HORTICULTURAL ENTOMOLOGY

## Effect of UV-Blocking Plastic Films on Take-Off and Host Plant Finding Ability of *Diaphorina citri* (Hemiptera: Liviidae)

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ABSTRACT The Asian citrus psyllid, Diaphorina citri Kuwayama (Hemiptera: Liviidae), is a major pest of citrus worldwide due to its ability to transmit the bacteria associated with huanglongbing. Vision, behavior, and performance of insect pests can be manipulated by using ultraviolet (UV)-blocking materials. Thus, the aim of our study was to evaluate how UV-blocking plastic films may affect the take-off and host plant finding ability of D. citri. To assess the effect of a UV-deficient environment on take-off, adult psyllids were released from a vial inside a screenhouse covered by a UV-blocking or standard (control) film and the number of insects remaining on each vial under each treatment was counted at different time intervals. Moreover, to assess the ability of D. citri to find citrus plants under a UV-deficient environment, two independent no-choice host plant finding assays with different plant arrangements were conducted. In each treatment, the number of psyllids per plant at different time intervals was counted. Both D. citri take-off and host plant finding ability was clearly disrupted under a UV-deficient environment. The number of psyllids remaining in the vials was significantly higher under UV-blocking than standard film in all periods recorded. Furthermore, psyllids were present in significantly higher number on citrus plants under standard film than under UV-blocking film in all of the periods assessed and experiments conducted. Our results showed that UV-blocking materials could become a valuable strategy for integrated management of D. citri and huanglongbing in citrus grown in enclosed environments.

KEY WORDS Asian citrus psyllid, huanglongbing, pest management, flight behavior

#### Introduction

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the main vector of a phloemlimited bacteria (*Candidatus* Liberibacter spp.), which causes huanglongbing (HLB), the world's most serious disease of citrus (Bové 2006). No curative measures are available to control this disease. The main strategies recommended to slow the spread of HLB are sanitary measures for the production of disease-free nursery trees in enclosed environments, inspection and eradication of symptomatic plants, and the control of the vector, *D. citri* (Belasque et al. 2010).

Nursery trees and foundation blocks in the major citrus-growing regions of the world are produced under enclosed environments such as screenhouses or greenhouses to prevent the spread of insect-borne diseases such as HLB (Bové 2006). São Paulo, Brazil, the major orange-producing region of the world, has around 17 million nursery trees per year grown under enclosed structures. In addition, citrus groves are produced commercially under protected environments (Japan, China, and South Korea) to produce high-quality cash crop citrus cultivars. This management strategy is growing worldwide, mainly in regions where HLB is present, because chemical control of the psyllid vector is not effective enough to prevent primary spread of this devastating disease (Bassanezi et al. 2013).

Most insects use both visual and olfactory cues to find their host plants, with visual cues of primary importance for most hemipteran insect pests studied so far, including D. citri (Moericke 1955, Kring 1972, Powell et al. 2006, Wenninger et al. 2009, Patt et al. 2011). Insects use two photoreceptors located in different parts of the compound eyes for spectral discrimination. Phytophagous insects are able to distinguish between vegetation and sky by the presence of two receptor types, one absorbing maximally before 500 nm, generally in the ultraviolet (UV)-A range (300-400 nm), and one absorbing beyond 500 nm (Prokopy and Owens 1983). Some insects use an additional third photoreceptor as visual stimuli. For example, Myzus persicae (Sulzer) photoreceptors react to the green region around 530 nm, to the blue-green region (490 nm), and to the UV region (330–340 nm) (Kirchner et al. 2005).

A strategy that has been developed to interfere with insect vision is the use of photoselective materials such as nets and plastic films that can selectively block or reflect radiation of specific wavelength regions. Some of these optical barriers are potent tools that have provided effective protection against several vector-borne

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virus diseases transmitted by aphids, whiteflies, and thrips (Costa et al. 2002, Antignus 2012, Paul et al. 2012, Rechner and Poehling 2014). One of the oldest strategies used to interfere with insect vision and protect vegetable crops from aphid-transmitted virus diseases is the use of reflective soil mulches (Kring 1964). When mulches such as aluminum foil are placed beneath the plant canopy insects are unable to locate their host plants. This results in reduction in the incidence of vector-borne virus diseases in peppers (Loebenstein et al. 1975), lupins (Jones 1991), squash (Brown et al. 1993), and several other vegetables (Greer and Dole 2003). More recently, Croxton and Stansly (2014) have found that metalized low-density polyethylene (PE) mulches can repel D. citri and delay the spread of HLB in young citrus plantings.

Another effective way to interfere with the vision of insect vectors of plant diseases is the use of UV-blocking barriers. These materials are known to reflect and absorb UV radiation, which reduces the immigration of insects vectoring virus diseases. The high contrast between the UV level in the outer and the inner sides of the enclosure reduces the propensity of insects to enter the greenhouse ("a-two-compartment effect" sensu Antignus 2012). At the same time, the spread of viruses once the vector enters a greenhouse with low-UV light is reduced due to the lack of stimuli for take-off once the insect lands on a plant (Antignus 2012). UV-blocking covers (films and nets) have been used to control the spread of several virus diseases transmitted by aphids, whiteflies, and thrips (Díaz et al. 2006, Antignus 2012, Legarrea et al. 2012a). However, there is a lack of information on the effect of UV-blocking materials in the take-off and flight behavior of psyllids such as D. citri. Consequently, the aim of our study was to evaluate how UV-blocking plastic films may affect the take-off and host plant finding ability of *D. citri*.

#### **Material and Methods**

The experiments were carried out at Fundecitrus (Araraquara, SP, Brazil; Latitude  $21^{\circ}$  48'31'' S, Longitude  $48^{\circ}$  09'48'' W, Altitude 670 m) in parallel independent screenhouses (5 m long by 2.5 m wide by 2 m height) placed on a grass surface facing southeast-northwest and separated by 1.5 m between each other. The screenhouses were covered by either a UV-blocking film or by a standard film (control). The screenhouses were identical except by the type of film covering each screenhouse. For all experiments, temperature and light intensity (outdoor) were recorded from 6:00 a.m. to 6:00 p.m. using the Priva Meteorological Station (Priva B.V., De Lier, The Netherlands) located at the experimental site (Fig. 1).

**Insect and Plant Material.** Psyllids (*D. citri*) were reared on *Murraya paniculata* L. (Rutaceae) plants in cages (40 by 40 by  $50 \text{ cm}^3$ ) located in a climate-controlled room at  $25 \pm 2^{\circ}$ C,  $60 \pm 10\%$  relative humidity (RH), and a photoperiod of 14:10 (L:D) h. Adults of mixed gender 10–15 d after emergence were used in all experiments.

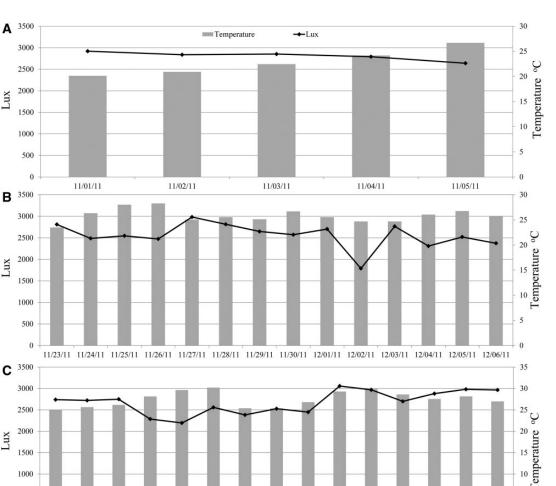
'Rangpur' lime (*Citrus limonia* Osbeck) seedlings, about 50 cm high, kept in plastic pots (2 liter) with *Pinus* substrates (MultplantCitrus; Holambra, SP, Brazil) were used as target plants for experiments to assess the ability of *D. citri* to find citrus.

**Plastic Films.** Two types of PE films were used to cover the screenhouses: a UV-blocking and a standard (non UV-blocking) film. The UV-blocking film also blocked part of the photosynthetically active radiation (PAR), and, therefore, the amount of PAR transmitted by the two types of films was not the same. Table 1 shows the UV and PAR) transmitted through the two types of films. The standard film was most commonly used in citrus nurseries. The films were provided by Electro Plastic company (São Paulo, SP, Brazil), both of them with identical properties, 0.15-mm-thick, low-density PE plus linear low-density PE, multilayer films but with different additives (to provide different UV-blocking properties).

Effects of UV-Blocking Film on the Take-Off of **D.** citri. For this experiment, two screenhouses were used, one covered by the UV-blocking and another by the standard film. About 20 min before release, 100 adult psyllids were placed in a plastic vial (5 cm in diameter and 6 cm in height), then the vial was closed by a lid and turned upside down to allow insects to move upward. Then, each vial was placed on a platform (1.5 m above ground) in the center of each screenhouse and turned right side up while the lid was opened. The number of live psyllids remaining in each vial under each type of film cover was counted every 5 min for 30 min after release. This procedure was repeated 11 times (replications) per treatment (UV-blocking and standard plastic film) over time on five consecutive sunny days. All releases were done from 8:30 a.m. to 9:30 a.m. and simultaneously for both treatments.

Effects of UV-Blocking Films on Host Finding Ability of Citrus Plants by D. citri. To assess the ability of *D. citri* to find citrus plants under a UVdeficient environment, two independent no-choice assays were done. These two experiments were conducted in four screenhouses, covered by the UV-blocking and by the standard film (two of each type in alternating order). In Experiment 1, psyllids were released (as described in the previous experiment) at the center of each screenhouse with five citrus plants scattered 1 m apart at each side of the release point (Fig. 2A). In Experiment 2, psyllids were released at one end of the screenhouse and 12 plants were placed in three rows of four plants spaced 1 m apart at one side of the release point (Fig. 2B). In both trials, all releases were done from 8:30 a.m. to 9:00 a.m. and 100 psyllids were released in each screenhouse (replication) and each experiment was repeated 10 times within a 2- and 5-wk interval always in sunny days, for Experiments 1 and 2, respectively. The number of psyllids alive present per plant was counted at 2, 5, 8, 24, 29, and 32 h after release (Experiment 1); and 2, 5, 8, 24, 32, 48, and 56 h after release (Experiment 2).

**Statistical Analysis.** The number of adults remaining in vials (take-off experiment) or landing on plants (host finding ability experiments) was compared for each time interval using a one-way ANOVA followed by Fisher least significant difference (LSD) test using



0 0 01/30/12 01/31/12 02/01/12 02/06/12 02/07/12 02/08/12 02/14/12 02/15/12 02/16/12 02/28/12 02/29/12 03/01/12 03/05/12 03/06/12 03/07/12 Date

Fig. 1. Mean temperature (°C) and light intensity (Lux; outdoor) recorded from 6:00 a.m. to 6:00 p.m., during take-off experiment (A); first and second host finding ability experiments (B) and (C), respectively.

Table 1. Percentage (%) of UV radiation transmitted by each type of PE films used in the experiments

JV (250–400 nm)	$\mathrm{PAR}\;(400700\mathrm{nm})$		
$0.8 \pm 0.1$	$59.2 \pm 3.8$ 78.7 ± 8.7		
	,		

Radiation measured using an Apogee Model UVM radiometer for measuring total UV radiation and an Apogee Quantum Flux meter (Model MQ-100) radiometer for measuring PAR (Logan, UT). Measurements were repeated four consecutive sunny days at midday from 3 October to 6 October 2011.

Statview 5.0 (Abacus Concepts 1989). All data were transformed by loge (x + 1) prior to analysis to decrease heteroscedasticity and achieve normal distribution.

In addition, the data on the number of psyllids landing on plants for the two host finding ability experiments were fitted into a generalized linear mixed model (GLMM) to study the main effects: type of plastic (standard or UV-blocking; fixed) and time after release (covariate) (Vonesh and Chinchilli 1997). Repeated measures (time), the main fixed factor (type of plastic), the covariate (time), and the random effects (replicates) were defined in the model, and the statistical procedure was performed using IBM Statistics SPSS 21.0 software (SPSS 2013, Chicago, IL).

#### Results

Effects of UV-Blocking Film on the Take-Off of **D.** citri. D. citri take-off behavior was disrupted under a UV-deficient environment. The number of D. citri adults remaining in the vials after opening the lid was significantly (P < 0.05) great under UV-blocking than in the standard film in all periods recorded (Table 2). Most psyllids (around 90%) left the vials rapidly (5 min) under standard film, whereas only about 60% left the

10 5

Lux

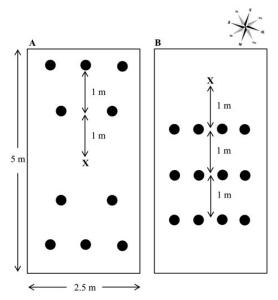
Lux

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Lux

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500



**Fig. 2.** Distribution of citrus plants and location of the *D. citri* release point in the first (A) and second (B) experiment. X represents the release point.

vial under UV-blocking film in the same period. Furthermore, most (98.5%) of the psyllids were able to take-off after 15 min under standard film, whereas about 18% of the insects were still in the vial under UV-deficient environment. At last (30 min after release), 7.36% of the psyllids remained in the vial under UV-blocking film, but only 0.36% were still in vials under the standard film. Moreover, psyllids were found only in two of the 11 replicates after 30 min under the standard film, whereas some insects were present in each of the 11 replicates under the UVdeficient environment.

Effects of UV-Blocking Films on Host Finding Ability of Citrus Plants by D. citri. The UV-blocking film impaired host finding ability of D. citri when searching for citrus plants. Psyllids were present in significantly (P < 0.05) higher numbers on citrus plants under standard than under UV-blocking film in all time periods recorded in both experiments (Tables 3 and 4). Results were similar when the psyllids were released in the middle (Experiment 1) or at the end (Experiment 2) of the screenhouse. For Experiments 1 and 2, the results of the GLMM analysis also indicated that the number of psyllids per plant was significantly higher under the standard than under the UV-blocking film (Experiment 1: F = 125.699; P < 0.0001; Experiment 2: F = 277.234; P < 0.0001). However, the time effect was highly significant for Experiment 1, according to the GLMM model (F = 20.955; P < 0.0001) but not significant for Experiment 2 (F = 1.184; P = 0.277). When we split each treatment (type of film) in Experiment 1, we found that there was a significant (F = 22.199; P < 0.0001)increase in the number of psyillids per plant over time under the standard film but not under the UV-blocking film (F = 0.003; P = 0.482). Furthermore, the GLMM analysis showed a significant interaction between the

two factors (type of film and time) only for Experiment 1 (F = 17.719; P < 0.0001) but not for Experiment 2 (F = 0.027; P = 0.868). The statistical analysis and Fig. 3 showed that the number of psyllids per plant remained almost constant over time under Experiment 2, but increased significantly over time in Experiment 1 under the standard film. Also, Figure 3 showed that the variation in the number of psyllids per plant throughout time was much smaller under the UV-blocking than under the standard film. This suggests that insects settled and remained on the plants after landing under the UV-blocking treatment. Conversely, the large oscillation in the counts of the number of psyllids per plant along time indicates that they moved from plant to plant after landing under the standard film.

#### Discussion

Host plant selection process by hemipterans includes a series of crucial steps initiated by visual attraction, olfactory, and tactile stimuli followed by gustatory cues (Powell et al. 2006, Martin et al. 2014). Both UV (320-400 nm) and visible radiation (400-700 nm) play key roles in the host finding ability of hemipterans. UV radiation, mainly in the UV-A range (320–400 nm), is known to play an essential role when aphids take-off from a solid surface (Kring 1972), as well as in navigation, orientation, and host finding abilities of most hemipterans (Antignus and Ben-Yakir 2004). It is known that both visual and olfactory cues are important and may play a synergistic role in the host plant finding and selection process by D. citri (Wenninger et al. 2009, Sétamou et al. 2012). However, no studies have been conducted so far on the role of UV radiation in the take-off and host plant finding ability of D. citri. Our work shows for the first time that *D. citri* was affected by the lack of UV radiation, both during the take-off phase and during the host searching and orientation process. Indeed, Experiment 1 showed that D. citri take-off was disrupted when insects were released under the UVblocking film (99% of ambient UV was blocked). Most psyllids (90%) were able to fly away in 5 min from the release point under standard UV conditions, while many (about 40%) did not take-off when UV radiation was blocked. Most psyllids (92.6%) finally left the vial 30 min after the lid was opened under UV-deficient conditions, but they spent several minutes walking around the vial before take-off. This type of behavior, characterized by walking in circles and wandering around the edge of the vial for several minutes before take-off, was never observed for psyllids placed under the standard UV-rich environment. Instead, psyllids took-off rapidly from the edge of the vial once the lid was open. Therefore, takeoff behavior by D. citri was clearly inhibited under a UV-deficient environment as previously reported for other hemipterans (Kring 1972, Coombe 1982, Legarrea et al. 2012b).

Our work also demonstrates that the lack of UV radiation disrupted the ability of psyllids to find and settle on their host plants. Actually, we observed in our host plant finding experiments that psyllids were not present in the vials 24 h after release (data not shown),

Table 2. Mean ± SE of D. citri adults remaining on the vial at different time intervals after insect release

Treatment (plastic films)	Sampling time (min)					
	5	10	15	20	25	30
UV-blocking	$41.18 \pm 4.12$	$26.82 \pm 3.05$	$18.27 \pm 2.08$	$13.55 \pm 1.70$	$9.46 \pm 1.56$	$7.36 \pm 1.51$
Standard F	$10.18 \pm 1.01$ 93.83	$4.91 \pm 1.05 \\ 45.69$	$1.46 \pm 0.71$ 76.28	$0.91 \pm 0.50 \\ 84.25$	$0.73 \pm 0.41 \\ 63.55$	$0.36 \pm 0.24$ 44.98
Р	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Table 3. Mean  $\pm$  SE of *D. citri* adults (number of insects per plant) present on citrus plants at different time intervals after insect release (Experiment 1)

Treatment (plastic films)	Sampling time (h)						Overall mean
	2	5	8	24	29	32	
UV - blocking	$0.77 \pm 0.11$	$0.82 \pm 0.11$	$0.89 \pm 0.12$	$0.87 \pm 0.13$	$0.85 \pm 0.12$	$0.89 \pm 0.12$	$0.84 \pm 0.04$
Standard	$3.56 \pm 0.33$	$4.70 \pm 0.41$	$5.02 \pm 0.47$	$5.34 \pm 0.48$	$5.72 \pm 0.48$	$5.99 \pm 0.51$	$5.05 \pm 0.18$
F	102.87	159.21	153.38	155.43	99.40	206.18	_
Р	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	_

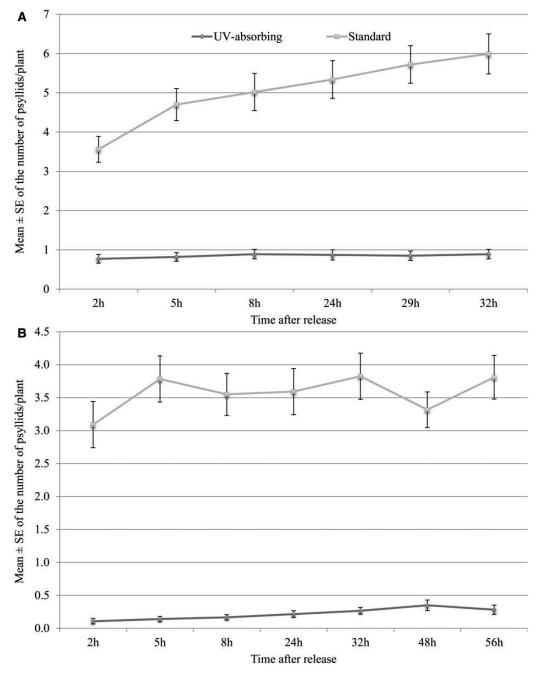
Table 4. Mean  $\pm$  SE of *D. citri* adults (number of insects per plant) present on citrus plants at different time intervals after release (Experiment 2)

Treatment (plastic films)	Sampling time (h)						Overall mean	
	2	5	8	24	32	48	56	
UV-blocking	$0.11\pm0.04$	$0.14\pm0.04$	$0.17\pm0.04$	$0.22\pm0.05$	$0.27\pm0.05$	$0.35\pm0.08$	$0.28\pm0.07$	$0.21 \pm 0.02$
Standard	$3.09 \pm 0.35$	$3.78 \pm 0.35$	$3.55 \pm 0.32$	$3.59 \pm 0.35$	$3.82 \pm 0.35$	$3.32 \pm 0.27$	$3.81 \pm 0.33$	$3.56 \pm 0.12$
F	207.39	299.19	262.65	222.22	229.71	191.66	247.79	-
Р	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	-

but very few psyllids settled on the target plants under the UV-deficient conditions. Our results suggest that psyllids had difficulties flying to their host plants in the absence of UV radiation. Our overall mean showed that more than a 5-fold and 16-fold reduction in the number of psyllids per plant was found under the UVdeficient environment, in Experiments 1 and 2, respectively. In fact, many psyllids were observed walking on the grass around the release point under UV-absorbing film. Conversely, several psyllids were often observed on the sides and ceiling of the screenhouse toward the sunlight under the standard film but none were walking on the grass around the release point. This positive phototactic response of *D. citri* has been previously reported by Sétamou et al. (2012). Our work is the first report showing that UV-blocking plastic films can interfere with the flight behavior of D. citri and disrupt their ability to find their host plants. A similar reduction in spread under UV-deficient environments has been reported for the aphid Macrosiphum euphorbiae (Thomas) on lettuce (Legarrea et al. 2012c) and for the whitefly Bemisia tabaci (Gennadius) on tomatoes (Antignus et al. 2001).

Our host plant finding experiments also showed some differences in the number of psyllids per plant depending on the location of the release point. Indeed, the number of psyllids per plant increased by fourfold when released in the middle versus at the end of the screenhouse under the UV-deficient environment. These results support that blocking UV radiation affects the dispersion ability of psyllids around the release point because more insects were able to find citrus plants when released from the middle than from one end of the screenhouse. Random displacement of psyllids from the middle of the screenhouse probably helped them to find a citrus plant nearby because plants were present on both sides from the release point as opposed to Experiment 2 where plants were present just in one direction. In a commercial citrus nursery and other protected environments, the UVblocking film could reduce entry or spread of psyllids as shown in our experiment where the insects were released on one side of the screenhouse.

The use of UV-cladding materials (films and nets) could become a valuable strategy for integrated pest management in commercial citrus groves as well as in citrus nurseries grown in enclosed environments. Our results show that the use of UV-blocking films modified the flight behavior of *D. citri* reducing their ability to find their host plants, which could limit the spread of HLB. It is important to stress that our results were obtained in small (5 by 2.5 m<sup>2</sup>) screenhouses, which could have increased the chances of D. citri to find their hosts, as olfactory cues are also involved in the host finding ability of D. citri (Wenninger et al. 2009, Patt and Sétamou 2010). Under a larger enclosure, psyllids might have even more difficulties to find their host plants under a UV-deficient environment. Therefore, further experiments should be conducted under larger enclosures to assess the effects of UV-blocking films on



**Fig. 3.** Number of the adult psyllids per plant in the screenhouses covered by the UV-blocking and standard films in the first and second host finding ability experiments (A) and (B), respectively.

the spread of *D. citri* and the incidence of vector-borne plant pathogens such as *Ca.* Liberibacter spp.

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