OLFACTORY RESPONSES OF ASIATIC CITRUS PSYLLID
(Diaphorina citri) TO MINERAL OIL TREATED MANDARIN LEAVES

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Abstract – The attractiveness of citrus leaves treated separately with an nC21 horticultural mineral
oil and an nC24 agricultural mineral oil to Diaphorina citri Kuwayama [Hemiptera: Sternorrhyncha:
Psyllidae] was evaluated under laboratory conditions. In the first of the two studies, the oils were
applied as 0.5% v/v aqueous emulsions to mandarin leaves, and responses of psyllid adults were
determined after they walked on surfaces saturated with either distilled water or 2% v/v aqueous
emulsions of the oils. Deposits of both oils significantly reduced the proportion of D. citri attracted to
citrus leaves. Both oils had similar effects, and prior exposure to oil deposits did not appear to
influence these responses. This outcome suggests that the response of psyllid adults was related to
their detection of oil volatiles by antennal olfactory receptors. In the second study, responses of adult
psyllids to host-leaf volatiles, and mineral oil volatiles emanating from leaves dipped in 2% v/v
aqueous emulsions of each oil, and similarly treated filter paper paired with water-dipped leaves were
determined in single linear-tube olfactometers. The results of the second study indicate that
application of the oils to the mandarin leaves may have: (a) suppressed the release of attractant host
plant volatiles; (b) masked attractant host plant volatiles; (c) led to the release of repellent volatiles
from leaves; and/or (d) to adults being repelled by oil volatiles. Oil volatiles emanating from filter
paper significantly reduced the attractiveness of water-dipped mandarin leaves to the psyllid. The
detection of mineral oil volatiles by adult D. citri contributed to their response to oil deposits on the
mandarin leaves.

Key words: behavior, citrus, Diaphorina citri Kuwayama, mineral oil deposits, olfactory responses,
petroleum spray oil

INTRODUCTION

In Asia, the Asiatic citrus psyllid, Diaphorina citri Kuwayama [Hemiptera: Sternorrhyncha:
Psyllidae] is the only known vector of huanglongbing (HLB), a devastating disease of
citrus that is known as citrus vein-phloem degeneration (CVPD) in Indonesia and more
widely as citrus greening (Tirtawidjaja, 1981; Aubert, 1990; da Graça, 1991; and Halbert &
Manjunath, 2004). In Asia, the disease is caused by ‘Candidatus Liberibacter asiaticus’ [α-
Proteobacteria] (Bové, 2006).

The impact of the disease has led to heavy use of pesticides for reducing populations of D.
citri, and spread of the pathogen. The emphasis was on contact and systemic synthetic insecticides
to kill psyllid eggs, nymphs or adults. However, use of other chemicals merely slow down the
inevitable death of citrus trees from the disease. Infected adult psyllids can also transmit the
pathogen while acquiring lethal doses of insecticide (Beattie & Barkley, 2009). Strategies
such as the use of mineral oils (petroleum spray oils) (Rae et al., 1997; Huang et al., 2005) and
guava interplants (Hall et al., 2008; Zaka et al., 2010) as strategies to reduce feeding, oviposition
and ingress of psyllids into orchards by altering the behaviour of adults were given very limited
emphasis.

Evidence of rejection or acceptance by phytophagous insect and mite pests of citrus plants
in Asia, the Asiat...
contact chemoreceptors of repellent oil or plant volatiles, masking of attractant volatiles, or suppression of attractant host plant volatiles. This paper reports the results of two studies undertaken to determine how mineral oil deposits on citrus leaves may influence feeding and oviposition behaviour of *D. citri*. In the first study, the response of adult psyllids with oil-contaminated tarsi to *n*C21 horticultural mineral oil (HMO) and *n*C24 agricultural mineral oil (AMO) deposits on leaves was determined. In the second study, single linear-track olfactometers we used to determine responses of adult psyllids to oil treated leaves and oil impregnated filter paper.

**MATERIALS AND METHODS**

**Psyllid cultures**

Disease-free cultures of *D. citri* were maintained on the common ornamental form of orange jasmine (*Murraya exotica* L. *sensu* Huang (1997) [Sapindales: Rutaceae: Aurantioidae: Aurantiaceae]) in nylon mesh cages in a controlled temperature greenhouse at 28°C ± 2°C and 60% RH respectively.

**Mineral oils**

Two oils were used in the study, an *n*C21 HMO, Sunspray UltraFine® (Amtrade Pty Ltd, Melbourne, Victoria, Australia) with an unsulfonated residue (UR) value ≥ 92%, and an *n*C24 AMO, SK Enspray 99® (Oilblend Pty Ltd, Sefton, New South Wales, Australia) with a UR value of ≥ 99.8%. The glossary in the study of Beattie *et al.* (2002) defined relevant spray oil terms used in this paper. Agnell (2002) described the chemistry of petroleum-derived spray oils while Kuhlmann and Jacques (2002) summarised their classifications, standards and nomenclature.

**Responses of *D. citri* adults to oil contaminated tarsi**

Freshly excised mandarin (*Citrus reticulata* Blanco [Sapindales: Rutaceae: Aurantioidae: Aurantiaceae]) leaves were dipped in water and in 0.5% aqueous emulsions of either (a) Sunspray UltraFine or (b) SK Enspray 99. Petioles of each pair of water and oil treated leaves were then inserted in 15 mm × 50 mm specimen tube bottle filled with water, and held in position with a thin piece of sponge so that the leaves did not touch. The tubes and leaves were then placed in a 120 mm wide × 150 mm high cylindrical cage covered by nylon mesh for 24 hours. Ten, 10-day old adult psyllids were then released into each cage (n = 15 for each oil) and allowed to choose to settle on either the water or oil treated leaves. Prior to their release, the *D. citri* adults were allowed to walk or stand for 10 minutes on a deposit of 2% oil emulsion on filter paper, or on saturated filter paper after it was dipped in distilled water. The position of adults was recorded 24 hours after they were released. Data were analysed using paired *t* test, with the level of significance set at *P* < 0.05.

**Olfactometer responses of *D. citri* adults to citrus and oil volatiles**

Each single linear-track olfactometer was constructed using a transparent glass tube which measures 31.5 mm internal diameter and 260 mm long and is sealed at the proximal end with a rubber plug. It is connected to a 2.3 mm diameter (internal) plastic tube to a 3 W aquarium aerator (Sunshine 2001, Sunshine Co. Ltd., China), that was used to inject air into the olfactometer at 141 mL/minute, as measured with a N 112-02G flowmeter, 150 mm flowtube with 1/8" diameter glass float inside (Cole-Parmer Instrument Company, 625 East Bunker Court, Vernon Hills, Illinois 60061-1844, United States of America). Air entering each olfactometer was filtered through activated charcoal and humidified by passing it through distilled water. The air then passed through a transparent plastic container measuring 80 mm long × 80 mm wide × 100 mm high which housed the odour source (treatment). Responses of adult psyllids of mixed gender were determined for paired treatment comparisons listed in Table 1. Healthy turgid leaves were used for the treatments were excised from healthy mandarin seedlings. The petiole of each leaf was wrapped immediately in wet cotton wool that was then inserted into a water-filled specimen tube measuring 20 mm × 50 mm. The leaves were then dipped in 2% aqueous oil emulsions, or distilled water, for 5 seconds then left to air-dry for 60 min. They were then placed vertically in the transparent plastic container. Strips of 20 × 20 mm filter paper used in some comparisons (Table 1) were also dipped in 2% aqueous oil emulsions, or distilled water for 5 seconds, then left to air-dry for 60 minutes. They were placed vertically beside leaves and held in place by styrofoam measuring 20 × 20 × 20 mm.

Adult psyllids used for each comparison were obtained from the culture maintained on orange jasmine. They were collected in specimen tubes measuring 31.5 mm internal diameter and 50 mm long. The specimen tube was open on one end and is covered by fine mesh at another end. The open end was immediately covered with fine mesh after the psyllids were collected. The psyllids, starved for 60 minutes before, were released into an olfactometer, by connecting the open end of the

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Table 1. Paired single tube olfactometer comparisons.

| Group | First test | Second test |
|-------|------------|-------------|
| I     | filtered air: no odour source (FA) | mandarin leaf dipped in water (WL) |
| II    | mandarin leaf dipped in 2% HMO (2HL) | mandarin leaf dipped in water (WL) |
| III   | mandarin leaf dipped in 2% AMO (2AL) | mandarin leaf dipped in water (WL) |
| IV    | mandarin leaf dipped in 2% HMO (2HL) | mandarin leaf dipped in 2% AMO (2AL) |
| V     | mandarin leaf dipped in water + filter paper dipped in 2% HMO (WL 2HFP) | mandarin leaf dipped in water (WL) |
| VI    | mandarin leaf dipped in water + filter paper dipped in 2% AMO (WL 2AFP) | mandarin leaf dipped in water + filter paper dipped in 2% AMO (WL 2AFP) |
| VII   | mandarin leaf dipped in 2% HMO (2HL) | mandarin leaf dipped in water + filter paper dipped in 2% HMO (WL 2HFP) |
| VIII  | mandarin leaf dipped in 2% AMO (2AL) | mandarin leaf dipped in water + filter paper dipped in 2% AMO (WL 2AFP) |

For each comparison, responses of 10 adult D. citri per replicate (n = 15) to odours from sources listed in the left hand column were recorded over 15 min intervals then, after an interval of 3 minutes in the absence of odours, for 15 min to odours in the right hand column. Treatment codes used in Figs 2 and 3 are given in parentheses.

Results and discussions

Responses of psyllid adults to oil contaminated tarsi

Prior tarsal contact with water or mineral oil did not appear to alter the response of D. citri adult to mandarin leaves dipped in 0.5% aqueous HMO or AMO emulsions (Figs. 1 and 2). After their release into cages enclosing a water dipped leaf and one oil dipped leaf, the number of D. citri adults that chose to land and rest or feed on water-dipped leaves was significantly (P < 0.05) greater than the psyllid adults that chose to land and rest or feed on oil treated leaves. Following prior contact with water and 24 hours after their release, ≥ 48% of the adults were observed on water-dipped and oil-dipped leaves, and compared to ≤ 8% of adults respectively (Fig. 1). Following prior contact with 2% HMO and 24 hours after their release, ≥ 23% of psyllid adults were on water-dipped leaves compared to 4.67% of adults were observed and HMO-dipped leaves. After prior contact with the AMO, ≥ 56% of the psyllid adults were observed on water-dipped leaves and 53.3% on AMO-dipped leaves (Fig. 2). These results suggest that responses of adult D. citri to mineral oil deposits are olfactory based and related to detection by antennal receptors of oil volatiles and/or increased levels of plant volatiles that either repel psyllids from treated surfaces or the reduced levels of volatiles attract psyllid adults to host plants.

Olfactometer responses of psyllid adults to citrus and oil volatiles

Significantly higher proportions (80.7% compared to 33.3%) of psyllid adults observed in comparison (i) moved towards the source of host plant volatiles than towards the source of clean air (Fig. 3i).
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This outcome showed that the olfactometer was an effective means of testing responses of *D. citri* adults to volatiles.

The proportions of psyllids attracted to host plant volatiles were significantly reduced when HMO was applied to leaves by 61.3% (Fig. 3i) and by 56.7% when AMO was applied to leaves (Fig. 3ii). This outcome indicates that oil application to the mandarin leaves may have: (a) suppressed the release of attractant host plant volatiles; (b) masked attractant host plant volatiles; (c) led to the release of repellent volatiles from leaves; and/or (d) the adults repelled by oil volatiles. Responses to both oils in paired comparisons (Fig. 3iv) were similar, with 30.7% of adults moving towards the volatiles from HMO dipped leaves, and 31.3% to AMO dipped leaves. This result indicates that both oils had similar impacts on psyllid behaviour.

Comparisons (v) and (vi) (Fig. 4) showed that oil volatiles emanating from oil-dipped strips of filter paper significantly reduced psyllid

![Fig. 2 Mean (± SE) proportions (%) of *D. citri* adults observed on mandarin leaves dipped in water or 0.5% v/v HMO (A), or in water or 0.5% v/v AMO (B). Differences between the water and oil treatments were not significant (t-test, *P* > 0.05). The observations were made 24 hours after adults had prior contact with deposits of either 2% v/v HMO (A) or 2% v/v AMO (B) before they were released into cages containing one water-dipped leaf and one oil-dipped leaf.](image)

**Fig. 2** Mean (± SE) proportions (%) of *D. citri* adults observed on mandarin leaves dipped in water or 0.5% v/v HMO (A), or in water or 0.5% v/v AMO (B). Differences between the water and oil treatments were not significant (t-test, *P* > 0.05). The observations were made 24 hours after adults had prior contact with deposits of either 2% v/v HMO (A) or 2% v/v AMO (B) before they were released into cages containing one water-dipped leaf and one oil-dipped leaf.

![Fig. 3 Mean (± SE) percentage of *D. citri* adults that moved towards volatiles entering single tube olfactometers: (i) response to clean filtered air vs response to volatiles from a normal mandarin leaf dipped in water (FA vs WL); (ii) responses to a mandarin leaf dipped in 2% aqueous horticultural mineral oil emulsions vs response to volatiles from a normal mandarin leaf dipped in water (2HL vs WL); (iii) responses to a mandarin leaf dipped in 2% aqueous horticultural mineral oil emulsions vs response to volatiles from a normal mandarin leaf dipped in water (2AL vs WL), and (iv) response to mandarin leaves dipped separately in 2% horticultural mineral oil or 2% agricultural mineral oil (2HL vs 2AL). Histograms marked with the same letter within comparisons are not significantly different (t-test, *P* > 0.05).](image)

**Fig. 3** Mean (± SE) percentage of *D. citri* adults that moved towards volatiles entering single tube olfactometers: (i) response to clean filtered air vs response to volatiles from a normal mandarin leaf dipped in water (FA vs WL); (ii) responses to a mandarin leaf dipped in 2% aqueous horticultural mineral oil emulsions vs response to volatiles from a normal mandarin leaf dipped in water (2HL vs WL); (iii) responses to a mandarin leaf dipped in 2% aqueous horticultural mineral oil emulsions vs response to volatiles from a normal mandarin leaf dipped in water (2AL vs WL), and (iv) response to mandarin leaves dipped separately in 2% horticultural mineral oil or 2% agricultural mineral oil (2HL vs 2AL). Histograms marked with the same letter within comparisons are not significantly different (t-test, *P* > 0.05).

![Fig. 4 Mean (± SE) percentage of *D. citri* adults that moved towards volatiles entering single tube olfactometers: (v) response to a mandarin leaf dipped in water + filter paper dipped in 2% horticultural mineral oil vs response to a normal mandarin leaf dipped in water (WL 2HFP vs WL); (vi) response to a mandarin leaf dipped in water + filter paper dipped in 2% agricultural mineral oil vs response to a normal mandarin leaf dipped in water (WL 2AFP vs WL); (vii) response to a mandarin leaf dipped in 2% horticultural mineral oil vs a mandarin leaf dipped in water + filter paper dipped in 2% horticultural mineral oil (2HL vs WL 2HFP); and (viii) response to a mandarin leaf dipped 2% agricultural mineral oil vs a mandarin leaf dipped in water + filter paper dipped in 2% agricultural mineral oil (2HL vs WL 2HFP). Histograms marked with the same letter within comparisons are not significantly different (t-test, *P* > 0.05).](image)

**Fig. 4** Mean (± SE) percentage of *D. citri* adults that moved towards volatiles entering single tube olfactometers: (v) response to a mandarin leaf dipped in water + filter paper dipped in 2% horticultural mineral oil vs response to a normal mandarin leaf dipped in water (WL 2HFP vs WL); (vi) response to a mandarin leaf dipped in water + filter paper dipped in 2% agricultural mineral oil vs response to a normal mandarin leaf dipped in water (WL 2AFP vs WL); (vii) response to a mandarin leaf dipped in 2% horticultural mineral oil vs a mandarin leaf dipped in water + filter paper dipped in 2% horticultural mineral oil (2HL vs WL 2HFP); and (viii) response to a mandarin leaf dipped 2% agricultural mineral oil vs a mandarin leaf dipped in water + filter paper dipped in 2% agricultural mineral oil (2HL vs WL 2HFP). Histograms marked with the same letter within comparisons are not significantly different (t-test, *P* > 0.05).
movement towards mandarin leaf volatiles. Movement towards the source of leaf volatiles in the absence of oil-treated filter paper was 43.5% and 52% higher respectively for the HMO and AMO treatments, than towards leaf volatiles when oil volatiles were present. These results suggest that: (a) oil volatiles repelled adult psyllids; (b) interfered with psyllid recognition of attractant leaf volatiles; and/or influenced the release of attractant or repellent plant volatiles from the water-dipped leaves. The results also suggest similar impact of the oils.

Comparisons (vii) and (viii) showed volatiles from oil-treated leaves were less attractive than volatiles emanating simultaneously from water-dipped leaves and oil-treated filter paper (Fig. 4). This could be due to possibly different concentrations of oil volatiles emanating from treated leaves and filter papers. It can also be related to oil deposits that are suppressed, masked or increased release of plant volatiles.

Discussion

Detection and processing of plant odours play an important role in host plant detection (Bichao et al., 2005) even if an insect’s central nervous system receives the volatile information at a fine-scale spatio-temporal resolution (Bruce et al., 2005). Mineral oils can also lead to the release of volatiles that are naturally released in response to feeding by herbivorous insect (Xue, 2007). Responses of Queensland fruit fly (Bactrocera tryoni Froggatt [Diptera: Tephritidae]) to mineral oil deposits have been attributed to several possible modes of action. These include physical disruption of epicuticular lipids, masking of oviposition stimulants and repellency, either directly or through mimicry of C6 and C8 green leaf volatiles (Liu et al. 2002a, Nguyen & Beattie, 2005, Nguyen et al., 2007). Resultz showed that detection of mineral oil volatiles by D. citri adult contributed to their response to oil deposits on the mandarin leaves. Further studies are required to determine if the direct impact of oil deposits on psyllid behavior is enhanced by indirect effects of deposits on the release of attractant or repellent plant volatiles.

CONCLUSION

The series of comparisons showed that HMO and AMO volatiles repel D. citri adult by: (a) suppressing the release of attractant host plant volatiles; (b) masking attractant host plant volatiles; (c) causing release of repellent volatiles from leaves; and/or (d) oil molecules directly repelling psyllid adults.

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