively caused by a gram-negative bacterium '*Candidatus* Liberibacter asiaticus' (Las) that is transmitted in Florida by the Asian citrus psyllid (*Diaphorina citri* (Kuwayama); Hemiptera: Liviidae) (Bové 2006). A key HLB management tactic to slow the spread of this disease is the intensive use of insecticides to kill the vector (Brlansky & Rogers 2007). Large scale field trials in Florida have demonstrated a 50% reduction in *D. citri* populations where aldicarb is applied (e.g. Qureshi & Stansly 2008). As a result of the need for enhanced control of *D. citri* and the many benefits provided by aldicarb described above, the use of aldicarb in Florida citrus nearly doubled from approximately 25,011 ha treated in 2005 to 47,354 ha treated in 2009 (NASS 2010).

Although aldicarb use was legally discontinued in Florida in 2011, there has been pressure from growers to reinstate its use. This pushback is due to the belief by growers that aldicarb is a useful tool to reduce *D. citri* and manage HLB. The goal of the current investigation was to begin to evaluate the effectiveness of aldicarb as a management tactic against HLB. Our specific objective was to test the effects of aldicarb soil-applied at a concentration and timing similar to that used in Florida citrus groves, on *D. citri* stylet probing behaviors. We were especially interested in effects on phloem ingestion and salivation behaviors, known to control acquisition and inoculation, respectively, of phloem-limited pathogens such as Las (Fereres & Moreno 2009; Bonani et al. 2010). Both acquisition and inoculation are critical stages of Las transmission to citrus.

Materials and Methods

Plants and Insects

Plants used in the experiments consisted of 'sweet orange' (*Citrus sinensis* (L.) Osbeck) seedlings (15-20 cm tall) planted in 120 mL tubes containing mix Fafard Citrus potting Mix (Fafard, Agawam, Massachusetts), grown in a pathogen-free greenhouse at 29 ± 3 °C and 60-80% RH. All seedlings were planted and maintained identically to minimize interplant variation.

Female psyllids (10-15 days post-emergence) were obtained from a greenhouse colony (free of Las) reared on citrus at 29 ± 3 °C and 12:12 h L:D.

EPG Recording and Waveform Analysis

Recordings of *D. citri* feeding for 12 h under constant light conditions on aldicarb-treated and untreated seedlings were made using a Giga-8 monitor (Department of Entomology, Wageningen Agricultural University, the Netherlands). EPG recordings were conducted as described in detail in Serikawa et al. (2012). In brief, psyllids were

wired using 18.5 µm (sold as 0.0010 in) gold wire (Sigmund Cohn Corp., Mt. Vernon, New York) and silver glue (recipe and description of wiring technique in Serikawa et al. (2012). When psyllid stylets probed the citrus leaf, a circuit was connected, electricity flowed across the stylets and variations in voltage were outputted as waveforms that have been previously correlated with psyllid feeding behaviors (Walker 2000). All EPG recordings used 75 mV DC applied signal, 50 × gain, and samples were digitized at 100 Hz using a DI-710UHB board and Windaq Lite software (Dataq Instruments, Akron, Ohio). Waveform event durations were measured using Windaq Waveform Browser software, v. 2.40 (Dataq) (Backus et al. 2007).

Experimental Design

Twenty days prior to EPG recordings, a subset of plants was treated with 0.0046 g of aldicarb (Temik 15G®) applied to the soil surface of each pot. After insecticide application, the pots were covered with a layer (1 cm) of potting soil to avoid insecticide spill during subsequent watering. A total of 15 plants were treated with aldicarb, and 15 additional, untreated plants were used as controls. Both the 20-day timing and aldicarb concentration were designed to directly mimic timing and rates of application in a typical Florida orange grove, where aldicarb is considered to be effective against *D. citri* for up to 60 days after application (Picó et al. 1990).

On the day of EPG recordings, a single, young leaf was randomly selected from each plant onto which a single psyllid was placed; feeding behavior was then recorded using EPG. All psyllids recorded fed during their recording periods, except one for each treatment. That insect was excluded from analysis; therefore, the final sample size was 14 insects per treatment.

Statistical Analysis

Diaphorina citri feeding behaviors were compared between aldicarb-treated and untreated plants, using biologically non-sequential variables described by Backus et al. (2007) and Bonani et al. (2010). These durations were tabulated by treatment, and averaged per insect, per probe (a single stylet insertion), and per waveform event (a single, uninterrupted duration of one waveform type). The variables analyzed at each of these levels were waveform duration per event (WDE), total number of waveform events (TNWE), total waveform duration (TWD), waveform duration per event per insect (WDEI), proportion of individuals that produced a waveform type (PPW), probing duration per insect (PDI), mean number of probes per insect (NPI), number of waveform events per probe (NWEP), number of probes by waveform (NPw), waveform duration per probe (WDP), number of waveform events per insect (NWEI) and waveform duration per insect (WDI). Psyllid waveform types used were C (pathway), D (phloem contact), E1 (putative phloem salivation), E2 (phloem sap ingestion), G (xylem ingestion), z (non-probing, standing still), np (non-probing, walking), and sometime znp (all non-probing) as further detailed in Bonani et al. (2009) and Serikawa et al. (2012).

Pearson's chi-square test was performed to test the goodness of fit and degree of heterogeneity (PROC GLIMMIX, SAS Institute 2001). Because insect probing behaviors measured with the fine-scale resolution of EPG are always highly heterogeneous, it is standard that data are transformed prior to further analysis; durations were log-transformed and frequencies were square root-transformed. Data were analyzed by mixed model ANOVA using restricted maximum likelihood estimation (REML) (PROC GLIMMIX, SAS Institute 2001), followed by protected least significant difference (LSD) (LSMEANS, SAS Institute 2001) for pairwise comparisons. Mixed model ANOVA (in contrast with conventional ANOVA) avoids problems with non-normality of data, another characteristic typical of EPG data, by using REML to model the actual distribution of the EPG dataset (Schabenberger & Pierce 2002). The above statistical methods are now standard for EPG research (e.g., Serikawa et al. 2012), rendering obsolete the older, less powerful use of nonparametric statistics. Means were considered significantly different between the aldicarb-treated and untreated plants at $\alpha = 0.05$.

Confirmation of Aldicarb in Treated Plants

After recordings of *D. citri* feeding behavior were completed, leaf samples were collected for analysis to confirm the presence of aldicarb in the plant tissue using HPLC/UV chromatography with detection at 205 nm (Cochrane & Lanouette 1981), the standard method used for aldicarb detection (Picó et al. 1990). Tests were performed by Waters Agricultural Laboratory (Camilla, Georgia). Because plants used in the recordings were small and a minimum of 5 g of leaf tissue was needed for proper analysis, leaves from 5 plants of each treatment were combined to obtain a sufficient quantity of leaf material.

Results

Summary of Results

Psyllids on aldicarb-treated plants performed all feeding behaviors typical of psyllids in a manner similar to insects on untreated control plants. Numerical differences between treated and un-

treated plants were small, with no significant differences for most variables analyzed, especially when averaged per insect. However, durations of individual events averaged on a cohort-wide basis (WDE, waveform duration per event), rather than per insect (WDEI, waveform duration per event per insect), showed some significant differences. For example, insects on aldicarb-treated plants stood still for longer periods (waveform np) per event compared with those on untreated plants. Similarly, phloem salivation and ingestion events were significantly longer for insects recorded on aldicarb-treated plants. The following in-depth analysis supports these overall findings.

Cohort Level Results

One hundred percent (PPW, proportion of insects that performed a specific waveform) of *D. citri* performed pathway/stylet penetration activities (waveform C) and non-probing/walking activities (waveform np) on both aldicarb-treated and untreated plants. On the untreated plants, 71.4% (PPW) of *D. citri* penetrated the phloem and salivated (waveforms D and E1, respectively) and 64.3% ingested phloem (waveform E2). Xylem ingestion (waveform G) was performed by 78.6% of the insects on untreated plants, and only 7.1% non-probing/standing still (waveform z). On aldicarb-treated plants, 78.6% (PPW) of *D. citri* penetrated the phloem (waveform D) and salivated into it (waveform E1), 57.1% ingested phloem sap (waveform E2), 85.7% ingested xylem sap (waveform G) and 64.3% stood still (Table 1).

Diaphorina citri had a total access period of 604,800 s, during which psyllids on untreated plants performed 217 probes (TNP, total number of probes) and spent 405,759.3 sec (TPD, total probing duration) with their stylets inserted into the leaf. Psyllids on aldicarb-treated plants probed 195 times (TNP) for which the TPD was 337,361.09 s. The percentages of TPD for each waveform are represented by the total waveform durations (TWD) shown in Fig. 1. There were only small numerical differences between *D. citri* feeding on treated and untreated plants. Those results were tested for significant differences between treatments first by examining an average insect's behavior (insect level), then dissecting an average probe (probe level) and the waveform events in it (event level).

Insect Level Results

Overall probing duration per insect (PDI) and number of probes per insect (NPI) were not significantly different between *D. citri* feeding on aldicarb-treated and untreated plants (Table 2). In addition, when probes were divided into their component waveforms, none of the waveform durations per insect (WDI) were significantly different between treatments (Table 1). Finally, analysis of time to first D (T1stD) showed that, although *D. citri* on untreated plants required on average 238.70 min to reach the phloem, and psyllids on aldicarb-treated plants took an average 252.23 min, this difference was not significant ($F = 0.03$; df = 1, 19; $P = 0.8721$).

Probe Level Results

Similar to observations at the insect level, the number of waveform events per probe (NWEP) did not differ significantly between aldicarb-treated and untreated plants for any of the waveforms (Table 3). In contrast, waveform duration per probe (WDP) differed significantly for waveform znp (all non-probing behaviors, combined) (Table 4), but not for any of the other waveforms.

Event Level Results

To best understand the performance of waveform events by the actual experimental unit, the insect, we averaged event durations within each individual insect (to give waveform duration per event BY insect, WDEi), then averaged across insects (to give waveform duration per event per insect, WDEI). This process allowed analysis of event durations from the point of view of an av-

TABLE 1. MEAN (± SE) WAVEFORM DURATION PER INSECT (WDI) (SECONDS) FOR *DIAPHORINA CITRI* FEEDING ON ALDICARB-TREATED AND UNTREATED PLANTS. FEEDING BEHAVIORS OF *D. CITRI* WERE RECORDED ON 'SWEET ORANGE' FOR 12 H UNDER CONSTANT LIGHT CONDITIONS.

	Control		Aldicarb				
Wave-form	WDI ± SE	N	WDI ± SE	N	*F*	df	*P*
z	5,750.40 ± N/A	1	10,273.44 ± 2,507.34	9	0.19	8	0.8771
np	16,040.72 ± 2,991.02	14	14,201.18 ± 2,412.40	14	0.18	26	0.9049
C	15,519.65 ± 2,786.05	14	14,407.25 ± 2,083.64	14	0.00	26	0.6542
D	998.22 ± 526.03	10	442.15 ± 132.11	11	1.15	19	0.3747
E1	622.69 ± 145.02	10	500.85 ± 103.65	11	0.23	19	0.4605
E2	15,595.10 ± 5,568.77	9	11,321.59 ± 4,204.89	8	0.01	15	0.5627
G	2,901.74 ± 411.41	11	3,228.84 ± 368.71	12	0.00	21	0.4627

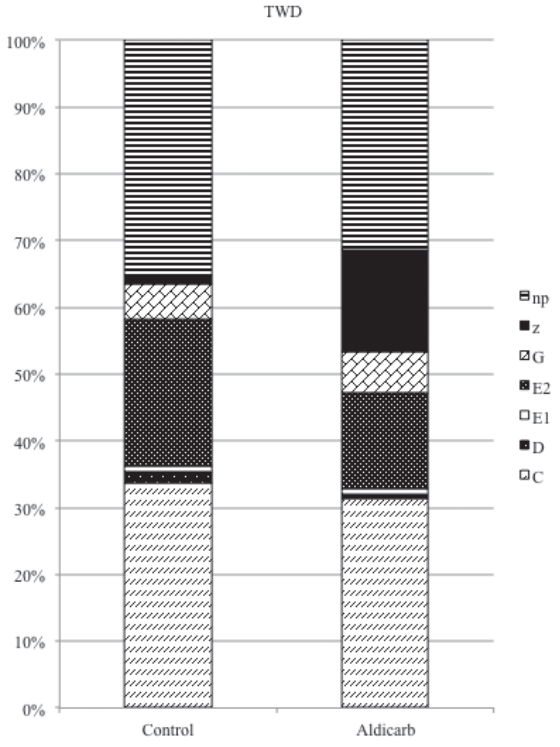

Fig. 1. Percentage of the total waveform durations (TWD) for *Diaphorina citri* feeding on aldicarb-treated and untreated 'sweet orange' citrus plants for 12 h under constant light conditions. Psyllid waveform types used were C (pathway), D (phloem contact), E1 (putative phloem salivation), E2 (phloem sap ingestion), G (xylem ingestion), z (non-probing, standing still), np (non-probing, walking), and sometime znp (all non-probing).

erage insect's feeding. However, when this was done, neither the number of waveforms events performed per insect (NWEI) nor WDEI were significantly different between *D. citri* on aldicarb-treated and untreated plants (Tables 5 and 6, respectively).

Waveform durations per event (WDE) were also analyzed in addition to per event per insect. Generalized *D. citri* probing durations per event (PDE) were significantly shorter on aldicarb-treated (677.43 ± 98.11 s) compared with untreated plants (678.79 ± 98.30 s) (F =7.76; df =1, 1,076; P = 0.0054). When probing durations were categorized by waveform, WDE was significantly shorter for walking (np; F = 7.42; df = 1, 435) and significantly longer for both phloem salivation (E1; F = 4.92; df = 1, 204), and phloem ingestion (E2; F = 4.61; df = 1, 47) on aldicarb-treated plants compared with untreated plants (Table 7).

Confirmation of Aldicarb in Treated Plants

Because there were no apparent differences in the variables analyzed for *D. citri* feeding on aldicarb-treated compared with untreated plants, analysis of aldicarb content within the leaves was conducted in the standard manner by a commercial laboratory, to confirm the presence of aldicarb in treated plants. The mean (±SE) content of aldicarb per 5 g of plant tissue for aldicarb-treated plants was 0.94 ± 0.62 ppm. In contrast, aldicarb was not detected in untreated plants.

Discussion

The objective of the current study was to examine the effects of soil-applied systemic applications of aldicarb on the feeding behavior of *D. citri* to estimate the potential effects of this insecticide on mediation of Las transmission. Analysis of *D. citri* feeding behavior indicated no effect of aldicarb on psyllid probing behavior, especially when considered from an average insect's point of view. It is also valuable to consider the plant's point of view, for which the number of bacterial cells injected in a short period of time is epidemiologically important, no matter how many vectors are involved. In the latter case, significant differences were found between treatments for each phloem activity (i.e, salivation [waveform E1] and ingestion [waveform E2]) when they were averaged across all such occurrences (E1 or E2 waveform events) in each treatment. Thus, when considered on a sample-wide basis, individual events of phloem salivation and ingestion were significantly longer on aldicarb-treated plants than untreated control plants.

During the 12 h access period, all *D. citri* survived and probed for 6.7 h on aldicarb-treated plants, during which time they were able to reach the phloem and salivate (wavefoms D and E1) for an average of 0.26 h, and ingest (Waveform E2) for more than 3 h, significantly longer than insects on untreated plants. Those results differ greatly from findings on *D. citri* feeding on imidacloprid-treated citrus (Serikawa et al. 2012), wherein all insects died within a 6 h access period. Also, phloem ingestion on imidacloprid-treated plants averaged (WDEI) only 1.0 h for psyllids on young leaves and did not occur on mature citrus leaves (Serikawa et al. 2012).

Accordingly, aldicarb appeared to stimulate increased phloem ingestion by *D. citri* feeding on treated plants. Aldicarb is known to change the physiology of plants, which may have contributed to results presented. For example, Ragab (1981) suggested that aldicarb might have a direct effect on mineral metabolism of cotton plants, mainly the ones that are involved in

TABLE 2. MEAN (± SE) PROBING (STYLET PENETRATION) DURATION PER INSECT (PDI) (SECONDS) AND MEAN NUMBER OF PROBES PER INSECT (NPI) FOR *DIAPHORINA CITRI* FEEDING FOR 12 H ON ALDICARB-TREATED AND UNTREATED 'SWEET ORANGE' CITRUS PLANTS UNDER CONSTANT LIGHT CONDITIONS.

	Control	Aldicarb	*F*	df	*P*
PDI ± SE	28,982.81 ± 3,297.99	24,097.22 ± 3473.27	0.92	26	0.3469
NPI ± SE	15.86 ± 3.12	14.36 ± 2.00	0.00	26	0.9804

nitrogen and phosphorous metabolism. Balayannis (1983) showed that aldicarb applications increased the leaf content of water-soluble sugars in tobacco and the concentration of iron, manganese and zinc in the leaves and roots. Also, aldicarb decreased leaf nitrate reductase activity and the concentrations of nicotine and crude protein. Plant nutrition has a direct effect on hemipteran feeding behavior. For example, studies on *Psylla pyricola* Foerster showed a higher production of honeydew when feeding on pear leaves with very low nitrogen content, indicating compensatory feeding effect due to the low nutrition of the leaves (Pfeiffer & Burts 1984). Previous studies have shown an increase of brix, yield, and peel color in citrus fruit sampled from trees treated with aldicarb along with an increase in calcium and potassium content in the citrus leaves (Wheaton et al. 1985). Although nitrogen content was not measured in our treated leaves, any increase in nitrogen content could stimulate increased *D. citri* feeding behavior (Tsagkarakis & Rogers, unpublished).

After 12 h of feeding on aldicarb treated plants, none of the *D. citri* were found dead. To confirm the presence of aldicarb within the plants, citrus test plants were sent for residual analysis, which confirmed insecticide presence. The actual residual concentration necessary to cause psyllid toxicity is unknown, but the levels we report here are similar to those found by Picó et al. (1990) to be present in citrus leaves approximately 30 days following an aldicarb application under typical field conditions. Thus, the aldicarb concentrations in our test are highly representative of the amounts of aldicarb typically found in citrus leaves following standard soil-applications. Nonetheless, studies on efficiency of aldicarb treatments for the control of *Trioza erytreae* (South African citrus psyllid) showed poor efficiency in egg and nymphal control even when applying 227 g of aldicarb per tree with trees averaging 23.2 m^2 canopy (Catling 1969). It was necessary to raise the concentration of aldicarb to 907.2 g per tree to achieve nymphal control (de Villiers 1969 cited by Catling 1969). However, such dosages have not been permitted in Florida due to concerns of groundwater contamination. In contrast, Qureshi & Stansly (2008) showed that aldicarb reduced field populations of *D. citri* when applied 2-3 months prior to spring flushes at recommended rates. Yet, when they caged adult psyllids for 25 days on the aldicarb-treated plants 1, 2 and 4 months after application, mortality was never greater than 45% indicating low levels of effects on adult *D. citri*. The authors hypothesized that the effects of consumption of aldicarb by developing nymphs may have contributed to decreases in field populations of *D. citri* that were greater than observed for caged adults (Qureshi & Stansly 2008).

Reliability of Conclusions

Our results revealed mostly non-significant differences among treatments. It can be argued

TABLE 3. MEAN (± SE) NUMBER OF WAVEFORMS EVENT PER PROBE (NWEP) AND NUMBER OF PROBES BY WAVEFORM (NPW) FOR *DIAPHORINA CITRI* FEEDING ON ALDICARB-TREATED AND UNTREATED SWEET ORANGE' CITRUS PLANTS FOR 12 H UNDER CONSTANT LIGHT CONDITIONS.

	Control		Aldicarb				
Wave-form	NWEP ± SE	NPw	NWEP ± SE	NPw	F	df	*P*
znp	1.01 ± 0.001	222	1.01 ± 0.01	201	N/A	421	N/A
C	1.47 ± 0.11	217	1.48 ± 0.10	195	0.08	410	0.7792
D	3.63 ± 0.67	24	3.33 ± 0.44	24	0.00	46	0.9902
E1	4.92 ± 0.85	24	3.67 ± 0.44	24	0.90	46	0.3488
E2	1.89 ± 0.31	19	1.27 ± 0.19	11	2.07	28	0.1613
G	1.00 ± 0.00	20	1.13 ± 0.07	23	2.86	41	0.0984

Note: Psyllid waveform types used were C (pathway), D (phloem contact), E1 (putative phloem salivation), E2 (phloem sap ingestion), G (xylem ingestion), z (non-probing, standing still), np (non-probing, walking), and sometime znp (all non-probing).

TABLE 4. MEAN (± SE) WAVEFORM DURATION PER PROBE (WDP) (SECONDS) FOR *DIAPHORINA CITRI* FEEDING ON ALDICARB-TREATED AND UNTREATED SWEET ORANGE' CITRUS PLANTS FOR 12 H UNDER CONSTANT LIGHT CONDITIONS.

Wave-form	Control WDP ± SE	Aldicarb WDP ± SE	*F*	df	*P*
znp	1,037.48 ± 174.12	1,469.20 ± 221.95	11.49	421	0.0008
C	994.74 ± 135.09	1,013.69 ± 144.13	2.95	410	0.0864
D	413.94 ± 225.64	202.65 ± 29.56	0.05	46	0.8264
E1	260.11 ± 45.00	229.55 ± 37.70	0.34	46	0.5609
E2	7,388.84 ± 3,063.89	8,233.89 ± 3,384.07	0.92	28	0.3469
G	1,666.76 ± 208.30	1,684.61 ± 219.85	0.09	41	0.7665

Note: Psyllid waveform types used were C (pathway), D (phloem contact), E1 (putative phloem salivation), E2 (phloem sap ingestion), G (xylem ingestion), z (non-probing, standing still), np (non-probing, walking), and sometime znp (all non-probing).

our sample sizes were small and degree of variability among insects so great that our results are questionable and not reliable. We counter this idea with the argument that our sample sizes of insects recorded are completely standard for EPG experiments, which (due to the time-consuming waveform measurement process) typically utilize 10-20 insects per treatment. A recently published study of the grain aphid, *Sitobion avenae* F., on phytohemagglutinin-treated wheat used a sample size of 10 aphids per treatment (Sprawka et al. 2013); likewise, Serikawa et al. (2012) recorded 14 ACP per treatment on imidacloprid-treated citrus. In fact, the present recording duration of 12 h is considered quite long (e.g., much longer than the 4-h recording time in Sprawka et al. [2013] or 6 h used in Serikawa et al. [2012]); some published EPG studies have used as short as a 2 h recording time, e.g. Calderon & Backus (1992). Our recording times were sufficient to generate many thousands of waveform events, the ultimate behavioral record measured.

In addition, variability in probing behavior among individual insects is always seen in EPG research. This is due not only to variation in plant growth and physiological stimuli perceived by the insect, but also due to highly variable differences in feeding within each insect, such as degree of hunger or satiety, physiological/reproductive state, prior experience on the host plant, and many other studied variables (Bernays & Chapman 1994, van Helden & Tjallingii 2000). The last decade of EPG research has seen development of effective statistical means to circumvent potential problems with sample size and heterogeneity, and has produced a highly reliable outcome from EPG statistical analysis. In other words, the same appropriate and rigorous steps taken in this study have shown many significant differences in other, similarly designed and analyzed EPG studies. There is no reason to believe that our results are any less reliable than those of many other, published EPG papers. In some cases, lack of significant differences can be as interesting and valuable as their presence. Given the importance of aldicarb to Florida citriculture, we felt that the lack of differences in psyllid feeding between treated and untreated plants was one of those cases.

Implication for Managing the Spread of HLB

The current results indicate that aldicarb has negligible effects on the feeding behaviors

TABLE 5. MEAN (± SE) NUMBER OF WAVEFORMS EVENTS PER INSECT (NWEI) FOR *DIAPHORINA CITRI* FEEDING ON ALDICARB-TREATED AND UNTREATED SWEET ORANGE' CITRUS PLANTS FOR 12 H UNDER CONSTANT LIGHT CONDITIONS.

Waveform	Control NWEI ± SE	Aldicarb NWEI ± SE	*F*	df	*P*
z	1.00 ± N/A	2.33 ± 0.53	0.87	8	0.3787
np	15.93 ± 3.13	15.29 ± 2.10	0.04	26	0.8495
C	22.93 ± 4.72	20.71 ± 3.33	0.00	26	0.9784
D	8.80 ± 3.42	7.27 ± 2.07	0.05	19	0.8328
E1	11.80 ± 4.96	8.00 ± 2.08	0.24	19	0.6359
E2	3.89 ± 1.81	1.75 ± 0.49	1.39	15	0.2567
G	1.81 ± 0.26	2.17 ± 0.40	0.42	21	0.5224

Note: Psyllid waveform types used were C (pathway), D (phloem contact), E1 (putative phloem salivation), E2 (phloem sap ingestion), G (xylem ingestion), z (non-probing, standing still), np (non-probing, walking), and sometime znp (all non-probing).

TABLE 6. MEAN (± SE) WAVEFORM DURATION PER EVENT PER INSECT (WDEI) (S) AND THE PROPORTION OF INDIVIDUALS THAT PRODUCED A WAVEFORM TYPE (PPW) FOR *DIAPHORINA CITRI* FEEDING ON ALDICARB-TREATED AND UNTREATED SWEET ORANGE' CITRUS PLANTS FOR 12 H UNDER CONSTANT LIGHT CONDITIONS.

Waveform	Control WDEI ± SE	PPW	Aldicarb WDEI ± SE	PPW	*P*
z	5,750.40 ± N/A	1/14	5,474.08 ± 1642.25	9/14	0.8771
np	1,664.81 ± 509.49	14/14	1,066.97 ± 201.14	14/14	0.9049
C	847.72 ± 145.90	14/14	784.42 ± 110.45	14/14	0.6542
D	602.18 ± 549.32	10/14	57.36 ± 7.85	11/14	0.3747
E1	120.36 ± 35.88	10/14	98.40 ± 30.54	11/14	0.4605
E2	8,916.69 ± 4,030.00	9/14	8,527.49 ± 3764.27	8/14	0.5627
G	1,738.52 ± 174.83	11/14	1,776.21 ± 257.55	12/14	0.4627

Note: Psyllid waveform types used were C (pathway), D (phloem contact), E1 (putative phloem salivation), E2 (phloem sap ingestion), G (xylem ingestion), z (non-probing, standing still), np (non-probing, walking), and sometime znp (all non-probing).

of *D. citri*, at least within 12-24 h of insect exposure to the compound. In addition, given the longer salivation and phloem ingestion events on aldicarb-treated plants, it is possible that aldicarb treatment might enhance overall Las transmission in a citrus grove. The efficiency of Las acquisition by adult *D. citri* is low (Pelz-Stelinski et al. 2010) and there is a latency period anywhere from 24 h to 25 days (Xu et al. 1988; Roistacher 1991). Nonetheless, Bonani et al. (2010), observed Las acquisition to occur 1 h after initiation of the ingestion waveform (E2) by *D. citri*. Mean phloem ingestion durations per insect in the present study were much longer than 1 h. Also, longer salivation would increase the probability of Las inoculation, once the latency period was completed. Aldicarb applications can result in less than 50% of adults being controlled under field conditions (Qureshi & Stansly 2008). Our results suggest that one reason control is poor may be that aldicarb, at standard rates used in Florida citrus groves, does not prevent *D. citri* from feeding. Furthermore, although we did not test actual Las transmission, our findings show that *D. citri* on aldicarb-treated plants can still perform the behaviors that are known to control both acquisition and inoculation of Las. While it is possible that aldicarb-exposed insects might die sometime after the first 24 h of exposure, by then it would be too late for the citrus tree to escape infection. Feeding by an infected psyllid would likely have already inoculated Las.

The current study underscores the importance of EPG studies for vector-transmission dynamics in order to improve management of insect-transmitted plant diseases. Investigations such as this one demonstrate that, unlike imidacloprid (Serikawa et al. 2012), not all soil-applied systemic insecticides can disrupt performance of Las-transmission-related behaviors by *D. citri*.

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TABLE 7. MEAN (± SE) WAVEFORM DURATION PER EVENT (WDE) (SEC) AND N OR TOTAL NUMBER OF WAVEFORM EVENTS (TNWE) FOR *DIAPHORINA CITRI* FEEDING ON ALDICARB-TREATED AND UNTREATED SWEET ORANGE' CITRUS PLANTS FOR 12 H UNDER CONSTANT LIGHT CONDITIONS.

Waveform	Control WDE ± SE	N	Aldicarb WDE ± SE	N	*P*
z	5,750.40 ± N/A	1	4,402.90 ± 1229.04	21	0.4568
np	1,007.04 ± 169.29	223	929.04 ± 96.40	214	0.0067
C	676.87 ± 78.92	321	693.12 ± 69.10	291	0.2039
D	113.43 ± 62.49	88	60.80 ± 3.73	80	0.7364
E1	52.77 ± 6.74	118	62.61 ± 9.81	88	0.0277
E2	4,010.17 ± 1752.20	35	6,469.48 ± 2787.80	14	0.0370
G	1,595.96 ± 218.89	20	1,490.23 ± 182.40	26	0.8607

Note: Psyllid waveform types used were C (pathway), D (phloem contact), E1 (putative phloem salivation), E2 (phloem sap ingestion), G (xylem ingestion), z (non-probing, standing still), np (non-probing, walking), and sometime znp (all non-probing).

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