Persistent of Superior-70 oil on peach leaves and its effect on plum pox virus transmission by green peach aphid and EPG monitoring of aphid probing behavior

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INTRODUCTION

Plum pox virus (PPV), is one of the most economically important viruses affecting stone fruits world-wide (Stobbs et al. 2005). PPV is transmitted in a non-persistent manner by the green peach aphid, *Myzus persicae* (Sulzer). Acquisition and virus transmission occurring optimally during the brief epidermal stylet penetrations (<1 min). (Labonne et al., 1995). Therefore, controlling the migratory aphid vectors during the growing season is the most effective approach for controlling the spread of the virus. Application of horticultural oils to peach seedlings were found to reduce the transmission rate of PPV by aphids significantly (Samara et al., MS. in preparation). The frequency at which oils need to be applied to minimize virus transmission and the effects of oils on aphid feeding behaviour are not clearly understood.

OBJECTIVES

- Assess the persistent of horticultural oil applications on rates of transmission of PPV by the green peach aphid (GPA)
- Monitor aphid probing behavior using Electrical Penetration Graph (EPG) to demonstrate aphid stylet plants penetration .

MATERIALS AND METHODS

***Persistent of horticultural oil on virus transmission rate*.**

Four week old peach seedlings were sprayed with 1% Superior-70 oil using a small hand held atomizer; oil was applied to all plant surfaces including the under sides of leaves until runoff (~12 ml/plant). For control treatment, another set of peach seedlings were sprayed with water only until runoff. Then seedlings were kept for 4 h to allow the plants to dry thoroughly.

Twenty-one leaves were randomly collected from both control and oil treated seedlings after 0, 2, 4, 7, 9, 11, and 14 days. A Standardized detached leaf technique was used to assess virus transmission rate by GPA for both control and oil treated leaves (Fig. 1).

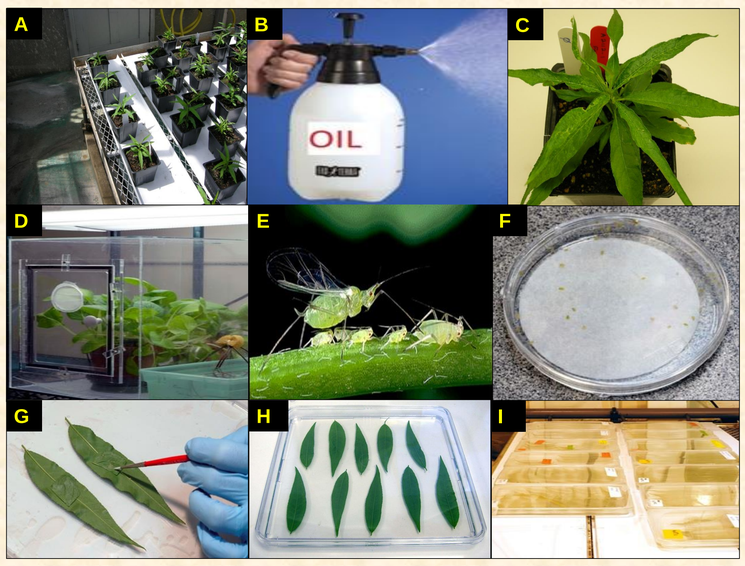

Fig. 1. Standardized detached leaf technique used for studying horticultural oil persistence on PPV transmission rate by GPA. (A) Four week old peach seedlings (B) Superior-70® Oil sprayed by hand-held atomizer. (C) PPV positive source of inoculum. (D) GPA colonies reared on Bok Choy. (E) GPA stages (adult and nymph). (F) Petri dish containing 4th instar GPA that were collected and left to starve for 2 h. (G) 25 GPA were transferred individually to a virus source plant placed on the treated leaves. (H) Treated leaves inoculated with PPV by viruliferous GPA were kept in sealed agar plates and held for a 48 h post-inoculation dark period. (I) Agar plates maintained in containment rooms for 4 weeks at18-20ºC and 16L: 8D. Leaves were then tested by RT-PCR for virus inoculation rate

Electrical monitoring of aphid probing and feeding behaviour

The EPG technique was used to monitor plant penetration activities by apterous GPA adults on peach seedlings treated either with 1% superior-70 oil or water as a control. Apterous adult aphids left to starve for 2 h, before a gold wire was attached to their dorsum, aphid was then placed on the abaxial surface of the leaf and connected to the DC-EPG device (Giga-4; EPG Systems, Wageningen, The Netherlands) (Fig. 2).

The EPG data acquisition was conducted using Stylet+ software. EPG signals of aphid stylet penetration activities were recorded for 1 h immediately after the aphid was placed on leaf under laboratory conditions (22–24ºC).

EPG waveforms described for aphids (Tjallingii, 1988) were identified as follows (Fig. 2.e.): non-probing (np), intercellular apoplastic stylet pathway (C), intracellular stylet puncture as potential drop (pd) which is associated with non-persistent virus acquisition and inoculation, and finally mechanical work associated to stylet penetration difficulties (F).

Statistical Analysis. For persistent oil applications on aphid rate of transmission studies, contingency tables generated using the Proc Freq procedure of SAS version 2.0.4 (SAS Institute Inc., Cary, NC) were used in chi-square analyses to compare percentages of virus infection rate in respond to application of horticultural oil, and Fisher' Exact test was used to separate between the means P-value.

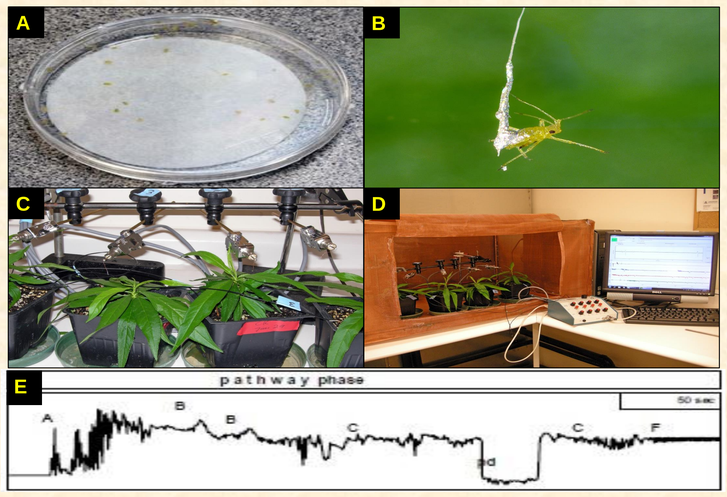

Fig. 2. EPG used to monitor aphid feeding behavior on peach seedlings treated either with 1% superior-70 oil or water. Apterous adult aphids were left to starve for 2 h. (A). A thin gold wire was attached to the dorsum of the aphid with a small droplet of silver glue(B). The opposite end of the gold wire was attached to copper electrode and connected to the EPG, and the other input electrode was inserted into the plant pot (C). EPG system inside Faraday cage to prevent electrical noises (D). EPG waveforms for aphids feeding activities (E)

Table 1. Persistent of horticultural oil application on plum pox virus transmission by GPA under greenhouse conditions. The detached leaf technique was used for control and oil treatment, with 25 starved aphids transferred to virus positive source placed on the previously treated leaves. After 4 weeks leaves were tested by direct real-time polymerase chain reaction (drtPCR) assay for inoculation rate

Days after oil application when viruliferous aphids applied to leaves	Treatment	N	Inoculation (%)	Chi Square	P ≤ 0.05	Contingency Coefficient**
0	CK	66	28.03	35.76	<0.0001 S	0.4617
	Oil		3.79			
2	CK	66	11.36	7.44	0.0064 S	0.2310
	Oil		3.03			
4	CK	66	20.45	9.46	0.0021 S	0.2586
	Oil		8.33			
7	CK	66	11.36	0	1.0000 NS	0
	Oil		11.36			
9	CK	66	28.79	30.03	<0.0001 S	0.4305
	Oil		6.06			
11	CK	66	23.48	8.54	0.0035 S	0.2465
	Oil		11.36			
14	CK	42	15.48	3.33	0.0679 NS	0.1954
	Oil		7.14			

RESULTS

Persistent of horticultural oil on virus transmission rate

Results of monitoring the persistent effect of horticultural oil "Superior-70" by the detached leaf technique showed a very low transmission rate (3 - 8%) for oil treated leaves compared to the water control (20-30%) during the first 4 days. These results indicated that under greenhouse conditions, oil application decreased the aphid transmission rate significantly compared to control plants, the lower transmission rate was found during 0-2 days and the inhibition effects of oil on virus transmission rate was found to last for 14 days after the initial oil application.

Boquel et al., (2013) found that application of horticultural oils decreased the aphids ability to acquire the virus, due to physical inhibition of virus binding to aphid stylet. This inhibition was observed to last only for one week. Our findings are in agreement with this, implying that horticultural oil should be applied weekly during spring and early summer and it can be repeated every two weeks. Moreover aphid population dynamics varies from year to year, therefore monitoring the aphid numbers is very important to assess the flight activity before applying oil.

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EPG monitoring of plants penetration behaviour

The intracellular punctures monitored using the DC electrical penetration graph (EPG) technique described by Tjallingii, (1988 and 1985) was used to monitor aphid stylet activities and their potential transmission of the non persistent viruses appears as distinct potential drops (pds). Results showed that peach treated with horticultural oil prevented aphids intracellular punctures associated with active acquisition and inoculation activities during the first week of oil application (Fig 3.).

During the monitoring period all aphids studied failed to record the (dp) on oil treated seedlings; stylet penetrations recorded were shorter and less frequent on oil treated seedlings compared to the control. These findings suggested that the mode of action of oil in inhibiting virus transmission may be interfering with the virus retention and inoculation processes.

These findings agree with lower virus transmission rates seen in the persistent of horticultural oil by the detached leaf technique recorded in the previous part of this study.

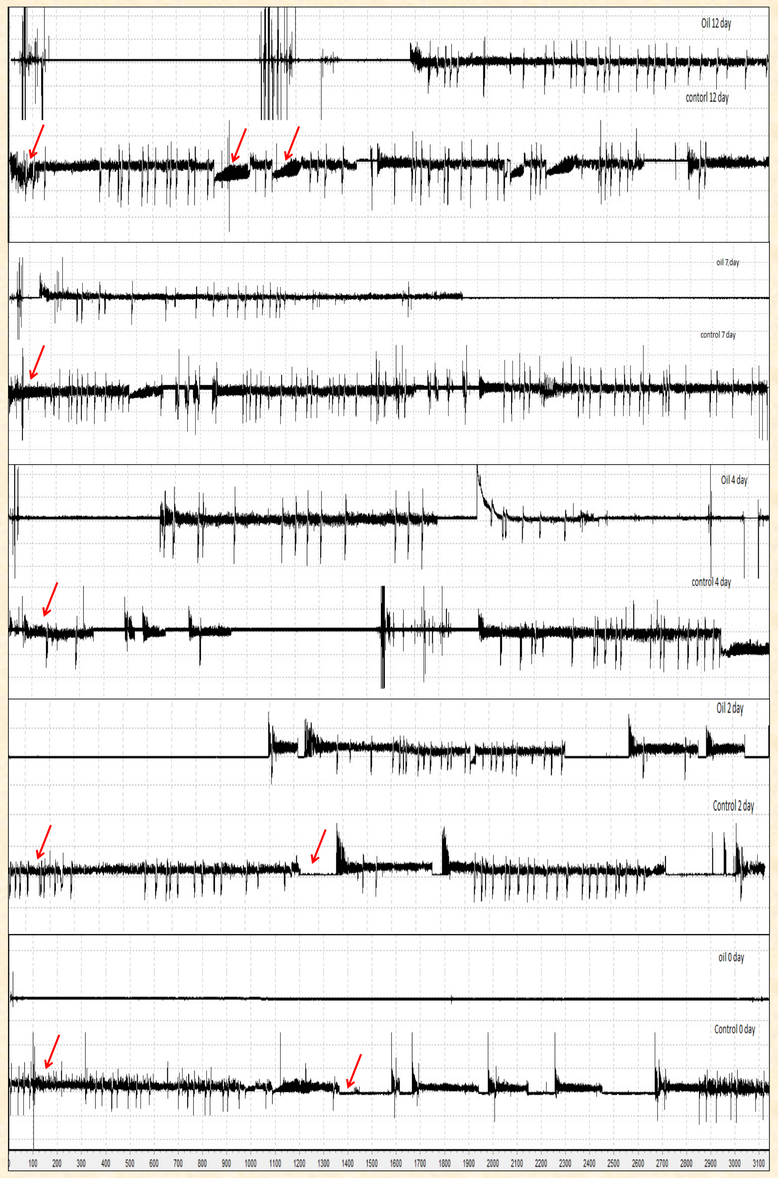

Fig. 3 Electrical penetration graph (EPG) variability in waveform and probing durations related to persistent effect of 1% Superior -70 oil or water during 60 min access period related to individual recordings of GPA on peach seedlings. Potential drop (dp) marked in red arrows

CONCLUSION:

- **Horticultural oil (Superior-70) reduced the PPV transmission rate by green peach aphids during the first week of oil application.**
- **EPG waveforms showed that oil interfered with retention and inoculation of PPV within the green peach aphid stylets.**
- **Further studies on the persistent of horticultural oil on PPV transmission rate by aphids under field conditions are binding before final recommendations can be made.**

References

Boquel, S., Giguere, M. Clark, C., Nanayakkara, U. Zhang, J. and Y. Pelletier. 2013. Effect of mineral oil on Potato virus Y acquisition by *Rhopalosiphum padi*. Entom. Exp. Appl.148:8-55

Labonne, G., Yvon, M., Quiot, J., Avinent, L., and G. Llacer. 1995. Aphids as potential vectors of plum pox virus: Comparison of methods of testing and epidemiological consequences. Acta Hort. 386: 207–218

Stobbs, L., van Driel, L., Whybourne, K., Carlson, C., Tulloch, M. and van Lier, J. 2005. Distribution of plum pox virus in residential sites, commercial nurseries, and native plant species in the Niagara region, Ontario, Canada. Plant Dis. 89: 822–827

Tjallingii, F.1985. Membrane potentials as an indication for plant cell penetration by aphid stylets. Entomol Exp Appl 38:187–195.

Tjallingii, F. 1988. Electrical recording of stylet penetration activities. In Aphids. Their Biology, Natural Enemies and Control, pp. 95-108. Edited by A. K. Minks & P. Harrewijn. Amsterdam: Elsevier