

**EFFECTS OF MINERAL OILS ON HOST SELECTION BEHAVIOR OF
*Diaphorina citri***

**PENGARUH MINYAK MINERAL TERHADAP PEMILIHAN INANG OLEH
*Diaphorina citri***

Mofit Eko Poerwanto*

Department of Agronomy, University of Pembangunan Nasional "Veteran" Yogyakarta

Y. Andi Trisyono, Siti Subandiyah, and Edhi Martono

Department of Crop Protection, Faculty of Agriculture, University of Gadjah Mada, Yogyakarta

Paul Holford and George Andrew Charles Beattie

Center for Plant and Food Science, University of Western Sydney

**To whom correspondence should be addressed. E-mail: mofitnuk@yahoo.com*

ABSTRACT

Response of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) on citrus leaves treated with horticultural and agricultural mineral oil (HMO & AMO) was evaluated under laboratory condition in choice and non-choice trials. Combination of HMO and AMO at concentration of 0 up to 2% was applied to citrus leaves. Mineral oil applications significantly reduced the proportion of *D. citri* stayed on citrus leaves for feeding with the proposed mechanisms: 1) oils covered leaves thus hampered it for releasing volatile compounds, 2) oil film preventing the direct contact of tarsus or palpus to leaf surfaces, and 3) the volatile compounds produced by mineral oils also blended with plant volatiles in the atmosphere and reduced the probability of olfactory receptors capturing the olfactory cues for locating the feeding site. Mineral oils also increased the mortality of *D. citri* due to the refusal for feeding on the treated citrus leaves. The effect of AMO was more prominent than HMO.

Key words: agricultural mineral oil, citrus, *Diaphorina citri*, feeding behavior, horticultural mineral oil, host selection

INTISARI

Tanggapan *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) pada daun jeruk yang diperlakukan dengan minyak mineral (HMO dan AMO) dievaluasi dengan metode pilihan maupun tanpa pilihan di laboratorium. Kombinasi antara HMO dan AMO pada konsentrasi 0% sampai dengan 2% diaplikasikan pada daun jeruk. Minyak mineral secara nyata menurunkan proporsi *D. citri* hinggap untuk makan pada daun jeruk, yang mungkin disebabkan oleh mekanisme: 1) minyak melapisi daun sehingga tidak mampu melepaskan senyawa volatil, 2) lapisan minyak menghalangi kontak langsung antara tarsus atau palpus pada permukaan daun, dan 3) senyawa volatil dari minyak bercampur dengan volatil tanaman di udara sehingga menurunkan probabilitas reseptor olfaktori menangkap sinyal penanda lokasi inang. Minyak mineral juga meningkatkan mortalitas, akibat dari penolakan makan pada daun jeruk yang diperlakukan. Pengaruh AMO lebih besar dari pada HMO.

Kata kunci: agricultural mineral oil, *Diaphorina citri*, horticultural mineral oil, jeruk, perilaku makan, seleksi inang

INTRODUCTION

Diaphorina citri Kuwayama (Hemiptera: Psyllidae) is a major pest in central and southern Asia due to its ability as the vector of *Huanglongbing* (Aubert, 1990). Fourth and fifth instars as well as adults are able to transmit diseases (Xu et al., 1988). *Huanglongbing* is caused by *Candidatus Liberibacter asiaticus* (Jagoueix et al., 1994), and it is noted as the most serious impediment to citrus culture in Asia.

Consideration on the role of *D. citri* as vector has led to abundant use of pesticides in many orchards since 1980. Recently, mineral oil was found to be highly effective against citrus pests. Mineral oil is able to provide population control on insect pests as effective as conventional pesticides (Rae et al., 1996; Cen et al., 2002). Behavioral effects (e.g. physical repellency, antifeeding and

oviposition deterrence) can be at least as important in reducing infestations as death (Beattie & Smith 1996). The oil film could provide a barrier by masking of feeding and oviposition stimulants preventing the insect from locating, accepting or using the host plant.

Mineral oils are consisted of horticultural mineral oil (HMO) and agricultural mineral oil (AMO). They are highly refined mineral oils originated from crude petroleum oils. HMO has a narrower range of distillation temperatures than AMO, and AMO tend to be heavier (Ag-nello, 2002; Beattie, 2005).

It is assumed that *D. citri* infestation also can be reduced by applying mineral oils. However, neither HMO nor AMO is known as the most effective oil, and the mechanism in reducing is still not known.

MATERIALS AND METHODS

Insect and mineral oils

D. citri were maintained on *Muraya paniculata* plant ed in pots (Ø 25 cm, 18 cm high) covered by nylon mesh cage (length: 200 cm, width: 100 cm, height: 100 cm) in the controlled temperature greenhouse. Eggs for rearing were collected initially from *M. paniculata* in the field. Rearing temperature was set at 28°C and humidity (RH) of 60%.

AMO: nC24 SK Enspray 99® (Oilblend Pty Ltd, Sefton, New South Wales, Australia) and HMO: nC21 Sunspray Ultra Fine® (Amtrade Pty Ltd, Melbourne, Victoria, Australia) in serial dilution of 2.0, 1.0, 0.5, 0.25, and 0% (v/v) of oil emulsion were used as treatments. Emulsion was thoroughly mixed before application.

Bioassays

1. The effect of HMO and AMO on *D. citri* host selection for feeding

A factorial experiment on mature citrus leaves was conducted in a choice trial with 16 replicates. The first factor was oil types consisting of HMO and AMO. The second factor was the concentration consisting of 2.0, 1.0, 0.5, and 0.25% (v/v) oil emulsion and one control (water). Each leaf was dipped into the oil emulsion for five seconds. Cut end of each citrus leaf was inserted into a plastic tube (Ø 1.0 cm, height 2.5 cm), filled with water, whose mouth was covered with a thin sponge to prevent insect to drop inside the tube. Leaves were left for 24 hours after dipping for evaporating water on leaf surfaces, before setting up the experiment. Leaves were placed in the nylon mesh cage (Ø 12 cm, height 15 cm). Each cage was consisted of 9 treated leaves and arranged randomly in circular fashion. Twenty *D. citri* adults (seven days old) were released into the middle part of each cage. The number of *D. citri* sitting on the treated leaves was recorded every day (24 hours) for two days.

2. The mortality of *D. citri* caused by the anti-feedant effect of HMO and AMO

A factorial experiment as mentioned above (number 1) was conducted on citrus twigs with four mature leaves in a non-choice trial with 16 replicates. Cut end of each twig was inserted into a glass bottle (Ø 5 cm, height 7 cm) whose mouth was covered with a thin sponge. Three quarters of the bottle was filled with water. Twigs were left for 24 hours after dipping for evaporating water on leaf surfaces, before placing them into the nylon mesh cages (Ø 12 cm, height 15 cm). Each cage consisted of one twig treated with one oil concentration and ten *D. citri* adults were released in each cage. The mortality of *D. citri* was recorded every day (24 hours) for three days.

3. The mortality of *D. citri* caused by starvation

Non-choice experiment was set up in five treatments and 16 replicates. First and second treatments were citrus twigs with 4 mature leaves dipped in 2% v/v of HMO and AMO emulsion. Third treatment was citrus twigs with 4 mature leaves dipped in water, as a control. Each twig was dipped for 5 seconds. Cut end of each citrus twig was inserted into a glass bottle (Ø 5 cm, height 7 cm) whose mouth was covered with a thin plastic wrap. Three quarter of the bottle was filled with water. Twigs were left for 24 hours after dipping for evaporating water on leaf surfaces. Fourth treatment was a glass bottle (Ø 5 cm, height 7 cm) full of water covered with cotton. Fifth treatment was a glass bottle with three quarters of the bottle filled with water and covered with nylon mesh. Each treatment was placed in the nylon mesh cage (Ø 12 cm, height 15 cm). Ten *D. citri* adults were released in each cage. The mortality of *D. citri* was recorded every day (24 hours) for three days.

Data analysis

Variance analysis was performed using SPSS® version 10.0.5. Significance different was arised at $P < 0.05$ (SPSS, 1999).

RESULTS AND DISCUSSION

1. The effect of HMO and AMO on *D. citri* host selection for feeding

Oil treatment on the citrus leaves could reduce the percentage of psyllid stayed for feeding (Fig. 1A). Only 3.0% of psyllids stayed for feeding on the leaves treated with oils. It was significantly lower ($P < 0.001$) to the percentage of psyllids stayed on leaves treated with water (29.2%) and psyllids stayed on the cage. Most of the psyllids did not stay for feeding on the citrus leaves but stayed on the cage (67.8%).

No differences were observed on the percentage of psyllid stayed for feeding between the treatments of various concentrations ($P = 0.231$) (Fig. 1B), but significant difference was appeared between type of oils ($P = 0.014$) (Fig. 1C). AMO and HMO could reduce the psyllid stay into 2.0% and 3.9% respectively. Interaction effect on the combination of concentration and type of oils was absent ($P = 0.694$).

It was suggested that the failure in host recognition made most of psyllids stayed on the cage, eventhough the leaves without oils on them existed in the arena inside the cage. Host finding behavior by phytophagous insects may range from random, to purposeful search (Miller & Strickler, 1984). It started from host habitat location into host location. Host location may involve responses to visual and chemical cues. Cues stimulate receptors, generating sensory input, processed in central nervous system

and decision made as to whether to make a certain behavioral response. Olfactory cues are likely to be signals that are more reliable. They may allow for host plant location even in a complex environment (Bruce *et al.*, 2005).

The result indicated that oils had triple actions in repelling psyllids infestation. First action was covering the leaves perform oil film on leaf surface and blocked the emission of leaves volatile. Citrus leaves might not provide sufficient cues (too low or too high) for psyllid to identify host plant that was appropriate for feeding. Insect needs specific compounds and intensity of volatiles as a cue for locating host plant (Campbell & Borden, 2006). Second action was volatile oil would also blend with plant volatiles in the atmosphere and reduced the probability of olfactory receptors captured the olfactory cues for locating the feeding site. Psyllid would avoid to land and feed on the plant if the specific odour from host plant can not be detected. The same repellent effect occurred when odours from non host plant blended into air (Camp-

bell & Borden, 2006). Third action was oil film also preventing the direct contact of tarsus or palpus to the leaves surface to get the specific chemical cues. When insects had direct access to the host plant material they were very well able to discriminate their host plant based on contact cues. They clearly preferred their host to the non-host (Heisswolf *et al.*, 2005). After landing on treated leaves, oil film would attach on tarsus and or palpus surface and induced the malfunction of those chemical receptors.

2. The mortality of *D. citri* caused by the antifeedant effect of HMO and AMO

Highest mortality of the psyllid was observed on the citrus leaves treated with oil on the non-choice trial (Fig. 2A). The mortality was 58.8% and it was significantly higher ($P < 0.001$) than on the leaves treated with water (15.0%). Oil treatment with various concentrations of AMO had significantly higher mortality (64.7 %) than HMO (53.0%) (Fig. 2B), but interaction effect on the combination of concentration and type of oils was not

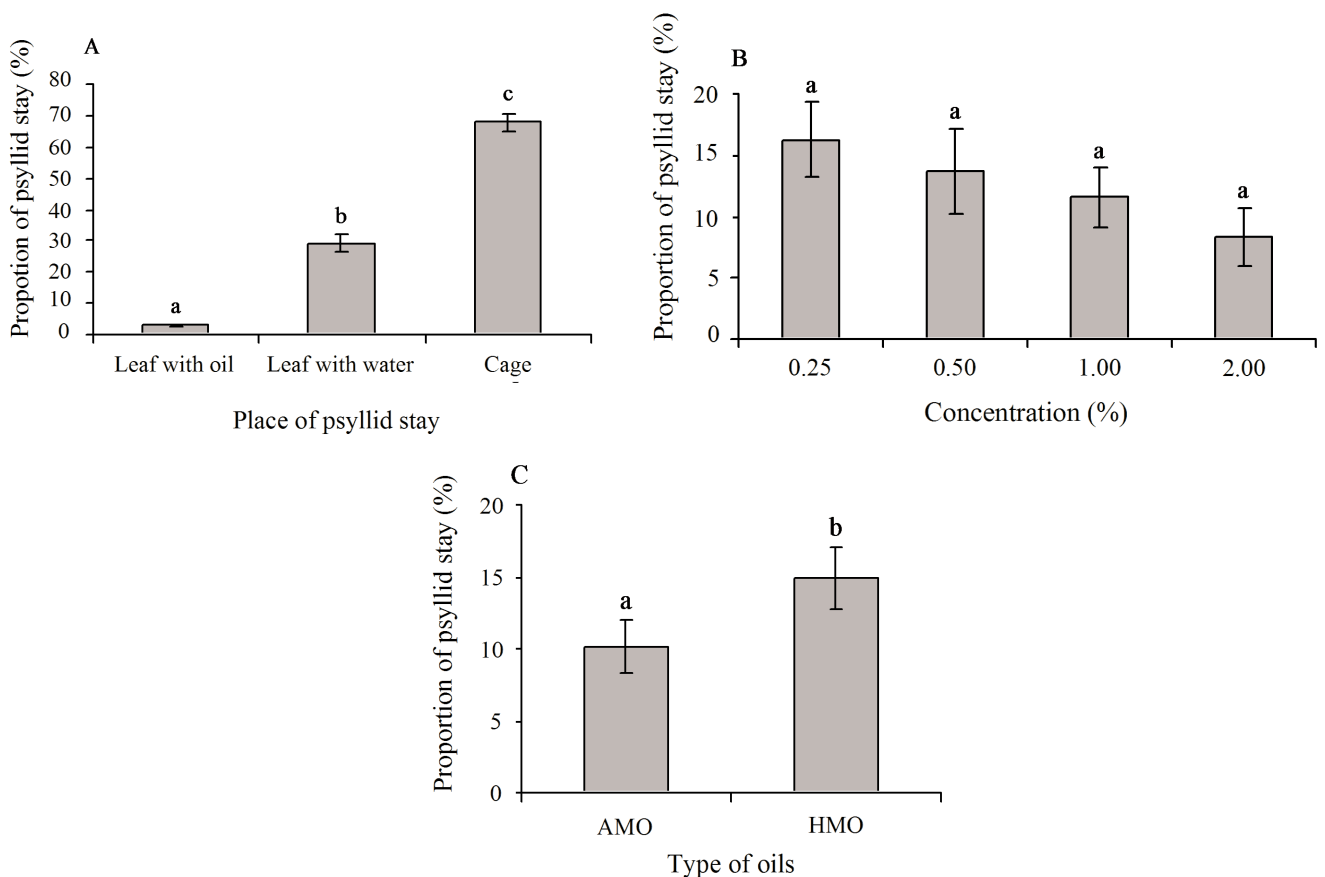


Fig.1. Average proportion of psyllids stayed on citrus leaf treated with oils (HMO and AMO), water and on the cage (A); on citrus leaf treated with oils in concentration of 0.25, 0.5, 1, and 2% v/v (B); and on citrus leaf treated with AMO and HMO (C) in a factorial choice trial. Interaction effect on the combination of concentrations and type of oils did not appeared ($P = 0.694$). Bars with the same letter are not significantly different (for A: $P < 0.001$; for B: $P = 0.231$; for C: $P = 0.014$).

appeared ($P=0.560$). Mortality of the psyllid significantly increased ($P<0.001$) along with the increase of oils concentration (Fig. 2C). Application of oil in concentrations of 0.25, 0.5, 1.0 and 2.0% significantly killed in average 45.9%, 55.0%, 63.8% and 70.6% of psyllid respectively.

Application of oil on the surface of citrus leaves would make barrier to the psyllid for feeding. AMO had wider range of distillation temperatures than HMO, and AMO tends to be heavier than HMO. AMO with nC value (nC24) higher than HMO (nC21) would stand longer performing oil film on the leaf surface (Agnello, 2002; Beattie, 2005). Higher oil concentration would perform thicker oil film and also covering more even on leaves surface. It would perform stronger barrier to feeding activity of the psyllid. Psyllid would spent longer time to find the site on the leaves surface that was not covered or covered by the thinnest oil film before decided to feed on it. The antifeedant effect appeared when more contaminant attached on their chemoreceptor and would govern them to the rejection of the feeding site (citrus leaves).

Feeding behavior of phytophagous insects is governed by the balance of phagostimulatory and deterrent inputs.

Saps from acceptable and unacceptable plants produced broadly similar levels of stimulation in the phagostimulatory cells of the galeal sensilla but the response of the deterrent cells was markedly higher with unacceptable plants (Chapman, 2003). Host selection depends on the balance of phagostimulatory and deterrent inputs with a dominating role of a host-related chemical. The contrast between net phagostimulatory inputs and net deterrent inputs governs the insect's responses.

3. The mortality of *D. citri* caused by starvation

Highest mortality of the psyllid was observed on the no food (no leaves and water) treatment on the non-choice experiment (Fig. 3). The mortality was 100% and it was significantly higher ($P<0.001$) to the others treatment. Treatment of citrus leaves with water, AMO 2%, HMO 2%, and only water without leaves gave 15.0, 75.7, 65.6, and 76.3% mortality to the psyllid respectively.

The result of oil application indicated that there was a barrier for psyllid to reach the food source in the phloem of the citrus leaves. It seems that the psyllid could only intake the water from the leaves since the difference between water and oil treatment was not quite far. Oil film

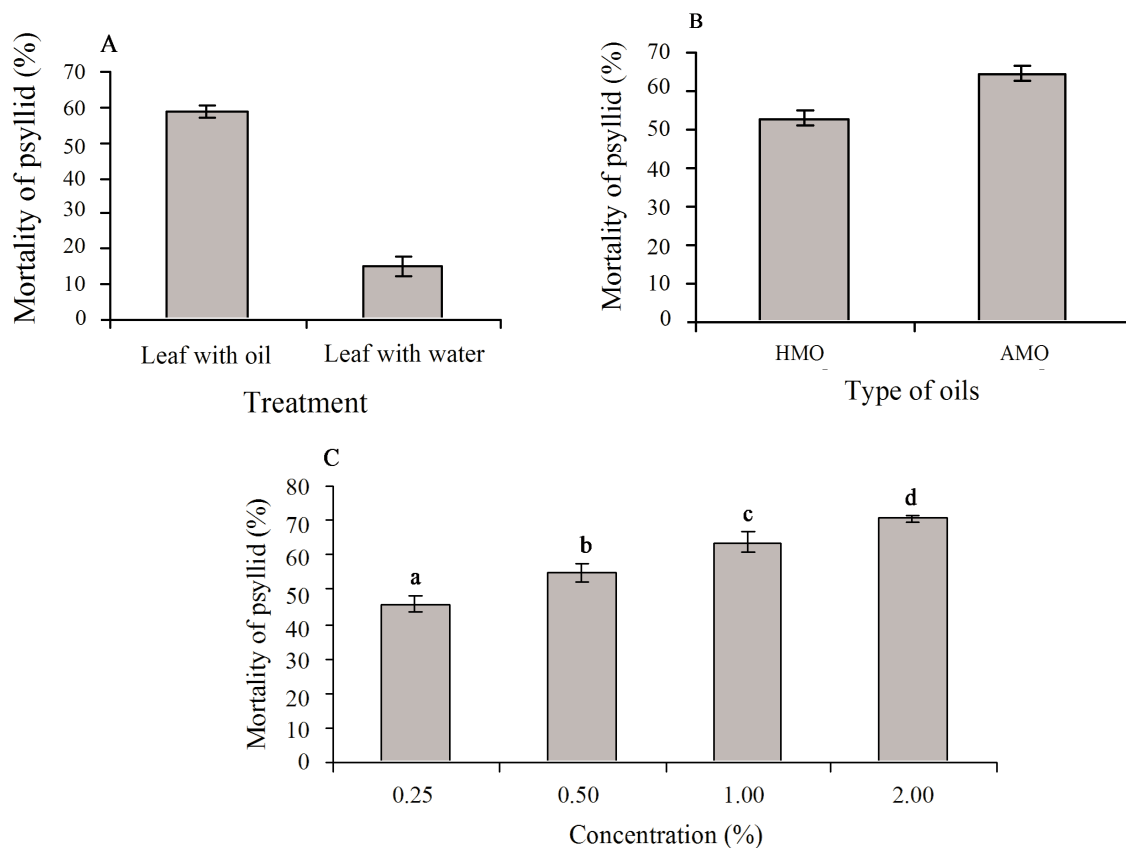


Fig.2. Average mortality of psyllids (%) feeding on citrus leaf treated with oils (HMO and AMO), and water (A); on citrus leaf treated with AMO and HMO (B); and on citrus leaf treated with oils in concentration of 0.25, 0.5, 1, and 2% v/v (C) in a factorial non-choice trial. Interaction effect on the combination of concentration and type of oils did not appeared ($P=0.560$). Bars with the same letter are not significantly different (for A, B, and C: $P<0.001$).

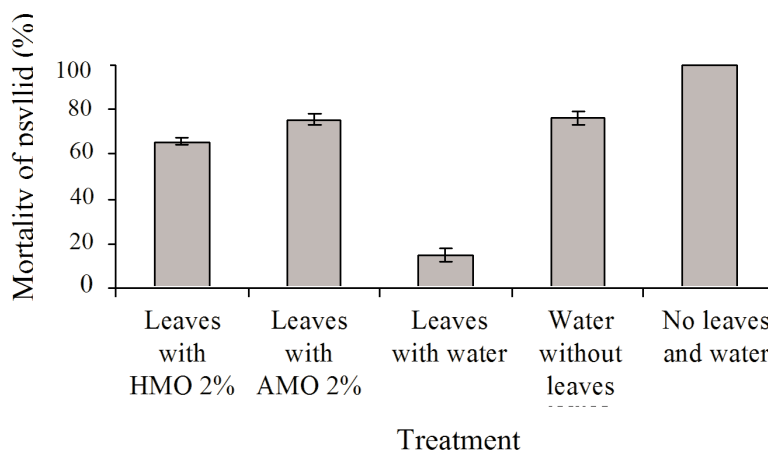


Fig.3. Average mortality of psyllids (%) on citrus leaves treated with HMO and AMO in concentration of 2% v/v, water, only water without citrus leaves and no food (no leaves and water) in non-choice experiment. Bars with the same letter are not significantly different ($P < 0.001$).

also could penetrate or diffuse into plant via both stomata and the cuticle (Tan *et al.*, 2005) covering the phloem, performing the barrier and decreasing the availability of phloem. It was suggested when stylet penetrated into leaves tissue, it would reach the oil layer inside leaves surface before entering phloem. The detection of strange contaminant by chemoreceptor in the stylet would retract the stylet for entering the phloem. This action would be repeatedly done up to they gave up and sucked only the water that was produced by the leaves. Higher mortality on water treatment than oils treatment also indicated that the oils had no poisoned effect on the psyllid when applied on the citrus leaves. Mortality merely caused by the change of feeding behavior that governs to the refusing of sucking the appropriate food sap from citrus leaves phloem.

The failure in host recognition and the refusing of feeding on citrus leaves treated with mineral oils were able to reduce the infestation *D. citri*. The uses of mineral oils are going to provide an efficacious alternative to chemical insecticides for managing citrus insect pests in the future. However, advance research on the mechanisms of oils influencing *D. citri* feeding behavior is still needed.

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