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EVALUATION OF THE EFFECTS OF LIGHT SOURCE AND PLANT MATERIALS ON ASIAN CITRUS PSYLLID (HEMIPTERA: PSYLLIDAE) TRAPPING LEVELS IN THE TRANSTRAP FOR CITRUS SHIPPING CONTAINERS

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Abstract

The Asian citrus psyllid (ACP), the principal vector of the pathogen of huanglongbing (HLB), has been reported to be transported in truckloads of oranges in Florida. Citrus, especially Key limes and lemons, are shipped to the U.S. from Mexican states that are heavily infected with HLB. Live, infected psyllids could spread the disease in orchards near inspection facilities or packing houses where trucks are unloaded. Experiments reported here tested the use of a sticky trap with light emitting diode(s) (LED) to detect possible contamination of fruit loads by ACP in containers. Experiments were performed in chambers maintained at temperatures and humidities similar to those in truck trailers arriving from Mexico. The effects of light intensity (no LED, 1 LED, 2 LEDs) and plant material (no material, fruit only, trees and fruit) were measured and analyzed to determine the relative efficacy of the trap types and to the role of plant material in a system to detect the ACP. Results showed that ACP could survive in containers with no plant material, fruit only, or a nursery tree as material. The majority of the insects were recovered from the traps with lower psyllid numbers surviving or dying in the container. The traps with 2 LEDs were the most effective, followed by 1 LED traps, then those with no lights. These results showed that the psyllids in these chambers were more likely to be trapped than to survive or die in the chamber. Thus, sticky traps with LEDs as a light attractant can be effective means to detect psyllid contamination in citrus shipping.

Key Words: *Diaphorina citri*, huanglongbing, detection, quarantine, light emitting diodes, citrus

Resumen

Se ha informado que el psílido asiático de los cítricos (PAC), el vector principal del patógeno Huanglongbing (HLB), se ha transportado en camiones de naranjas en la Florida. Los cítricos, especialmente los limones verdes y los limones amarillos, se envían a los EE.UU. de los estados mexicanos que están infectados con HLB y los psílidos vivos infectados pueden transmitir la enfermedad en los huertos cercanos a las instalaciones de inspección o empacadoras donde se descargan los camiones. Experimentos reportados aquí probaron el uso de una trampa pegajosa con diodo emisor de luz (s) (DEL) para detectar la posible contaminación de las cargas de frutas por PAC en contenedores. Se realizaron los experimentos en cámaras mantenidas a temperaturas y humedades similares a las de remolques de camiones procedentes de México. Se midieron y analizaron los efectos de la intensidad de la luz (sin DEL, DEL 1, DEL 2) y material de la planta (sin material, sólo fruta, árboles y frutos) para determinar la eficacia relativa de las clases de trampas y el papel de material vegetal en el sistema para detectar el PAC. Los resultados mostraron que el PAC podría sobrevivir en recipientes sin material de plantas, sólo frutas, o fruta más material de la planta. La mayoría de los insectos fueron recuperados de las trampas con números más pequeños de psílidos supervivientes o muertos en el recipiente. Las trampas con 2 DELs fue la más efectiva, seguido de la trampa con 1 DEL, y luego aquellas sin luces. Estos resultados mostraron que los psílidos en estas cámaras tenían un mayor probabilidad de ser atrapados que para sobrevivir o morir en la cámara. Por lo tanto, las trampas pegajosas juntas con los DELs como un atrayente de luz puede ser un medio eficaz para detectar la contaminación por psílidos en el transporte de cítricos.

Palabras Clave: *Diaphorina citri*, huanglongbing, detección, cuarentena, diodos emisores de luz, cítricos

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Large areas of the Yucatan states and western states of Mexico are infested by the Asian citrus psyllid (ACP) (*Diaphorina citri* Kuwayama; Hemiptera: Psyllidae), a vector of '*Candidatus* Liberibacter asiaticus', the pathogen thought to cause huanglongbing (HLB) which is also known as citrus greening disease (Trujillo-Arriaga 2010). This pathogen is lethal for *Citrus* (Sapindales: Rutaceae) (da Graça 1991). Lemons (*Citrus limon* (L.) Burm.f.) and Mexican Key limes (*Citrus aurantifolia* Swingle) are exported to the U.S. from Colima which one of the most heavily HLB infested states in Mexico (Flores et al. 2010). Mexican Key limes are grown along the Pacific coast in the states of Colima, Michoacán, Guerrero, and Oaxaca. This cultivar is not considered by USDA APHIS to be a host for fruit fly pests (*Anastrepha* spp.; Diptera: Tephritidae) infesting this region and quarantine treatments for internal pests are not required. Other citrus species including oranges (*Citrus sinensis* (L.) Osbeck), sweet limes (*Citrus limettioides* Tanaka), mandarins (*Citrus reticulata* Blanco) and grapefruit (*Citrus* × *paradise* Macfad.) require postharvest treatments for fruit fly pests; however, the security screens and procedures for protecting the fruit after treatment are designed for fruit flies, which could allow entry of the smaller adult psyllids

Halbert et al (2010) showed that untreated oranges, shipped by truck from orchards in ACP infested and HLB infected locations to juicing plants in Florida transported Asian citrus psyllids in all loads they examined; with counts ranging from 31 to 268 psyllids per load. Of the 116 psyllids tested, 4 were positive and 9 were suspected of being infected by '*Candidatus* Liberibacter asiaticus'. The reports from the west coast states of Mexico, which export lemons and limes to packing houses in Texas, suggest to us that trucks of untreated citrus could provide a mode of entry for psyllids infected with HLB.

'Eureka' lemons and Mexican Key limes are not treated or waxed and Persian (sweet) limes (*Citrus latifolia* Tanaka) are fumigated but not waxed. These practices, plus the frequent contamination of load with other plant material are likely to allow adult psyllids to be shipped in loads of lemons and limes to the Texas packing houses. These fruit are classified in trade records (provided to us by the U. S. Agricultural Marketing Service, USDA) under the Harmonized Tariff Schedule (HTS) 0805.5020.00. According to these records, Mexico exported 22,338 metric tons (MT) of these fruits during 2008-2009 season, and 21,555 MT during the 2009-2010 harvest season mostly to the USA. During the 2009 season, the USDA Marketing Service recorded that 48,770,000 pounds (22,121,700 kg) (= 1,219 truckloads) of lemons and 709,380,000 pounds (321,769,355 kg) (= 17,734 truckloads) of limes and that during 2010, 50,520,000 pounds (22,915,487 kg) (= 1,260 truckloads) of lemons and 622,430,000 pounds (282,329,499 kg) (= 15,560 truckloads) of limes entered the Texas ports of entry. Postharvest quarantine treatments are not required for the lemons. The lime cultivars were about 70% Persian limes, which are receive fumigation treatments for fruit flies, and 30% Mexican Key limes, which are not treated. Most of these shipments crossed into the USA at bridges in Pharr and Progresso, Texas, the citrus production zone of the Rio Grande Valley. Texas has been infested by ACP since 2001 (French et al. 2001) and as of the summer of 2011 no '*Candidatus* Liberibacter asiaticus' infected trees or psyllids had been detected in Texas. South Texas has an area-wide program to manage psyllid populations. Arizona and California has had programs to eradicate outbreaks as they were detected. In January 2012 HLB was confirmed in San Juan, about 1.5 km from the Pharr International Bridge in Hidalgo County in Texas and in Apr 2012 an HLB infected tree was reported on private property in Los Angeles County, California.

Materials and Methods

Experiments were conducted in three 223.5 × 156.2 × 152.4 cm controlled walk-in temperature and humidity chambers (Hotpack, SP Industries, Warminster, Pennsylvania) which had previously been used for tropical fruit storage experiments. Each chamber contained a cage (137.5 × 137.5 × 55.8 cm) with 6 sticky traps arranged with one trap on each end and 2 traps at the front and back sides. Prior to use the temperature and humidity systems were cleaned and calibrated under the maintenance contract. Each unit had controls and digital readouts of current, high and low humidity and temperature on a secure panel outside the door. The temperature was set at 24.4 °C (= 76 °F) and relative humidity at 40%. Temperature and humidity in each chamber were recorded at approximately 3 hour intervals during the working day (7:00 AM-4:00 PM) during the testing period. Psyllids for testing were collected from various host plants (mostly orange jasmine, *Murraya paniculata* (L.) which we maintained in screen cages. The colony cages were maintained in a glass greenhouse at 26 °C and about 80% RH. A total of 100 psyllids were placed in each cage on vegetation if present, otherwise on the bottom of the cage. Immediately after releasing psyllids, lights were extinguished, the doors were shut and sealed with duct tape around the edge of each door, and the control panel was locked. All tests were run for 48 h with no external lights. After the 48 h exposure, the 6 traps from each cage were removed and Asian citrus psyllids were counted. In addition, all live and dead psyllids in the cage were tabulated.

The commercial traps consisted of a sticky insert with a single light-emitting diode (LED) located in the center. This was mounted into a cardboard shallow container. The first models used in preliminary tests were labeled "Transtraps" provided by Alpha Scents (West Linn, Oregon) with which we performed preliminary testing for battery life and psyllid catching effectiveness. The box portion measured 32.5 cm wide × 20.5 cm high × 3 cm deep and the sticky insert sheet measured 30 × 20 cm. A battery socket for 2 batteries (AA alkaline) was attached to the inside bottom of the trap. For our laboratory tests reported here, we used a second version smaller trap labeled "Trans Trap" having 22.5 × 14 × 3.5 cm box dimensions and a 20 × 13.5 cm sticky panel. The battery socket was mounted on the right interior side of the box. Both trap boxes had lids with insertable tabs that could securely fasten the lid closed for transport to the lab or for shipping.

For our tests with the Trans Trap, we tested only the inserts and lighting system by removing the sticky card and battery mount from the box, and attaching the battery mount with batteries to the back of the card or directly to the side of the cage. The sticky panels were removed from the box and attached to the side frames of the cages with sticky tape in the first replicate, then with wire hooks in all following tests. Plant material tested included no plant material, a box of lemons, or a small nursery lime tree in each cage. The box of lemons was a commercial plastic field box, which typically contains about 18 kg (= 40 lb) of fruit. Lemons were harvested from the ARS orchard in Weslaco. The lime trees were purchased from nurseries and maintained in the plastic containers (2 gallon) and trimmed to about 90 cm tall (container + vegetation) and occupied about 25% of the cage.

Experiment 1

This experiment compared numbers of psyllids captured on traps, each with 0, 1, or 2 LED type lights, in cages containing no vegetation, a plastic field box of lemons, or a Mexican lime tree.

The traps at the ends of the cages each contained either unlit or contained a single LED. The back side of the cage had traps with either 2 or zero LED and the front side of the cage had traps with either 1 or 2 LED. In the first trial of Experiment 1 traps were attached to the sides of the cages with double sided sticky tape, in all subsequent trials traps were attached to the sides of the cages with metal hooks. For the 3 trap types in each cage, the positions were rotated clockwise by one position for each date replicate. The capture data were analyzed by SYSTAT 12 (2007), ANOVA procedure with total capture on each trap type as the dependent variable and plant material (box of lemons, lime tree, none) and trap type (1 light, 2 lights, no light) and the interaction (plant material × trap type) as categorical factors. The experiments each with 3 chambers were replicated on 3 dates.

Experiment 2

This test compared trap capture of psyllids in a cage containing a field plastic box of 'Eureka' lemons. The 3 chambers used the same cages as experiment 1, but had identical plant material, and 2 of each trap type (1, 2, 0 LEDs). The experiments were performed in triplicate. Analysis was performed by the ANOVA procedure of SYSTAT 12 (2007) using the 3 trap types as categorical factors. This analysis compared trapping of ACP in traps with 3 levels of localized light in the same cage.

Experiment 3

This test compared trap capture of psyllids in the 3 cages with each containing the same plant material. In 3 replicates the cage contained a field plastic box of lemons and in 2 replicates the cage contained a Mexican lime tree. In all 5 replicates each cage had 6 identical traps each with either 0, 1, or 2 LEDs. The test was used to compare numbers of psyllids captured related LED numbers (total light in cage) from all the traps. Analysis was performed by the ANOVA procedure of SYSTAT 12 (2007) using the 3 trap types as categorical factors. This analysis compared total trapping among cages with the different total levels of light.

Results

The temperature controls in the chambers were set at 24.4 °C and maintained temperatures within 0.1 °C of this setting. Relative humidity was set at 40% but ranged from 31% to 60%. We could detect no temporal pattern in these fluctuations over the 48 h period, but we noted that the chambers with no plant material had consistently lower humidity, usually below 40%, than those with fruit or trees.

Results of the Experiment 1 are given in Table 1 for the concurrent comparison of 3 plant materials and 3 trap types. During these experiments, one trap containing 2 LEDs fell from the side of the Control (no plant material) and both traps with 2 LED lights fell in the cage with the Mexican lime tree in the first replication (Experiment 1-1). We could not determine when the traps fell but the LED's were still illuminated and the fallen traps collected some psyllids in both the cages. We performed ANOVA tests to determine the significance of the influence of plant material and numbers of LED per trap on numbers of captured psyllids. This analysis is shown at the bottom of

TABLE 1. NUMBERS OF PSYLLIDS TRAPPED IN SEALED, DARK CAGES PLACED IN ENVIRONMENTAL CHAMBERS CONCURRENTLY TESTING EFFECTS OF PLANT MATERIALS AND NUMBER OF LEDS PER TRAP. EACH CAGE CONTAINED 2 OF EACH TYPE OF TRAP. TRAPS WERE TESTED FOR 48 H WITH ABOUT 100 LIVE, UNSEXED ASIAN CITRUS PSYLLIDS PER CAGE.

Experiment	Date	Plant Material	Light Source (#LED)	Psyllids on trap	Psyllids live in cage	Psyllids dead in cage
1-1 Three materials 3 lights	02/15/11	Mexican Lime Tree	1	31	4	0
			2	*24**		
			None	4		
		Box of Lemons	1	27	4	10
			2	46		
			None	3		
		No material	1	22	0	6
			2	*40***		
			None	4		
1-2 Three materials 3 lights	02/23/11	Mexican Lime Tree	1	19	27	10
			2	24		
			None	3		
		Box of Lemons	1	22	0	40
			2	54		
			None	2		
		No material	1	16	3	6
			2	59		
			None	5		
1-3 Three materials 3 lights	05/02/11	Mexican Lime Tree	1	19	20	4
			2	54		
			None	4		
		Box of Lemons	1	32	2	2
			2	62		
			None	1		
		No material	1	26	0	3
			2	72		
			None	5		

* both traps with 2 LEDs fell off cage side; **one 2 LED trap fell off cage side

Analysis of Variance

Source	Sum of Squares	df	Mean Square	*F* Ratio	*P*-value
Number of LEDS	9,094296	2	4,547.148	56.604	< 0.001
Plant Material	332.519	2	166.259	2.070	0.155
LED × Plant	667.259	4	166.815	2.077	0.126
Error	1,446,000	18	80.333		

Table 1 and shows that the LED number per trap was significant ($p < 0.001$) but the plant material and the plant material × LED number interaction was not.

Experiment 2 for the repeated direct comparison of the numbers of LED per trap in competition among traps in the same cage is given in Table 2. In this series of experiments we had learned to attach the traps to the cage mesh fabric more securely and no traps fell. The performance of the traps as shown by the relative numbers of psyllids per trap was consistent over the tests. The single LED traps averaged 29.5 (SD 10.05) psyllids, traps with 2 LEDs averaged 54.83 (SD 10.11), and traps without lights averaged 5.17 (SD 1.94). This consistency is shown in the analysis at the bottom of Table 2 showing highly significant influence of the light number on the trap catches.

Experiment 3 tested cages with either a box of lemons or a lime tree in each cage with 6 traps of the same type so traps in each cage had the same level of light but different cages had different total levels of light. Table 3 shows that total numbers of psyllids on traps were highest for traps with 2 LEDs, then 1 LED and lowest for traps without LED lights. The numbers of psyllids on traps with lights exceeded those collected dead in the cage in both boxed lemon tests but trap capture was less than dead psyllids number for the no LED traps. However in the completely dark cages (Table 3, light source none) some psyllids (> 10) were captured on the 6 traps with no lights. The analysis at the bottom of table 3 shows that the number of LED lights is a significant factor in psyllid capture rate, but there was no effect of type of plant material. This pattern agrees with experiments 1 and 2 (in which traps competed) and the lack of significance for plant material in psyllid capture shown in Table 1.

Discussion

The 3 experiments demonstrated that the presence of light emitting diodes on the sticky board traps greatly increased numbers of ACP collected on traps. The results also showed that in the containers, the majority of psyllids moved to the traps in all 3 experiments, a much smaller proportion died in the cage, and in containers with adequate food (lime tree) a significant number of psyllids survived. These results are comparable to those of Hall and McCollum (2011) who found about 50% mortality ACP in cages with no host material after 2 days and much lower mortality in cages with fruit or leaves of a variety of different citrus species and cultivars.

The concurrent comparison of traps and plant material in experiment 1 showed that the plant material (or lack thereof) in the cage had little effect on numbers of psyllids trapped on any of the trap types. Experiment 1 also had a significant effect of trap type despite the fact that the 2 LED traps fell in the first replicate. We did not know when the 2 traps fell on the lime tree cage or in the no material cage, but the 2 LED capture total was still much higher than the no-LED light trap capture indicating that either the traps fell near the end of the trial or were attractive while lying on the cage floor. The effects of light number in experiment 2 was clarified by using 2 different collections of psyllids and a total of 6 trials; and the 2 LED traps always captured the most psyllids and the traps with no lights captured the least.

In design of experiment 3 with each cage containing 6 traps with the same number of LEDs, each trap type was tested in a container with a different amount of total light. The no-LED trap cages were completely dark (or at least as dark as a transport container), and the 2 LED trap cages had twice (in total 12 LEDs) as much light as the 1 LED cage (in total 6 LEDs). Although the zero light cage caught the fewest psyllids in each trial, psyllids were apparently moving in the dark since all dark cages had more than 10 psyllids on the 6 traps. The traps and lights used in these tests were produced by a commercial supplier and their product contained a single white LED. These results showed that additional sensitivity is likely if more than one LED per trap were used. The commercial trap used the same yellow sticky card used in field sampling traps for psyllids, different colors of LEDs or sticky cards might have improved trapping rates.

Phototaxis as part of the orienting stimuli in Asian citrus psyllid is usually listed as an important factor in mating and host finding behavior (Patt & Setamou 2010; Wenninger & Hall 2007; Wenninger et al. 2009) and in other psyllid species (Samways 1977). Host recognition odors are probably strong stimuli (review in Patt & Setamou 2010) but in closed containers, such as our chambers tested here and in trailers used to transport commercial citrus with greater than 90% of the volume of the trailer packed with citrus, the air is likely to have been saturated with these odors. The failure of the type, or absence, of plant material to significantly influence trap catches suggests that these traps can be effective monitoring devices for detecting these psyllids in empty trucks, those with fruit, or those contaminated by plant debris or with leaves and stems attached to the fruit.

In all tests the numbers of psyllids on traps were higher than the numbers surviving after the tests, which could indicate a possible role of using light traps as a method to kill psyllids in transported fruit. However the rates of survival (Table 3) for either cage—each with a box of lemons or with a lime tree—were greater than 7% and as high as 45%. These survival (non-trapping) rates suggest that the trans trap, while reducing numbers, is not a solution for protection from transport and introduction of Asian citrus psyllids.

TABLE 2. TRAPPING TESTS IN SEALED, DARK ENVIRONMENTAL CHAMBERS. NUMBER OF PSYLLIDS CAPTURED IN CAGES CONTAINING FRESH HARVESTED LEMONS COMPARING NUMBERS OF PSYLLIDS CAPTURED ON TRAPS WITH EITHER 1, 2, OR 0 LEDS PER TRAP. EACH CAGE CONTAINED 2 OF EACH TYPE OF TRAP. TRAPS WERE TESTED FOR 48 H WITH ABOUT 100 LIVE, UNSEXED ASIAN CITRUS PSYLLIDS PER CAGE.

Experiment	Date	Plant Material	Light Source (#LED)	Psyllids on trap	Psyllids live in cage	Psyllids dead in cage
2-1 Trap competition	05/04/11	Box of Lemons	1	25	8	6
			2	47		
			None	4		
		Box of Lemons	1	34	1	6
			2	44		
			None	6		
		Box of Lemons	1	24	5	24
			2	46		
			None	2		
2-2 Trap competition	05/09/11	Box of Lemons	1	33	8	11
			2	65		
			None	7		
		Box of Lemons	1	45	1	20
			2	63		
			None	7		
		Box of Lemons	1	16	25	24
			2	64		
			None	5		

Analysis of Variance

Source	Sum of Squares	df	Mean Square	F Ratio	P-value
Number of LED	7,401.333	2	3,700.667	53.624	<0.001
Error	1,035.167	15	69.011		

TABLE 3. TRAPPING TESTS IN SEALED, DARK ENVIRONMENTAL CHAMBERS. NUMBER OF PSYLLIDS CAPTURED IN CAGES CONTAINING HOST MATERIAL, AND ONE TYPE OF TRAP (1, 2, OR 0 LEDS) WITH 6 TRAPS PER CAGE. TRAPS WERE TESTED FOR 48 H WITH ABOUT 100 LIVE, UNSEXED ASIAN CITRUS PSYLLIDS PER CAGE.

Experiment-replicate	Date	Plant Material	Light Source (#LED)	Psyllids on traps	Psyllids live in cage	Psyllids dead in cage	Percent Psyllids Survival
3-1 same lights/cage	02/28/11	Box of Lemons	1	71	0	10	0.00
		Box of Lemons	2	71	2	16	2.25
		Box of Lemons	None	16	5	53	6.76
3-2 same lights/cage	03/02/11	Box of Lemons	1	60	7	33	7.00
		Box of Lemons	2	87	11	9	10.28
		Box of Lemons	None	22	9	65	9.38
3-3 same lights/cage	05/17/11	Box of Lemons	1	106	3	4	2.65
		Box of Lemons	2	96	6	12	5.26
		Box of Lemons	None	29	35	21	41.18
4-1 same lights/cage	03/07/11	Lime Tree	1	39	26	18	31.33
		Lime Tree	2	65	17	10	18.48
		Lime Tree	None	13	23	50	26.74
4-2 same lights/cage	03/09/11	Lime Tree	1	68	5	32	4.76
		Lime Tree	2	79	5	23	4.67
		Lime Tree	None	35	22	20	28.57

Analysis of Variance-Psyllids on Traps

Source	SS	df	MS	F	p-value
Number of LED	8,072.867	2	4,036.433	15.660	0.001
Plant Material	532.900	1	532.900	2.067	0.184
LED x Plant	443.267	2	221.633	0.860	0.455
Error	2,319.833	9	257.759		

Analysis of Variance-Percent Psyllids Survive

Source	SS	df	MS	F	p-value
Number of LED	608.126	2	304.063	2.203	0.166
Plant Material	336.903	1	336.903	2.441	0.153
LED × Plant	52.881	2	26.440	0.192	0.829
Error	1,242.191	9	138.021		

Conclusions

Trapping Asian citrus psyllids in a closed dark container indicated several patterns and conclusions concerning their activity. In the completely dark cage, psyllids were captured on traps indicating that they were moving and encountering the traps. Light from light emitting diodes (LEDs) greatly increased the rates of trap capture. In cages containing traps with 0, 1, or 2 diodes, those with the most diodes captured the most psyllids, indicating that psyllids responded to the localized intense light. In cages with different traps all containing the same number of LEDs, total trap capture was greatest in cages with the most LEDs, but some psyllids were trapped in cages with traps having no lights. Presence of plant material increased psyllid survival but some psyllids survived and were trapped in cages with neither fruit nor plant foliage.

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