

Screening organic miticides for spider mite control for organic greenhouse
vegetable production

FINAL REPORT with revisions as per BCGGA Research Committee
to:

BC Greenhouse Growers Association
Organic Sector Development Program
Origin Organics

from
Renée Prasad, Karina Sakaloukas, and Megan Willems
ES Cropconsult Ltd
3041 W. 33rd Avenue
Vancouver, BC, V6N 2G6

EXECUTIVE SUMMARY

Four pesticides suitable for organic vegetable production were tested under a variety of conditions for control of two-spotted spider mites on greenhouse pepper, tomato and cucumber. The four products were JMS Stylet Oil a paraffin-based horticultural oil; Pure Spray Green a horticultural mineral oil; AzaDirect a botanical pesticide with the active ingredient derived from neem trees; and Naturalis-L a formulation of the entomopathogenic fungus *Beauveria bassiana*. All four products were tested in a screening trial on tomato, pepper and cucumber. Products were applied onto plants twice, at a seven day interval. A greenhouse efficacy trial was conducted using AzaDirect, as this is the product most likely to be registered and available to growers in the near future. Finally, a study of the toxicity of the four miticides to natural enemies was also conducted. The key findings of this study were:

- In the screening trials on all three crops JMS Stylet Oil and Pure Spray Green effectively suppressed the mite population within 12 days, compared to No Spray or Water based control treatments.
- AzaDirect performed as well as JMS and Pure Spray on peppers and tomatoes, but did not suppress mites as well as the oils on cucumber.
- Naturalis-L had moderate levels of efficacy in the screening trials
- No phytotoxicity, on any crop from any of the four products, was observed during the trials however greenhouse temperature did not exceed 30°C.
- In a follow up efficacy trial AzaDirect was tested at 0.5X, 1X and 1.5X the label rate on tomato. At 1X and 1.5 X rates mite suppression was not observed until 5-days after the second spray. There was no suppression of mites observed with 0.5X rate.
- There was no toxic effect of the four miticides on *Phytoseiulus persimilis*, either applied directly to mites in vermiculite carrier or as 24 and 48-h old residue on leaves.
- Thus the miticides used in the trial could be followed up with predator mite releases within 24-h to effectively suppress a spider mite hot-spot using an integrated approach.
- Label directions for all four products recommend repeat applications on a 5 to 10-day interval; however follow-up sprays may not always be necessary for if *P. persimilis* can be released 24-h after sprays.
- There was no toxic effect of direct application of the four miticides on *Amblyseius cucumeris*, but JMS Stylet Oil and Pure Spray Green did suppress emergence of *Encarsia formosa* adults. Thus caution should be used to avoid direct exposure of other enemies.
- Registration of more than one of these products is critical in order for growers to have access to products with different modes of action in order to delay resistance.

EXTENSION AND OUTREACH SUMMARY

The results of this project were presented to growers at the COABC Annual Meeting (February 21, 2009, Abbotsford, BC). Greenhouse growers have access to this report through the BCGGA website and organic growers will have access through the COABC website. This report and raw data have been shared with Petro Canada (registrant for Pure Spray Green) and Gowan (registrant for Azadirect).

INTRODUCTION AND OBJECTIVES

Spider mites, *Tetranychus urticae*, are a ubiquitous agricultural pest capable of causing significant yield loss and death of plants. Fortunately, the natural enemies of spider mites are equally common and can be utilized for biological control either through enemy conservation or through releases of mass-reared natural enemies (van Lenteren and Woets, 1988). In the greenhouse environment the efficacy of released natural enemies depends on a number of different factors. First, natural enemy performance varies on different crops, for example predator mites like *Phytoseiulus persimilis* are less effective on tomatoes, because of leaf hairs, than pepper and cucumber. Second, enemy effectiveness also depends on the size of spider mite population - enemies are often not able to bring down a large spider mite outbreak quickly enough to prevent economic damage. Different approaches can be used to improve natural enemy impact on spider mites. One effective tactic is to treat spider mite "hot spots" - small areas with large pest populations - first with miticides sprays and then follow up with natural enemy releases in the hot spot. This approach can be especially efficient if miticides with low toxicity to predatory mites are used, e.g. Floramite and Forbid, as predators can be released shortly after sprays to attack any remaining spider mites or their eggs. For organic greenhouse vegetable production, there are no OMRI (Organic Material Research Institute) approved miticides currently available in Canada. Thus organic greenhouse growers do not have the ability to employ the highly effective tactic of miticide followed by enemy release to suppress hot spots. Several OMRI-approved miticides are available to greenhouse growers in the US and we selected four of these products JMS Stylet Oil (organic formulation), Pure Spray Green, Naturalis-L and AzaDirect for evaluation of spider mite control on greenhouse plants. Information about each product is summarized below and in Table 1.

Oils: The mode of action of insecticidal oils like JMS and Pure Spray Green is primarily physical - by coating the exterior of the body arthropods oils interfere with respiration (Johnson 1988). Work conducted on pears in Washington state showed that a season long spray program with Pure Spray Green provided good control of 2-spot spider mites with approximately 1 adult or nymph/leaf by the end of the season in Pure Spray plots compared to almost 12 adults or nymphs/leaf in control plots (Brunner et al. 2007). Stansly and Conner (2005) found that Pure Spray Green suppressed whitefly, broad mites and aphids on tomato and pepper. In tomato trials in Florida, Pure Spray Green caused only slight phytotoxicity on tomatoes, i.e. phytotoxicity score of 0.1 to 0.4 on a 1 to 4 scale (Stansly and Conner 2005).

JMS Stylet oil is a paraffinic oil registered for mite control in the US and recommended for 2-spot control on several crops in California. JMS Stylet oil was shown to be highly repulsive to 2-spot spider mites on grape leaves in a trial by Walsh and Grove (2001). Another advantage of this oil is that it has also been shown to suppress some diseases, for example it caused a significant reduction in powdery mildew lesions on cucumber (McGrath and Shishkoff, 2000).

Biological control: The active ingredient in Naturalis-L is the entomopathogenic fungus *Beauveria bassiana*. Work conducted in the UK has shown that Naturalis-L was more effective than the synthetic miticide Vendex in controlling 2-spot spider mites on greenhouse tomatoes (Chandler et al. 2005). Further, Naturalis-L was not harmful to spider mite predator mites which could be released shortly after Naturalis-L application. Currently Naturalis-L is registered for

greenhouse use in Italy and Spain, with registrations pending in UK, Holland and France (S. Franceschini, personal communication).

Botanicals: Neem-based products have been shown to be effective at reducing spider mite populations. For example, Cote et al. 2002 found that neem oil caused significant mortality to spider mites, 40% dead compared to less than 10% dead in control. The effect of neem oil however was short-lived, as mortality was equal in control and neem treatments by 3 days. Neem oil residues were not toxic to predator mites (Cote et al. 2002). It is important to note that AzaDirect has a unique extraction process that results in no neem oil being present in the formulation (M. Brossom, personal communication). AzaDirect was shown to be more effective than neem oil in suppressing pests of potatoes and eggplants (El Shafie and Basedow, 2003)

The objectives of this study are

1. Screen four potential organic miticide products - JMS Stylet Oil, Pure Spray Green, Naturalis-L and AzaDirect - for spider mite control on greenhouse cucumber, pepper and tomato.
2. Examine the efficacy of one of the products - AzaDirect - at two different application rates.
3. Determine the toxicity of the miticides to predatory mites and other natural enemies.

Table 1. Additional information on the four organic miticides examined for control of spider mites on greenhouse vegetables.

Product	Manufacturer/Registrant - future status in Canada	Registration status (US and elsewhere)	Toxicity to natural enemies ¹
JMS Stylet Oil - Organic formulation	JMS Flower Farms Ltd. - manufacturer contacted via email and phone but no response	EPA exempt OMRI Approved	None available
Naturalis-L	Troy Biosciences - manufacturer is intending a Canadian registration but no timeline provided (See Appendix 1)	Registered for greenhouse use in Italy, UK and US EPA exempt OMRI Approved	None available
Pure Spray Green	Petro Canada - manufacturer wants Canadian registration but discouraged by registration requirements	EPA exempt OMRI Approved	Petroleum and mineral oils are moderately harmful to predator mites and harmless to <i>Encarsia formosa</i>
AzaDirect	Pronatex – to be registered in Canada for use in ornamentals in 2009 Gowan – to submit food use package in 2010	Registered as Azadirect in the US. EPA exempt OMRI Approved	Harmless to predator mites and <i>E. formosa</i> pupae, but moderately harmful to <i>E. formosa</i> adults

1. Information obtained from Koppert's Side Effects web site

MATERIALS AND METHODS

Changes to the originally proposed methodology: There were several changes from the original proposal to the project methodology. However, these changes did not prevent us from addressing the three objectives of the study. The rationale behind each change is summarized below.

1. Protocol for applying products. Originally we proposed to spray all products just once. However all of the labels recommended repeat sprays in order to achieve efficacy and indeed in our first set of trials on pepper we noticed that mite levels rebounded 5 days after the first spray. Thus in all of the trials all products were sprayed twice at seven day intervals.
2. Product to test for the efficacy trial. Originally we proposed to conduct the efficacy trial on the most effective product - which would have been either Pure Spray Green or JMS Stylet Oil. However, information from the manufacturer of Pure Spray Green indicated that they were not considering a Canadian registration at the time this study was being conducted (K. Chandler, personal communication). Despite many attempts we were not able to get any information from the manufacturer of JMS Stylet Oil on their plans for Canadian registration. The second most effective product on all crops was AzaDirect and this was the product we decided to pursue for the efficacy work, as a Canadian registration (for greenhouse ornamentals) is imminent.
3. The design of Objective 3 - Natural enemy toxicity trial - has been modified from that originally proposed in part because we encountered continual difficulties in recovering predator mites from tomato stems using the originally proposed methods - thus we were never able to confirm toxicity on predators. We modified the trial to first directly assess the impact of directly spraying *Phytoseiulus persimilis*, *Amblyseius cucumeris* and *Encarsia formosa* with miticide solutions. Second we examined survival of *P. persimilis* on tomato leaves 24-h and 48-h after application of miticides.

Screening trial: The miticide screening trial was conducted at the research greenhouses at the University College of the Fraser Valley (UCFV) Chilliwack campus. The trials were conducted from December 18, 2007 to January 25, 2008; average greenhouse conditions during this time were 21 to 25°C and 50%RH. All lighting was ambient, i.e. no supplemental lighting. Prior to the trial there was no significant spider mite activity in the greenhouse, thus mites introduced for this trial were the main source of pests. Other than those used for the trial no pesticides were used in the greenhouse during the trial.

Plants used for the trial were obtained from Bevo Farms (Langley, BC), and were approximately 50cm high at the time of the trial. Trials with each species - tomato (var. Starbuck), cucumber (var. Camaro), and pepper (var. Fantasy) - were conducted at different times and are thus considered to be three independent trials. Plants were infested with spider mites on the day that they were set up at the UCFV greenhouse. Spider mites were obtained from The Bug Factory (Nanose Bay, BC) and were on bean leaves. Bean leaves with mites were placed on plants and mite infestation levels were checked every 48-h until a sufficient number of mites were observed on plants, the amount of time from infestation to start of trial was

approximately 1 week. Prior to making the first (pre-spray count) of spider mites on plants we randomly assigned all plants to treatments.

The four miticide treatments for the trial were JMS Stylet Oil, Pure Spray Green, Naturalis-L and AzaDirect; all products were mixed with water (100 ml/m²) according to the label direction. The rates for each product per meter square were: JMS 1.4 ml/m², Pure Spray Green 1 ml/m², AzaDirect 0.2 ml/m² and Naturalis-L 0.11 ml/m² - these rates were determined based on label directions. The two control treatments for the study were Water and No Spray. All three trials had a Water control but because of a shortage of plants the pepper trial did not have the No Spray treatment. For each crop there were 10 plants/ treatment. As the size of plants and amount of foliage varied among the three crops we varied the size of the treatment area in order to have a sufficient volume of spray to thoroughly cover all plants. For peppers amount of area required to adequately cover 10 plants was 2 m and for cucumber and tomato 3 m. All sprays were mixed into hand-held sprayers, and sprayer nozzles were set to hollow cone. To ensure that the entire volume of spray solution was distributed equally over all 10 plants in each treatment, we sprayed the 10 plants for each treatment at one time, rather than pulling plants out and individually spraying them. Plants were placed back on greenhouse benches in random order after spraying. We sprayed all products, including water, twice, at a seven day interval. All sprays were applied as per label directions, except that all products recommended solid cone nozzles while we used hollow cone.

In all three trials mite levels on plants were counted five times: 1) prior to sprays, 2) 1 day after treatment (1-DAT), 3) 5-DAT, 4) 1 day after second treatment (1-DAST) and 5) 5-DAST. Mites were counted on a single randomly selected leaf on each plant. The leaf counted for the pre-spray count was marked with a pen, and this leaf was then used for all other subsequent counts. All live mites were counted, including nymphs and adults. We did not count eggs or dead mites. In the tomato trial we observed a reduction in mite counts (1DAT) in Control treatments as well as miticide treatments. This mostly likely reflects the poor reproduction of mites on older tomato leaves (D. Gillespie, personal communication). Thus for the remaining three counts in the tomato trial we also marked a new leaf and followed mite levels on these leaves after the second miticide treatment. Although the data are univariate, i.e. only a single dependent variable (mite number), the data were analyzed using repeated-measures MANOVA. A multivariate approach is recommended for the analysis of univariate data if the repeated-measure has more than two levels, in order to avoid violating the assumptions of compound symmetry and sphericity (StatSoft 2003). In the case of a significant treatment x time interaction a follow-up profile analysis (one-way ANOVA for each count) was performed and post-hoc analysis using Tukey-Kramer HSD ($\alpha = 0.05$). Analyses were conducted using Systat 9.

Efficacy trial: The efficacy trial was conducted on tomato (var. Starbuck) with AzaDirect (see above for rationale). The trial was conducted in the UCFV greenhouses and plants were infested with mites in a similar manner to the screening trial described above. The four treatments for this trial were: AzaDirect at 0.5X label rate (0.1 ml/m²), 1X label rate (0.2ml/m²), 1.5X label rate (0.3ml/m²), and Water control. Mite levels were counted and analyzed in the same manner as in the screening trial.

Natural enemy toxicity: Toxicity of the four products was assessed by applying spray solutions directly to enemies and by examining predator survival on foliage after sprays were applied.

Toxicity of direct spray was assessed using *P. persimilis* (in vermiculite carrier), *Amblyseius cucumeris* (in bran carrier) and *E. formosa* pupae. For *P. persimilis* and *A. cucumeris* a 1/4 teaspoon of carrier was sprinkled in a Petri dish and then a 0.3 ml of one of the four miticide solutions or water was applied with a syringe to the carrier material. We used a syringe rather than directly spraying the carrier as the impact of the spray may have had a detrimental impact on mite survival. Survival of mites was assessed after 24-h by counting both the live and dead mites in each Petri dish. Cards with *E. formosa* pupae were dipped for 1 second in miticide or water solution. The number of pupae with emergence holes 5 days after treatment was counted. There were six replicates for each combination of natural enemy and spray.

Survival of *P. persimilis* on leaves sprayed 24 and 48-h prior to predator release was also assessed. The purpose of this test was to determine how quickly after spraying with these products could enemies be released into hot spots. Leaves from a single tomato plant were sprayed with one of the four solutions or water, one plant for each treatment. Six leaves were removed from each plant after 24 and 48-h after the spray. Each leaf was placed in a Petri-dish on top of a moist square of gauze. The purpose of the gauze was to keep the leaf from desiccating and provide water to the predator mites. Three *P. persimilis* were added to each Petri-dish via a paint brush. After 24-h the number of live *P. persimilis* were counted. Direct toxicity data, for each predator species, were analyzed using one-way ANOVA and leaf residue results analyzed using two-way ANOVA (time X treatment).

RESULTS

Screening - Pepper: The effect of sprays on mite populations varied over the five counts leading to a significant treatment X time interaction (Table A1). Prior to the application of miticides there were significantly more mites on plants from the AzaDirect treatment than in the other treatments. This reflects very high counts on three of the 10 plants. As plants were randomly assigned to treatments prior to mite counts it is unlikely that this difference reflects any intentional bias in assignment of treatments. One day after sprays we saw a significant reduction in mite counts on all treated plants compared to the Water control, with no difference among the four products (Fig.1), however 5 days after the first treatment mite levels are similar across all five treatments. Again we see a significant reduction in mite numbers following the second spray, but this time the effect of sprays last longer for some of the treatments (Fig. 1). Mite counts remain significantly lower in JMS, Pure Spray and AzaDirect treatments compared to Water, with counts in Naturalis-L treatments being higher than the oils or botanical but lower than Water.

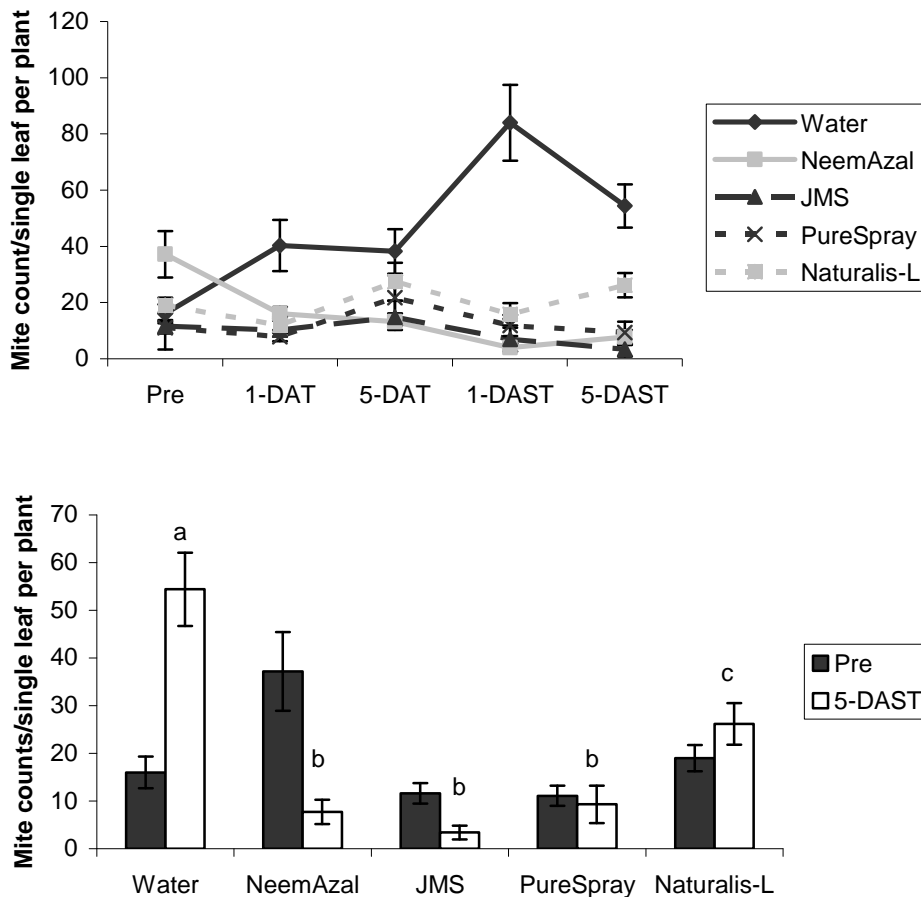


Figure 1. Top) Effect of four different miticides on spider mite control (mean \pm sem number of spider mites per one leaf/plant) on pepper plants over a 12 day period, following two sprays applied at 7 days intervals. Bottom) Comparison of mite counts among the five treatments prior to first spray (pre) and 5 days after the second spray (5 DAST). Bars represent mean \pm sem number of spider mites per one leaf/plant and clear bars with different letters are significantly different based on Tukey-Kramer HSD ($\alpha=0.05$).

Screening - Tomato: Interestingly, on tomato leaves we do not observe a significant effect of sprays until five days after the second treatment when all four miticides caused significant reductions in the mite populations on plants compared to the Water or No Spray treatments (Fig. 2, Table A1). The delay could reflect the slow population growth of spider mites on older tomato leaves (D. Gillespie, personal communication) as well as movement within each plant. In other words it took 5 days to see the negative effect of miticides separate from the effect of poor leaf quality. By the final count all treated groups of plants had significantly fewer mites than either of the control groups of plants (Fig. 2).

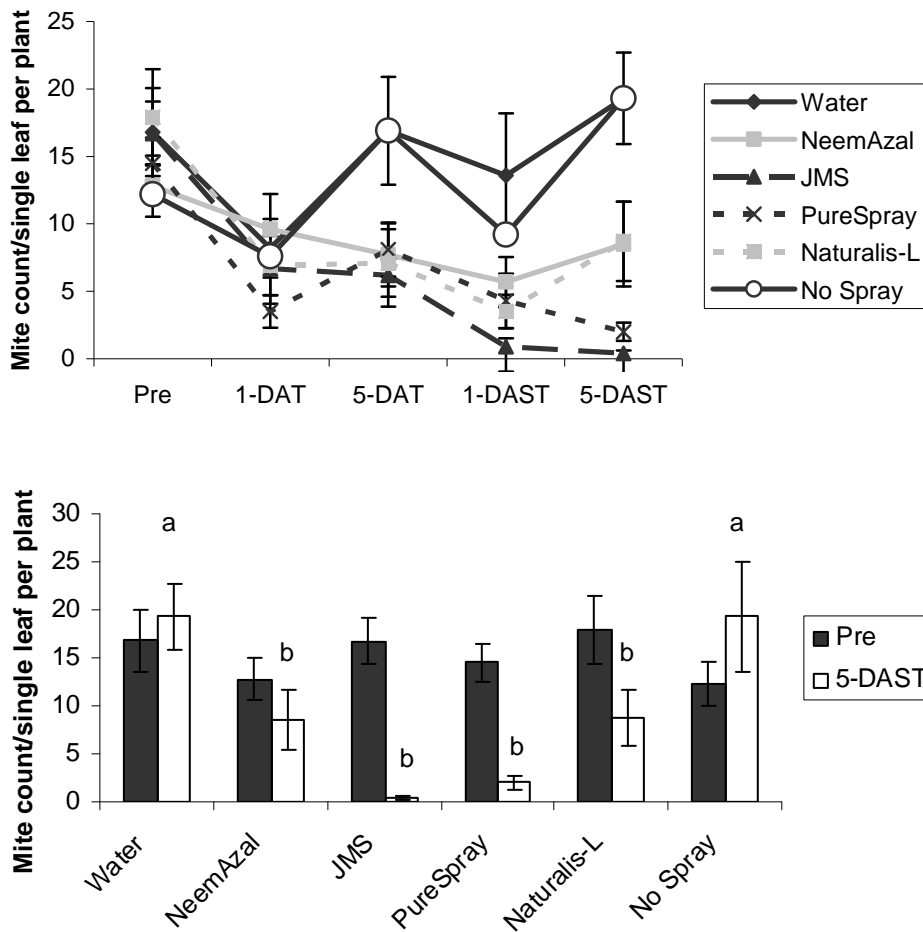


Figure 2. Top) Effect of four different miticides on spider mite control (mean \pm sem number of spider mites per one leaf/plant) on older tomato leaves over a 12 day period, following two sprays applied at 7 days intervals. Bottom) Comparison of mite counts among the five treatments prior to first spray (pre) and 5 days after the second spray (5 DAST). Bars represent mean \pm sem number of spider mites per one leaf/plant and clear bars with different letters are significantly different based on Tukey-Kramer HSD ($\alpha=0.05$).

On younger leaves there were significantly fewer mites on JMS and Pure Spray Green treated plants than on those treated with Water one day after the second treatment (Fig. 3, Table A1). However neither the AzaDirect or Naturalis-L treatments differed from the controls. Five days after the second treatment JMS and Pure Spray Green treatments continued to have much lower counts than the controls. Interestingly, mite counts on AzaDirect and Naturalis-L treatments began to show significantly lower levels than on the controls by the final count.

Thus by the final count results on the older leaves and younger leaves were similar: lowest levels of mites on Pure Spray Green and JMS treated plants, and intermediate - but still lower than controls - levels on AzaDirect and Naturalis-L treatments.

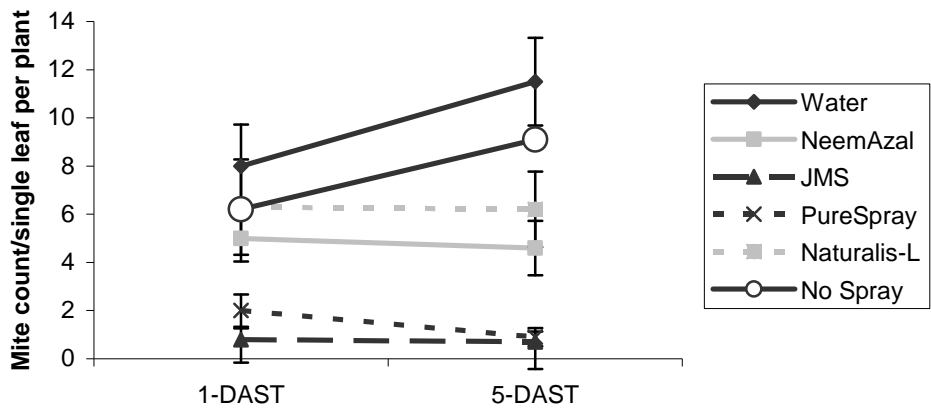
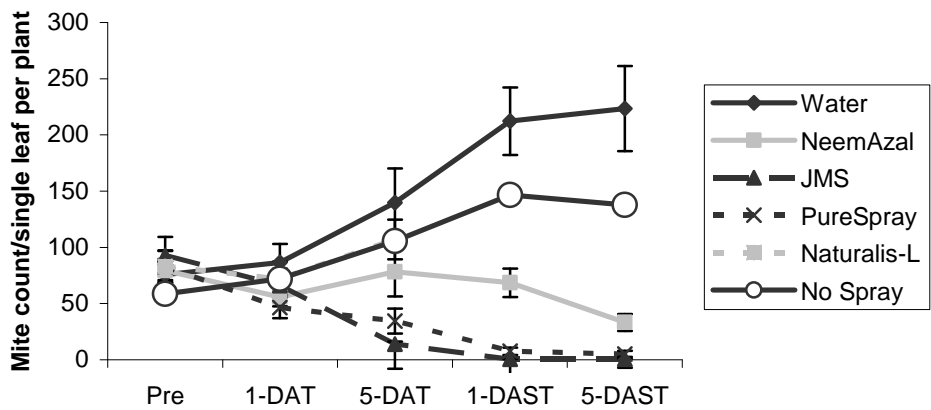


Figure 3. Effect of four different miticides on spider mite control (mean \pm sem number of spider mite per one leaf/plant) on newer tomato leaves following second spray. Mite levels on newer leaves were not counted at the beginning of the trial or following the first spray.

Screening - Cucumber: We did not count mite levels on Naturalis-L treated plants after the second spray because of an error in mixing product resulted in the second spray being applied at 10X the label rate. Thus the cucumber data was analyzed two ways - only the first three counts with Naturalis-L and then all five counts without Naturalis-L.

Comparison of all six treatments following the first spray show that significant differences were not observed in mite counts until five days after the spray (Fig. 4, Table A1). Both JMS and Pure Spray Green caused a significant decline in mite counts compared to the Water and No Spray control, while AzaDirect was different from the Water but not the No Spray control treatment. Mite levels on Naturalis-L treated plants did not differ from either of the control treatments. When data were analyzed for the remaining two counts (excluding Naturalis-L treatments) we see that one day after the second spray JMS and Pure Spray Green continue to have the lowest mite populations and AzaDirect treatment results in intermediate mite levels. However, by the fifth day after the second treatment all three products cause a significant reduction in mite numbers compared to either control.



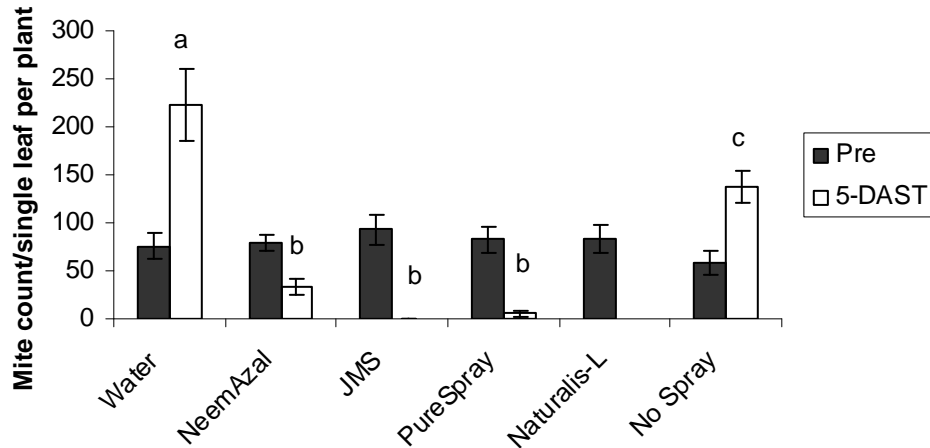
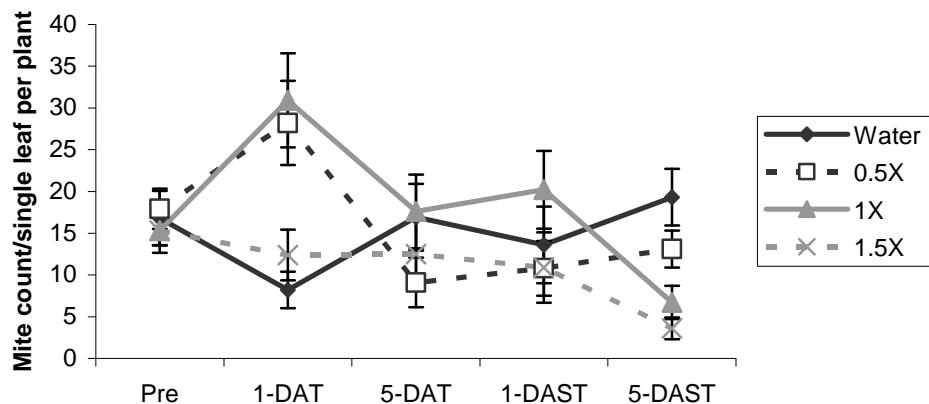


Figure 4. Top) Effect of four different miticides on spider mite control (mean \pm sem number of spider mite per one leaf/plant) on cucumber plants over a 12 day period, following two sprays applied at 7 days intervals. Bottom) Comparison of mite counts among the six treatments prior to first spray (pre) and 5 days after the second spray (5 DAST). Note there is no 5DAST count for Naturalis-L. Bars represent mean \pm sem number of spider mite per one leaf/plant and clear bars with different letters are significantly different based on Tukey-Kramer HSD ($\alpha=0.05$).

Efficacy: On both older and younger tomato leaves we did not see an effective suppression of mite populations until 5 days after the second spray (Fig.5 and Table A2). On older leaves there was a significant difference in mite counts one day following the first spray however in this case the Water control was significantly lower than the 0.5X and 1X label rate treatments, but prior to the second spray mite levels were similar among all four treatments (Fig. 5). By the end of the trial, on both old and young leaves, there was no significant difference between the 0.5X and 1X label rate or between 1X and 1.5X label rate (Fig 5 & 6). However, 05X label rate did not suppress mites in comparison with the Water control.



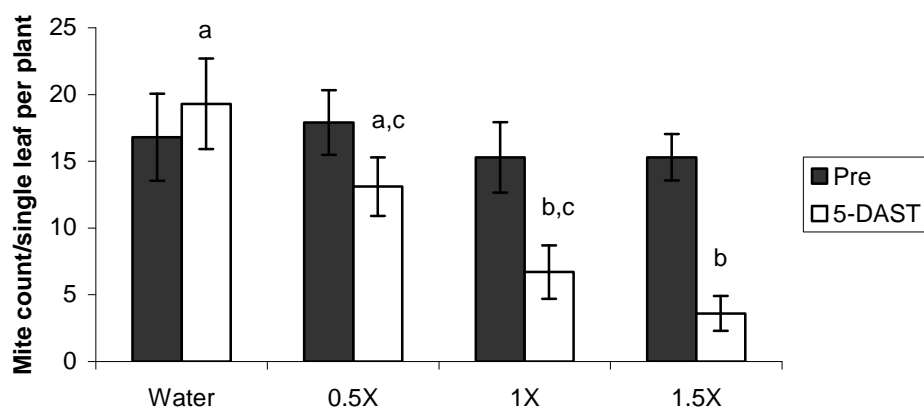


Figure 5. Top) Effect of three rates of AzaDirect on spider mite control (mean \pm sem number of spider mite per one leaf/plant) on tomato plants (older leaves) over a 12 day period, following two sprays applied at 7 days intervals. Bottom) Comparison of mite counts among the three rates prior to first spray (pre) and 5 days after the second spray (5 DAST). Bars represent mean \pm sem number of spider mite per one leaf/plant and clear bars with different letters are significantly different based on Tukey-Kramer HSD ($\alpha=0.05$).

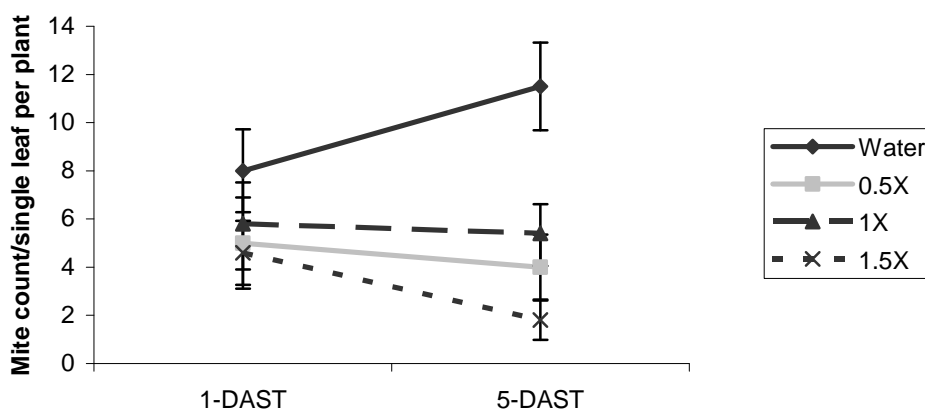


Figure 6. Effect of three rates of AzaDirect on spider mite control (mean \pm sem number per one leaf/plant) on newer tomato leaves following second spray. Mite levels on newer leaves were not counted at the beginning of the trial or following the first spray.

Natural enemy toxicity: Directly applying the miticide solutions to *A. cucumeris* (in bran carrier) or *P. persimilis* (in vermiculite carrier) did not cause significant reductions in the survival of either these predators (Fig. 7; Table A3). *Encarsia formosa* emergence was however negatively impacted by both JMS and Pure Spray (Fig. 8; Table A3). Leaf residues of the four miticides were not toxic to *P. persimilis* after 48 or 24-h from spraying (Fig. 9; Table A3).

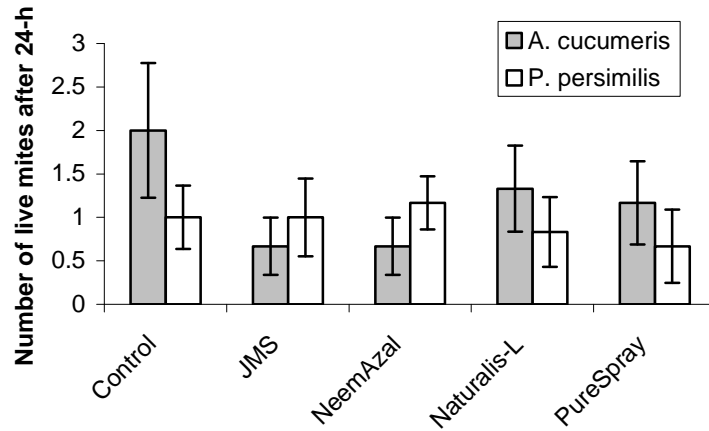


Figure 7. Effect of direct application of miticide solutions on survival of *Amblyseius cucumeris* and *Phytoseiulus persimilis*. The maximum number of mites in a Petri-dish was 4 and each bar represents the mean \pm sem of six replicates (N = 30 for each species).

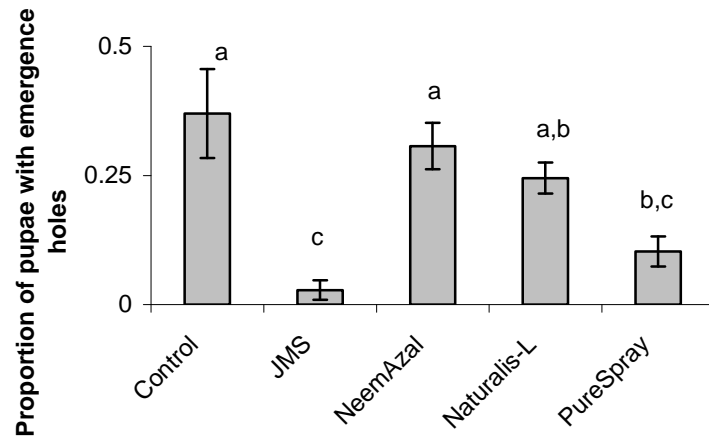


Figure 8. Effect of miticide solution or water Control on emergence of *Encarsia formosa* adults 5 days after treatment. Bars represent mean \pm sem of 6 replicates for each treatment (N=30). Bars with the same letter(s) are not significantly different based on Tukey-Kramer HSD ($\alpha=0.05$).

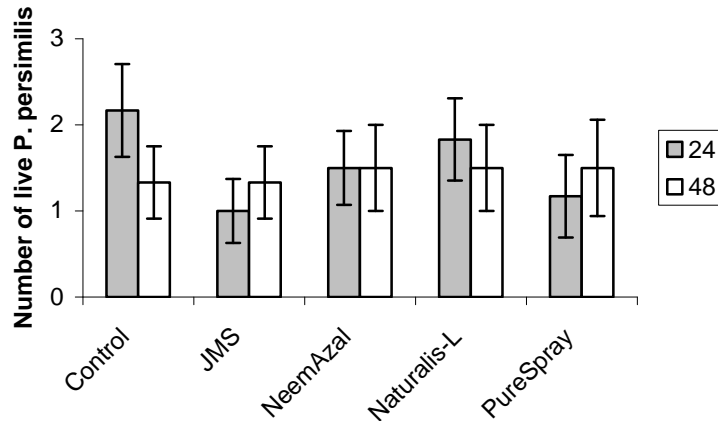


Figure 9. Survival, after 24-h, of *Phytoseiulus persimilis* adults on tomato leaves with miticide residue. Miticides were sprayed either 24 or 48-h prior to the addition of three *P. persimilis* to a single leaf/replicate. Bars represent the mean \pm sem of 6 replicates (Total N=60).

Phytotoxicity: No phytotoxicity was observed during the trial, however as the study was conducted during December and January and highest greenhouse temperatures were only in the low 30°C for a brief period of time, thus the conditions leading to phytotoxic effects of sprays on plants were not present.

DISCUSSION AND RECOMMENDATIONS

In all three of our trials we observed that both JMS Stylet Oil and Pure Spray Green were able to effectively reduce the spider mite population by the end of the trial. Performance of AzaDirect was more variable, but again for all three crops final mite counts showed that AzaDirect treated plants had lower mite counts than Control plants. In terms of efficacy, AzaDirect applied at rates lower than the label did not effectively suppress mites compared to Water control, but using a higher rate, i.e. 1.5X label rate did not result in better mite suppression than the label rate. Performance of Naturalis-L was the most variable across the three trials, usually with final mite levels between the other three products and the controls.

Despite the very consistent performance of products by the end of the trial i.e. on the last count, mite counts showed lots of variation over the course of the trial. For example, in peppers treatment effects were noticeable 24-h after the sprays but then diminished four days later, justifying a second spray. In contrast in tomatoes, miticide effects were not apparent until five days after the spray, however this observation may be an artifact of poor leaf quality. But, even on the younger leaves we do not see a suppressive effect of Naturalis-L or AzaDirect on mite until five days after treatment. We observed a similar effect in cucumbers for all three products. Two possible explanations for these results are differences in initial spider mite levels and plant interactions with chemicals.

First, differences observed between cucumber and pepper may be due to the much higher initial spider mite levels on cucumber than on pepper. In this case then, these products take longer to have an effect on a higher spider mite population, regardless of the crop. The product label for all four of the products emphasize that efficacy is best achieved if control begins when

spider mite populations are low. Another explanation for the different performance of products on the crops is that physical or chemical interactions between the leaf surfaces and the active ingredients that may influence efficacy. Although we adjusted the amount of active ingredient and volume of spray used in the tomato and cucumber trials to account for the additional volume of foliage, the structure of tomato (hairy) and cucumber (tented) leaves may require additional amounts of active ingredient or spray volume to ensure that a sufficient amount of product comes into contact with mites. Since adequate coverage is essential for the efficacy of all products growers will have to monitor coverage closely.

We did not observe direct toxicity of any of the four miticides on the survival of either *A. cucumeris* or *P. persimilis*. Naturalis-L has been reported to be safe on predator mites (Chandler et al. 2005). These authors propose that Naturalis-L be used as a hot spot treatment to compliment biological control of spider mites in tomato greenhouse crops in the UK, as *P. persimilis* and Naturalis-L in combination resulted in significantly lower spider mite levels than Vendex alone (Chandler et al. 2005). Cote et al. (2004) also found that spraying miticides like mineral oil prior to predator mite releases was effective at suppressing spider mites. All four products could be used in this manner: initially spray miticides to bring down a hot spot then follow with predator mite releases 24 h later to further control spider mites in the hot spot and surrounding plants. If this practice were used in commercial houses, then repeat applications of miticides may not be necessary. Results with *E. formosa* however do suggest that some of the products, like JMS Stylet Oil and Pure Spray Green, should be used with caution to prevent direct spraying other natural enemies.

Based on the results of this study we make the following recommendations

- A small assay to determine phytotoxicity of products in commercial greenhouses during the summer. If a grower is interested then we can provide samples of all four products for this work.
- A Low-risk Working Group is apparently being formed to advise PMRA on identifying priority pesticides for registration and research. All four products would be good candidates. Both Petro Canada and Troy Biosciences have indicated interest in pursuing Canadian registrations. Ideally more than one organic product will be available so that growers have choices and can rotate among products.

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APPENDIX - STATISTICAL RESULTS TABLES

Table A1. Statistical results of miticide screening trial on pepper, tomato and cucumber. All significant (≤ 0.05) p-values are highlighted. Where treatment X time interaction in repeated-measures MANOVA is significant, a profile analysis is conducted. Profile analysis consists of one-way ANOVA for each count.

	Repeated-measures MANOVA	Pre-count	1DAT	5DAT	1DAST	5DAST
Pepper	Treatment: $F_{4,45}=12.61$ $P < \mathbf{0.001}$ Time: Wilks' $\lambda = 0.58$ $F_{4,45}=12.61$ $P < \mathbf{0.001}$ Treatment X Time: Wilks' $\lambda = 0.12$ $F_{16,128}=8.14$ $P < \mathbf{0.001}$	$F_{4,45}=5.95$ $P = \mathbf{0.001}$	$F_{4,45}=7.26$ $P < \mathbf{0.001}$	$F_{4,45}=2.34$ $P=0.064$	$F_{4,45}=24.30$ $P < \mathbf{0.001}$	$F_{4,45}=21.58$ $P < \mathbf{0.001}$
Tomato (older leaves)	Treatment: $F_{5,54}=10.37$ $P < \mathbf{0.001}$ Time: Wilks' $\lambda = 0.89$ $F_{2,53}=3.39$ $P = \mathbf{0.041}$ Treatment X Time: Wilks' $\lambda = 0.61$ $F_{10,106}=2.97$ $P < \mathbf{0.002}$	$F_{5,54}=4.15$ $P = \mathbf{0.003}$	$F_{5,54}=4.15$ $P = \mathbf{0.003}$	$F_{5,54}=4.15$ $P = \mathbf{0.003}$	$F_{5,54}=4.15$ $P = \mathbf{0.003}$	$F_{5,54}=4.15$ $P = \mathbf{0.003}$
Tomato (younger leaves)	Treatment: $F_{5,54}=10.37$ $P < \mathbf{0.001}$ Time: Wilks' $\lambda = 0.89$ $F_{2,53}=3.39$ $P = \mathbf{0.041}$ Treatment X Time: Wilks' $\lambda = 0.61$ $F_{10,106}=2.97$ $P < \mathbf{0.002}$	-	-	-	$F_{5,54}=4.15$ $P = \mathbf{0.003}$	$F_{5,54}=11.94$ $P < \mathbf{0.001}$
Cucumber (with Naturalis-L)	Treatment: $F_{5,51}=2.38$ $P = \mathbf{0.05}$ Time: Wilks' $\lambda = 0.86$ $F_{2,50}=3.98$ $P = \mathbf{0.03}$ Treatment X Time: Wilks' $\lambda = 0.51$ $F_{10,100}=3.98$ $P < \mathbf{0.001}$	$F_{5,54}=0.72$ $P = 0.62$	$F_{5,54}=1.14$ $P = 0.35$	$F_{5,51}=6.58$ $P < \mathbf{0.001}$	-	-
Cucumber without (Naturalis-L)	Treatment: $F_{4,43}=23.59$ $P < \mathbf{0.001}$ Time: Wilks' $\lambda = 0.81$ $F_{4,40}=2.43$ $P = 0.06$ Treatment X Time: Wilks' $\lambda = 0.24$ $F_{16,127}=4.66$ $P < \mathbf{0.001}$	$F_{4,45}=0.88$ $P = 0.49$	$F_{4,45}=1.47$ $P = 0.23$	$F_{4,43}=7.41$ $P < \mathbf{0.001}$	$F_{4,43}=23.71$ $P < \mathbf{0.001}$	$F_{4,43}=28.64$ $P < \mathbf{0.001}$

Table A2. Statistical results of AzaDirect efficacy trial on tomato. All significant ($p \leq 0.05$) p-values are highlighted. Where treatment X time interaction in repeated-measures MANOVA is significant, a profile analysis is conducted. Profile analysis consists of one-way ANOVA for each count.

	Repeated-measures MANOVA	Pre-count	1DAT	5DAT	1DAST	5DAST
Tomato (older leaves)	Treatment: $F_{3,36}=1.224$ $P=0.315$ Time: Wilks' $\lambda = 0.60$ $F_{4,33}=5.573$ $P=0.002$ Treatment X Time: Wilks' $\lambda = 0.25$ $F_{12,87}=5.05$ $P < 0.001$	$F_{3,36}=0.242$ $P = 0.87$	$F_{3,36}=7.18$ $P = 0.001$	$F_{3,36}=1.01$ $P = 0.40$	$F_{3,36}=1.09$ $P = 0.36$	$F_{3,36}=8.81$ $P < 0.001$
Tomato (younger leaves)	Treatment: $F_{3,36}=1.21$ $P=0.32$ Time: Wilks' $\lambda = 0.77$ $F_{2,35}=5.59$ $P=0.01$ Treatment X Time: Wilks' $\lambda = 0.65$ $F_{6,70}=2.86$ $P=0.02$	■	■	■	$F_{3,36}=0.82$ $P = 0.49$	$F_{3,36}=9.77$ $P < 0.001$

Table A3. Statistical results of the natural enemy toxicity assays. All significant p-values are highlighted.

Trial	Analysis and results
Direct toxicity <i>A. cucumeris</i>	One-way ANOVA: $F_{4,25} = 1.180$, $p = 0.344$
Direct toxicity <i>E. formosa</i>	One-way ANOVA: $F_{4,25} = 8.897$, $p < 0.001$
Direct toxicity <i>P. persimilis</i>	One-way ANOVA: $F_{4,25} = 0.236$, $p = 0.916$
Leaf residue toxicity <i>P. persimilis</i>	Two-way ANOVA: Treatment: $F_{4,50} = 0.509$, $p = 0.730$ Time: $F_{1,50} = 0.112$, $p = 0.740$ Treatment X Time: $F_{4,50} = 0.546$, $p = 0.703$