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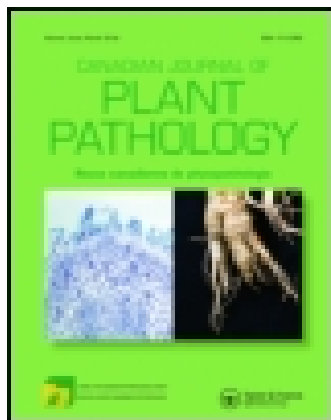
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Virology/Virologie

Influence of horticultural oil (Superior-70) on *Plum pox virus* (PPV) detection in treated and untreated PPV-infected peach (*Prunus persicae* L.) leaves

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Abstract: The effect of foliar application of horticultural oil on the detection of *Plum pox virus* (PPV) in infected peach leaves was examined. No significant differences in virus detection using ELISA or DRT-qPCR were found between matching detached half leaves treated either with oil or water immediately after oil application, and up to 3 weeks post application. Similarly, *in vitro* amendments of oil to dilutions of PPV-infected leaf macerates did not affect DRT-qPCR detection of virus, and only reduced detection of virus by ELISA at the lowest virus dilutions containing 5 $\mu\text{L mL}^{-1}$ of oil. Application of horticultural oil by growers to reduce aphid transmission of PPV should have no impact on virus detection in regulatory surveys associated with monitoring the quarantine zone.

Keywords: horticultural oil, peach, *Plum pox virus*, virus detection

Résumé: Les effets de l'application foliaire d'huile horticole sur la détection du virus de la sharka sur des feuilles de pêcher infectées ont été étudiés. Aucune différence significative quant à la détection du virus par ELISA ou DRT-PCRq n'a été notée entre des demi-feuilles détachées assorties traitées soit avec de l'huile ou de l'eau immédiatement après l'application d'huile, et ce, jusqu'à trois semaines après application. De la même façon, l'ajout d'amendements à base d'huile, *in vitro*, à des dilutions de macérations de feuilles infectées par la sharka n'a pas influencé la détection du virus par DRT-PCRq et a seulement réduit la détection du virus par ELISA dans les plus faibles dilutions contenant 5 $\mu\text{L mL}^{-1}$ d'huile. L'application d'huile horticole par les producteurs qui cherchent à réduire la transmission du virus de la sharka par les pucerons ne devrait pas avoir d'influence sur la détection du virus au cours d'enquêtes réglementaires associées à la surveillance des zones de quarantaine.

Mots clés: détection des virus, huile horticole, pêcher, virus de la sharka

Introduction

Plum pox virus (PPV) or Sharka is a devastating disease of cultivated *Prunus* stone fruits including peaches, plums, apricots and nectarines. The Dideron strain (PPV-D) was first detected in Ontario and Nova Scotia in 2000 (Thompson et al. 2001), threatening not only stone fruit but also ornamental *Prunus* nursery production

(Brethour et al. 2005). The Niagara region represents about 85% of Canada's stone fruit production and was the most highly impacted by the disease (Brethour 2001). Trees infected with PPV-D, depending on variety, can show reduced growth and vigour with loss in yield and fruit quality (Travis et al. 2001; Errampalli et al. 2004; Huisman 2008). The green peach (*Myzus persicae* (Sulzer)), spirea (*Aphis spiraeicola* (Patch)) and soybean

(*Aphis glycines* (Matsumura)) aphids were shown to be the most efficient vectors of PPV-D; transmission rates were approximately 27%, 16% and 9%, respectively (Lowery & Vickers 2007). Partial control of the disease was accomplished through establishment of an eradication programme in 2001 which included detection surveys, diseased tree removals and quarantine of affected areas. Over 10 years, the eradication programme successfully eradicated PPV from all but the Niagara quarantine zone. Here, significant reductions in the levels of detectable virus infections were achieved in less heavily infected areas (Annual Report of the PPV International Expert Panel 2009).

Horticultural oils have been used in various parts of the world to limit the spread of non-persistent, aphid-borne viruses (Vanderveken 1977; Simons & Zitter 1980; Lowery et al. 1990; Umesh et al. 1995; Asjes & Blom-Barnhoorn 2002; Furness & Combella 2002; Boiteau et al. 2009). It is believed that oils prevent the attachment or release of virus particles from the aphid stylet (Vanderveken 1977). Studies have demonstrated that foliar applications of horticultural oils effectively inhibited the transmission of PPV by aphids (Migliori et al. 1998; Lowery & Vickers 2007; Vidal et al. 2010). Based on these findings, in 2009 the PPV International Expert Panel recommended that as part of the Canadian PPV eradication programme, season-long application of foliar oil sprays be made to peach orchards within the quarantine zone (IEP Report 2009). This would reduce the natural spread of virus by aphids and improve the chances for successful eradication by 2015. Since eradication surveys would also need to be done over this period, concern was expressed that stylet oil residues on the leaves might adversely affect virus detection in both ELISA and RT-PCR methods (IEP 2009). This study examines virus detection in PPV-infected detached peach leaves, each half either treated with oil or left untreated as the control. ELISA and DRT-PCR was compared between the two treatments.

Materials and methods

Plant material and virus source

Two year peach whips (*Prunus persica* L. 'Elberta') infected with PPV were used as source material for harvesting infected leaves. A Canadian peach isolate of the Dideron strain (#2630, PPV-D) was used in this study.

Oil treatment of leaves

Earlier trials on detached and dissected peach leaves did not demonstrate any variance in virus titre between two

halves of leaves separated at the midrib (unpublished data). Therefore, leaves were first cut in half along the midrib, the midrib was removed, and the two halves were arranged, abaxial surface up, on moistened paper towels in 24.5 × 24.5 × 2.5 cm (*l* × *w* × *h*) Nunclon™ polystyrene covered trays (VWR Scientific, Mississauga, ON). Corresponding halves were numbered and placed in separate trays such that half leaf pairs could be matched up later. Half leaves in one tray were sprayed to runoff with 1% Superior-70 oil (Bartlett Emulsifiable Insecticide, United Agri Products Canada Inc., Dorchester, ON) delivered from a hand-held atomizer (Plant Smart EZ Sprayer, Walmart, Canada). Water was sprayed on corresponding half leaves in the second tray as a control. Leaves were placed in a fume hood to dry for approximately 2 h. Leaf halves were then macerated in ELISA extraction buffer (0.5 g tissue: 3 mL extraction buffer) in 12 × 14 cm sample extraction bags (Bioreba AG, Reinach, Switzerland). Samples were tested in duplicate wells by ELISA and DRT-RT-PCR as previously described (Kim et al. 2008).

In a second experiment, the effect of oil dissipation on the leaf surface over time on the detection of virus was examined. Two hundred detached peach leaves from PPV infected whips were treated with oil or water as previously described and half leaves transferred abaxial surface up to the polystyrene covered trays containing 4% agar. The trays were then sealed with Parafilm and incubated in a growth room (20 °C, 4100 lux halide lighting, 16 h photoperiod) for 4 weeks. Thirty of the oil-treated and corresponding untreated half leaves were each sampled immediately after treatment, and after 1 h, and 1, 2 and 3 weeks and assayed for virus as previously described.

Studies on oil and virus suspensions in vitro

The approximate amount of residual oil left on leaves when sprayed to runoff with the recommended 1% formulation was calculated using the method of Baudoin et al. (2006). With fully expanded peach half leaves, approximately 6.25 µL of oil residue was retained per gram of leaf tissue. Thus for each gram of oil-treated leaf macerated in extraction buffer (1:6 w:v:tissue:buffer) for assay, 1.04 µL oil residue would be expressed in each mL of buffer. To examine the effect of oil on various concentrations of virus in suspension, 10 g of positive peach leaf tissue was first macerated in 50 mL of ELISA extraction buffer. Filtrate (15 mL) was placed in a glass test tube, and serially diluted (2×) into two sets of 8 tubes each. Superior Oil 70 concentrate was added to each tube in one set of dilutions to give a final oil concentration of

Table 1. Effect of oil application to half leaf surfaces of peach on the detection of *Plum pox virus* by ELISA and DRT-PCR assays. One half of each leaf was treated with oil, the other half treated with water.

Trial	n =	ELISA ^{1,3}				DRT-PCR ^{1,3}			
		Water		Oil		Water		Oil	
		X ± sd (t value)	[log(x + 1)] (t value)	X ± sd (t value)	[log(x + 1)] (t value)	X ± sd (t value)	[log(x + 1)] (t value)	X ± sd (t value)	[log(x + 1)] (t value)
1	36	4.85 ± 2.50 (11.63)	0.73 ± 0.18 (24.42)	3.74 ± 2.30 (9.75)	0.63 ± 0.20 (19.11)	26.78 ± 6.51 (20.57)	1.44 ± 0.09 (83.14)	27.05 ± 6.02 (22.48)	1.45 ± 0.09 (78.84)
2	40	17.73 ± 15.40 (7.28)	1.11 ± 0.41 (17.12)	12.75 ± 13.02 (6.19)	0.94 ± 0.44 (13.52)	17.71 ± 6.26 (16.00)	1.21 ± 0.33 (20.85)	17.23 ± 7.05 (13.82)	1.17 ± 0.39 (16.86)
3	36	12.34 ± 7.18 (10.31)	1.05 ± 0.32 (19.40)	10.63 ± 6.85 (9.31)	0.94 ± 0.38 (14.77)	16.59 ± 6.25 (14.26)	1.15 ± 0.40 (15.45)	18.76 ± 3.69 (27.34)	1.29 ± 0.08 (89.96)
4	40	13.85 ± 10.27 (8.53)	1.05 ± 0.33 (19.73)	8.25 ± 7.64 (6.83)	0.82 ± 0.38 (13.64)	23.79 ± 4.77 (26.37)	1.39 ± 0.09 (82.31)	22.74 ± 5.01 (24.00)	1.37 ± 0.10 (71.63)
5	28	14.86 ± 7.09 (11.09)	1.14 ± 0.32 (18.52)	19.00 ± 13.72 (7.32)	1.18 ± 0.34 (18.58)	19.13 ± 5.20 (18.38)	1.28 ± 0.11 (58.04)	21.73 ± 5.91 (18.38)	1.35 ± 0.09 (73.61)
6	32	9.41 ± 6.77 (7.86)	0.92 ± 0.32 (16.09)	15.03 ± 11.78 (7.22)	1.03 ± 0.46 (12.72)	18.36 ± 4.14 (17.75)	1.27 ± 0.10 (50.03)	20.29 ± 4.57 (17.77)	1.32 ± 0.09 (57.93)
7	29	8.76 ± 8.02 (5.88)	0.82 ± 0.44 (10.10)	14.03 ± 10.29 (7.34)	1.04 ± 0.40 (14.12)	20.55 ± 4.59 (23.68)	1.32 ± 0.08 (83.99)	21.86 ± 5.56 (20.79)	1.35 ± 0.09 (77.4)

¹ Log transformed data were used to compare the mean values. Mean value, standard deviation and t values are shown.² ELISA absorbance value (log(x + 1)) at 405 nm.³ DRT-PCR ct value, detection threshold cut-off = ct.⁴ NS: no significant effect on oil on virus detection ($P = 0.05$, Student–Newman–Keuls test, SNK).

Table 2. Influence of the time interval between oil or water application on detached PPV-infected leaves and the detection of virus by ELISA and DRT-PCR assays.

			ELISA ^{2,3}			DRT-PCR ^{2,4}					
Time ¹	Trial	n =	Water		Oil	Sig. test ⁵	n =	Water		Oil	Sig. test ⁵
			X ± sd (t value)	[log(x + 1)] (t value)	X ± sd (t value)			[log(x + 1)] (t value)	X ± sd (t value)	[log(x + 1)] (t value)	
0 hr	1	10	9.13 ± 4.23 (6.82)	0.97 ± 0.20 (15.01)	9.00 ± 4.63 (6.15)	NS	14	23.81 ± 7.80 (11.41)	1.38 ± 0.12 (42.91)	18.88 ± 9.30 (7.60)	NS
	2	10	24.05 ± 13.14 (5.66)	1.27 ± 0.46 (8.76)	22.54 ± 14.45 (4.93)	NS	12	19.77 ± 7.30 (9.38)	1.23 ± 0.39 (10.83)	17.57 ± 8.91 (6.83)	NS
1 day	1	10	14.21 ± 6.7 (6.73)	1.13 ± 0.25 (14.31)	12.75 ± 4.75 (8.49)	NS	10	19.30 ± 2.57 (23.74)	1.31 ± 0.05 (82.71)	19.81 ± 2.88 (21.74)	NS
	2	15	10.96 ± 5.25 (8.08)	1.03 ± 0.23 (17.18)	9.98 ± 5.83 (6.63)	NS	15	19.28 ± 2.44 (30.60)	1.30 ± 0.05 (20.35)	19.75 ± 3.76 (101.07)	NS
1 wk	1	11	18.30 ± 10.70 (83.28)	1.19 ± 0.34 (11.48)	15.42 ± 7.75 (89.85)	NS	11	20.57 ± 2.60 (26.25)	1.33 ± 0.05 (5.67)	19.35 ± 2.20 (29.15)	NS
	2	12	18.44 ± 10.29 (6.21)	1.23 ± 0.25 (16.73)	17.80 ± 7.80 (7.80)	NS	13	16.76 ± 5.44 (11.10)	1.18 ± 0.36 (11.91)	19.68 ± 5.00 (14.19)	NS
2 wk	1				NA		14	20.72 ± 6.53 (11.87)	1.27 ± 0.37 (12.89)	18.81 ± 8.22 (8.56)	NS
	2						15	23.41 ± 4.84 (18.74)	1.38 ± 0.08 (66.81)	20.83 ± 5.92 (13.63)	NS
3 wk	1	9	7.07 ± 4.66 (4.55)	0.82 ± 0.35 (7.11)	10.01 ± 4.24 (7.08)	NS	9	20.45 ± 1.34 (45.91)	1.33 ± 0.03 (141.07)	19.92 ± 1.54 (38.73)	NS
	2	7	3.69 ± 2.44 (4.00)	0.62 ± 0.22 (7.32)	4.31 ± 2.63 (4.34)	NS	9	20.00 ± 12.08 (4.97)	1.10 ± 0.63 (5.26)	22.19 ± 8.71 (7.64)	NS

¹ Elapsed time after treatment and before assay² Log transformed data were used to compare the mean values. Mean value, standard deviation and t values are shown.³ ELISA absorbance value (log(x + 1)) at 405 nm.⁴ DRT-PCR ct value, detection threshold cutoff = ct.⁵ NS : no significant effect on oil on virus detection ($P = 0.05$, Student–Newman–Keuls test, SNK).

NA: Data not available

Table 3. Effect of oil added to virus suspensions on PPV detection by ELISA and DRT-PCR.

Trial	Virus Dilution	Water			Oil			
		ELISA ^{1,2}	DRT-PCR ^{1,2}	($\mu\text{L mL}^{-1}$)	ELISA ^{1,2}	DRT-PCR ^{1,2}	($\mu\text{L mL}^{-1}$)	DRT-PCR ^{1,2}
1	0	42.8 (+)	28.2 (+)	1.0	44.2 (+)	29.9 (+)		
1	2x	65.0 (+)	32.0 (+)	1.0	48.5 (+)	27.8 (+)		
1	4x	45.8 (+)	27.4 (+)	1.0	41.4 (+)	25.8 (+)		
1	8x	33.7 (+)	26.5 (+)	1.0	30.0 (+)	25.7 (+)		
1	16x	26.5 (+)	28.5 (+)	1.0	25.9 (+)	26.7 (+)		
1	32x	21.6 (+)	27.9 (+)	1.0	18.5 (+)	27.8 (+)		
1	64x	14.4 (+)	30.7 (+)	1.0	13.3 (+)	28.4 (+)		
1	128x	10.0 (+)	33.4 (+)	1.0	7.6 (+)	32.4 (+)		
1	256x	5.8 (+)	36.4 (S)	1.0	5.4 (+)	33.2 (+)		
2	0	9.6 (+)	20.9 (+)	2.0	8.0 (+)	21.0 (+)	5.0	21.4 (+)
2	2x	8.7 (+)	20.8 (+)	2.0	6.6 (+)	21.4 (+)	5.0	21.1 (+)
2	4x	7.3 (+)	20.9 (+)	2.0	4.6 (+)	21.9 (+)	5.0	22.4 (+)
2	8x	5.6 (+)	22.6 (+)	2.0	4.0 (+)	22.7 (+)	5.0	22.9 (+)
2	16x	4.7 (+)	23.6 (+)	2.0	2.7 (+)	23.5 (+)	5.0	24.2 (+)
2	32x	3.9 (+)	24.9 (+)	2.0	2.7 (+)	24.8 (+)	5.0	25.1 (+)
2	64x	3.2 (+)	25.2 (+)	2.0	2.4 (+)	25.6 (+)	5.0	26.6 (+)
2	128x	2.9 (+)	26.6 (+)	2.0	2.4 (+)	27.8 (+)	5.0	28.7 (+)
2	256x	2.6 (+)	29.1 (+)	2.0	2.1 (S)	30.0 (+)	5.0	30.7 (+)

¹ ELISA values measured at 405 nm, positive threshold absorbance considered as 2 times negative control, values expressed as ratio infected/healthy control absorbance. DRT-PCR threshold cut-off for positive samples set at 36 ct.

² Values represent average of two tests.

1 $\mu\text{L mL}^{-1}$ and designated as the oil treatment. The second set of control tubes were adjusted by adding a corresponding volume of buffer. Tubes were shaken to mix and ELISA and DRT-PCR were run on the dilutions as described earlier. In a subsequent trial, the oil concentration was adjusted to 2 and 5 $\mu\text{L mL}^{-1}$ virus suspension, the tubes shaken and assayed as described.

Results and discussion

Initial trials investigating the application of Superior 70 oil to detached PPV-infected peach half leaves revealed no significant differences in virus detection from the half leaves of the water-treated controls by either ELISA or DRT-PCR (Table 1). When water- and oil-treated half leaves were compared immediately after oil application, and 1 day, or 1, 2 and 3 weeks post oil application (Table 2), no significant differences in the ELISA and DRT-PCR values were apparent (Table 2). A slight drop in the percentage of positive leaf detection on oil-treated leaves was seen at time 0 in both trials using DRT-PCR detection. For *in vitro* trials, where dilutions of virus-infected macerates were amended with increasing concentrations of oil, ELISA failed to detect virus at high dilutions at the highest concentration (5 $\mu\text{L mL}^{-1}$) of oil (Table 3).

Horticultural oil application to orchard trees is unlikely to cause significant reductions in ELISA absorbances affecting virus detection. Inhibitory effects of oil on virus detection was only seen for *in vitro* trials at the highest oil concentration. There was no evidence that oil application to leaves affected DRT-qPCR detection of virus in any of the studies. This is particularly important in an eradication programme or regulatory quarantine boundary surveys of a quarantine zone where orchards may be treated with oil to reduce the spread of PPV. Oils may also be used on younger more susceptible trees for protection against aphid transmission. From this study, there does not appear to be any significant effect of oil residue on leaves on the detection of PPV.

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