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REPELLENCY OF FIVE MINERAL OILS AGAINST *DIAPHORINA CITRI* (HEMIPTERA: LIVIIDAE)

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ABSTRACT

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the vector of huanglongbing, a destructive citrus disease worldwide. Horticultural mineral oils (HMOs) are commonly used for management of *D. citri*. In the past, repellency of HMOs against *D. citri* was reported. The primary objective of this study was to determine the repellency characteristics of 5 HMOs against *D. citri*. The settling and oviposition responses of *D. citri* to aqueous emulsions of the HMOs were assessed by bioassay. The relationships between repellency and carbon number distribution, as well as the emulsifying efficiency of each HMO were analyzed. The results showed that the various HMOs had significantly different behavioral repellent effects against *D. citri*. Correlation analysis using Pearson's correlation coefficient indicated that the repellent effects of the HMOs against *D. citri* were significantly correlated with the mass percentages of C22, C23 and C24, but not with their emulsifying efficiencies. These results can help to select and develop HMOs with high repellencies for *D. citri* control. The mechanism of HMO repellency against *D. citri* is unclear and needs to be investigated.

Key Words: horticultural mineral oil, carbon number distribution, emulsifying efficiency, repellency

RESUMEN

El psílido asiático de los cítricos, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), es el vector del huanglongbing, una enfermedad destructiva de los cítricos en todo el mundo. Se utilizan aceites minerales hortícolas (AMH) para el manejo de *D. citri*. En el pasado, se informó de repelencia de las AMH contra *D. citri*. El objetivo principal de este estudio fue determinar las características de repelencia de 5 AMH contra *D. citri*. Se evaluó la respuesta del asentamiento y de oviposición de *D. citri* a emulsiones acuosas de las AMH por medio de un bioensayo. Se analizó la relación entre la repelencia y la distribución del número de carbonos, así como la eficacia emulsificaante de cada AMH. Los resultados mostraron que los diferentes AMH tenían significativamente diferentes efectos repelentes sobre el comportamiento del *D. citri*. El análisis de correlación mediante el coeficiente de correlación de Pearson indicó que los efectos repelentes de las AMH contra *D. citri* se correlacionaron significativamente con los porcentajes de masa de C22, C23 y C24, pero no con la eficiencia de los emulsionantes. Estos resultados pueden ayudar a seleccionar y desarrollar los AMH con altas repelencias para el control de *D. citri*. El mecanismo de la repelencia de AMH contra *D. citri* no está clara y se necesita investigar.

Palabras Clave: aceite mineral horticultura, la distribución del número de carbonos, emulsionar eficiencia, repelencia

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the vector of huanglongbing (HLB), a destructive citrus disease worldwide (Bové 2006). Horticultural mineral oils (HMOs) have the potential in ACP integrated pest management (Rae et al. 1997; Hilders & Rogers 2005; Stansly 2010; Leong et al. 2012). It is generally considered that HMOs

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are less damaging to the environment, and less disruptive to biological pest control than chemical pesticides (Stansly et al. 2002; Rae et al. 2006). HMOs have been commonly used to kill small, relatively immobile insects by suffocation (Riehl 1969). HMOs are also repellent to many insect pests (Larew & Locke 1990; Beattie et al. 1995; Liu & Stansly 1995; Riedl et al. 1995; Rae et al. 1997; Stansly et al. 2002; Nguyen et al. 2007). Although the mechanisms of action of HMOs against insects are still unclear, their effects are associated with factors such as physical disruption of epicuticular lipids, masking of oviposition stimulants, suppression of release of attractant host plant volatiles, induction of the release of repellent volatiles from plants, and direct repellency of insect pests (Liu & Beattie 2002; Poerwanto et al. 2012). Liu et al. (2001) reported that level of oviposition deterrence of the citrus leafminer [*Phyllocnistis citrella* Stainton (Gracillariidae)] by an HMO was related to the number of carbon molecules in the mineral oil. These authors found that oviposition increased with increasing molecular weight of oil molecules but not with their aromaticity and emulsifier concentration. This indicated that different HMOs with different numbers of carbon atoms may have different repellent effects against insect pests. To understand the repellency of HMOs against ACP, the mineral oil properties that are related to the repellent effects should be analyzed.

The molecular weight of oil molecules can be characterized by the boiling point of an equivalent *n*-paraffin carbon number (*n*Cy), where *n* means normal alkane and *y* denotes the number of carbon atoms (Kuhlmann & Jacques 2002). *n*Cy is used instead of distillation temperature to describe the distillation properties of HMOs. HMOs typically are composed of molecules anywhere from *n*C16 to *n*C32, with corresponding differences in molecular weights. A single *n*Cy can be assigned to a particular HMO sample based on the relative proportions of the different *n*Cy entities it is found to contain. However, the complete equivalent carbon number distribution provides a more complete picture of the product's physical and chemical properties, which are likely to determine biological effects including pest repellency, although these relationships are still poorly understood.

HMOs contain emulsifiers that allow oil molecules to form semi-stable emulsions when mixed with water. The types and concentrations of emulsifiers used in a mineral oil formulation have impacts on its pesticidal efficacy (Ebeling 1936; Campbell 1972). Generally speaking, increasing the concentration of an emulsifier leads to less oil being deposited on sprayed surfaces (Campbell 1972), and to more uniform distribution of the oil. Thus emulsifiers may influence the repellent effects of HMOs against insect pests.

In order to select HMOs with high repellency for controlling the ACP, the behavioral responses of the ACP to several HMOs were compared in this study. Additionally, the mineral oil properties related to the repellent effects against ACP, such as carbon number distribution and emulsifying efficiency, were evaluated.

Materials and Methods

Asian Citrus Psyllid and Mineral Oil Sources

Two trials were conducted during Aug, 2011 at the Guangdong Entomological Institute (GEI), Guangzhou, China. The seedlings used in the experiments were potted 3-yr old sweet orange [*Citrus sinensis* (L.) Osbeck (Sapindales: Rutaceae)], selected and pruned to have similar growing conditions and canopy size. The seedlings were watered by drip irrigation once every 3 days before and during the experiment. These seedlings were places inside net tents (1.5 × 1.0 × 2.0 m). All tents were arranged in a line in an open yard. Temperatures inside the tents ranged from 26~35 °C during the experiment, which were determined by mercury thermometers hung in the tents.

ACP adults were obtained from a population mass-reared on orange jasmine [*Murraya exotica* L. (Sapindales: Rutaceae)] trees in a large greenhouse (5.0 × 4.0 × 3.0 m) at GEI. HMOs used in the experiment are listed as Table 1. They were Bioclear® Spray Oil, Caltex® Summer Spray Oil, Citrole® Paraffinic Oil, and Sunspray® Ultra Fine Oil, Yueyang® Spray Oil.

Choice Trial

Two potted seedlings with no fresh flushes were placed 1.0 m apart from each other inside a tent. One seedling was sprayed with 0.67% oil-in-water emulsion (0.67 units of oil dispersed in 99. 33 units of water) by a knapsack pressure sprayer (5319-5L, Qiaojuan Gardens Facility Co. Ltd., Shanghai, China), the other plant was sprayed with water. The pressure in the sprayer was maintained at 2.0×10^2 KPa. After allowing the oil-in-water emulsion on the seedlings to dry for 24 h, a plastic tube (10 cm long and 1 cm diam) containing 40 ACP adults was placed on the floor midway between the 2 seedlings, and the adults were allowed to exit the tube freely. The numbers of ACP adults on the citrus seedlings were observed and recorded daily at 24 h after the release. These ACP adults were then manually removed from the seedlings and 40 new ACP adults were again released into the tent. This procedure was repeated daily until no significant difference in the number of ACP adults between the 2 seedlings was observed based on a paired-sample t-test ($\alpha = 0.05$) performed using SPSS for Windows 16.0 (SPSS Inc., Chicago, Illinois, USA).

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TABLE 1. MINERAL OILS EVALUATED FOR REPELLECY AGAINST THE SETTLING OF ASIAN CITRUS PSYLLID FEMALES ON SWEET ORANGE SEEDLINGS.

Mineral oil	Manufacturer	Franchiser
Bioclear® Spray Oil	Caltex Australia Petroleum Pty. Ltd., Australia	
Caltex® Summer Spray Oil	Caltex Australia Petroleum Pty. Ltd., Australia	
Citrole® Paraffinic Oil	Total Fluides Co. Ltd., France	JiangSu Rotam Chemistry Co. Ltd., China
Sunspray® Ultra Fine	Sunoco Inc, USA	China Agent of Agricultural Resources Service Center of Strongwill Group
Yueyang® Spray Oil	Yueyang Petroleum Refining Manufacturer of Sinopec Group, China	Guangdong Entomological Institute, China

The repellent effect of each HMO against ACP settling was determined as an index of behavioral tendency (Ibt):

$$\text{Ibt} = \frac{\text{ACP adults on the oil sprayed seedling}}{\text{ACP adults on the oil spryed seedling + ACP adults on the water check seedling}} \quad (1)$$

A low Ibt indicated high mineral oil repellency against ACP adult settling.

Each oil listed in Table 1 was regarded as a treatment, we made 6 replicates per treatment, and we regarded each tent as a replicate. A completely randomized design was used to assign the replicates.

No-Choice Trial

Potted seedlings were pruned and fertilized to encourage new flushes 2 wk before the trial commenced. Four potted seedlings with new flushes were placed in a tent, and sprayed with 0.67% oil-in-water emulsion. The tents with 4 potted seedlings sprayed with water served as the check (CK). The sprayer was maintained at 2.0×10^2 KPa during spraying. After allowing the aqueous emulsion on the seedlings to dry for 24 h, 40 gravid female adults were released into a tent, i.e., 1 plastic tube (10 cm long and 1 cm diameter) with 10 adults released per each potted seedling. The number of live females on each seedling was recorded. Oviposition and the development of eggs and nymphs were observed daily at a regular time. Six days after spraying, all flushes (either with eggs and nymphs or not) on the seedlings were collected in plastic sealed bags. The numbers of ACP eggs and nymphs on the flushes were counted under a microscope. The mean number of offspring (4th - 5th instars, 1st - 3rd instars, eggs) per female was calculated by dividing the total number of offspring by the total number of adult females alive on the corresponding day. The number of flushes on each seedling, the length of each flush in millimeters and the number of leaves in each flush were recorded.

Each specific mineral oil was regarded as a treatment, 5 replicates were made per treatment, and each tent was regarded as a replicate. A completely randomized design was used to assign the replicates. The treatment means were compared by one-way analysis of variance (ANOVA). The differences between the means were separated using the Fisher's least significant difference test (LSD) at 5% significance performed using SPSS for Windows 16.0.

Analysis of Carbon Number Distribution of HMOs

The carbon number distributions of the 5 HMOs were determined by gas chromatography based on ASTM D5442, the American Standard of Testing Method for Analysis of Petroleum Materials (American Society for Testing and Materials 2003). Qualitative analyses were performed by gas chromatograph–mass spectrophotometer (GCMS–QP2010, SHIMADZU Corporation, Kyoto, Japan). Quantitative analysis was performed by gas chromatograph (GC–9750, FULI Analytical Instrument CO. LTD., Zhejiang, China) with a flame ionization detector and chromatography workstation (FL9500, FULI Analytical Instrument Co. Ltd., Zhejiang, China) using a chromatographic column (50 m × 320 μm × 0.17 μm) (HP–5, Agilent Technologies Inc., California, USA). The results were analyzed using GCMS solution version 2.50 analysis software (Shimadzu Corporation, Kyoto, Japan) and the carbon number distributions of the 5 HMOs were obtained.

The chromatographic conditions were performed in splitless injection mode as follows: injection volume, 0.2 μL; initial vaporizing temperature, 100 °C for 10 min, increased incrementally to 350 °C at 10 °C/min for 30 min; column temperature, 330 °C; detector temperature, 380 °C; flow rate of the carrier gas (high purity helium), 1 mL/min; flow rate of the combustion gas

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(high purity hydrogen), 35 mL/min; flow rate of the combustion-supporting gas (purified air), 350 mL/min; and flow rate of the equilibrating gas, 30 mL/min.

The *n*Cy values of the HMOs were transformed from carbon number distributions as follows:

$$n\text{Cy} = \sum(\text{carbon number} \times \text{mass percent of corresponding carbon nmber}) \quad (2)$$

Correlations between repellent effects against ACP and carbon number distributions of the mineral oils were analyzed by Pearson's correlation coefficient performed using SPSS for Windows 16.0.

Analysis of Emulsifying Efficiency of HMOs

The emulsifying efficiencies of the HMOs were determined by infrared spectrophotometry based on the national environmental standards of China HJ 637-2012 (The Ministry of Environmental Protection of the People's Republic of China 2012). The gross amount of mineral oil from 5 mL aqueous emulsion of the substrate was determined by infrared spectrophotometry after the aqueous emulsion was shaken and then allowed to stand for 1 min. A large value for the gross yield of mineral oil from the procedure indicated a high emulsifying efficiency of the mineral oil. The procedure was as follows: 0.5 mg mineral oil was mixed with 50 mL distilled water and shaken on a MMV-1000W EYELA separatory funnel shaker (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at a shake frequency of 150 rpm and shake amplitude of 40 mm for 3 min. After allowing the mixture to stand for 1 min, 5 mL of the aqueous emulsion was collected and mixed with 50 mL of carbon tetrachloride (extractant), and then transferred into a 4 cm quartz colorimetric cuvette. The gross oil from the emulsion was analyzed using an infrared photometric oil analyzer (IR-200A, Xiamen Astar Technology Development Co., Ltd., Fujian, China) at 2930, 2960, and 3030 cm^{-1} with 4 cm^{-1} spectral resolution.

Correlations between the repellent effects against ACP and the emulsifying efficiency of mineral oil were analyzed by Pearson's correlation coefficient performed using SPSS for Windows 16.0.

Results

Choice Trial

Significantly fewer ACP adults settled on oil-sprayed seedlings than on water-sprayed seedlings as seen by low Ibt values (Table 2). Durations of the significant repellent effects varied from a minimum of 2 days for Yueyang and Citrole, to 9 days for Caltex after oil spraying.

No-Choice Trial

Significantly fewer ACP adults were seen on seedlings sprayed with any oil compared to the water-sprayed check 2 days after spraying (Table 3). The fewest adults had settled on Caltex-treated plants, which were significantly fewer than on seedlings sprayed with Yueyang or Citrole (Table 3). Only Caltex-treated plants had significantly fewer adults than the water-treated check at 3 days after treatment. No significant treatment effects were seen during 4-6 days after treatment.

Significantly more 4th-5th instars were seen 6 days after oil treatments compared to the water

Table 2. Ratio of *Diaphorina citri* adults settling on an oil-treated orange seedling to number settling on a treated plus untreated plant (Ibt) in a choice trial, the smaller the Ibt, the greater the repellency.

	Index of behavioral tendency (Ibt)				
Days after spraying	Bioclear	Caltex	Citrole	Sunspray	Yueyang
2	0.164 ± 0.076*	0.128 ± 0.041*	0.247 ± 0.076*	0.188 ± 0.057*	0.295 ± 0.078*
3	0.196 ± 0.065*	0.176 ± 0.058*	0.437 ± 0.149	0.234 ± 0.049*	0.433 ± 0.108
4	0.236 ± 0.061*	0.201 ± 0.053*		0.269 ± 0.079*	
5	0.309 ± 0.084	0.209 ± 0.073*		0.334 ± 0.019*	
6		0.235 ± 0.086*		0.463 ± 0.082	
7		0.214 ± 0.079*			
8		0.307 ± 0.054*			
9		0.360 ± 0.051*			
10		0.387 ± 0.107			

*: The number of ACP adults on the oil-sprayed orange seedlings is significant less than that on water-sprayed orange seedlings by paired-sample t-test ($\alpha = 0.05$). Otherwise, there is no significant difference between the ACP adults on the oil and water treatments. The times when the treatment effects no longer were apparent are shown as blanks in the table.

$$\text{Ibt} = \frac{\text{Adults on the oil sprayed seedling}}{\text{Adults on the oil sprayed seedling} + \text{Adults on the water check seedling}}$$

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TABLE 3. NUMBER OF THE 40 RELEASED *DIAPHORINA CITRI* ADULT FEMALES FOUND ON SWEET ORANGE SEEDLINGS SPRAYED EITHER WITH A MINERAL OIL OR WATER (CHECK) IN NO-CHOICE TRIAL.

	Number of ACP female adults on sprayed seedlings				
Treatment	2nd day	3rd day	4th day	5th day	6th day
Bioclear	12.200 ± 2.498 ab	9.800 ± 2.538 ab	8.000 ± 2.627 a	6.400 ± 1.720 a	4.400 ± 1.631 a
Caltex	8.000 ± 1.975 a	7.200 ± 1.463 a	6.600 ± 1.249 a	5.400 ± 1.122 a	3.800 ± 0.970 a
Citrole	14.000 ± 2.000 b	12.000 ± 2.121 ab	10.400 ± 1.631 a	6.600 ± 1.077 a	4.800 ± 1.241 a
Sunspray	11.400 ± 1.166 ab	9.400 ± 1.435 ab	9.600 ± 1.749 a	7.400 ± 1.166 a	4.200 ± 0.583 a
Yueyang	13.600 ± 2.205 b	12.600 ± 2.502 ab	9.600 ± 1.249 a	6.800 ± 1.428 a	5.800 ± 1.530 a
CHECK	19.600 ± 1.123 c	13.200 ± 0.735 b	11.400 ± 1.435 a	8.400 ± 0.980 a	6.200 ± 1.200 a
df	5, 24	5, 24	5, 24	5, 24	5, 24
F	4.053	1.438	0.996	0.625	0.577
P	0.008	0.217	0.441	0.682	0.717

Numbers of ACP female adults were analyzed by one-way ANOVA and means followed by the same letter in the same column are not significantly different ($P > 0.05$, LSD).

CHECK: Untreated control (sprayed with water).

control in the no-choice test (Table 4). Among the oil treatments, significantly the fewest 4th-5th instars were seen on seedlings sprayed with Caltex oil, and numerically most on Citrole sprayed plants, but the latter treatment results were not significantly different from the Yueyang treatment. These results showed that Caltex oil was the most repellent oil and was still the most repellent of the 5 oils at 2 to 3 days after being sprayed when the 4th-5th instar individuals would have been deposited as eggs. In contrast, there were no significant differences among the treatments in the numbers of 1st-3rd instars and eggs, suggesting that the repellency had already dissipated when these individuals were laid as eggs on the 3rd to the 5th day after the oils had been sprayed.

Mean numbers of 4th-5th instar offspring per female (Table 5) were significantly greater in all of HMO treatments than in the check. Numerically the fewest 4th-5th instars per female were in the Caltex treatment, but this result did not differ significantly from that in the Bioclear, Sunspray and Yueyang treatments. Numerically among the HMO treatments the most 4th-5th instars per female oil occurred in the Citrole treatment, but these offspring were not significantly more than in the Bioclear, Sunspray and Yueyang treatments. Clearly Caltex oil had significantly stronger oviposition repellency than Citrole oil during the initial 2 to 3 days after the oil had been sprayed.

However, no significant differences in flush development of the orange seedlings were observed. Number of flushes varied from 24.600 ± 3.723 of the Caltex oil-treated seedlings to 32.200 ± 2.782 of Citrole-treated seedlings. The mean length of the flushes varied from 37.413 ± 2.343 cm in the Caltex treatment to 44.566 ± 2.511 cm in the CK. The number of leaves per flush varied from 4.076 ± 0.212 in the Yueyang treatment to 4.479 ± 0.235 in the Sunspray treatment. These results suggested that

TABLE 4. NUMBER OF OFFSPRING FROM THE 40 *DIAPHORINA CITRI* ADULT FEMALES RELEASED ON SWEET ORANGE SEEDLINGS SPRAYED EITHER WITH A MINERAL OIL OR WATER (CHECK) IN A NO-CHOICE TRIAL. THE DATA WERE RECORDED ON THE 6TH DAY AFTER TREATMENT.

	Mean number of ACP offspring on oil-treated citrus seedlings		
Treatment	4th - 5th instars	1st - 3rd instars	Eggs
Bioclear	22.400 ± 2.112 b	350.000 ± 68.311 a	125.600 ± 25.069 a
Caltex	7.400 ± 1.568 a	233.000 ± 84.165 a	135.000 ± 51.584 a
Citrole	35.400 ± 2.750 c	503.400 ± 107.275 a	275.800 ± 58.420 a
Sunspray	21.800 ± 1.960 b	288.200 ± 42.843 a	127.400 ± 9.973 a
Yueyang	25.000 ± 2.074 bc	329.000 ± 82.902 a	209.800 ± 71.230 a
CHECK	82.800 ± 4.673 d	330.000 ± 55.466 a	142.400 ± 23.209 a
df	5, 24	5, 24	5, 24
F	92.881	1.409	1.793
P	0.000	0.257	0.152

The numbers of ACP offspring were analyzed by one-way ANOVA; and means followed by the same letter in the same column are not significantly different ($P > 0.05$, LSD).

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TABLE 5. TOTAL NUMBER OF 4TH AND 5TH INSTAR OFFSPRING PER *D. CITRI* FEMALE THAT HAD SETTLED ON THE SWEET ORANGE SEEDLINGS 6 DAYS AFTER BEING SPRAYED EITHER WITH A MINERAL OIL OR WATER (CHECK) IN A NO-CHOICE TRIAL.

Treatment	Mean number of 4th and 5th instars per female
Bioclear	2.415 ± 0.547 ab
Caltex	1.144 ± 0.239 a
Citrole	3.244 ± 0.645 b
Sunspray	2.342 ± 0.329 ab
Yueyang	2.114 ± 0.317 ab
CK	5.206 ± 0.439 c

Numbers of 4th -5th instars per female were analyzed by one-way ANOVA ($F_{5, 24}$ = 9.749, P =0.000); and means followed by the same letter are not significantly different (P > 0.05, LSD)

the flush development was not a factor contributing to the differences in ACP oviposition success on treated seedlings.

Repellency vs. Certain Other Properties of Mineral Oils

The carbon number distribution, *n*C*y* value, and emulsifying efficiency of each oil are listed in Table 6. Caltex oil had the greatest C23 and C24 carbon mass percentages, and Sunspray oil had the greatest C22 carbon mass percentage. Although Caltex and Bioclear oils had very similar *n*C*y* values, they had quite different carbon number distributions, i.e., Caltex oil had greater C22, C23 and C24 carbon mass percentages than Bioclear oil.

Analysis of the corresponding repellency data using Pearson's correlation coefficient (Table 7) indicated no significant effects on repellency of carbon numbers C12-C21 or C25-C29. However, the numbers of ACP adult females on treated seedlings at 2 days after spraying, the numbers of 4th-5th instars and the mean numbers of 4th-5th instar offspring per female on treated seedlings at 6 days after spraying were significantly negatively correlated with the mass percentages of C22, C23, C24, and C22+ C23+C24, but not with the emulsifying efficiencies of mineral oils (Table 7). These results indicated that the carbon mass percentages in C22, C23 and C24 molecules, or in C22 + C23 + C24 molecules probably were responsible for the repellency of mineral oil against ACP.

DISCUSSION

HMOs are excellent insecticides, acaricides and fungicides for controlling a wide range of citrus pests (Rae et al. 1997; Childers 2002; Chen et al. 2009; Hoy 2011). Contemporary HMOs are relatively selective and compatible with biological control, and they are considered as "biorational" pesticides (Stansly et al. 2002). HMOs demonstrated significant ACP mortality and oviposition deterrence (Rae et al. 1997) and feeding reduc-

TABLE 6. CARBON NUMBER DISTRIBUTIONS, EQUIVALENT *N*-PARAFFIN CARBON NUMBER (*n*CY) VALUES, AND EMULSIFYING EFFICIENCIES OF MINERAL OILS (HMOS) EVALUATED FOR REPELLENCY TO *DIAHORINA CITRI* ADULTS.

	Mass percent of carbon number (%, m/m)				
Carbon number	Caltex	Bioclear	Sunspray	Yueyang	Citrole
C12	0.000	0.000	0.000	0.030	0.000
C13	0.000	0.000	0.000	0.370	0.000
C14	0.000	0.000	0.000	0.890	0.020
C15	0.000	0.000	0.000	1.370	0.190
C16	0.000	0.270	0.040	2.250	0.730
C17	0.000	0.560	0.450	4.370	3.070
C18	0.060	1.210	1.420	6.100	9.680
C19	0.460	2.410	5.020	8.740	25.940
C20	1.690	4.430	10.730	10.970	28.780
C21	5.450	7.820	16.100	13.040	20.090
C22	14.850	11.550	20.240	14.780	8.630
C23	24.810	16.030	20.950	15.310	1.970
C24	24.650	19.800	15.970	12.660	0.570
C25	18.870	17.050	7.340	7.450	0.260
C26	8.010	11.800	1.750	1.400	0.060
C27	1.150	5.600	0.000	0.170	0.010
C28	0.000	1.370	0.000	0.040	0.000
C29	0.000	0.110	0.000	0.050	0.000
*n*Cy	23.581	23.578	22.211	21.287	19.888
Emulsifying efficiency (mg/L)	844.617	625.594	1,168.278	333.322	1,862.361

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TABLE 7. CORRELATION ANALYSIS OF REPELLENT EFFECTS AGAINST *DIAPHORINA CITRI* ADULTS AND CARBON NUMBER DISTRIBUTIONS, AND EMULSIFYING EFFICIENCY OF MINERAL OILS (HMOS).

Mass percent of carbon number	Female adults on seedlings at 2 days after spraying		Total 4th - 5th instar offspring on seedlings at 6 days after spraying		4th-5th instar offspring per female on seedlings at 6 days after spraying	
	r	*t*	*r*	*t*	*r*	*t*
C22	-0.816*	0.048	-0.871*	0.024	-0.849*	0.033
C23	-0.882*	0.020	-0.844*	0.035	-0.902*	0.014
C24	-0.861*	0.028	-0.815*	0.048	-0.878*	0.021
C22 + C23 + C24	-0.900*	0.014	-0.882*	0.020	-0.923**	0.009
Emulsifying efficiency	-0.413	0.416	-0.429	0.396	-0.337	0.514

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level by Pearson's correlation coefficient test (2-tailed); *r* is the correlation coefficient, and *t* is its significance level by the t-test.

tion (Yang et al. 2013). Results of this study demonstrated that certain HMOs tested have repellency against the settling on citrus seedlings and/or oviposition of ACP. Reduction in the fraction of ACP adults that settle, feed and oviposit on citrus should reduce the probability that they will spread HLB.

This study demonstrated that different HMOs can have significantly different repellent effects against the ACP. This implies that selecting the right HMO with high repellency against the ACP should be considered in ACP and HLB management programs. The repellent effects were significantly correlated with the carbon mass percentages of C22, C23, C24, or C22 + C23 + C24, but not with the emulsifying efficiencies of HMOs. Although Caltex and Bioclear oils had very similar *n*Cy values, they had different C22, C23, C24, and C22 + C23 + C24 carbon mass percentages. Correspondingly, their repellencies against the ACP differed to a certain degree. This indicated that carbon number distribution may be a more reliable index than *n*Cy of insect pest repellency of a mineral oil. This finding is helpful in selecting and developing the right HMOs for ACP management. For example, a *n*C23 HMO with narrow range of carbon mass numbers may have higher repellency to ACP than a *n*C23 HMO with broad range, because it may has more C22, C23, C24 carbon mass percentages.

Further investigation should be conducted to determine why the insect repellency of HMOs is significantly correlated with C22, C23, and C24 carbon mass percentages. Cen et al. (2005) studied ACP behavioral response to D-C-Tron® NR HMO by using a 4-armed olfactometer. The result indicated that the volatile component of HMOs was not the main cause of the behavioral repellency of mineral oil against the ACP. The electroantennogram responses of *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) to HMOs suggested that oil deposits probably block the release of plant volatiles that attract *B. dorsalis* to lay their eggs (Ouyang et al. 2008). A solid phase microextraction test to determine the effect of Caltex Canopy® mineral oil on volatiles released by cotton plants showed that the quantity of volatiles released by the treated cotton plants was less than from water-treated plants (Mensah et al. 2005). Their study indicated that mineral oil may suppress or mask the leaf-surface volatiles of the cotton plants, thereby deterring oviposition by *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae).

The chemical characteristics of plants affect host-plant selection by phytophagous insects. Commonly phytophagous insects acutely perceive green odor volatile molecules, which are composed of C6 alcohols, aldehydes and derivative acetates, and which are emitted from plant stomata (Visser 1979, 1983, 1986). Indeed plant volatiles are the main factor in host selection by insects (Visser 1979, 1983). If oil molecules are too small, they should volatilize or penetrate into plants more easily through plant stomata (Knight et al. 1929; Hodgkinson et al. 2001). This type of compound should have few effects on blocking the release of plant volatiles. If oil molecules are too large, they cannot enter plant stomata, and it is likely that they have few effects on blocking the release of plant volatiles. We speculate that C22, C23, and C24 oil molecules may have the optimum molecular size, which allows them to enter plant stomata, but prevent them from moving quickly within the plants. Hence, they block the release of plant volatiles. Probably, there are other factors responsible for mineral oil repellency against insect pests, such as causing releases of repellent volatiles from plants, or mineral oil molecules directly repelling insects. Further study on the subject is needed.

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