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ORIGINAL CONTRIBUTION

Horticultural mineral oil influences *Plum pox virus* transmission by *Myzus persicae*

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detached leaf technique, electrical penetration graph, green peach aphid, horticultural mineral oil, *Plum pox virus*

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Abstract

The residual activity of horticultural mineral oil (HMO) on the ability of green peach aphids, *Myzus persicae* (Sulzer), (GPA) to transmit *Plum pox virus* (PPV) to peach was measured by infection rates of detached leaves from plants sprayed with either HMO or water as a control that were inoculated using transfer of 25 viruliferous aphids per leaf at 0, 2, 4, 7, 9, 11 and 14 days after treatment (DAT). Persistent effects of HMO residue on the probing and feeding behaviours of GPA were also monitored with the electrical penetration graph (EPG) system. For glasshouse-grown peach seedlings, the residual activity of HMO reduced PPV infection rates by more than 58% for up to 4 DAT following an initial reduction of approximately 81%. EPG recordings of GPA feeding behaviour showed that HMO significantly delayed first feeding probes and first intracellular punctures by more than 50 min without changing the ensuing stylet penetration behaviour. Applying HMO reduced virus infection rates for up to a week depending on the environmental conditions. EPG monitoring of aphid probing showed that HMO reduced the mean duration and mean number of potential drop (PD) phase feeding occurrences, compared with the water control. A reduction in the PD that has been shown to be related to the transmission of non-persistently transmitted viruses may partly explain the reduction in PPV infection rates.

Introduction

Plum pox virus (PPV) is one of the most economically important viruses affecting stone fruits (*Prunus* sp.) worldwide (Roy and Smith 1994; Cambra et al. 2006; Capote et al. 2006; Garcia and Cambra 2007; Barba et al. 2010). PPV is transmitted in a non-persistent manner by numerous aphid species after relatively short acquisition and inoculation periods (Labonne et al. 1995; Isac et al. 1998; Moreno et al. 2009). Therefore, management of this disease relies in part on a reduction in inoculum and disruption of virus infection by migratory aphid vectors during the growing season. Mineral oils have been widely used on various crops to prevent the spread of non-persistently and semi-persistently transmitted viruses (Bradley et al. 1962; Bradley 1963; Loebenstein et al. 1964; Simons et al. 1977; Zitter and Simons 1980; Asjes 1984; Lowery et al. 1990; Powell 1992). While earlier use of oils to mitigate the spread of viruses was limited due to their phytotoxicity, newer horticultural mineral oil (HMO) formulations are less phytotoxic (Lowery et al. 2012). Although the exact modes of action of HMO are not fully understood, it is believed that they change aphid probing and feeding behaviours (Simons and Zitter 1980; Ameline et al. 2009; Fereres and Moreno 2009). Reductions in aphid populations slowed development, and reduced virus infection have also been linked to the insecticidal activity of HMO (Powell 1992; Martin-Lopez et al. 2006; Najar-Rodriguez et al. 2007). Others have reported that it inhibits development or movement of

virus in the plant after inoculation (Loebenstein et al. 1964; Peters and Lebbink 1975). A more plausible finding was that HMO interferes with virus retention. During aphid probing and virus acquisition, the oil film formed on the plant cuticle prevents the aphids from retaining virus in the stylet (Qiu and Pirone 1989; Wang and Pirone 1996; Wrobel 2009). Such viruses are retained in the common duct of the aphid stylet and during probing are inoculated into host cells in watery salivations (Uzest et al. 2007). Effective aphid acquisition of the virus was confirmed whether the virus was detected in their stylet (Singh et al. 1996). Thus, prevention of virus infection by HMO could be due to their effect on aphid feeding and probing behaviour (Boquel et al. 2013), or because they interfere with virus retention on the aphid stylet (Wang and Pirone 1996). Most studies acknowledge the efficiency of oil in reducing spread of non-persistently transmitted plant viruses (Bradley et al. 1962; Simons and Zitter 1980; Qiu and Pirone 1989; Wrobel 2009). In the present study, the effect of HMO on the feeding behaviour of the green peach aphid, *Myzus persicae* (Sulzer), (GPA) was investigated using the electrical penetration graphing (EPG) method. EPG has been used as a useful tool to study the feeding behaviour of piercing and sucking insects. Because it quantifies individual behaviours (e.g. salivation, ingestion) occurring during interactions between the stylet and different tissues of the host plant, it demonstrates the potential ability of aphids to transmit viruses (Perez et al. 1996; Martin et al. 1997; Moreno et al. 2012). This technique has also been used to determine the effects of insecticides and antifeedants and their mode of action on sap-feeding insects (Tjallingii and Gabrys 1999; Alvarez et al. 2007; Tjallingii et al. 2010).

Understanding the degree and length of protection provided by HMO is very important for the management of non-persistently transmitted plant viruses. The purpose of this study using GPA as the virus vector was to determine the residual activity of an HMO on PPV infection rates of peach and to provide a better understanding of its mode of action in order to structure recommendations for a PPV management programme.

Materials and Methods

Virus source

The Canadian isolate of the Dideron strain of PPV (PPV-D) used in this study, characterized by Rochon et al. (2003), was isolated from an infected peach tree in Niagara, ON, Canada. Virus was aphid-inoculated and maintained in peach (*Prunus persica* L. cv. Elberta) seedlings for use in infection trials following the methodology described by Stobbs et al. (2015). The infected seedlings were maintained in a containment room at 22 ± 2°C, and a 16-h photoperiod provided by 4100 lux halide lighting. They were fertilized with a dilute solution of 20 : 20 : 20/N : P : K (~100 ppm, Plant Products Co. Ltd, Brampton, ON, USA) on alternating days until they were discarded after 12 weeks. Virus presence in the plants was confirmed by enzyme-linked immunosorbent assay (ELISA) (Kim et al. 2008).

Aphid colonies

GPA were reared in ventilated Plexiglass cages on Bok Choy (*Brassica rapa* L., subspecies *pekinensis var.* Heavy (422E, Stokes Seeds, St. Catharines, ON, USA) and maintained under fluorescent lighting (16-h photoperiod) at 20 ± 2°C. Ceramic plant watering spikes (Blumat, Rittenhouse Growers Supply, St. Catharines, ON, USA) were inserted in each pot to minimize exposure of the plants to outside aphid contamination through hand watering. Third- and fourth-instar nymphs and apterous adult green peach aphids were transferred using a fine artist's brush to 5-cm Petri dishes (VWR Scientific, Mississauga, ON, USA) with tight fitting lids and starved at ambient temperature (22°C) for 2 h prior to the virus acquisition probes.

Detached leaf procedure

For the detached leaf procedure described by Stobbs et al.(2015), detached peach leaves were supported on a 0.4% agar gel bed, midrib up, in a 24.5 × 24.5 × 2.5 cm (l × w × h) Nunclon TM polystyrene culture dish with lid (VWR Scientific, Mississauga, ON, USA). Pirliss® 50DF (50% pirimicarb ai, Plant Products Ltd., Brampton, ON, USA) was added to the agar (0.063% Pirliss, w/v) to minimize aphid escape and was found to result in complete aphid mortality within 48 h without adversely affecting PPV infection, nor aphid feeding behaviour (Stobbs et al. 2015). Twenty-five starved GPA were transferred individually to each 1 × 1 cm piece of PPV-infected peach leaf, cv. Elberta that had been placed on the top of each detached leaf, as PPV is not considered to be mechanically transmissible. Plates were then sealed with Parafilm® to minimize moisture loss and stored in the dark for 48 h. Leaves were subsequently transferred to agar-only culture dishes, re-sealed and incubated for 4 weeks in the contain-

ment room at 20 ± 2°C, 16-h photoperiod. Leaves were then assayed for PPV infection by direct real-time reverse-transcriptase Taqman probe-based polymerase chain reaction assay (DRT-qPCR) as described by Kim et al. (2008).

Horticultural mineral oil persistence under glasshouse conditions

The HMO used in this study (Superior-70 Oil, N.M. Bartlett, Beamsville, ON, Canada, PCP # 24999) is a commercial light weight emulsifiable oil registered in Canada for, among other uses, the control of European red mite and scale on peaches and for the control of *Turnip mosaic virus* infection of rutabaga, *Brassica napus L. var. napobrassica*. It is a mixture of C18–C32 hydrocarbons (C23 on average) (Petro Canada, Mississauga, ON, Canada; CAS# #8042-47-5) with an 80% distillation temperature and an unsulfonated residue (UR) of 99%. The emulsifier (T-Mulz A02 FS802) is a solution of fatty alcohols and oleic acid. Four-week-old peach seedlings (cv. Elberta) placed in a fume hood were sprayed to runoff (~12 ml/plant) with 1% HMO or water as a control using a small hand-held atomizer. Twelve seedlings used in each treatment were maintained in a glasshouse at 20 ± 2°C under fluorescent lighting with a 16-h photoperiod. Twenty-one leaves were randomly collected from plants of each treatment at 0, 2, 4, 7, 9, 11 and 14 days after treatment (DAT) and exposed to PPV using the detached leaf procedure described above. Leaves for day 0 were collected approximately 4 h after the spray applications to allow the solutions to dry thoroughly before use. Leaves were assayed by DRT-qPCR for PPV infection after 3 weeks. Experiments were repeated four times.

Persistence of horticultural mineral oil under field conditions

The persistent effect of HMO residue on the inhibition of virus infection by aphid inoculations was also investigated under field conditions. Foliar spray applications of 1% HMO or water as a control were made with a Rittenhouse truck-mounted sprayer equipped with a Spraying Systems handgun fitted with a D-6 orifice plate and operated at 200 psi (M.K. Rittenhouse 2003, Air blast sprayer 2898 R, St. Catharines, ON, Canada). Three replicated plots of 64 peach trees each containing 6 treated and 6 untreated trees (cv. Loring) arranged in a randomized complete block design were used for the HMO and water control treatments. Growing shoot tips were tagged prior to spraying to avoid collecting non-treated newly emerged leaves. Eighteen leaves from trees of each treatment were collected following the same timeline described above for the glasshouse study. Twenty-five starved green peach aphids were used to inoculate each detached leaf with PPV as previously described. The experiment was replicated three times.

Electrical penetration graph (EPG)

Following the method of Tjallingii (1988), EPG was used to monitor plant penetration and feeding activities of apterous adult GPA on 5-week-old peach seedlings sprayed with HMO or water as the control. A thin (20 μm dia) gold wire 4 cm long was attached to the aphid dorsum with water-soluble conductive silver glue (EPG systems, Wageningen, the Netherlands) and the other end to a copper input electrode (2–3 cm × 1 mm dia). The output electrode was a copper post (10 cm × 2 mm dia) inserted into the soil. Both electrodes were connected to the direct current electrical penetration graph (DC-EPG device, Giga-4; EPG System, Wageningen, the Netherlands). The EPG acquisition procedure was performed inside a grounded copper mesh Faraday cage to prevent stray electrical interference. Since aphids can only retain PPV for less than 1 h in their stylets after feeding is initiated (Fuchs et al. 2008; Celetti et al. 2009); feeding access periods were standardized at 1 h immediately after aphids were placed on the peach leaf. All experiments were carried out under laboratory conditions (22–24°C) with at least twelve replicates for each treatment date. Data acquisition was performed using Stylet$^+$ software (Moreno-Delafuente et al. 2013) and data analysed using Microsoft Excel. Of the six EPG waveforms defined by Tjallingii (1988) for categories of aphid probing and feeding, our focus was on those that relate to the acquisition and inoculation of non-persistently transmitted plant viruses. This included formation of the stylet pathway (combination of waveforms A, B and C) and the intracellular potential drop (waveform PD) and its two subphases, waveform II-1 (salivation) and II-3 (ingestion) that, respectively, relate to inoculation and acquisition of non-persistent viruses (Tjallingii et al. 2010). HMO's inhibitory effect on aphid probing and feeding would also be reflected in the time spent not probing (NP) and mechanical work and penetration difficulties (waveform F). Total duration time and number of occurrences of each of the patterns were compared between treatments. Also, sequential feeding parameters were compared, including time required until first probe and first PD.

Data analysis

All data on infection rates were arcsine transformed prior to ANOVA test using the general linear models (PROC GLM) procedure (SAS Institute 1998). EPG parameter values were established for each individual aphid, and then, the mean and standard error of the mean (SEM) of the total duration time, mean number of occurrences and time per occurrence were calculated. The time needed to initiate the first pathway, intracellular punctures potential drop (PD) and phloem feeding phases since the start of the experiment were also calculated. Parametric differences were analysed using Duncan's multiple range test.

Results

Horticultural mineral oil persistence under glasshouse conditions

In the glasshouse study, mean PPV infection rates for the water control treatment plotted and fit to a polynomial linear regression curve, showed a steady decline over the 2 weeks as the peach seedlings grew older (fig. 1), perhaps reflecting a change in susceptibility of peach to PPV over the growing season as we have demonstrated in other studies (Stobbs et al. 2012, 2013). Infection rates for plants treated with 1% HMO were significantly lower ($P < 0.05$) compared with the controls for up to 4 days after treatment (DAT) (fig. 1). Following an initial reduction of approximately 81% relative to the control on day zero, infection rates for the HMO treatment rose steadily and were not statistically different from the control by day seven in spite of an ~35% reduction. ($F = 1.14$, $P = 0.3265$). Although the infection rate regression lines for the two treatments did not intersect until day 14 (fig. 1), HMO did not afford significant protection from day 7 until the end of the study.

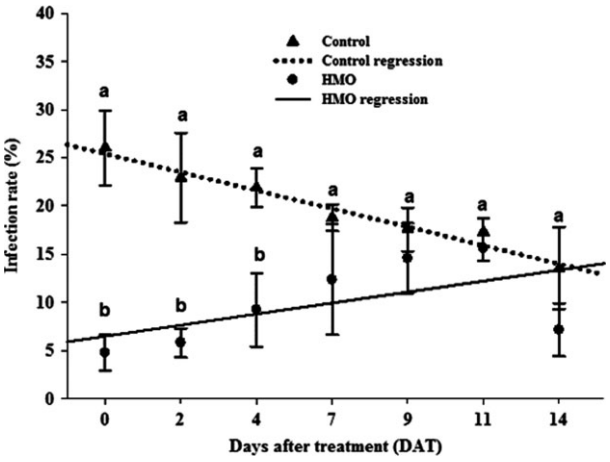

Fig. 1 Persistent effect of a 1% solution of horticultural mineral oil (HMO) applied to peach seedlings in the glasshouse on infection of detached leaves with PPV under laboratory conditions using 25 *M. persicae*, per leaf as the vector. Virus replication assessed by DRT-qPCR 4 weeks after leaves were inoculated. For a given day, means and standard errors ($P > 0.05$) based on Duncan's multiple range test were plotted and a best-fit curve was determined using polynomial liner regression. Equations for the regression lines are $Y = 6.758 + 0.472*X$ in HMO; $Y = 25.21 + (-0.82)*X$ in control. For each day, means followed by the same letter are not significantly different ($P > 0.05$) based on Duncan's multiple range test.

Horticultural mineral oil persistence under field conditions

Environmental conditions such as temperature, sunlight and rain might enhance the degradation of HMO under field conditions. Our field results demonstrated a significant 70% to 75% reduction in PPV infection rates of peach leaves treated with HMO compared with the water controls on zero DAT ($F = 17.97$; $P = 0.0133$) and two DAT ($F = 12.47$; $P = 0.0242$) (fig. 2). Infection rates from 4 to 14 DAT did not differ significantly ($P > 0.05$) between treatments. Persistence of the HMO may have been reduced somewhat under these field conditions due to the ~5 mm of rain recorded the day after the treatments were applied (fig. 2).

Electrical penetration graph (EPG)

Time until initiation of the first pathway probing and PD feeding is shown in fig. 3. HMO may delay the onset of pathway probing by more than 27 min on day zero and 20 min on 4 DAT ($F = 1.76$; d.f. = 4; d.f. = 4; $P = 0.1746$), compared with less than a minute for GPA to begin feeding on the control ($F = 1.15$; d.f. = 4; $P = 0.361$). HMO delayed initiation of the first PD more than 20 min at 0 and 4 DAT ($F = 0.98$; d.f. = 4; d.f. = 4; $P = 0.4402$) compared with 1 and 3 min, respectively, for the controls ($F = 1.72$; d.f. = 4; d.f. = 4; $P = 0.1831$).

The total duration and number of occurrences of the various GPA probing and feeding behaviours recorded on the EPG over the first 5 min are shown in fig. 4. A five-minute recording was used in this study to examine the impact of HMO on aphid transmission of non-persistent viruses that occurred during short-duration epidermal probes sufficient to inoculate and acquire the virus. Application of 1% HMO significantly reduced the mean total time GPA spent

pathway probing and not probing (NP) compared with the control and caused a significant increase in penetration difficulty (PD) time (fig. 4). Pathway duration times for the control treatment ranged from 200 to 250 s (F = 11.944; d.f. = 4; P = <0.001) compared with 30–50 s (F = 57.95; d.f. = 4; P = <0.001) for the HMO and amounted to a 75–86% reduction. On average, aphids conducted 3–6 pathway probes on control leaves compared with only 0.25 to 0.8 for the HMO treatments (F = 10.509; d.f. = 4; P = <0.001) on individual days of the study.

Although there were no significant differences in the frequency of probing difficulties (P > 0.05), HMO significantly increased by 11- to 14-fold the total length of time aphids experienced difficulty penetrating leaf tissues (245–263 s; F = 9.615; d.f. = 4; P = <0.001) compared with the control (18–23 s; F = 29.615; d.f. = 4; P = <0.001). The total time that aphids were not probing was reduced significantly on HMO to less than 1–2 s (F = 0.602; d.f. = 4; P = 0.663) compared with 14–66 s on the control (F = 11.578; d.f. = 4; P = <0.001) (fig. 4).

Recordings of the intracellular punctures of the potential drop (PD) subphases II-1 and II-3 associated with inoculation and acquisition of non-persistent viruses, respectively, showed that application of 1% HMO significantly reduced the total duration time (F = 15.338; d.f. = 4; P = <0.001) and the mean number of occurrences (F = 6.413; d.f. = 4; P = <0.001) of these feeding behaviour patterns compared with the water control (fig. 5). At zero and 2 DAT, the PD salivation feeding subphase II-1 on HMO was less than 1 s (F = 2.994; d.f. = 4; P = 0.045) compared with 8.6 and 5.3 s for the water control (F = 3.135; d.f. = 4; P = 0.052). Subphase II-3 for HMO-treated plants was also less than 1 s (F = 3.310; d.f. = 4; P = 0.045) compared with 6.2 and 4 s for the water control (F = 3.005; d.f. = 4; P = 0.030). The amount these behaviours were reduced by HMO relative to the control gradually decreased over time until they were 22% and 50% lower by DAT 7 and 12, respectively. Thus, the average duration time per occurrence for HMO was also significantly lower (F = 3.005; d.f. = 4; P = 0.030) (fig. 5).

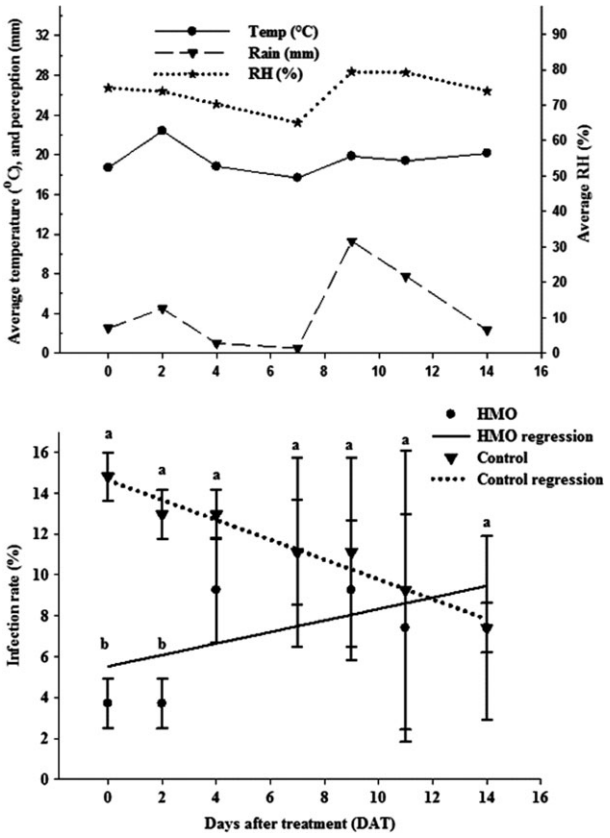

Fig. 2 Persistent effect of a 1% solution of horticultural mineral oil (HMO) applied to peach trees in the field on subsequent infection under laboratory conditions of detached leaves with PPV using 25 by *M. persicae* per leaf as the vector. Virus replication assessed 3 weeks after each inoculation date by DRT-qPCR. For a given day, means and standard errors (P > 0.05) based on Duncan's multiple range test were plotted and a best-fit curve was determined using polynomial liner regression. Equations for the regression lines are $Y = 5.519 + 0.281*X$ in HMO; $Y = 14.635 + (-0.486)*X$ in control. For each day, means followed by the same letter are not significantly different (P > 0.05) based on Duncan's multiple range test.

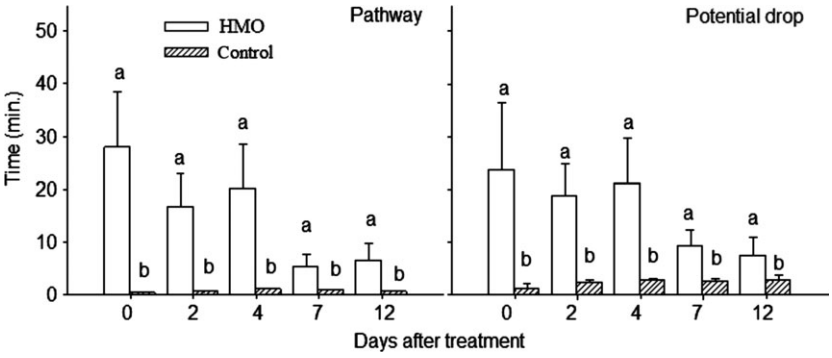

Fig. 3 Time until the first pathway probe (PP) and first potential drop (PD) activities for *M. persicae* feeding for 1 h on leaves of peach seedlings treated with a 1% solution of horticultural mineral oil (HMO) or water as a control as recorded by electronic penetration graph system. For each test day, means followed by the same letter are not significantly different (P > 0.05) based on Duncan's multiple range test. Vertical bars represent the standard errors of the means.

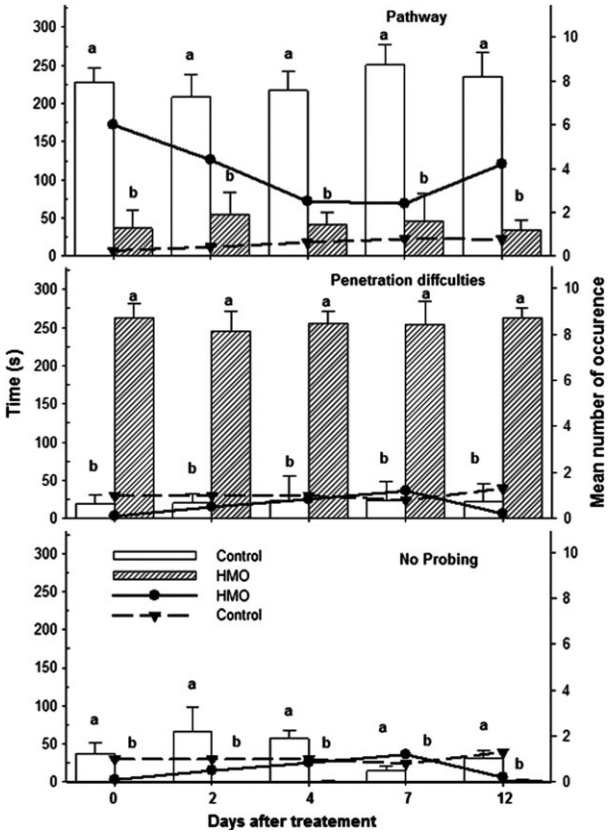

Fig. 4 Mean number of occurrences and total time spent pathway probing, probing with difficulty and not probing during 5 min of electronic penetration graph monitoring of *M. persicae* on detached leaves of peach from seedlings treated with a 1% solution of horticultural mineral oil (HMO) or water as a control up to 12 days after treatment. Means on each day followed by the same letter are not significantly different ($P > 0.05$) based on Duncan's multiple range test. Vertical bars represent the standard errors of the means, bars represent total duration time, and lines represent mean number of occurrence.

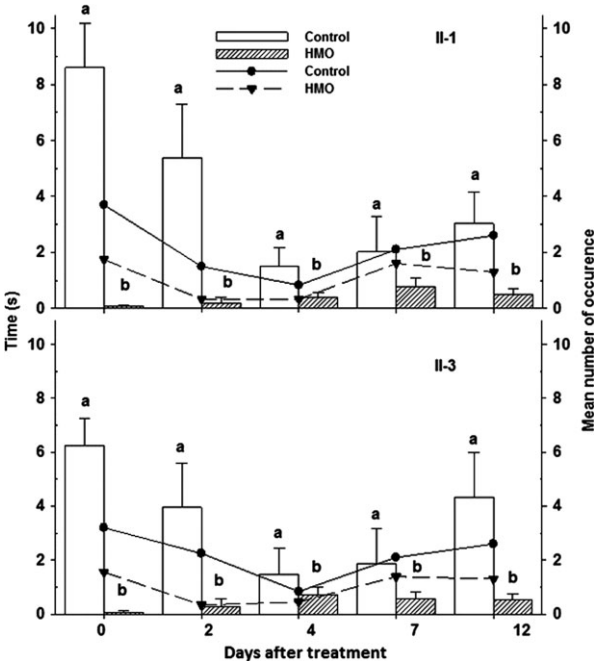

Fig. 5 Mean number and total duration of intracellular potential drop subphase II-1 (salivation) and II-3 (ingestion) waveforms that relate to inoculation and acquisition of non-persistent viruses, respectively, as recorded using an electronic penetration graph for *M. persicae*, during 5 min of feeding on leaves from peach seedlings treated with a 1% solution of horticultural mineral oil (HMO) or water as a control for up to 12 days after treatment. For each day, means followed by the same letter are not significantly different ($P > 0.05$) based on Duncan's multiple range test. Vertical bars represent the standard errors of the means, bars represent total duration time, and lines represent mean number of occurrence.

Discussion

Plum pox virus (PPV) is spread by migratory aphids in a non-persistent manner (Wallis et al. 2005). Aphids conduct short test probes on leaves and fruit prior to feeding, and these short probes lasting often less than 30 s are sufficient to introduce the virus particles and cause infection (Martin et al. 1997; Collar and Fereres 1998; Gildow et al. 2004; Tjallingii et al. 2010). For this reason, insecticides are generally considered to be ineffective for the control of non-persistent viruses (Lowery and Boiteau 1988). Although the virus does not persist in the aphid stylet, it can remain there for at least 1 h after feeding (Fuchs et al. 2008; Celetti et al. 2009). Therefore, reducing the spread of non-persistent viruses such as PPV depends on reducing virus inoculum and disrupting feeding by migratory aphids during early spring when aphid populations usually peak (Gaborjanyi and Basky 1995). In our glasshouse studies, HMO reduced PPV infection of peach by 70–80% compared to the controls during the first 4 days. Although infection rates for plants treated with HMO remained lower than the controls during the entire two weeks of study, the difference was not significant by day 7. We have shown that peach trees become less susceptible to infection over the summer (Stobbs et al. 2012, 2013), such that protective HMO sprays might be most effectively used early in the season when trees are most susceptible and numbers of migrant aphids are high. Applying mineral oils decreases an aphid's ability to acquire virus, probably through physical inhibition of binding of virus to the aphid stylet (Boquel et al. 2013), but this effect has been shown to not last more than a week (Margaritopoulos et al. 2010). Our results from laboratory and field studies similarly demonstrated that HMO was not effective beyond 1 week. Environ-

mental conditions such as temperature, ultraviolet light and rain enhance the degradation of HMO (Blank et al. 1995; Boquel et al. 2013), which is apparent in the differences recorded between our glasshouse and field studies. It has been theorized that oil particles acquired during aphid probing could alter the metabolism of the inoculated cell, resulting in reduced infection rates (Bradley 1963; Loebenstein et al. 1964; Peters and Lebbink 1975; Qiu and Pirone 1989). Oils are thought to suppress acquisition and inoculation of non-persistent viruses (Loebenstein et al. 1970) by preventing the attachment of the virus to the aphid stylet (Powell 1992; Wang and Pirone 1996). Our results indicate that HMO also alters aphid feeding behaviours that are related to the infection of plant viruses, delaying the onset of feeding and altering the frequency and duration of particular probing behaviours.

During EPG recording, HMO caused a significant delay in initiation of leaf tissue penetration, which is in agreement with other findings (Wyman 1971; Simons et al. 1977; Powell 1992). HMO may prevent aphids locating suitable sites for stylet insertion or alter sensory perception. Powell (1991) suggested that HMO affects the process of virus inoculation and acquisition or the retention of transmissible virus between acquisition and inoculation. Aphids insert their stylets into the plant epidermis and the mesophyll prior to reaching the intracellular membranes and producing watery saliva that carries virus particles into the protoplast. Virus is also acquired during ingestion from the protoplast (Martin et al. 1997).

Delayed intracellular membrane feeding and probing caused by the presence of HMO might lead to the loss of virus particles from the aphid stylets. The number of PD subphases and time per occurrence were significantly lower for HMO-treated seedlings compared with the control, suggesting that aphids were still puncturing the intracellular membrane but were unable to transmit or acquire virus during the first hour of exposure. As PPV is retained in the aphid stylet for a short period of time, this delay could be responsible for the reduction in the infection rate. However, EPG recordings of GPA feeding showed that HMO might have contributed to the reduction in PPV infection by altering stylet penetration behaviour and duration. The mean number of intracellular punctures was 3–4 times higher during the initial 5 min of recording for the controls as compared to HMO.

According to Moreno et al. (2009), five intracellular stylet punctures of frequent and short duration would lead to higher PPV infection rate than one longer duration intracellular puncture. Similarly, short intracellular penetrations lasting 1–2 s provided the highest infection rates of *Cucumber mosaic virus* and *Potato virus Y* (Martin et al. 1997). Using an artificial membrane feeding system, Garrett (1973) reported that 15 to 60 s of feeding was optimal for acquisition and inoculation of non-persistent viruses. In our study, HMO applied to peach seedlings resulted in a significant reduction in the duration of intracellular puncture activities for salivation into (II-1) and ingestion from protoplast (II-3), which is associated with non-persistent virus inoculation and virus acquisition, respectively (Collar et al. 1997). However, in addition to interfering with feeding phases related to virus acquisition and inoculation, our results suggest that HMO interferes with virus retention and subsequent inoculation because of a delay in the initiation of the pathway probing phase when aphids select their feeding site. A significant delay initiating probing of leaves caused by HMO agrees with the findings of other studies (Loebenstein et al. 1970; Simons et al. 1977; Powell et al. 1995; Martin et al. 1997). Powell et al. (1999) found that mineral oil reduced aphid transmission of PVY without altering stylet penetration behaviour. As the reduction in virus infection was not associated with a reduction of membrane punctures, it appears that oil is affecting the process of virus acquisition and/or inoculation during stylet cell penetration.

Based on our findings, HMO should be applied at least weekly in spring and early summer for the management of non-persistent viruses depending on weather conditions. Moreover, aphid population dynamics varies between years and it would be beneficial to monitor numbers of migratory aphid to assess flight activity and the need for sprays before an oil spray programme is initiated.

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