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Effect of selected oils and insecticides on beneficial insect species

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Previous trials within this Sustainable Farming Fund project (SFF11-058) have highlighted the potential for oil-based products to act as both repellents and slow-acting insecticides against the tomato potato psyllid (*Bactericera cockerelli*; TPP). These properties may enable oils to be used instead of agrichemicals at times of low TPP pressure and/or low crop risk. Alternatively, oils may be used with agrichemicals to enhance or prolong action. Either scenario could contribute to reduced agrichemical use within potato crops (the overall aim of the current SFF project). However, little is known about the effects of oil-based products on beneficial insect species.

Previous SFF work has also showed that a number of relatively new insecticides have potential to disrupt TPP behaviour (especially egg-laying) for at least 14 days after application. The disruptive effects of these insecticide residues may be sufficient to protect the crop for extended periods, enabling longer spray intervals and fewer total spray applications across the growing season. Some information on beneficial insect impacts is already available for these insecticides (Table 1), but further trials using key New Zealand beneficial insects are required. In particular, the impact of insecticides on hoverflies is poorly understood.

To address these knowledge gaps, a series of laboratory-based assays were conducted to investigate the effects of a selection of oil-based products and one insecticide on representative psyllid predators.
Three oil-based products were selected for investigation: Sapsucker Plus (and, once available, a newer formulation of this product known as Thunderbolt), Excel® Oil and Organic JMS Stylet Oil®. Water and Tamaron® were also included in assays for comparison. Limited testing was also carried out using the insecticide Avid®. All products were used at maximum recommended field rates. Three beneficial insect species were examined in these trials: Tasmanian lacewing (*Micromus tasmaniae*), eleven-spotted ladybird (*Coccinella undecimpunctata*) and the small hoverfly (*Melanostoma fasciatum*). Two types of bioassay were conducted: direct spray bioassays and residue bioassays. In the direct spray bioassays, insects were sprayed with product mixtures using a Potter Spray Tower. In the residue bioassays, 40 mm potato leaf discs were dipped for 5 seconds in product mixtures. Twenty-four hours after dipping, a single insect was added to the centre of each leaf disc. In both types of assay, mortality was assessed at 72 h. There were at least 30 individual insects tested for each product/beneficial species combination.

All the oil-based products tested caused significantly less mortality than Tamaron®, the broad spectrum insecticide used as a “high” control (i.e. a treatment that was expected to cause severe mortality). None of the oil-based products tested caused mortality that would place them in the highest International Organisation for Biological and Integrated Control of Noxious Animals and Plants (IOBC) side effect category (i.e. did not cause mortality of >79%). However, some products were moderately harmful to some species (i.e. caused mortality of 41-56%). Sapsucker Plus had the most consistent effects, never causing more than 20% mortality. Hoverflies were the most tolerant of the beneficial species tested, surviving well in all direct spray assays and most residue assays. The results are encouraging and suggest that some oil-based products could be used in TPP management programmes without jeopardising biological control.

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1 Introduction

Biological control (i.e. mortality caused by beneficial insects and other natural enemies) is a crucial component in most long-term stable integrated pest management (IPM) programmes. In order to retain biological control in crop systems effectively, the side effects of insecticides and other products must be well understood and, whenever feasible, “biorational” insecticides should be employed. The term “biorational” is used to describe insecticides that are effective against their target pest, but less detrimental to natural enemies, and therefore non-disruptive to biological control (Schuster & Stansly 2005). A range of characteristics can make a product “less detrimental”: for example, a product may have no direct toxicity to beneficial insects, or it may be broad spectrum but have a short field residual time, thereby minimizing the exposure of beneficial insects to the product. Biorationals are defined by their effects, not their origins, and can include both synthetic and naturally derived products (in contrast to “biopesticides” or “botanicals”). To determine the biorational (or IPM-compatible) products available for a crop, side-effect testing for key beneficial insects is often required, especially when new products are brought to market and/or incorporated into crop management practices. This study focused on the side effects of three oil-based products, Sapsucker Plus (and, once it became available, a new formulation of this product known as Thunderbolt), Excel® Oil and Organic JMS Stylet Oil®. One insecticide (Avid®) was also examined in this study. Details of all products tested are given in Tables 2 and 3.

Previous trials within this SFF programme have highlighted the potential for these oil-based products to act as both repellents and slow-acting insecticides against the tomato potato psyllid (*Bactericera cockerelli*; TPP) (Dohmen-Vereijssen et al. 2012). These properties may enable oils to be used instead of agrichemicals at times of low TPP pressure and/or low crop risk. Alternatively, oils may be used with agrichemicals to enhance or prolong action. Either scenario could contribute to reduced agrichemical use within potato crops. However, the impact of oils on beneficial insects is largely unknown (Table 1) and this area requires investigation, as New Zealand field work has indicated that beneficial insects can contribute to TPP control, especially in the early growing season (Walker et al. 2011; Walker et al. 2012). Previous SFF work has also showed that a number of relatively new insecticides have potential to disrupt TPP behaviour (especially egg-laying) for at least 14 days after application (Gardner-Gee et al. 2012). The disruptive effects of these insecticide residues may be sufficient to protect the crop for extended periods, enabling longer spray intervals and fewer total spray applications across the growing season. Some information on beneficial insect impacts is already available for these insecticides (Table 1), but further trials using key New Zealand beneficial insects are required. In particular, the impact of insecticides on hoverflies is poorly understood.

1.1 Aim

To investigate the acute direct and residual effects of a selection of oils and insecticides on representative TPP predators (e.g. lacewings, ladybirds, hoverfly)

Table 1. Summary of published studies on the impacts of selected agrichemicals and oils on key predators of tomato potato psyllid in New Zealand potato crops. Entries in grey refer to related species, given if no information available on New Zealand species.

Agrichemicals					
Active ingredient (a.i.) (example of product)	IRAC group (exemplifying a.i.)	New Zealand beneficial insect species			
		Tasmanian lacewing (*Micromus tasmaniae*)	Small hoverfly (*Melanstoma fasciatum*)	Eleven-spotted ladybird (*Coccinella undecimpunctata*)	Other
Spinetoram (e.g. Sparta™)	5 (spinosyn)	Residues of related a.i. (spinosad) caused < 39% mortality to larvae in acute tests but no long-term effects (Cole et al. 2010)	Residues of related a.i. (spinosad) caused 68% mortality to *Episyrphus balteatus* larvae and adults from surviving larvae did not lay eggs (Moens et al. 2011)	Residues of related a.i. (spinosad) caused low mortality to *C. transversalis* larvae in acute tests and no long-term effects (Cole et al. 2010), *C. septempunctata* numbers little affected in Spinosad field trials (Misra & Mukherjee 2012)	Residues caused 100% mortality to *Tamarixia triozae* (Liu et al. 2012)
Abamectin (e.g. Avid®)	6 (abamectin)	Abamectin derivative (emamectin benzoate) caused < 25% mortality to larvae in acute tests and no long-term effects (Cole et al. 2010)		Abamectin derivative (emamectin benzoate) caused less than 10% mortality to *C. transversalis* larvae in acute tests and had no long-term effects (Cole et al. 2010)	Residues caused 100% mortality to *Tamarixia triozae* (Liu et al. 2012)
Pymetrozine (e.g. Chess®)	9B (pymetrozine)	Caused no mortality to larvae in acute tests and no long-term effects (Cole et al. 2010)		Caused < 20% mortality to *C. transversalis* larvae in acute tests and in long-term tests 98% killed before maturity (Cole et al. 2010), no adverse effects on *C. undecimpunctata* (Cabral et al. 2008)	Residues harmless to *Tamarixia triozae* (Liu et al. 2012)
Spirotetramat (e.g. Movento®)	23 (tetronic and tetramic acid derivatives)	Harmless to *Chrysopa* spp. (Schnorbach et al. 2008)	Harmless to larvae of *Episyrphus balteatus*, fertility of adults treated as larvae not negatively affected (Moens et al. 2011)	Harmless to moderately harmful to *Coccinella* spp. and *Chilocorus nigritus* (Schnorbach et al. 2008)	Harmless to spiders and parasitoid wasps, moderately harmful to some mites (Schnorbach et al. 2008); residues harmless to *Tamarixia triozae* (Liu et al. 2012)
Cyantraniliprole (alternative name for a.i. is Cyazypyr™) (e.g. Benevia®)	28 (diamide)			Field trials indicate little impact on *C. septempunctata* numbers (Misra & Mukherjee 2012)	Harmless to parasitoid wasps (Brugger et al. 2010), residues harmless to *Tamarixia triozae* (Liu et al. 2012)
Oils					
Field tests of a range of oils showed that most had strongly detrimental effects on lacewing numbers (*Chrysoperla carnea*) and ladybird numbers (*Coccinella undecimpunctata*) in the crops 14 days post-application (Simmons & Abd-Rabou 2011). Chenopodium oil residues did not cause significant mortality to *Tamarixia triozae* (Liu et al. 2012).					

2 Methods

2.1 Products tested

Following consultation with Potatoes New Zealand and growers, three oil-based products and one insecticide were selected for investigation. Product details and concentrations used are detailed in Tables 2 and 3. Water was used as a low control (i.e. a treatment expected to cause little mortality), while Tamaron® was used as a high control (i.e. a treatment expected to cause severe mortality). Products were used at maximum recommended field rates.

Table 2: Oil-based products used in bioassays.

Trade name	Active ingredient/s	Mode of action	Recommended field rates	Application rate used (product/L spray)
Organic JMS Stylet Oil®	Mineral Oil + adjuvant	Inhibits insect respiration	1.5 litre/100 L	15 ml
Excel® Oil	Mineral Oil	Inhibits insect respiration	1 litre/100 L	10 ml
Sapsucker Plus	Mixed monoterpenes (gamma-T-ol), neem oil, isopropanil, non-ionic surfactant	Neem: Antifeedant and insect growth regulator	240 g in 12 L water	20 g
Thunderbolt	Mixed monoterpenes (alpha-Tops), neem oil, beta cyclodextrin, non-ionic surfactant	Neem: Antifeedant and insect growth regulator	1 kg/ha in 300-400 L water	3.3 g

Table 3: Insecticides used in bioassays. International Resistance Action Committee (IRAC) insecticide groups are given, along with active ingredients (a.i.), mode of action and application rates.

Trade name	Active ingredient (IRAC group)	Mode of action	Recommended field rates	Application rate used (product/L spray)
Tamaron®	Methamidophos (1B)	Contact and stomach poison. Penetrates plant tissue and enters sap, killing sucking insects.	800 ml (aphids) - 1 L (potato tuber moth)/ha in 500 - 1000 L water	2 ml
Avid®	Abamectin (6)	Paralyses insect, eventually causing death (may take 7 days to reach maximum effectiveness). Moves into leaves and remains there for several weeks.	600 ml/ha	1.2 ml (used both in isolation and with recommend partner Eco-Oil®).

2.2 Species tested

Three beneficial insect species were examined in these trials: Tasmanian lacewing (*Micromus tasmaniae*), eleven-spotted ladybird (*Coccinella undecimpunctata*), and the small hoverfly (*Melanostoma fasciatum*).The first lacewing colony used in the bioassays was established from wild lacewings collected from unsprayed potato crops at the Pukekohe Research Station. A second lacewing colony was established from lacewings that invaded a TPP colony held in a glasshouse at the Mt Albert Research Centre, Auckland. Two ladybird colonies were established, the first from a single gravid female collected in lucerne paddocks in South Auckland in 2012, and the second from five adults collected in Canterbury in January 2013. Additional ladybird adults were collected from unsprayed potato crops in February 2013 at the Pukekohe Research Station and were used in assays. Both the ladybird and lacewing colonies were maintained on a diet of green peach aphid (*Myzus persicae*), TPP and occasional honey. Hoverfly eggs were collected as opportunity arose from potato crops in Waiuku and Matamata, and hatched larvae were maintained on green peach aphids. Hoverfly and lacewing larvae (5-7 days old) were used in the bioassays, whereas ladybird adults were used in the assays, as this is the life stage most commonly found in potato crops.

2.3 Types of bioassay

Two types of bioassay were conducted: direct spray bioassays and residue bioassays. In the direct spray bioassays, larvae were sprayed with product mixtures using a Potter Spray Tower (Burkard Manufacturing). Prior to spraying, batches of 10 larvae were briefly exposed to CO_2 gas. The anoxiated larvae were then transferred to a shallow 55-mm Petri dish lid and sprayed. For each batch of larvae, a 5-ml aliquot of mixed product was used, resulting in a wet spray deposit of 8-12 mg /cm^2. A coarse spray nozzle was used with pressures in the range of 6-8 psi. Once each product had been sprayed, the Potter Spray Tower was cleaned with ethanol, triple rinsed with water, and settings were re-calibrated to ensure delivery was as consistent as possible. After spraying, each larva was transferred to a separate 60-ml vented container and provisioned with food (i.e. a capsicum leaf disc with approximately 20 tomato potato psyllid nymphs on it). Larvae were then held in controlled-temperature room (20°C; 16:8 L:D). Mortality was assessed 72 h after larvae were exposed to the products (i.e. 72 hours after spraying). Assays using ladybirds were the same as described above, except that adult ladybirds were used. At least 30 individual insects of each species were tested with each product (e.g. 30 lacewing larvae, 30 hoverfly larvae and 30 adult ladybirds were sprayed with Excel® Oil). At least two trials (typically with 15 insects per product in each trial) were carried out for each product to gain some measure of inter-trial variability.

In the residue bioassays, 40-mm potato leaf discs were dipped for 5 seconds in product mixes and allowed to air dry for at least one hour. The potato plants (‘Moonlight’) used for the leaf discs were field grown and had received fungicide applications but no insecticide applications prior to the assay. Once dry, the leaf discs were mounted on beds of agar in 55-mm Petri dishes and 20 TPP (fourth and fifth instars) were added to each leaf disc to provide food for the larvae. Twenty-four hours after dipping, a single larva was added to the centre of each leaf disc. Mortality was assessed 72 h after larvae were exposed to residues (i.e. 72 hours after they were added to the leaf disc). Once again ladybird adults and lacewing and hoverfly larvae were used, and there were generally 30 replicates for each product and for each beneficial species.

For both direct and residue assays, mortality was assessed 72 h after spraying. Larvae were considered dead if they were unresponsive when prodded gently with a fine brush. Ladybird adults were considered dead if they remained unresponsive after 15 min of observation and occasional prodding with a fine brush.

2.4 Data analysis

Generalized Linear Models (GLMs) were used in R (R Core Team 2013) to estimate mean mortality (and confidence intervals) of the predators in the presence of the various products. A quasi-binomial model was used to compensate for the high degree of underdispersion. Fisher's Exact tests were used to make comparisons with water and with Tamaron® (as low and high controls respectively). Data from each trial were graphed separately to provide a visual indication of inter-trial variability. Horizontal lines were added to all graphs to indicate side-effect thresholds, as determined by the International Organisation for Biological and Integrated Control of Noxious Animals and Plants (IOBC) (Table 4).

Table 4: International Organisation for Biological and Integrated Control of Noxious Animals and Plants (IOBC) classification of side-effects of pesticides (mortality and/or reduction in beneficial capacity) (Boller et al. 2005).

	IOBC classification		
	N (harmless or slightly harmful)	M (moderately harmful)	T (harmful)
Laboratory test results	<30% mortality	30-79% mortality	>79% mortality
Field/ semi-field test results	0-50% reduction	51-75% reduction	>75% reduction

3 Results

All the oil-based products caused significantly less mortality than Tamaron®, the broad spectrum insecticide used as a high control ($P < 0.05$ in all Fisher Exact tests comparing product with Tamaron®). None of the oil-based products tested caused mortality that would place them in the highest IOBC side effect category (i.e. did not cause mortality of <79%). However, some products were moderately harmful to some species (i.e. caused mortality of 41-56%) (Table 5). Sapsucker Plus had the most consistent effects, never causing more than 20% mortality. Hoverflies were the most tolerant of the beneficial species tested, surviving well in all direct spray trials and most residue trials.

Table 5: Mean percent mortality (95% confidence intervals) for each product. Means that differ significantly ($P < 0.05$) from the water control in that column are marked with an asterisk (*). NA (not applicable) indicates that a species/product combination was not tested.

	Tasmanian lacewing larvae		Eleven-spotted ladybird adults		Small hoverfly larvae	
	Direct	**Residues**	**Direct**	**Residues**	**Direct**	**Residues**
Water (low control)	16 (3-58)	4 (1-20)	0 (0-0)	0 (0-0)	0 (0-0)	2 (0-23)
Tamaron® (high control)	98* (71-100)	96* (85-99)	97* (93-99)	97* (76-100)	97* (83-100)	100* (100-100)
Organic JMS Stylet Oil®	55* (28-85)	23* (12-41)	0 (0-0)	56* (32-83)	7 (1-28)	3 (0-33)
Excel® Oil	47* (22-80)	11 (4-28)	0 (0-0)	41* (20-71)	0 (0-0)	0 (0-0)
Sapsucker Plus	19 (4-61)	7 (2-23)	0 (0-0)	9 (2-44)	14 (5-36)	7 (1-32)
Thunderbolt	NA	7 (1-49)	0 (0-0)	NA	NA	NA
Avid®	NA	NA	NA	NA	7 (1-28)	44* (29-64)
Avid® + Eco-Oil®	NA	NA	NA	NA	14 (5-36)	63* (43-83)

3.1 Tasmanian lacewing

Lacewing larvae were tolerant of residues of most of the oil-based products (except Organic JMS Stylet Oil®), but were moderately susceptible to direct spray applications of Organic JMS Stylet Oil® and Excel® Oil (Figure 1, Table 5).

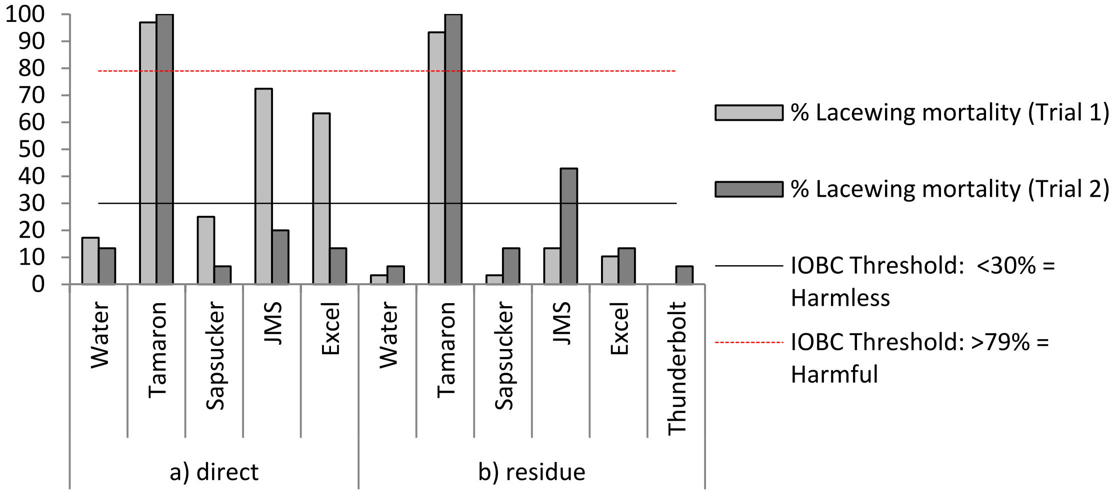

Figure 1. Mortality of Tasmanian lacewing larvae following a) direct spray application of oil-based products and b) exposure to residues on leaf surfaces. Assessments were carried out after 72 hours.

3.2 Small hoverfly

Direct sprays and residues of oil-based products had little effect on the larvae of the small hoverfly (Figure 2, Table 5). The insecticide Avid® caused little mortality as a direct spray, but Avid® residues were moderately harmful (Figure 3, Table 5). The addition of Eco-Oil® did not substantially alter the effects of Avid® (direct assay P = 0.42, residue assay P = 0.16).

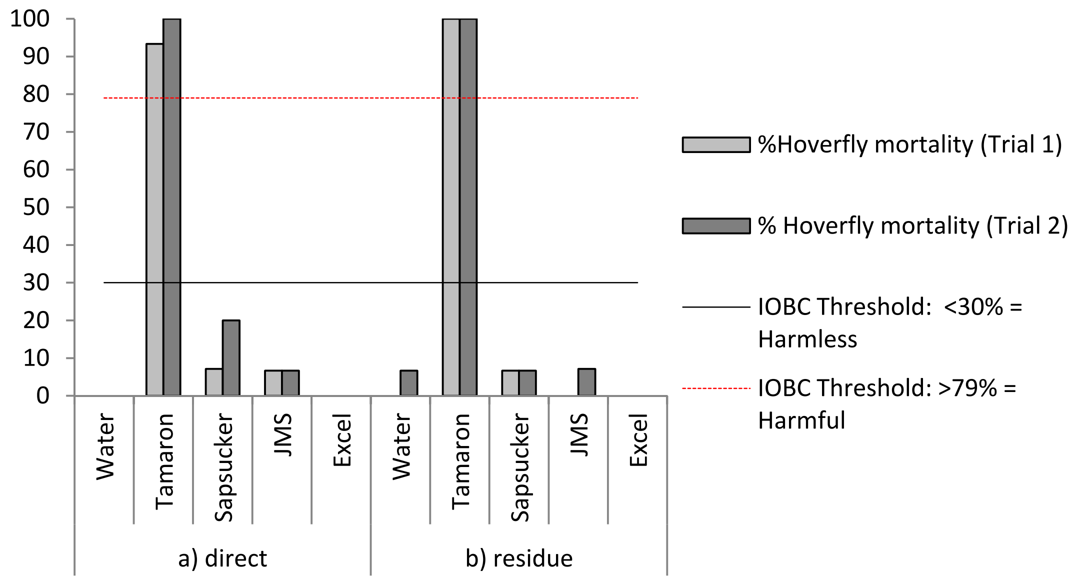

Figure 2. Mortality of small hoverfly larvae following a) direct spray application of oil-based products and b) exposure to residues on leaf surfaces. Assessments were carried out after 72 hours.

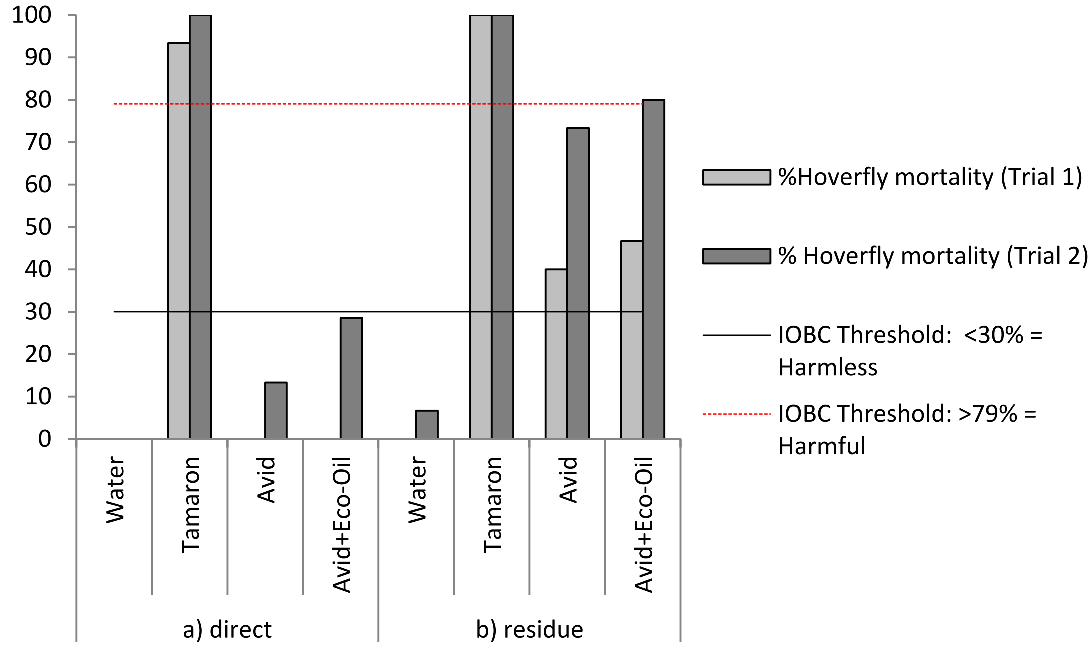

Figure 3. Mortality of small hoverfly larvae following a) direct spray application of Avid® and b) exposure to residues on leaf surfaces. Assessments were carried out after 72 hours.

3.3 Eleven-spotted ladybird

Direct spraying of oil-based products had no discernible effect on adult ladybirds (Figure 4, Table 5). Residues of Organic JMS Stylet Oil® and Excel® Oil did affect the ladybird adults, but results varied considerably between trials (Figure 4).

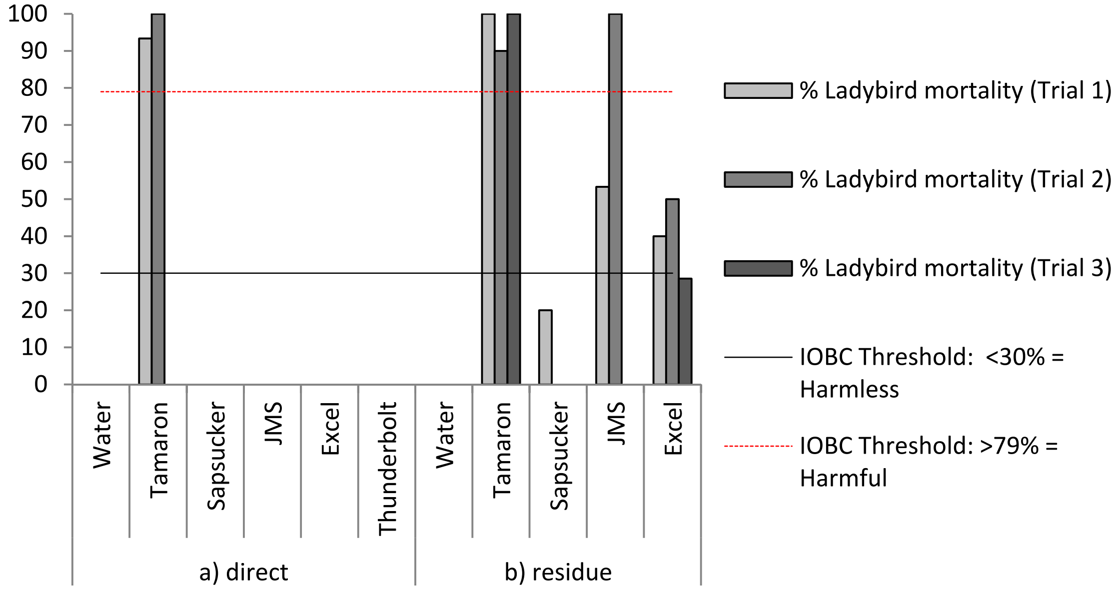

Figure 4. Mortality of eleven-spotted ladybird adults following a) direct spray application of oil-based products and b) exposure to residues on leaf surfaces. Assessments were carried out after 72 hours. Note that there were two direct trials and three residue trials.

4 Discussion

Mineral oils (derived from the petroleum industries) have long been used in the management of insect pests, both as adjuvants and as insecticides. Mineral oils kill insects by physically interfering with their respiration and their effectiveness as insecticides depends on the physical properties of the oil (e.g. viscosity) as well as the timing and coverage of the oil applications (Perring et al. 1999). Mineral oils (especially paraffinic mineral oils such as Organic JMS Stylet Oil®) have also been used extensively to control plant viruses, especially non-persistent aphid-transmitted viruses such as PVY in potato crops (Perring et al. 1999; Al-Mrabeh et al. 2010). Mineral oils can reduce virus transmission in a variety of ways: by reducing the density of the insect vector, by altering the feeding behaviours of the insect vector when on the plant, or by repelling the insect vector from the plant (Perring et al. 1999; Al-Mrabeh et al. 2010). In contrast, essential oils are derived from plants and contain a variety of secondary plant metabolites (including monoterpenes) that protect the plant from herbivores and pathogens. Essential oils affect biochemical processes in insects in a variety of ways: they may be neurotoxic or they may act as insect growth regulators, disrupting the normal processes of development within the insect (Rattan 2010).

In recent years a range of oil products have been investigated in relation to the tomato potato psyllid (TPP), which vectors the bacterium *Candidatus* Liberibacter solanacearum (Lso). Yang et al. (2010) showed that a mineral oil (Sunspray®) and an essential oil mixture (BugOil) were both strongly repellent to TPP adults and reduced egg-laying. Field trials indicated that BugOil significantly reduced the incidence of Lso in potato tubers and plants (compared with untreated plants) (Liu & Zhu-Salzman 2010). Recent New Zealand SFF studies showed that two other mineral oil products (Organic JMS Stylet Oil® and Excel® Oil) and a monoterpene-based product (Sapsucker Plus) also repelled TPP and reduced nymph survival in laboratory trials (Dohmen-Vereijssen et al. 2012).

In this study we investigated the effects of oil-based products on beneficial insects, as non-target effects need to be well understood before these products are incorporated into TPP management programmes. It is difficult to make generalisations about oil impacts on beneficial insects, as information is patchy and effects vary between species (Tables 1 and 6). Of the products examined in the present study, Sapsucker Plus (and its re-formulation Thunderbolt) was the most benign, causing little or no mortality to any of the beneficial species tested (equivalent to an IOBC classification of N). The small hoverfly was the most resilient species tested, as larvae showed little or no mortality when exposed to the three oil-based products tested. Adult eleven-spotted ladybirds tolerated direct spraying well, but mortality was higher when exposed to residues of Organic JMS Stylet Oil® and Excel® Oil. This may have been because of differences in the health of the ladybird adults used in the assays, as adults collected directly from the field were used for the direct assays, whereas colony-reared adults were used for the residue assays. Both ladybird colonies established for this study declined in vigour over time (possibly because of pathogen infection), with high mortality in late-stage instars. Although the adults from these colonies survived the water treatment in the assays, colony adults may have been more susceptible to the oil treatments than normal if they were in poor health.

The insecticide Avid® (active ingredient: abamectin) was also examined in the present study. Abamectin is able to kill TPP adults rapidly and also has anti-feeding effects (Butler et al. 2011). As a result, it is used for TPP control in New Zealand and elsewhere. However, residues of abamectin are extremely toxic to some beneficial insect species (especially parasitic wasps; Tables 1 and 6). In the present study, one-day-old residues were found to be moderately harmful to hoverfly larvae, causing mortality of 44%-63% (with and without the adjuvant Eco-Oil® respectively). Previous work by Cole et al. (2010) showed Tasmanian lacewings (important for TPP control in New Zealand potato crops) are not harmed by abamectin. No information is available specifically on the eleven-spotted ladybird (another important predator in New Zealand), but related *Coccinella* species are not harmed by abamectin (Tables 1 and 6).

Table 6: International Organisation for Biological and Integrated Control of Noxious Animals and Plants (IOBC) classification of side effects of pesticides for selected oil-based products and insecticides. N= harmless or slightly harmful (field reduction 0-50%, laboratory mortality <30%); M = moderately harmful (field reduction 51-75%, laboratory mortality 30-79%); T= harmful (field reduction >75%, laboratory mortality >79%). Flower bug data from Biondi et al. (2012); remaining data from Boller et al. (2005).

Beneficial insect species	**Oil and monoterpene products**			**Insecticides**
	Rape seed oil (Codacide® for flower bugs, Telmion for others)	Paraffinic mineral oil (Ufo®)	Para-menthene (Nu-film-P®, a monoterpene adjuvant)	Abamectin (Cal-EX EW® for flower bugs, Vertimec® for others)
Predatory mites (*Phytoseiulus persimilis*)	M			T
Flower bugs (*Orius laevigatus*)	1 h residues: M 7 d residues: M 14 d residue: N	1 h residues: M 7 d residues: M 14 d residue: N	1 h residues: N 7 d residues: N 14 d residue: N	1 h residues: T 7 d residues: T 14 d residue: T
Lacewings (*Chrysoperla carnea*)	N			N
Ladybirds (*Coccinella septempunctata*)	N			N
Parasitoids (*Thrichogramma cacoeciae*)	T			T

Overall, results from the present study are encouraging, as they suggest that some oil-based products could be used in New Zealand TPP management programmes without jeopardising biological control. Further work is needed to examine the effects of field applications of these products. The results of this and previous studies also highlight the need for knowledge about the beneficial species present in a given crop, as effects of both oils and insecticides vary considerably between species.

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