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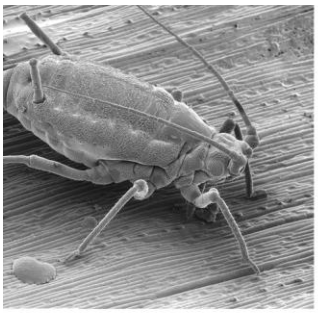
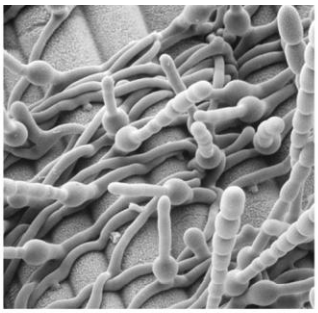
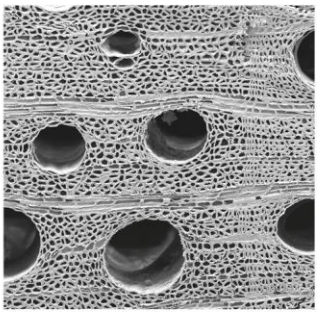
Effect of selected biorational insecticides and conventional insecticides on transmission of *Candidatus* Liberibacter solanacearum by tomato potato psyllid (*Bactericera cockerelli*) on potato plants

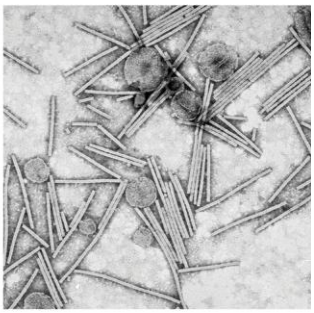
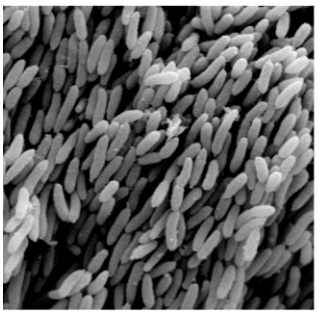
Barnes AM, Butler RC & Vereijssen J

June 2013

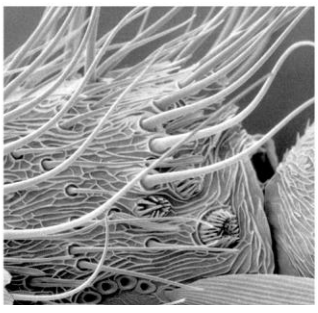
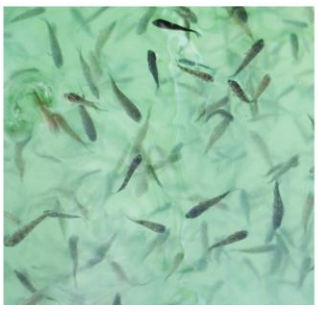
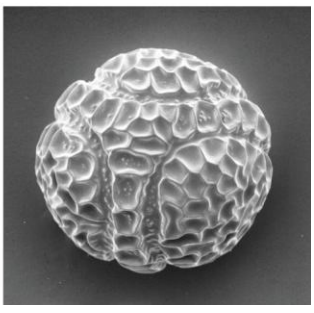






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June, 2013

Report for:
Potatoes New Zealand

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PFR SPTS No. 8516

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Executive summary

Effect of selected biorational insecticides and conventional insecticides on transmission of *Candidatus* Liberibacter solanacearum by tomato potato psyllid (*Bactericera cockerelli*) on potato plants

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June 2013, SPTS No. 8516

This study was conducted in Milestone 6a 'Liberibacter transmission studies' in the second year of SFF 11/058 'IPM tools for psyllid management'. The two trials were carried out in the period November 2012 until May 2013 at Plant & Food Research Lincoln.

Previous studies in this SFF programme showed that selected conventional and biorational insecticides have potential to disrupt tomato potato psyllid (TPP) feeding and behaviour, and increase mortality. However, the potential for these (biorational) insecticides to disrupt *Candidatus* Liberibacter solanacearum (Lso) transmission, which leads to zebra chip disease in potato, is not clearly understood and most studies only investigate the repellent effect on the psyllids. If biorational insecticides can replace some conventional insecticide applications in the growing season or can be added to increase insecticide effectiveness, the aim to reduce insecticide applications, reduce the risk of insecticide resistance and develop a successful Integrated Pest Management (IPM) programme in potatoes can be achieved. Therefore, conventional and biorational insecticides that showed promising results in the previous behavioural and mortality studies were further tested in the second year of this project to investigate the potential of selected biorational insecticides and conventional insecticides to disrupt Lso transmission.

A preliminary trial, with Avid® and water only, was conducted to test if the set-up and protocol would work. In the main trial, individual potato plants were sprayed with Organic JMS Stylet Oil®, Excel Oil®, Sap Sucker Plus (re-formulated as Thunderbolt), Sparta™, Movento™ and Benevia Cyazypyr™, Avid® (positive control) and water (negative control) which were then placed in a controlled environment growth room. TPP were taken from an Lso-positive colony in Lincoln and a subsample was tested for Lso quantity using qPCR. Twenty-four hours after applying the treatments, 10 TPP were released on the individual plants, and sprayed off with Avid® after 24 h. Four weeks after spraying off the psyllids, stem and stolon samples were tested for Lso using qPCR. This growth-room trial showed that (biorational) insecticides can decrease Lso inoculation of potato plants by TPP. The quantity of Lso transmitted to the potato plants was lowest with the Excel Oil® and Avid® treatments and highest with the Benevia Cyazypyr™ and Water treatments. Lso quantities and ratios were significantly lower than water (negative control) for Excel Oil® and Avid® ($P < 0.05$), and only Water and Benevia Cyazypyr™ were significantly higher than Avid® (positive control) ($P < 0.05$). Within this current SFF programme and the previous programme (SFF09-143) several bioassays have been conducted to test (biorational) insecticides on TPP behaviour and mortality and their effect on beneficials (summarised in Table 2). There are still some gaps for the different treatments which have to be filled in to be able to formulate spray advice at different times in the growing season. A field trial in Canterbury in 2012–13 showed there was no phytotoxicity from Organic JMS Stylet Oil® or Excel Oil® applications, which is a promising result for use of these oils in an IPM programme. No single treatment scores positively across all studies, but Avid® showed promise for inclusion in an IPM programme, as did Excel Oil®, Sap Sucker Plus and Organic JMS Stylet Oil® (in order of promise).

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1 Introduction

Since the tomato potato psyllid, *Bactericera cockerelli* (Šulc) (TPP), was first recorded in New Zealand in 2006 it has caused problems for the capsicum, tomato, potato and tamarillo industries. TPP has led to a considerable increase in insecticide applications in the horticultural industry and thus presents a serious challenge to the implementation of Integrated Pest Management (IPM) strategies (Teulon et al. 2009). TPP vectors the bacterial pathogen *Candidatus* Liberibacter solanacearum (Lso) which has been identified as the cause of 'zebra chip' disease in potato tubers (Munyaneza et al. 2007; Liefting et al. 2009). Current TPP pest management practices in New Zealand potato crops rely on regular applications of often broad-spectrum insecticides. These practices are not only costly but are likely to have a negative impact on the environment and beneficial insect species, while increasing the potential for insecticide resistance in pest populations. As part of a sustainable IPM approach, the use of selective, cost-effective and environmentally sensitive products is therefore preferred. Biorational insecticides fit this description and are described "as any type of insecticide active against pest populations, but relatively innocuous to non-target organisms, and, therefore, non-disruptive to biological control" (Stansly et al. 1996). Traditionally, soaps/detergents, oils and botanicals have been termed biorational, but the biorational status of new insecticides is also determined (Schuster & Stansley 2005). Biorational insecticides may affect life stages of psyllid species by direct mortality, or by repellence of adults, deterrence from settling, feeding and/or ovipositing (Al-Jabr 1999; Berry et al. 2009; Marčić et al. 2009; Yang et al. 2010; Boina et al. 2011; Tiwari et al. 2012; Prager et al. 2013). A number of biorational options are already on the market in New Zealand, but to date they have mainly been considered for use in control of TPP on greenhouse crops (Walker et al. 2010; Walker et al. 2011).

This study was conducted in year 2 of SFF 11/058 'IPM tools for psyllid management'. In year 1 it was shown that selected conventional and biorational insecticides have potential to disrupt psyllid feeding and behaviour, and increase mortality (Dohmen-Vereijssen et al. 2012; Gardner-Gee et al. 2012). However, the potential for these (biorational) insecticides to disrupt Lso transmission is not clearly understood and most studies only investigate the repellent effect on the psyllids (Yang et al. 2010; Butler et al. 2011; Peng et al. 2011; Diaz-Montano & Trumble 2013; Prager et al. 2013). If biorational insecticides can replace some conventional insecticide applications in the growing season or can be added to increase insecticide effectiveness, the aim to reduce insecticide applications, reduce the risk of insecticide resistance and develop a successful IPM programme in potatoes can be achieved. Therefore, conventional and biorational insecticides that showed promising results in the previous behavioural and mortality studies were further tested in the second year of this project. Concurrently, Dr Robin Gardner-Gee studied the effect of the selected biorational insecticides on the main beneficial insects that control TPP in New Zealand.

1.1 Aim

To investigate the potential of selected biorational insecticides and conventional insecticides to disrupt Lso transmission.

2 Material and methods

Two trials were conducted, a preliminary and a main trial. The preliminary trial, with Avid® and water only, was conducted to test if the set-up and protocol would work. The outcome of the preliminary trial would ideally have determined the main trial set-up, but due to delayed analyses this was not possible.

2.1 Insect source and rearing

Adult TPP used in the trials were sourced from a laboratory colony at Plant & Food Research, Lincoln, Canterbury. This colony originated from field-collected adult TPP from a potato crop in Pukekohe. TPP were reared on tomato plants (*Lycopersicon esculentum* 'Money Maker') in a controlled environment growth room at 20.5°C, 16:8 light:dark photoperiod and 41% RH. The Lso status of this colony was determined by qPCR testing just before the psyllids were used in the trials. They were confirmed Lso positive for each trial (data not shown). TPP were not sexed before use in either trial.

2.2 Plant material

In vitro tissue culture potato plantlets (*Solanum tuberosum* 'Russet Burbank' – guaranteed Lso free) were transplanted into individual 1.5 L pots containing standard potting mix and placed in white 2 L plastic containers to allow watering (preliminary trial – 26 November 2012; main trial – 19 February 2013). The plants were then grown on to a vegetative growth stage (6–10 leaves) over 5 weeks in a controlled environment growth room at 25°C, 16:8 day:night photoperiod and 41% RH. Plants were watered twice-weekly.

2.3 Insecticide selection and application

After the 5-week growth period, whole individual potato plants were sprayed with either a biorational insecticide or conventional insecticide treatment. Insecticide selection was based on the results of the mortality and behaviour bioassays in Year 2 (Dohmen-Vereijssen et al. 2012; Gardner-Gee et al. 2012) and the biorational insecticide review conducted in Year 1 of this project (Berry & Bourhill 2012).

The insecticide Avid® was applied in the preliminary trial, with water as a control. Biorational insecticides applied in the main trial were Organic JMS Stylet Oil®, Excel Oil®, Sap Sucker Plus (re-formulated as Thunderbolt), and the insecticides were Sparta™, Movento™ and Benevia Cyazypyr™. Avid® (as a positive control) and water (as a negative control) were also included, giving a total of eight treatments (Table 1). One litre of each treatment was prepared according to label rates (Table 1) and applied to the plants via a 2 L hand sprayer.

2.4 Introduction of psyllids to plants

Treatments were applied to the potato plants after the 5-week growth period (preliminary trial – 14 January 2013; main trial – 25 March 2013). After 24 h, fibreglass support stakes and fine insect-proof mesh bags were used to enclose each plant. Seven (preliminary trial) or ten (main trial) Lso-positive TPP were then introduced to the individually bagged plants and the bags were secured around the base of each pot with tape (preliminary trial – 15 January; main trial – 26 March (Figure 1)). TPP were subsequently left on the plants for a 24-h period. The bags were then removed and the adults and eggs sprayed off with Avid®, allowing the plants to continue to grow without psyllid or Lso pressure. Twenty-four hours after spraying off the insects, the plants were inspected for any surviving TPP before being moved back to the growth room. The plants were grown for a further 4 weeks before sampling (Figure 2).

Table 1: Treatments used in Lso transmission studies.

Trade name	Abbrev-iation	Active ingredient(s)	Mode of action	Formulation	Field rate
Organic JMS Stylet Oil®	J	Mineral oil + adjuvant	Inhibits insect respiration	971 ml/L EC	1.5 L/100 L
Excel Oil®	E	Mineral oil	Inhibits insect respiration	843 g/L EC	1 L/100 L
Sap Sucker Plus (re-formulated as Thunderbolt)	S	Oxygenated monoterpenes, neem oil, dispersants and adjuvants	Antifeedant and insect growth regulator	Information not accessible	240 g/12 L
Benevia Cyazypyr™	C	Cyantraniliprole	Impairs insect muscle function; induces feeding cessation	100 g/L OD	100 ml/100 L
Sparta™	Sp	Spinetoram	Nerve disruptor; causes hyperexcitation of insect nervous system	120 g/L	100 ml/100 L
Movento™	M	Spirotetramat	Lipid biosynthesis inhibitor	240 g/L SC	112 ml/100 L
Avid® (positive control)	A	Abamectin	Nerve disruptor; induces feeding cessation	18 g/L EC	90 ml/100 L
Water (negative control)	W	-	Negative control	-	-

Figure 1: Caged potato plant used in transmission trials.

Figure 2: Potato plant at maturity just prior to harvest.

Stem and stolon samples were taken from the potato plants 4 weeks after the TPP were sprayed off (preliminary trial – 18 February; main trial – 26 April). Plants were taken from their pots individually, with the soil removed carefully from the root system. For the preliminary trial, approximately 1 cm of stem tissue was removed from the portion of the main stem found just below soil level, using a pre-sterilised scalpel, which was re-sterilised between plants. For the main trial, where tubers were present, approximately 3 cm

of stolon tissue was excised from the region between the tuber and the main stem. Where tubers had not yet formed, approximately 1 cm of below-ground stem tissue was removed as described for the preliminary trial. Additionally, the growing tip of each plant (approximately 5 cm, including 2–3 of the youngest leaves) was removed and placed in a labelled plastic bag. This was a back-up sample, in case the other sample would give an unreliable result. Each of the tissue samples was placed in a separate labelled plastic bag and immediately frozen at -20°C prior to qPCR analysis.

To prepare the stolon / below-ground stem tissue samples for qPCR analysis, each tissue section was placed on a Petri dish and the soiled outer skin scraped off. Sub-samples of the sample were tested using qPCR and Lso-specific primers. All samples were tested in triplicate.

2.5 Trial design

2.5.1 Preliminary trial

Each treatment was applied to a single potted potato plant and replicated 10 times (20 pots total). Pots were laid out on benches in a completely randomised design (Figure 3). Controlled environment growth room conditions were held at 20.5°C, 16:8 day:night photoperiod and 41% RH for the duration of the trial.

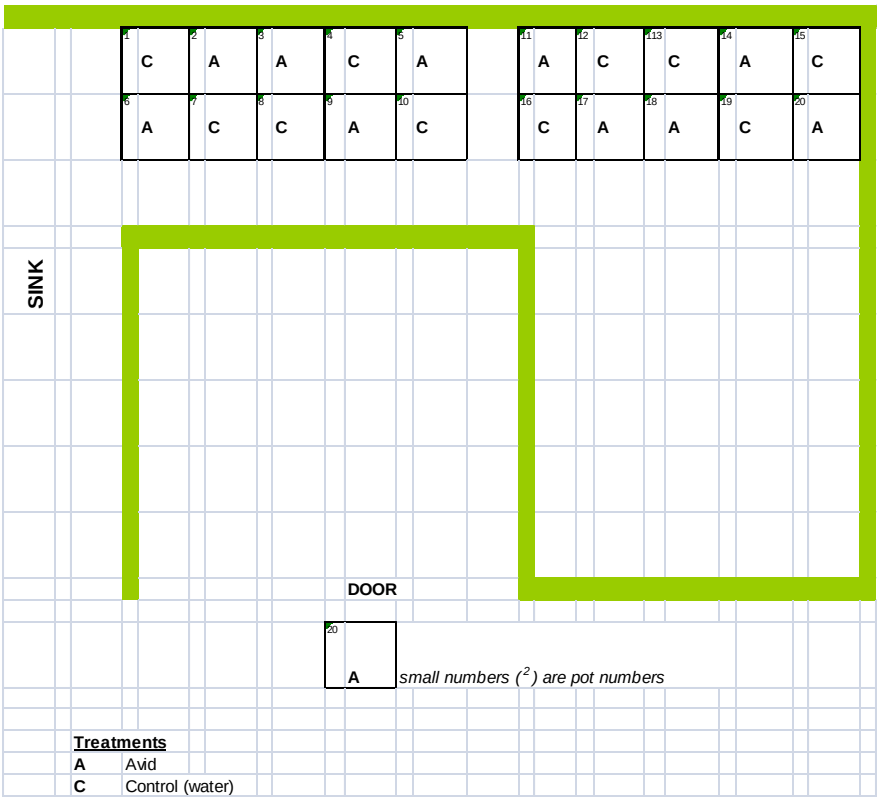

Figure 3: Layout of pots in the controlled environment growth room for preliminary trial. Green lines indicate bench edges.

2.5.2 Main trial

Each of the eight treatments was applied to a single potted potato plant and replicated four times (32 pots total). Pots were laid out on benches in a Latinized resolvable block design (Figure 4), constructed using

CycDesigN (CycSoftware 2009). Controlled environment growth room conditions were as in the preliminary trial.

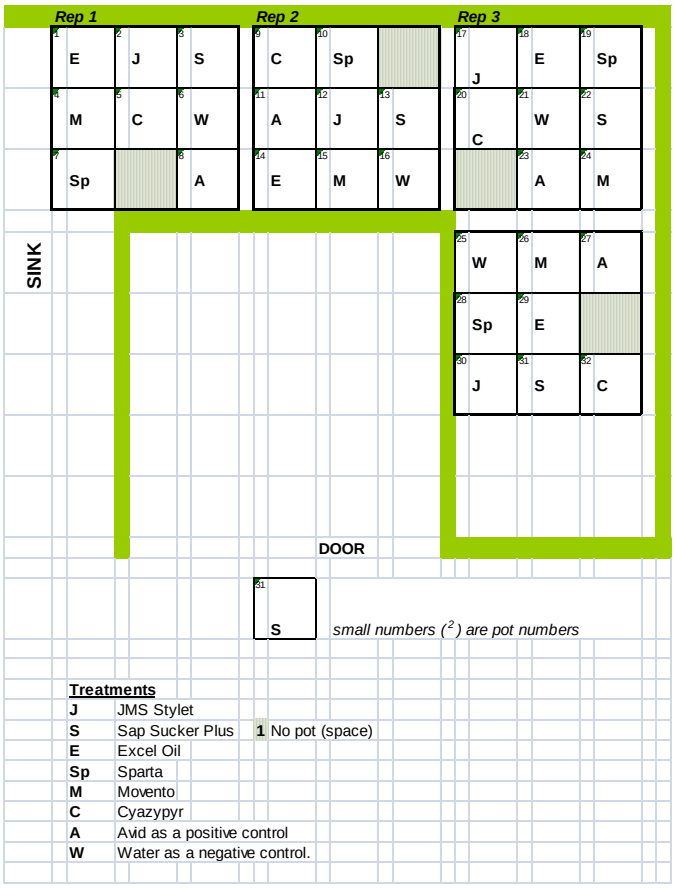

Figure 4: Layout of pots in the controlled environment growth room for main transmission trial. Green lines indicate bench edges.

2.6 Statistical analysis

For both trials, qPCR was used to estimate the quantity of Lso and the reference gene EF1α was used in the main trial only. In the main trial, two runs per gene were carried out, with material for pots 1–16 placed on one plate per gene and pots 17–32 on the second plate. Material from each pot was inoculated in triplicate (three wells per pot). Each plate also contained a set of six calibration standards, as well as the appropriate controls, all in triplicate.

The analysis of the qPCR data was carried out in two stages: calibration regressions/conversion of cycle threshold (Ct) numbers to quantities, and analysis of the sample quantities. This gave the quantities in each well as copies per microlitre (µL), since the quantities for the standards were also in these units. Wells with 'Undetermined' Ct were assumed to have Quantity = 0. For the data analysed in this trial, the ratio of Lso to EF1α quantities was of most interest.

The Poisson-Gamma hierarchical generalised linear model approach (HGLM, Lee et al. 2006) was used, with logarithmic links. This enables potential sources of extra variation, such as spatial position and between pots ('random effects') to be taken into account. It also allows the undetermined (quantity = 0)

data to be included, whilst still carrying out an analysis on the log(Quantity) scale. The importance of the random effects was assessed with a X^2 test of the change in likelihood on dropping the effect, as implemented in GenStat's HGRTEST procedure (GenStat Committee 2012), and fixed effects (treatments) were assessed with a similar test using GenStat's HGFTEST procedure. Only pot to pot variation was found to be important so only pots were included in the final analyses as a random effect.

For the main trial, data for both genes was analysed simultaneously, using Treatment, Gene and the interaction between them as fixed effects. Ratios become differences on the log scale: therefore the analysis of both genes together allowed the ratio to be indirectly analysed in addition to effects of the two genes separately. The variance for each of the two genes was estimated separately, as was the pot to pot random effect. In the results presented, means were obtained on the transformed (log) scale along with 95% confidence limits and were back-transformed for presentation. All analyses were carried out with GenStat (GenStat Committee 2012).

3 Results

3.1 Preliminary trial

The qPCR calibration regressions fitted the data well (R^2 > 99%). Of the 10 plants treated with water, eight plants tested positive for Lso using qPCR, one plant tested negative and one plant gave an unreliable result. Of the 10 plants treated with Avid®, two plants tested positive, five plants tested negative and three plants gave an unreliable result.

Although the results above allowed for discrimination between treatments, Lso quantities did not vary significantly between Avid® and water treatments in this trial (P = 0.456).

3.2 Main trial

The qPCR calibration regressions fitted the data well (R^2 > 99%) (data not shown), and the regressions for the two plates for each gene were very similar. There were no undetermined values for the EF1α gene. For the Lso gene, there were 25 undetermined values, and five that were missing (the qPCR process had not worked correctly). However, there was no treatment for which *all* values were undetermined or missing, indicating that the Lso quantity was not completely zero for any treatment. Ct variation was considerably smaller for the Ef1α gene than for the Lso gene, with the EF1α Ct being quite similar both within and between pots. Lso Ct varied noticeably between the wells for different pots for the same treatment, but varied much less between wells from the same pot. This most likely reflects the fact that the EF1α gene is naturally present in the plant, whilst the presence of the Lso gene depends on the behaviour of TPP and also that Lso titre varies between individual TPP.

DNA quantities did not vary substantially between the treatments for the reference gene EF1α (P = 0.898), but there were some significant treatment differences in quantities for the Lso gene (P = 0.028). Consequently, there were also some differences in the ratio of the quantities for the two genes (P = 0.051). Lso quantities were smaller than those for EF1α in all cases, ranging from just 0.16% to over 50% of the quantities for EF1α.

The quantity of Lso transmitted to the potato plants was lowest with the Excel Oil® and Avid® treatments and highest with the Benevia Cyazypyr™ and Water treatments. Lso quantities and ratios were significantly lower than water (negative control) for Excel Oil® and Avid® (P < 0.05), and only Water and Benevia Cyazypyr™ were significantly higher than Avid® (positive control) (P < 0.05) (Figure 5).

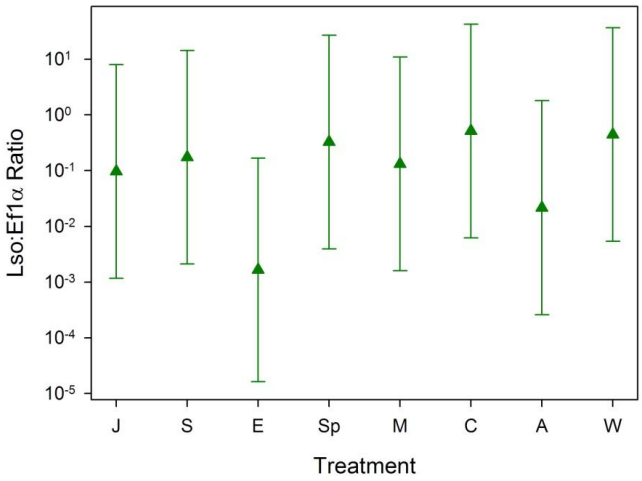

Figure 5: The ratio between Lso and EF1α genes per treatment, where a smaller ratio indicates a lower quantity of Lso gene present in the sample. Error bars are approximate 95% confidence intervals for the means. Please refer to Table 1 for treatment abbreviations.

4 Discussion

This study showed that (biorational) insecticides can decrease Lso inoculation of potato plants by TPP. The most effective treatments were Excel Oil®, a biorational option, and Avid®, a conventional insecticide, although no treatments completely reduced inoculation of potato plants with Lso. The reduced transmission observed for Excel Oil® is a great addition to the already promising results obtained in the other bioassays (Table 2). Avid® is non-systemic, translaminar product which mode of action consists of paralysing the insect (nerve disruptor), eventually causing death (may take 7 days to reach maximum effectiveness). Additionally, it induces feeding cessation, which may have led to the results obtained in this study.

The results with Benevia Cyazypyr™ may have been caused by the age of the product we used (the same batch has been used for the Residual activity and the TPP soft chem studies (Table 2)) or the fact that Benevia Cyazypyr™, although being systemic, is xylem mobile. TPP is a phloem feeder, but does access the xylem (Pearson et al. 2010), but this is very unpredictable and only a small percentage feed on xylem, especially after being put from one host plant species onto another (Sandanayaka M, pers. comm.). If an adult goes to phloem straight away, they will not go into xylem. If they do not go into phloem immediately, then they are more likely to feed on xylem (Sandanayaka M, pers. comm.). Since the TPP were reared on tomato and the trials were carried out using potato, some xylem feeding could have been expected.

Chemical applications are not always effective and can lead to increased transmission and disease spread and there is increased risk of insecticide resistance because of incorrect use and mismanagement (Weintraub 2012). In case of TPP, insecticides are applied often (calendar spraying on a weekly interval) and also prophylactically, which may not always lead to the best results. For semi-persistent and persistent transmitted pathogens, which is very highly likely the case for TPP and Lso, the vector needs more time feeding on a plant to be able to transmit the pathogen and it is thus exposed to the insecticide for longer, which increases the effectiveness of the insecticide (Weintraub 2012). Oils have been reported to reduce vector numbers which subsequently led to a decrease in disease incidence of persistently and semi-persistently transmitted pathogens (references in Perring et al. 1999), but whether this was due to vector mortality or to avoidance by vectors of treated plants is unclear (Perring et al. 1999).

Table2: Summary of studies conducted on (biorational) insecticides in the SFF11/058 and SFF09/143 programmes.

Trade name	This study[a]	SFF11/058 Effect on beneficials[b]	SFF11/058 Residual activity[c]	SFF09/143 Residual activity[d]	SFF11/058 TPP soft chem[e]
Organic JMS Stylet Oil®	Very slight reduction in Lso transmission	Direct mortality lacewing (55%), residue mortality lacewing (23%) and ladybird (56%)	Not tested	Not tested	Highly repellent, high nymph mortality after 2 sprays
Excel Oil®	Reduced Lso transmission	Direct mortality lacewing (47%), residue mortality ladybird (41%)	Not tested	Not tested	Repellent, high nymph mortality after 2 sprays
Sap Sucker Plus (re-formulated as Thunderbolt)	Very slight reduction in Lso transmission	<20% mortality in tested beneficials	Not tested	Not tested	Highly repellent, high nymph mortality after 2 sprays
Benevia Cyazypyr™	No reduction	Not tested	Reduced egg lay 1+14 DAT, increased adult mortality 1+14 DAT	Not tested	Not tested
Sparta™	No reduction	Not tested	Reduced egg lay 1 DAT only, reduced feeding 1 DAT only. Increased adult mortality 1 + 14 DAT	Reduced egg lay up to 21 DAT, increased adult mortality up to 28 DAT	Not tested
Movento™	Very slight reduction in Lso transmission	Not tested	Reduced egg lay up to 7 DAT, no significant effect on adult mortality at 1, 7 or 14 DAT	Not tested	Not tested
Avid® (positive control)	Reduced Lso transmission	Residue mortality hoverfly (44%)	Not tested	Reduced egg lay up to 7 DAT, increased adult mortality up to 17 DAT	Not tested

[a] reduction compared to water treatment
[b] please refer to Gardner-Gee et al. (2013)
[c] please refer to Gardner-Gee et al. (2012)
[d] please refer to Page-Weir et al. (2012)
[e] please refer to Dohmen-Vereijssen et al. (2012)

In light of very mixed or unexplained results using oils to control vectors, two alternative hypotheses have been proposed by Perring et al. (1999). The first hypothesis is that oils change the pathogen/vector relationship, since the effect of oils on transmission depends largely on the transmission characteristics of the pathogen. The second one is that the infection process is impeded even though the pathogen is transmitted and may even lead to altered plant physiology so that symptoms are not expressed.

A good understanding of the host-vector-pathogen relationship is needed to understand whether the insecticide is going to be applied to primary or secondary infective vectors. Most insecticides are not fast enough to kill the primary vectors, but secondary vector numbers may be reduced and thus secondary pathogen transmission. An epidemic usually consists of these two types of infective vectors, but one of these will predominate. If spread is mainly primary, an insecticide application to the crop is rarely effective (in Perring et al. 1999). This is mostly valid for non-persistently transmitted pathogens, vectored by a non-colonising, transient vector. Applying insecticides is more effective when spread is mainly secondary and vectors accept the plants in the field as host plants. Then the vector will colonise the plants and is less likely to fly out again and consequently infect more plants. This latter situation seems to be true for TPP.

Systemic insecticides that reside in the phloem could kill the vector of persistently transmitted pathogens before pathogen acquisition or inoculation (e.g. Powell & Mondor 1973). Non-persistent transmitted pathogens are acquired from and inoculated to epidermal cells, so the vector does not ingest the insecticide and control is therefore inconsistent.

A successful insecticide not only kills the vector, but also disrupts the relationship among vectors, plants and pathogens (e.g. repellence, modified behaviour). In conclusion, the effectiveness of insecticides against vectors of plant pathogens is variable and the assessment by Broadbent (1957) of the limitations of insecticides in these systems still holds today (Perring et al. 1999).

Within this current SFF programme and the previous programme (SFF09-143) several bioassays have been conducted to test (biorational) insecticides on TPP behaviour and mortality and their effect on beneficials (summarised in Table 2). There are still some gaps for the different treatments which have to be filled in to be able to formulate spray advice at different times in the growing season. A field trial in Canterbury in 2012–13 showed there was no phytotoxicity from Organic JMS Stylet Oil® and Excel Oil® applications (Munro P, unpubl. data), which is a promising result for use of these oils in an IPM programme. No single treatment scores positively across all studies, but Avid® showed promise for inclusion in an IPM programme, as did Excel Oil®, Sap Sucker Plus and Organic JMS Stylet Oil® (in order of promise).

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