

# **Using selected acaricides to manipulate *Tetranychus urticae* Koch populations in order to enhance biological control provided by phytoseiid mites.**

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This thesis is being submitted to the faculty in the department of entomology at Virginia Polytechnical Institute and State University as part of the requirements for a Master's degree in Life Sciences, with an emphasis in entomology.

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## Abstract

The twospotted spider mite, *Tetranychus urticae* Koch, is a serious pest of many ornamental plants (Johnson and Lyon, 1991). Pesticide resistance, the high cost of pesticides and loss of production time have raised interest by growers to introduce predatory phytoseiid mites to manage twospotted spider mites and reduce their need for acaricide applications (Sabelis, 1981). The predatory mite *Phytoseiulus persimilis* Athias-Henriot has been used successfully in integrated pest management programs for *T. urticae* suppression. Despite the success of *P. persimilis* in reducing populations of *T. urticae*, acaricide applications may still necessary due to limitations associated with the effectiveness of *P. persimilis* introductions. The objectives of this study were to;

1. Measure the effects of acaricides on the density and age structure of *T. urticae* populations.
2. Determine the compatibility of acaricides in an IPM program by measuring the toxicity of residues to *P. persimilis* and *T. urticae* adults.
3. Study the feeding behavior of *P. persimilis* on *T. urticae*.
4. Measure the effects of combinations of acaricides followed by release of *P. persimilis* on *T. urticae* populations using greenhouse trials.

The effects of ten acaricides on *T. urticae* populations were measured on infested *Buddleia x davidii* 'White Profusion' cuttings. Acaricides did not alter age structure in predictable manner. Initial analysis of results demonstrates that

cuttings treated with acaricides had age structures that were different from control treatments. However, these differences were not distinguishable from natural fluctuations in the age structure. Chlorfenapyr may have changed the age structure of *T. urticae*. Azadirachtin, pyridaben and spinosad did not suppress *T. urticae* populations at the rates and formulations tested in this trial. Abamectin, bifenthrin, chlorfenapyr, Gowan 1725, oil and neem oil suppressed *T. urticae* populations. Hexythiazox suppressed *T. urticae* populations but these results were not seen until two weeks after application.

The effects of acaricide residues were tested on adult *P. persimilis* and *T. urticae* 1, 3, 7, and 14 days after application using a leaf disk system. Abamectin, Gowan 1725, hexythiazox, horticultural oil, neem oil, pyridaben and spinosad were not toxic to *P. persimilis* adults while bifenthrin and chlorfenapyr residues were toxic to *P. persimilis*. *Tetranychus urticae* mortality from chlorfenapyr residues was significantly greater than the control 1, 3, 7 and 14 days after application. *Tetranychus urticae* mortality from bifenthrin and abamectin residues was significantly greater than the control 3, 7, and 14 days after application. *Tetranychus urticae* mortality caused by Gowan 1725, horticultural oil, and neem oil residues was significantly greater than the control 1 day after application, while mortality from hexythiazox and spinosad residues was not significantly greater than the control at any of the times tested in this study.

*Phytoseiulus persimilis* feeding behavior studies examining life stage preference tests and functional response studies were conducted on bean leaf disks. We found *P. persimilis* functional response to be a type II response for

both eggs and adults with handling times of 0.079 hours for eggs and 3.399 hours for adults.

The effects of a combination of acaricides followed by release of *P. persimilis* on *T. urticae* populations was tested using greenhouse studies conducted on infested *Buddleia* plants. In the first trial, severe plant damage occurred despite a reduction in the mean number of mites per leaf in treatments with oil+ predator treatments 7 days after release. Results from the second greenhouse trial produced plants with less visual damage compared to those in the first greenhouse trial. Treatments with predators alone and predators + acaricides produced similar results. However, treatments with predators had a mean numbers of mites per leaf that were significantly less than treatments with acaricides alone. The results demonstrate that the acaricides tested in the second greenhouse trial allowed the predators to provide suppression of *T. urticae* populations. A high release rate was used in the second greenhouse trial and lower release rates as well as different acaricide predator combinations need be tested to explore the possibility of new management techniques.

Our results suggest that the number of pest mites present in the crop may be the most important factor affecting the success of biological control with predators. Combinations of oil applications followed by introduction of *P. persimilis* 3 days after release provided suppression of *T. urticae* populations in a meaningful time frame. I was not able to shape the age structure of *T. urticae* populations in a predictable manner with acaricide applications. *Phytoseiulus persimilis* does not have a prey-stage preference when feeding on *T. urticae*, but

the shorter handling time for eggs may indicate that they are better able to suppress populations with higher proportions of eggs. However, we cannot determine if *P. persimilis* can keep *T. urticae* populations composed of predominately of eggs below threshold levels because our greenhouse trials did not test this hypothesis. *Phytoseiulus persimilis* feeding on adult *T. urticae* may suppress *T. urticae* populations below threshold levels because a reduction in the number of adults will lead to a reduction in the number of *T. urticae* eggs deposited on a plant. Our research suggests that abamectin and oil are two acaricides that would be less detrimental to the survival of *P. persimilis*. Additional greenhouse trials with compatible acaricides should be conducted as well as research on the threshold density of *T. urticae* that will allow *P. persimilis* to provide adequate control.

## Chapter 1

### Introduction

Production of ornamental crops is becoming an increasingly important part of agriculture as the demand for installation of ornamental plants in urban landscapes increases with urbanization of rural landscapes. Customers of ornamental plant producers and landscape installation businesses demand high quality crops. The twospotted spider mite, *Tetranychus urticae* Koch, is a serious pest of ornamental plants (Johnson and Lyon, 1991).

Despite the fact that some species and certain cultivars of plants are more resistant than others, numerous pesticide applications may be required to produce the high quality crop demanded by consumers. An aesthetic threshold can be defined as a pest density that causes visible plant injury which is undesirable to a grower or consumer. Aesthetic thresholds differ from economic thresholds because they are not based on crop yield, but are based on visual plant damage. Aesthetic thresholds can differ among plants species and are usually based on a person's perception of plant damage (Sadof and Alexander, 1993). The density of pests that exceeds an aesthetic threshold may depend on the species of pest, type of plant, and environmental conditions in which a plant is growing. Surveys conducted at retail nurseries have shown that 50% of people recognize plant damage with a change of less than 5 % leaf discoloration (Sadof and Alexander, 1993). Even within the same species of plant, there can be difference in cultivar susceptibility (Gillman et al., 1999).



Increasing public concern about pesticide applications and the widely-occurring problem of pesticide resistance in mites justifies the need for alternative strategies for mite control. *Tetranychus urticae* populations have developed resistance to hexythiazox in Australia (Herron et al., 1993) and resistance to other chemicals can occur with numerous pesticide applications (Helle and Sabelis, 1985). There are also concerns about public and worker exposure to greenhouse and nursery grown crops that are treated with pesticides. Cultural techniques used to grow ornamental plants require numerous manual tasks, which can increase the risk of pesticide exposure to workers. This can cause cumulative exposure, which can lead to illness, especially to hypersensitive individuals who can have allergic reactions. In the early 1990's, the Occupational Safety and Health Administration enacted the Worker Protection Standard to reduce the threat of cumulative exposure to pesticides by workers in crop production. Restricted reentry intervals associated with the worker protection standard have made use of toxic acaricides more costly due to loss of production time in treated greenhouses. These limitations have led many growers to attempt to introduce phytoseiid mites to manage *T. urticae* as an alternative to numerous acaricide applications. Successful biological control programs using *P. persimilis* for *T. urticae* suppression have been established in some greenhouse ranges in The Netherlands (Sabelis, 1981).

Biological control programs on ornamental plants have had varying degrees of success. *Encarsia formosa* is a parasitic wasp that has been used to

control greenhouse whitefly and has been successful in certain situations (Gill and Sanderson, 1998). Predatory mites in the family Phytoseiidae have been used to suppress *T. urticae* populations (Gill and Sanderson, 1998). One species of phytoseiid mite that is widely used and is commercially available is *Phytoseiulus persimilis* Athias–Henriot. Trials conducted in Florida utilizing *P. persimilis* to control *T. urticae* on Crotons and Areca palms reduced the number of acaricide applications by 87% -92% in Crotons, and 100% in Areca palms (Cashion et al., 1994). Releases of *P. persimilis* in interiorscapes to suppress mite populations have performed with varying degrees of success (Lindquist, 1981).

However, chemical applications are often necessary to decrease spider mite densities to acceptable levels, even after predator release (Helle and Sabelis, 1985). Biological control failures can occur for many reasons. Common reasons include releasing a species not adapted to the environmental conditions and pest species present, releasing the biological control agent at an incorrect time in the development of the infestation, or using chemical pesticides that are not compatible with the biological control agent. To be effective, predatory mites must be released at a rate that provides a high predator to prey ratio; the larger the predatory-prey ratio, the better the biological control from releases of beneficial mites (Strong and Croft, 1995). Treatment thresholds vary, depending on the type of crop, the species of predatory mite, the environmental conditions present in the system, and the amount of visual plant injury a grower or consumer will perceive as acceptable. When pest mite populations reach a level

that begins to cause unacceptable plant injury it is necessary to utilize chemical applications to prevent plant damage.

Acaricide applications may be necessary to suppress *T. urticae* populations in ornamental crop systems. However, selective use of acaricides that are compatible with *P. persimilis* may preserve predator populations and enhance control (Osborne and Petiitt, 1985). To combine *P. persimilis* with acaricide applications, chemical residues must be non-toxic to the predators. The effects of chemical classes on *P. persimilis* from most harmful to least harmful are; organophosphates, pyrethroids, organochlorines and carbamates (Pratt and Croft, 2000). However, the effects of individual products and formulations can vary greatly and new products remain to be evaluated.

In certain situations, releases of *P. persimilis* combined with compatible acaricides can produce an additive benefit that results in increased suppression of spider mite populations (Trumble and Morse, 1993). Studies conducted by Trumble and Morse (1993) on the harvest value of strawberries demonstrated that a combination of predator releases followed by applications of abamectin after pest mite threshold levels were reached provided a higher value crop than abamectin alone. The same could be true for certain ornamental crops, but studies need to be conducted.

The objective of this study was to study the feeding behavior of *P. persimilis*, and gather data to recommend acaricides that have residues which are safe for *P. persimilis* and provide suppression of *T. urticae*. Acaricides that have few negative effects and/or positive effects may enhance biological control

provided by *P. persimilis*. Several studies were conducted to examine how *P. persimilis* and selected acaricides could be used in compatible combinations. First, I measured the toxicity of various acaricides on the age structure by applying acaricides to infested *Buddleia x davidii* cuttings. Then, the compatibility of residues from these acaricides were determined using a leaf disk assay system. Feeding studies were conducted to measure the functional response of *P. persimilis* to mobile and egg forms of *T. urticae*, and the presence of a prey-stage preference in *P. persimilis* that feed on *T. urticae*. Finally, greenhouse trials were conducted using a combination of compatible acaricides followed by release of *P. persimilis* to determine if the combination of compatible acaricides and *P. persimilis* can enhance suppression of *T. urticae* populations provided by *P. persimilis*.

## **Chapter 2: Compatibility of Selected Acaricide Residues with *Phytoseiulus persimilis* and their Effects on *Tetranychus urticae***

### **Introduction**

The twospotted spider mite, *Tetranychus urticae* Koch, is a serious pest of numerous greenhouse plants, nursery-grown ornamentals and field crops.

Twospotted spider mite damage includes webbing, fine stippling, leaf yellowing, leaf drop, and even plant death (Helle and Sabelis, 1985). Species in its host range include numerous herbaceous and woody landscape plants such as rose, ivy, and winged euonymus (Johnson and Lyon, 1991). Female *T. urticae* can develop from egg to adult in approximately 6.5 days at 30 ° C (Sabelis, 1981), and females can lay as many as 60 eggs in five days (Helle and Sabelis, 1985). The expense of new acaricides and the loss of production time associated with pesticide applications has made frequent acaricide applications costly.

Development of resistance by *T. urticae* to numerous acaricides has caused difficulties in controlling outbreaks (Carbonaro et al., 1986). These conditions have raised interest by growers in using predatory phytoseiid mites to manage twospotted spider mites to reduce their need for acaricide applications (Sabelis, 1981).

*Phytoseiulus persimilis* Athias-Henriot can be an effective tool of an integrated pest management program for *T. urticae*. Despite successful suppression of *T. urticae*, limitations to the effectiveness of *P. persimilis* arise under certain conditions because their fecundity may be reduced. The optimum conditions for rapid population development of *P. persimilis* is a temperature of

27° C and relative humidity (RH) of 60%-85% (Stenseth, 1979). A temperature of 27° C with RH less than 40% reduces the reproductive rate of *P. persimilis* by increasing egg mortality (Stenseth, 1979). This is an important disadvantage because most greenhouses have temperatures and humidity levels that are outside these optima for part of the day. Another limitation to *P. persimilis* effectiveness is related to *T. urticae* density. When *T. urticae* density is too high, *P. persimilis* predation will not reduce *T. urticae* populations to acceptable levels (Helle and Sabelis 1985). Trumble and Morse (1993) demonstrated that adequate suppression of *T. urticae* populations was achieved by releasing *P. persimilis* in combinations with abamectin applications. In their study, after threshold levels are surpassed, predator release combined with compatible acaricides was more effective than using chemical or biological control tactics alone.

To combine *P. persimilis* with acaricide applications, chemical residues must be non-toxic to the predators. Our objective was to determine the toxicity of residues of ten new or commonly used acaricides to *P. persimilis* 1, 3, 7, and 14 days after application. In addition, residual toxicity to *T. urticae* was recorded.

## **Materials and Methods**

Twospotted spider mite colonies were maintained on lima beans (*Phaseolus vulgaris*) in rearing cages at 30 °C and 14:10 (L:D) photoperiod. The individuals which were used to initiate the colony, originated from an infested rose plant that was purchased at a local nursery. Each rearing cage was a 20 x 40 x 30-cm Plexiglas™ box with an open top, fitted with thrips-proof screening. A

ring of double-sided sticky tape on the outside rim and petroleum jelly on the inside rim was used to prevent mite escape and contamination of colonies.

Acaricides were mixed with tap water at the manufacturer's recommended rates and applied with a hand sprayer to whole bean plants under a fume hood (Table 1). Control plants were left unsprayed. Treated plants were left in the fume hood 30-45 minutes until leaf surfaces dried, after which they were placed under high intensity discharge (HID) lights emitting 250 fc, 14:10 (L:D) photoperiod without overhead watering. Twenty leaf disks, each with a surface area of approximately 10 cm<sup>2</sup>, were cut from plants treated with each chemical 1, 3, 7 and 14 days after plants were sprayed.

Table 1. Acaricides tested

Common Name	Trade Name + Formulation	Mix Rate
Abamectin	Avid 0.15 EC (Novartis, Greensboro, NC)	4 oz/ 100 gallons
Bifenthrin	Talstar GH 0.67F (FMC, Philadelphia, PA)	40 oz/ 100 gallons
Chlorfenapyr	Pylon 2SC (American Cyanamid, Parsippany, NJ)	5.2 oz/ 100 gallons
Gowan 1725	Gowan 1725 0.1% EC (Gowan, Yuma, AZ)	20 oz / 100 gallons
Hexythiazox	Hexygon 50 WP (Gowan, Yuma, AZ)	1.5 oz / 100 gallons
Horticultural oil	Sunspray Ultra-Fine (Sun Company, Inc., Philadelphia, PA)	250 oz / 100 gallons
Neem oil	Triact 70 EC (Thermotriology Corporation, Columbia, MD)	250 oz/ 100 gallons
Pyridaben	Sanmite 75 WP (BASF Corporation, Research Triangle Park, NC)	4 oz/ 100 gallons
Spinosad	Conserve SC (Dow AgroSciences, Indianapolis, IN)	600 ml/ 100 gallons



Survival tests were conducted on treated and control leaf disks using a modified Huffaker cell system (Munger, 1942; Huffaker, 1948; Lester et al., 1999). Cells were made from three 7.6 x 7.6 X 0.6 cm Plexiglas pieces bolted together like a sandwich. A 4.5-cm diameter hole was cut in the middle piece of Plexiglas created a small chamber to hold a leaf disk for the assay.

*Phytoseiulus persimilis* adults obtained from Koppert Biological (Ann Arbor, Michigan) were brushed into a container of bean leaves infested with *T. urticae*, and were allowed to feed on prey for 18-24 hours before testing. For each test, one *P. persimilis* adult was placed on the leaf disk in a modified Huffaker cell with two *T. urticae* adults to provide food for the predators. Cells were closed immediately after mite introduction. The total number of mites that died in all cells in each treatment was recorded after 24 hours. Temperatures averaged 28 °C with a range of 25.2- 32.5 °C during the test period. For each acaricide tested, mite mortality was compared on 20 non-treated leaf disks and 20 treated leaf disks for each of the four time periods. Data were analyzed by contingency table ( $\alpha= 0.05$ ). Fourteen day old residues were not tested when mortality from residues was not significantly greater than controls for seven day old residues.

## **Results**

The duration of acaricide residue toxicity varied among the compounds tested. *Phytoseiulus persimilis* mortality on leaf disks treated with abamectin, Gowan 1725, hexythiazox, horticultural oil, neem oil, pyridaben and spinosad

was not significantly greater than on untreated leaf disks at any time after application. Mortality of *P. persimilis* from exposure to residues of bifenthrin and chlorfenapyr was significantly greater than observed on the controls 1, 3, 7, and 14 days after application (Figure 1).

The response of *T. urticae* to residue exposures was more variable than that of *P. persimilis*. *Tetranychus urticae* mortality from chlorfenapyr residues was significantly greater than the control 1,3,7 and 14 days after application. After two weeks, chlorfenapyr residues caused 55% mortality to adult *T. urticae* compared with 6% mortality in the control. *Tetranychus urticae* mortality from bifenthrin and abamectin residues was not significantly greater than the control 1 day after application. However, *T. urticae* mortality for both bifenthrin and abamectin residues was significantly greater than the control 3, 7, and 14 days after application. *Tetranychus urticae* mortality caused by Gowan 1725, horticultural oil, and neem oil residues was significantly greater than the control 24 hours after application but not at the other times tested. *Tetranychus urticae* mortality from hexythiazox and spinosad residues was not significantly greater than the control at any time tested (Figure 1).

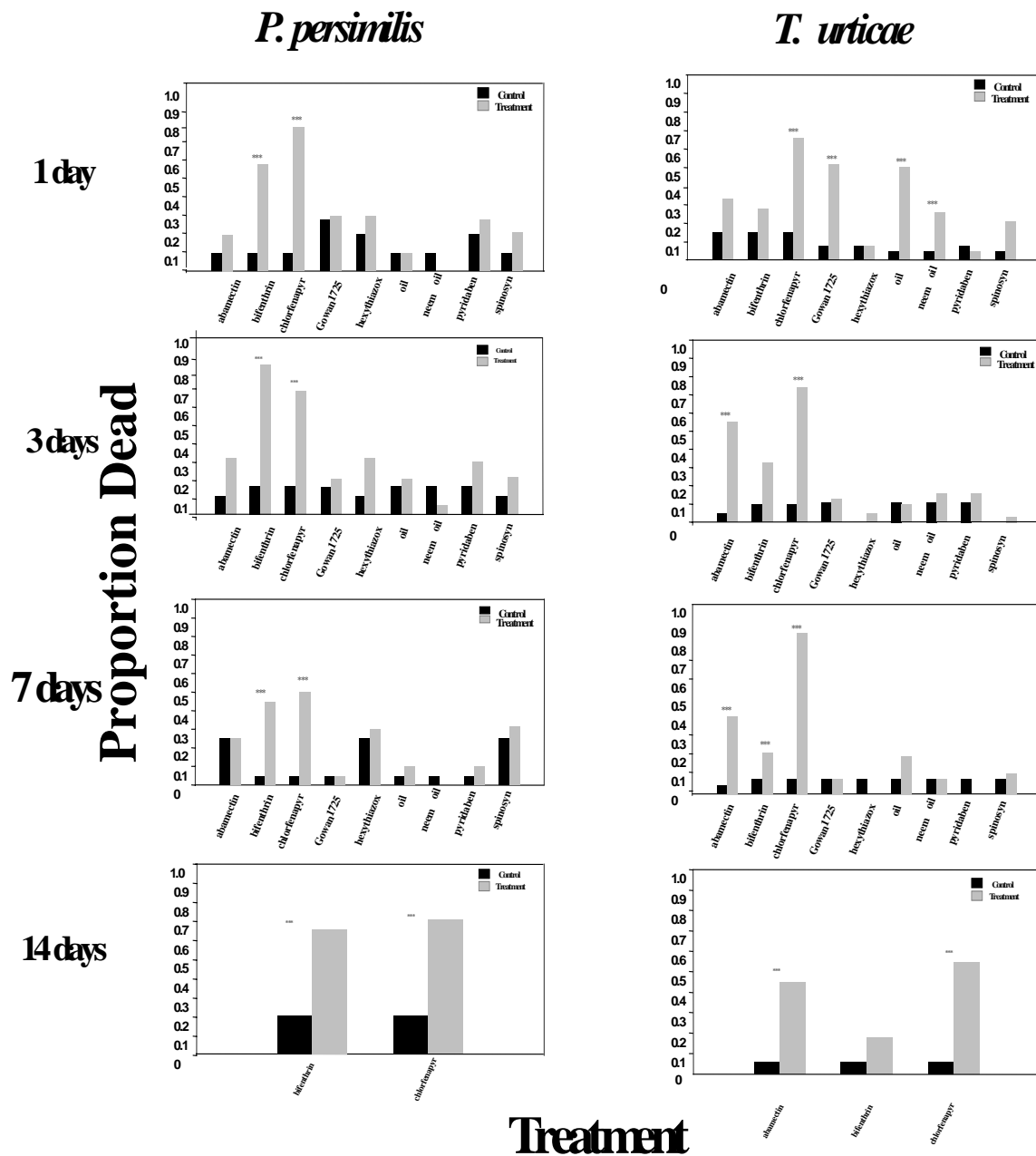


Fig. 1. Residual toxicity to *Phytoseiulus persimilis* and *T. urticae* (A) 1 day after application, (B) 3 days after application, (C) 7 days after application, (D) 14 days after application

\*\*\* Indicates significant difference ( $\alpha = 0.05$ ) between treatments and control

## Discussion

The objective of this study was to determine the compatibility of selected acaricides with releases of predatory mites for management of *T. urticae*. For our purposes, a compatible acaricide can be defined as a product that has residual activity that does not kill *P. persimilis*. The level of compatibility will usually depend, at least partly, on the post application interval. We measured toxicity of acaricide residues to commercially available *P. persimilis*. We did not consider sublethal effects, which can occur from acaricide residues (Oomen et al., 1991).

Abamectin residue caused significant mortality to adult *T. urticae* 3, 7 and 14 days after application. Several other studies have found that exposure to abamectin residues does not have a significant effect on *P. persimilis* survival (Oomen et al., 1991; Zhang and Sanderson, 1990; Shipp et al., 1999). However, abamectin has been shown to cause significant mortality and reduction in the mobility and fecundity of *T. urticae* (Zhang and Sanderson, 1990). After two weeks, in our experiments abamectin residue killed *T. urticae* adults. Residual activity of abamectin is likely to decrease more quickly in outdoor environments than indoor environments (Wright et al., 1984). Fourteen-day-old residues from abamectin applications of 3 ppm on cotton grown in greenhouses resulted in 97% mortality after 72 hours of exposure (Wright et al., 1984). Trials conducted with plants treated similarly under field conditions caused 47% mortality with the same exposure (Wright et al., 1984). In our experiments, 14-day-old abamectin residues from applications of 6.25 ppm, with 24 hours of exposure resulted in

45% *T. urticae* mortality compared to 6% mortality on untreated leaf disks in the control.

Abamectin residues can have sublethal effects on *P. persimilis*. Eight days of exposure beginning one hour after 4 ppm abamectin applications reduced *P. persimilis* egg laying by as much as 50% (Zhang and Sanderson, 1990). Exposure of *P. persimilis* to abamectin residues does not decrease egg hatch rate, but feeding on *T. urticae* intoxicated with abamectin can reduce egg production (Zhang and Sanderson, 1990). Despite its sublethal effects on *P. persimilis*, abamectin may be a good candidate for IPM programs because of its high toxicity to *T. urticae* and its relatively low impact on *P. persimilis*. However, additional studies are necessary to confirm this idea.

Mortality of *P. persimilis* caused by Gowan 1725 and hexythiazox residues one day after application was not significantly greater than the control. Our results were consistent with other studies of hexythiazox on *P. persimilis* (Oomen et al., 1991). However, a duration of hexythiazox exposure which is longer than ours may have a negative impact on *P. persimilis*. Residues of Gowan 1725 and hexythiazox may not provide rapid suppression of adult *T. urticae* in some situations. Gowan 1725 has a chemical structure that is similar to abamectin, but its residues were not effective against adult *T. urticae*. Although hexythiazox has a low toxicity to adult female *T. urticae*, it still may suppress mite populations by reducing *T. urticae* egg production (Chapman and Marris, 1986). Hexythiazox residues have been shown to have ovicidal effects on *Panonychus ulmi* 30 days after application (Pree et. al., 1992). Unlike hexythiazox, Gowan 1725 residue

has a short period of adulticidal activity on *T. urticae* under laboratory conditions and it is probably even shorter in field environments.

Neem products and parafinic horticultural oils may be a useful part of IPM programs for management of *T. urticae* populations. I found these products to be compatible with *P. persimilis* because they are active for only a short period however, their short residual toxicity may not suppress high density populations. Based on our studies, residues from these products caused mortality to *T. urticae* 24 hours after application, but no mortality thereafter. All neem products may not be equally compatible with *P. persimilis*. We applied a formulation of 70% neem oil at a rate of 1.4 %. Although the residues had low toxicity to *P. persimilis*, topical applications may be harmful. Direct application of a neem formulation containing 80% neem oil at a rate of 3% was highly toxic to *P. persimilis* and only moderately toxic to *T. urticae* (Papaioannou-Souliotis et al., 2000). Horticultural oil can control *T. urticae* eggs and mobile stages (Haitas, 1997) and reduce female fecundity (Osman, 1997).

Spinosad and pyridaben residues did not cause direct harm to either *P. persimilis* or *T. urticae*. Pratt and Croft (2000) found that spinosad did not have a highly toxic effect on *P. persimilis*. Formulations of spinosad containing 11.6% spinosad A and D, can cause 100% mortality when applied directly to mites at 400 ppm (DeAmicis et al., 1997). Our application rate was 181 ppm and residues were allowed to dry before exposing mites. This application rate did not suppress *T. urticae* populations under laboratory conditions (Cote, chapter 4, unpublished data). We recorded 28% and 30% mortality after 24 hours of

exposure to 1 and 3- day old pyridaben residue, respectively. Shipp et al. (1999) found pyridaben to cause *P. persimilis* mortality as high as 71% 4 days after application with 48 hours of exposure under laboratory conditions.

Bifenthrin and chlorfenapyr were toxic to *P. persimilis* up to two weeks after treatment, and would be poor choices for use in an IPM program that uses *P. persimilis*. However, the prolonged residual activity may be useful for controlling high-density populations of *T. urticae*. Chlorfenapyr provided excellent control of *T. urticae* infestations without short-term population resurgence (Allen and Kharboutli, 1999; Cote unpublished data). This is the first published account of the effects of chlorfenapyr residues on *P. persimilis*. Our results with bifenthrin were consistent with other studies (Oomen et al., 1991, Pratt and Croft, 2000).

## **Conclusion**

The acaricides tested in this study varied greatly in their toxicity to *P. persimilis* and *T. urticae* adults. *Phytoseiulus persimilis* releases alone are unlikely to prevent *T. urticae* populations from reaching economic injury levels on ornamental crops (Helle and Sabelis 1985). Selective use of acaricides may create a favorable environment for release of *P. persimilis* by reducing *T. urticae* to manageable levels, provided that other environmental conditions are suitable. While treatment with acaricides that have long residual toxicity may be required to suppress high-density spider mite populations, their use may promote spider mite resistance. Acaricides that have short residual toxicities can be used in combination with predators to reduce large populations of spider mites, but the

timing of application and predator release is critical (Osborne and Petitt, 1985). Applications of insecticidal soap three days after release of *P. persimilis* did not adversely affect predator populations and provided enhanced suppression of spider mite populations (Osborne and Petitt, 1985). Biological control may be enhanced through careful selection of acaricides and releasing predators into the crop once residues are no longer toxic to them. Our research suggests that abamectin and oil are two acaricides that would be less detrimental to the survival of *P. persimilis*. Their use in an integrated program on ornamental crops should be considered.



### **Chapter 3: The Prey-Stage Preference of *Phytoseiulus persimilis* on *Tetranychus urticae***

#### Introduction

*Phytoseiulus persimilis* Athias-Henriot locate their prey using chemical cues from the infested plant, the prey, and materials associated with prey such as webbing and fecal material (Helle and Sabelis 1985). *Phytoseiulus persimilis* respond to volatile odors from *T. urticae* by arresting forward movement and turning towards the direction of the odor source (Sabelis et al. 1984; Sabelis and van der Weel 1993). Experiments using Y tubes conducted by Sabelis and Van der Baan (1982) demonstrated that *P. persimilis* does not respond to kairomones produced by *Panonychus ulmi*, but responds positively to kairmones emitted from *T. urticae*. Positive responses to prey kairomones may not occur when predators are satiated; air currents which do not contain kairomones cause positive anemotaxis in hungry predators and negative anemotaxis in satiated predators (Sabelis and Van der Weel, 1993). Once predatory mites have encountered and consumed their first prey item, they tend to remain near the point of prey capture regardless of prey density (Eveleigh and Chant 1981). *Phytoseiulus persimilis* exhibits a reduction in forward searching speed and an increase in the number and angle of turns after the first prey capture, which restricts the search area in order to maintain close proximity to prey patches (Eveleigh and Chant 1981).

The number of prey a phytoseiid mite is able to consume over time can be described by its functional response. The functional response is defined as the number of prey consumed per predator as prey density increases. Typically, as

prey density increases, attack rate will increase. There is a prey density at which a predator's attack rate reaches a maximum because the predator can no longer feed on all available prey. In laboratory experiments, *P. persimilis* shows a Type II functional response (Everson, 1979). The Type II functional response is described by the Hollings disk equation,

$$N_a = \frac{a' T N}{1 + a' T_h N}$$

where  $N_a$  is the number of prey attacked,  $a'$  is the search rate,  $T$  is the total time,  $N$  is the prey density, and  $T_h$  is the handling time (Wiedmann and O'Neil, 1991).

Our objective was to examine the feeding behavior of commercially available *P. persimilis* on mobile versus immobile stages of *T. urticae*. There has been some controversy as to whether a constant prey stage preference exists in predatory mites or if prey-stage preferences are just random predatory events triggered by environmental and food source changes such as differences in prey quality (Sabelis 1990). Researchers claim that prey stage preference is regulated in each individual phytoseiid mite by gut fullness and attack rate (Sabelis 1990). If the gut of a predator is empty, it will likely have a higher attack rate and seek out the prey stage that provides the highest net caloric value, or the one that is easiest to capture (Sabelis 1990). The handling time for each prey stage is likely to be different. If the handling time of a prey stage is short, a predator can consume more prey over a period of time. The short handling time associated with egg feeding may allow *P. persimilis* to provide better suppression of *T. urticae* populations which are comprised of predominately eggs compared

to those primarily composed of adults. However, further studies are necessary to make this conclusion.

## Materials and Methods:

### Preference Trials

Twospotted spider mite colonies were maintained in on lima beans using the system described in chapter 2. Preference tests were conducted in modified Huffaker cells (Huffaker, 1948; Munger, 1942; Lester et al., 1999). The cells were made from three 7.6 x 7.6 X 0.6 cm Plexiglas pieces held together with four bolts and nuts. A 4.5-cm diameter hole was made in the middle piece of Plexiglas™ to create a small chamber in which the assay was performed.

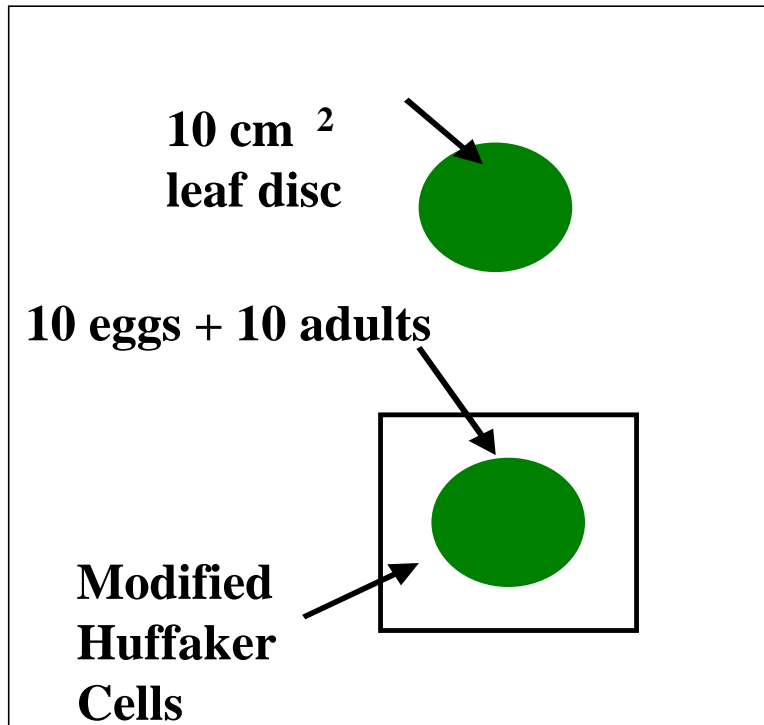
Twenty 10 cm<sup>2</sup> leaf disks were cut from bean plants grown in the laboratory under high intensity discharge mercury vapor lights with 250 fc, 14:10 (L:D) photoperiod. Leaf disks were placed in the cells to prevent desiccation until prey were introduced. Ten *T. urticae* eggs in a single patch and ten *T. urticae* adults were placed on each leaf disk with a #000 fine, sable hair paintbrush. (Figure 1)

Predatory mites were acclimated to room temperature and one predator was released into each cell directly from the shipping container. Huffaker cells were sealed and the number of *T. urticae* adults and eggs were counted. The number of prey in each arena was counted before the observations began. Cells were left undisturbed for 10-15 minutes to allow predators to settle before initial observations were made. We recorded walking, resting, attacking an egg or attacking a non-egg stage at 0, 30, 60, 90 and 120 minutes after initiation of the experiment. Observations were not taken after 120 minutes because there was

a high probability of egg laying and egg hatching by the prey, which changed prey age structure.

Additional experiments were conducted to determine whether *P. persimilis* developed a search image when exposed to a single life stage of *T. urticae*. Leaf disks were prepared by the same methods previously described. Predators were exposed to *T. urticae* adults or eggs before being placed in an arena with a mixed population of *T. urticae*. Predators were exposed to prey monocultures by placing 30 predators on a leaf disk in a Petri dish with 100 egg or adult prey for 10-15 minutes. After exposure to prey monocultures, a single predator was removed from the monoculture and placed in a Huffaker cell with a mixed population of *T. urticae*. The cells were sealed and the prey were counted. Observations were taken 15, 30, 60, 90 and 120 minutes after prey were placed in arenas. An additional experiment was conducted to determine whether the transfer itself from prey monocultures to mixed population arenas affected the behavior of the predators was conducted. In this experiment, *P. persimilis* were placed directly into mixed arenas, or exposed to a pest free bean leaf in a Petri dish before being transferred to arenas with a mixed population. Three blocks with 20 cells in each block were conducted at different times to make a total of 60 replications. Proportional data of all observed behaviors were analyzed by contingency table analysis, the mean numbers of events observed were analyzed by ANOVA and the relationship between resting and adult feeding and walking and egg feeding was analyzed using simple correlation ( $\alpha= 0.05$ ).

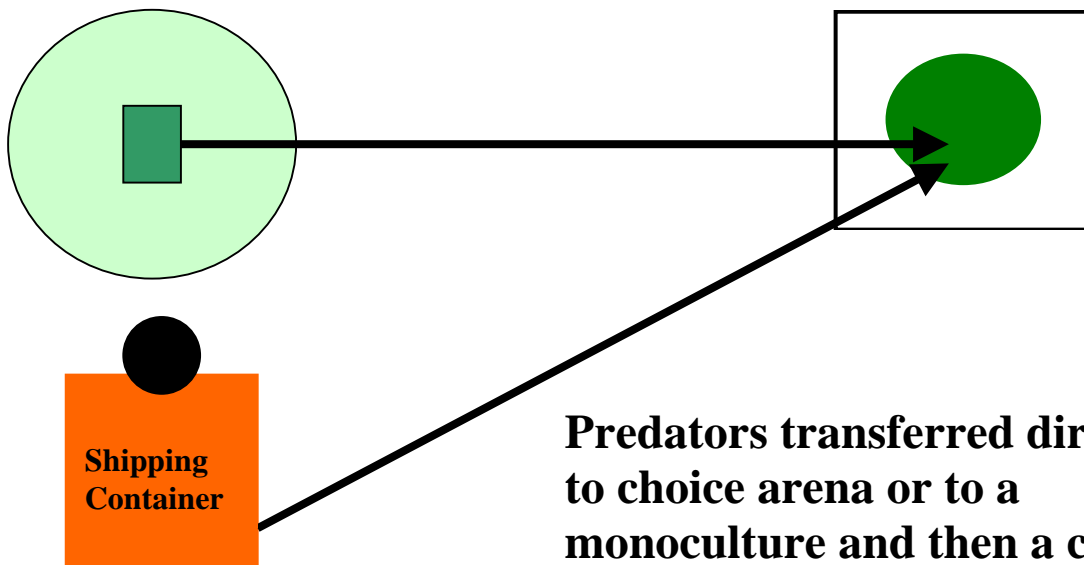
Figure 1: Predator handling during choice tests.



Petri dish with egg or adult  
monoculture on bean leaf

Choice Arena

10 eggs and 10 adults  
*T. urticae*



Predators transferred directly  
to choice arena or to a  
monoculture and then a choice  
arena

## Functional Response Experiments

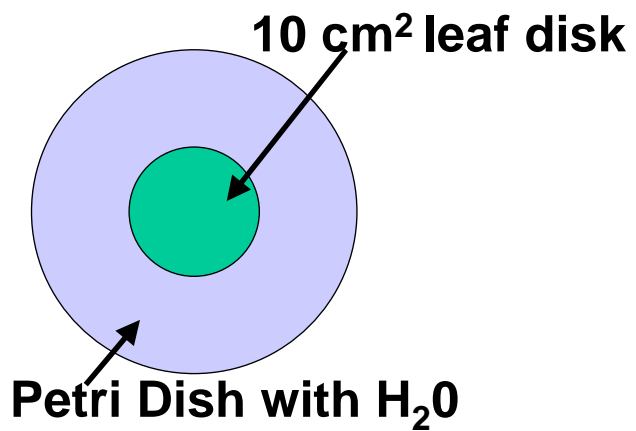
*Tetranychus urticae* were reared on bean plants as previously described. Experimental arenas were constructed by placing a ten cm<sup>2</sup> bean leaf disk excised from uninfested bean plants on top of a small piece of clay in the bottom of a 15 mm Petri dish filled with enough tap water to float the disk. (Figure 2)

*Tetranychus urticae* eggs or adults were placed on leaf disks at densities of 5, 10, 20, 40 and 60 per disk. Adult *T. urticae* were transferred directly to arenas from the colonies on the day of the experiment. Eggs were obtained by placing 25 *T. urticae* adults on each of five pest free bean leaves inside a Petri dish for two days at 30 °C prior to the experiment. On the day of the experiment, eggs were transferred from the bean leaves inside the Petri dish to the arenas. At least ten replications were conducted for each prey density. The arenas were left undisturbed for one hour and then inspected to see if any eggs had hatched or adults had laid eggs. *Phytoseiulus persimilis* adults were removed from the shipping container and one was placed in the center of each leaf disk and allowed to feed for eight hours. Eggs were removed from adult arenas every hour for the duration of the experiment. Egg arenas were inspected hourly for the presence of newly hatched mobile forms, which were removed from the arena and replaced with an egg. After eight hours, the number of adult *T. urticae* cadavers were counted in the adult arenas and the number of viable eggs in the egg arenas to determine the number of prey that had been consumed. Temperature and relative humidity readings were recorded hourly. Data were analyzed using least squares regression by fitting a line through the data points

predicted by the descriptive functional response equation as described by Fujii et. al. (1986).

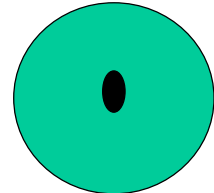
Figure 2: Functional Response Assay Arenas

## Arena Design

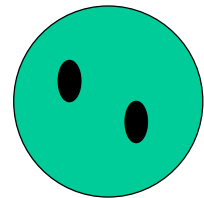


## Egg Placement

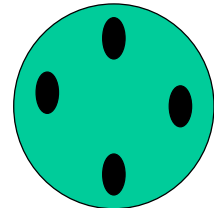
5 and 10



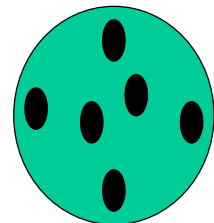
20



40



60



Eggs or adults: 5, 10, 20, 40 and 60 per leaf disk. (10 disks per treatment).



## Results

### Preference Trials

*Phytoseiulus perismilis* did not demonstrate a prey stage feeding preference consistently throughout the duration of the experiment. Analysis revealed only a few significant instances in which more adults were eaten than eggs, and at no time was the reverse true. A significantly higher proportion of adult *T. urticae* were consumed by predators that were exposed to prey monocultures (either adults or eggs) prior to being placed in choice arenas versus predators that were not placed in prey monocultures before being placed in choice arenas. *Phytoseiulus persimilis* that were exposed to prey monocultures also had a higher proportion of observed predator resting events compared to adult feeding events. Treatments with predators exposed to egg monocultures had a significantly greater proportion of adult feeding versus egg feeding compared to predators that were not exposed to prey egg monocultures. There was no a significant difference in the proportion of adult feeding to egg feeding between treatments with exposure to egg and adult prey monocultures.

Predator feeding decreased over the duration of the experiment. Observed feeding events at 90 and 120 minute observations were significantly fewer than those observed at 0 and 30 minutes ( $F=4.49$   $P>F=0.0039$ ). There was no significant difference among the mean number of non-feeding behaviors observed at all times tested ( $F=1.00$   $P>F= 0.4152$ ).

There was a positive correlation between resting and adult feeding ( $r=0.40368$ ,  $DF= 49$ ) and no correlation between adult feeding and walking ( $r=$

0.023208, DF=49). There was no correlation between egg feeding and walking ( $r=0.209293$ , DF=49) and a negative correlation between egg feeding and resting ( $r=-0.41373$ , DF=49). (Table 1)

**Table 1: Total number of events observed in 300 observations in choice arenas.**

<b>Exposure Treatment</b>	<b>Eggs</b>	<b>Adults</b>	<b>Resting</b>	<b>Walking</b>
<b>Eggs</b>	9	29	177	85
<b>Adults</b>	15	35	155	88
<b>No Exposure Direct transfer</b>	43	52	82	123

**Eggs**= attacking *T. urticae* egg

**Adults**=attacking adult *T. urticae*

**Resting**=predator not running or walking.

**Walking**= predator running or walking in search of prey

Pre-exposure to monocultures had no effect on the mean number of walking and resting events 0, 30 and 60 minutes after introduction of predators. However, at 90 and 120 minute after predator introduction, there were more resting events than walking events.

#### Functional Response Experiments

The functional response of *P. persimilis* to eggs and adults was a Type II curve. The type of functional response curve was determined using the

facilitation coefficient (C term) from the descriptive functional response equation described by Fuji et. al. (1986). The calculations were performed by fitting Holling's disk equation to the data points to derive the search rate and handling time. Then, these terms were substituted into the descriptive equation to derive a C value, which was used to interpret the type of functional response curve. At a density of 60 prey per arena, *P. persimilis* consumed an average of 21.7 eggs and 2.5 adults over an eight-hour period. Calculated handling times of *T. urticae* were approximately 4 minutes and 3.4 hours for *T. urticae* eggs and adults respectively (Fig. 3 and 4).

*T. urticae* Eggs

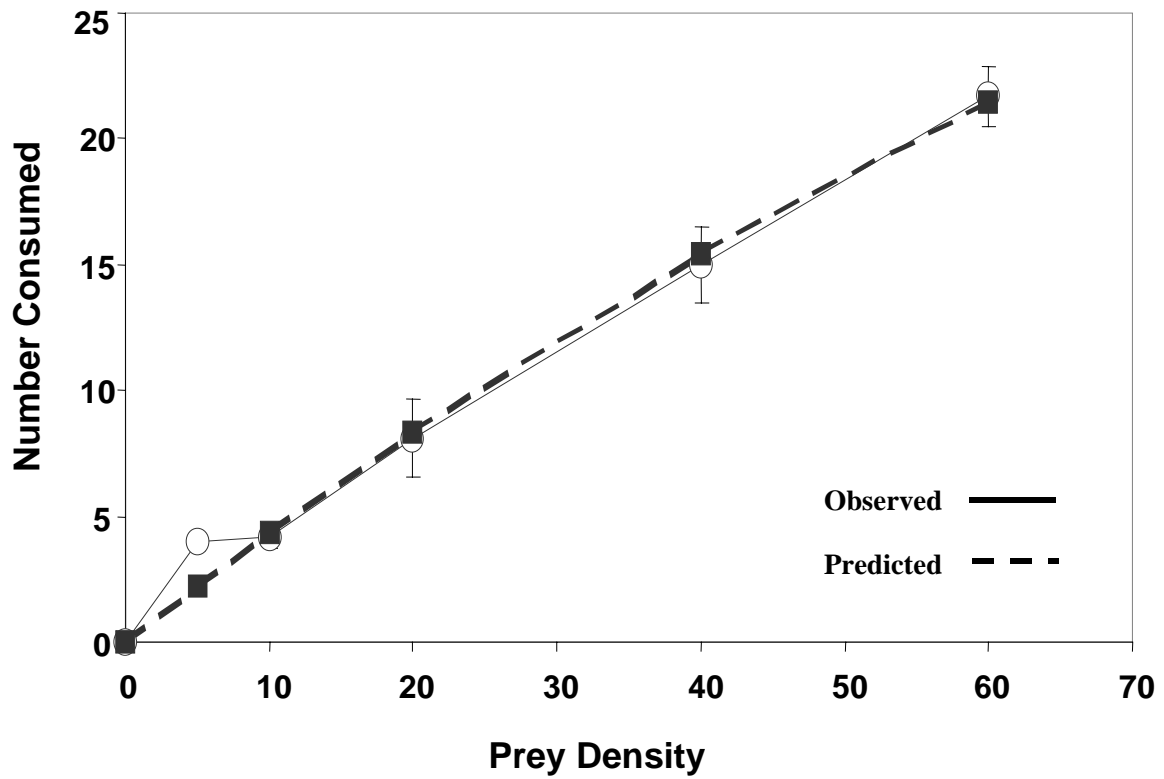


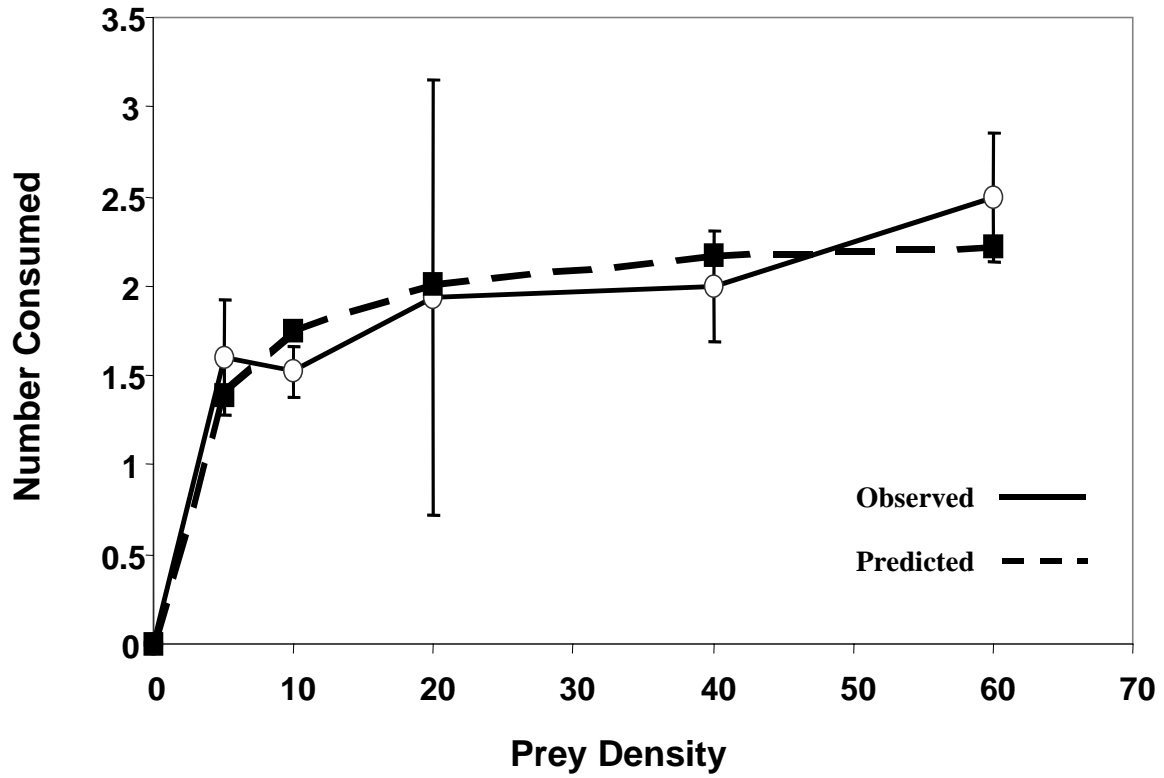
Figure 3: The functional response of commercially available adult *P. perisimilis* on *T. urticae* eggs.

$a'$  = search rate = 0.056

$T_h$  = Handling Time = 0.079

$R^2$  = 0.985

### *T. urticae* Adults



**Figure 4: The functional response of commercially available adult *P. persimilis* to *T. urticae* adults.**

$a'$  = Search Rate = 0.085

$T_h$  = Handling Time = 3.399

$R^2$  = 0.944

## Discussion

In these experiments, we tested adult *P. persimilis* that were in shipping for 1-2 weeks without a food source, which may explain the slight preference observed for adults over eggs. During initial observations, we observed *P. persimilis* adults aggressively attacking *T. urticae* adults. Previous research illustrates that protonymph *P. persimilis* prefer larval *T. urticae* over deutonymphs, while adult *P. persimilis* prefer deutonymphs over larvae (Fernando and Hassel, 1980). *Phytoseiulus perismilis* protonymphs, deutonymphs and adults all preferred twospotted spider mite eggs over deutonymphs in previous studies. Fernando and Hassel used predators that were well fed.

Experiments exposing *Amblyseius fallacis* to a mixture of eggs, larvae, protonymph and deutonymphs of *T. urticae* demonstrated that earlier stages of prey were most commonly attacked (Burnett, 1971). In this study, the percentages of prey eaten were 52% eggs, 32.5% larvae, 19.5% protonymphs and 9% deutonymphs. Additional experiments conducted in a similar manner comparing consumption of larvae, protonymphs, deutonymphs and adults revealed that the highest percentage of predation occurred on prey larvae (Burnett, 1971). Preference for younger prey stages may exist because younger prey are easier to handle (Burnett, 1971). Our functional response data agree with this concept indirectly. *Phytoseiulus persimilis* handling times of approximately 4 minutes and 3.4 hours for eggs and adults respectively, suggest that adult *T. urticae* may not be an ideal prey item.

Based on the data collected, we cannot conclude if *P. persimilis* has a feeding preference for younger prey in a complex prey age structure. Studies conducted by Takafuji and Chant (1976) demonstrated that adult *P. persimilis* attack larvae and protonymphs of *T. pacificus*, but preferred eggs even when the availability of mobile prey stages were abundant. Alternatively, Chant (1961) observed that *P. persimilis* feeding on *Tetranychus telarius* dispersed from prey patches once active prey forms were consumed, leaving the eggs uneaten. *Phytoseiulus persimilis* may have a preference for prey adults in low-density populations of *T. urticae*, and switch to eggs as prey density increases (Mori and Chant, 1965). The density in our experimental arenas may not have been the correct density to induce a behavioral feeding preference for eggs, which may occur in a natural age structure, which would have a larger number of eggs compared to mobile forms. Eggs may be preferred or easier to locate because they are more abundant than other available prey stages (Mori and Chant, 1965). This may not be a behavioral preference or a choice, but a behavioral event that occurs because of increased probability of encountering a specific stage of *T. urticae*. Research conducted on entomopathogenic nematodes demonstrated that nictating species are more effective at finding mobile forms of prey while actively searching species of nematodes are more effective at finding stationary prey (Campbell and Gaugler, 1997). The same may be true for *P. persimilis* preying on *T. urticae* populations; *P. persimilis* may eat the first prey that they encounter while searching regardless of the life stage.



A test was conducted to determine if the handling of *P. persimilis* in the experiments significantly affected the observed behaviors. Results demonstrated that transfer of *P. persimilis* from arenas exposing predators to prey monocultures or from shipping containers did not significantly affect their behavior. This means that the data collected were a representation of how the predators behaved in the system and not how transfer affected their behavior. There was significantly less feeding observed in the 90 and 120 minute observations than observations at 0 and 30 minutes. This was expected because predators feed less as they become satiated.

It is not likely that *P. persimilis* develops a search image when feeding on a prey monoculture for short time periods. Regardless of the type of monoculture exposure, there was not a significantly higher proportion of feeding on a single life stage in mixed populations. It is possible that a search image can develop when *P. persimilis* is exposed to a prey monoculture for longer duration than was conducted in this study. Prey monocultures are not likely to exist for long periods of time in natural populations because *T. urticae* has such a rapid developmental rate.

Our study and Bancroft and Margolies (1996) found that adult *P. persimilis* usually spend more time moving or resting than feeding. Treatments with pre-exposure to prey monocultures had a higher proportion of resting and a higher proportion of adult feeding compared to treatments without pre-exposure. This may have occurred because stationary predators have a higher chance of encountering mobile prey. However, we found that resting and adult feeding

were negatively correlated. These seemingly conflicting results may have occurred because we did not observe a single predator for a prolonged period of time, thus we may not have been able to observe all behavioral aspects related to feeding. The mean number of resting events observed at 90 and 120 minute observations was significantly higher than the mean number of walking events recorded. The increase in resting behavior probably occurred because predators became satiated over time.

I found *P. persimilis* to exhibit a Type II response for both egg and adult *T. urticae*. Adult prey have a larger food content compared to eggs, but they are more difficult to capture (Sabelis, 1990). Thus, predators should eat more eggs than adults to survive and maintain a steady fecundity rate. *Phytoseiulus persimilis* must consume six prey eggs for every egg they lay and they can oviposit up to four eggs per day (Helle and Sabelis, 1985). Theoretically, *P. persimilis* individuals can consume 24 eggs per day. *Phytoseiulus persimilis* in our functional response experiments consumed an average of 21.7 eggs in an eight-hour period. This may be artificially high because the temperatures in our experiment averaged 28° C with relative humidity levels between 30 and 40%. In low relative humidity situations predators feed to acquire liquids (Helle and Sabelis, 1985). Mori and Chant (1965) also found that lower relative humidity levels lead to higher prey consumption rates.

The mean number of adult *T. urticae* consumed by *P. persimilis* at densities of 20, 40 and 60 per leaf disk remained relatively constant. The rate of predation increase with increasing prey density was not constant due to

interference by prey. Prey were seen bumping into predators that were attempting to feed. This caused the predators to abandon the prey and move quickly to another area of the arena. Disturbance has been observed by other researchers to cause the functional response curve to become dome shaped (Mori and Chant, 1965), although this did not occur over the densities tested in the study.

High-density populations of *T. urticae* usually have considerable amounts of webbing. Arenas with densities of 20, 40 and 60 *T. urticae* adults had significant amounts of webbing which could be the cause for similar predation rates at these densities. Webbing can increase or decrease the capture success rate by phytoseiid mites. The capture rate by a species of phytoseiid mite depends on its ability to maneuver across leaf surfaces with webbing (Sabelis and Bakker, 1992). Setal arrangement on the dorsal surface of the soma and tarsi greatly influence the ability of phytoseiid mites to maneuver through webbing (Sabelis and Bakker, 1992). Species that are able to move through webbing have a higher predation rate because the slower forward walking speed increases the chance of prey encounter within a patch (Helle and Sabelis, 1985). However, extremely dense webbing may prevent predation by allowing predators to walk over top of patches, and reduce the chance of prey encounter (Helle and Sabelis, 1985).

The substrate on which mites search for prey affects their behavior and their functional response. Experiments conducted with *P. persimilis* and *T. urticae* indicate that artificial substrates, such as glass, increased the activity of

both species, thus increasing their contact with each other, which caused a Type IV (dome shape curve) functional response to occur (Everson 1980). Even though we used a natural surface to conduct our experiments, the predator behavior observed in this study may not always occur. The functional response of *P. persimilis* can be affected by different morphological features of leaf surfaces (Skirvin and Fenlon, 2001). Leaves with waxy surfaces or numerous trichomes can make predator mobility difficult by impeding movement or grip to the leaf surface which will inherently reduce prey capture rate (Skirvin and Fenlon, 2001).

#### Conclusion

The commercially available strain of *P. persimilis* we examined did not exhibit a consistent prey-stage preference. The results demonstrated a preference for adults over eggs in only a few instances and a consumption of one life stage over another did not occur consistently throughout the study. A persistent preference for a single life stage over a long duration in changing age structures is not likely to occur with *P. persimilis* feeding on *T. urticae* populations. Preference can be affected by prey density (Mori and Chant, 1965), levels of gut fullness and predator motivational state. Any prey-stage preferences that occur in *P. persimilis* are likely to have a short duration. *Phytoseiulus persimilis* that are extremely hungry capture and feed on adult *T. urticae*, but this may not be an efficient long-term strategy. Adults are more difficult to capture and require a longer handling time compared to eggs. Although adults may possess a higher quantity of digestible material, it is more

difficult for *P. persimilis* to extract those materials (Helle and Sabelis, 1985). Predatory mites feed until they are satiated and leave the prey, even if valuable material remains (Helle and Sabelis, 1985). Sandness and McMurtry (1972) found that regardless of the amount of starvation, when a predator feeds on a second prey item after a period of starvation, its duration of feeding for each additional captured prey is similar to previous feeding durations for each prey (Sandness and McMurtry, 1972). If a predator can reach its desired gut fullness level with eggs, it would be inefficient for it to handle adults. Predatory mites appear to feed on the stage that is most abundant at the time (Helle and Sabelis, 1985). Regardless of the strategy utilized by a predator it is usually one that is efficient for predator survival.

## Chapter 4: Using Selective acaricides to Manipulate *Tetranychus urticae* Populations

### Introduction

The twospotted spider mite, *Tetranychus urticae* Koch, is a serious pest of many greenhouse plants, nursery-grown ornamentals and field crops. The host range of *T. urticae* includes numerous species of herbaceous and woody landscape plants such as rose, ivy, and winged euonymus (Johnson and Lyon, 1991). Development of resistance by *T. urticae* to numerous acaricides has caused difficulties in controlling outbreaks (Carbonaro et al., 1986). Some growers have attempted to use the predatory mite *Phytoseiulus persimilis* to control *T. urticae* populations. However, results from release of phytoseiid mites are variable (Linquist, 1981). When *T. urticae* density is too high, *P. persimilis* predation will not reduce *T. urticae* populations to acceptable levels (Helle and Sabelis 1985). Acaricide applications are often necessary because of the demand for high quality ornamental crops and the inability of *P. persimilis* to reduce high-density populations of *T. urticae*.

Many new acaricides are available on the market but they have a high cost associated with their use and associated application restrictions listed on the label to prevent the development of resistance. Thus, the release of predators and the selective use of acaricides to conserve natural enemy populations may be an important part of reducing the number of acaricide applications. Numerous studies have been conducted on the compatibility of acaricides with *P. persimilis*

(Cote chapter 2, unpublished, Oomen et. al., 1991 and Zhang and Sanderson 1990).

Researchers have also examined the effectiveness of combinations of releases of *P. persimilis* with compatible acaricides. Studies conducted by Trumble and Morse (1993) on the harvest value of strawberries demonstrated that a combination of predator releases followed by applications of abamectin after pest mite threshold levels were reached provided a higher value crop than abamectin alone. Applications of insecticidal soap three days after release of *P. persimilis* did not adversely affect predator populations and provided enhanced suppression of spider mite populations (Osborne and Petitt, 1985). Acaricides that have short residuals can be used in combination with predators to reduce high-density populations of spider mites, but the timing of application and predator release is critical (Osborne and Petitt, 1985).

Selective use of acaricides may create a favorable situation for release of *P. persimilis* by reducing *T. urticae* to manageable levels, provided other environmental conditions are suitable. Treatment with acaricides that have long residual toxicity may be required to suppress high-density spider mite populations. However, use of acaricides with long residual periods may promote resistance in spider mite populations. Low-density populations may be suppressed with acaricides that have short residual toxicity. Biological control may be enhanced through careful selection of acaricides and releasing predators into the crop once residues are no longer toxic to them.

We hypothesized that selective acaricides may manipulate the age structure and density of *T. urticae* populations in a manner that better enables predatory mites to use the pest population as a food resource. There is controversy regarding the existence of prey stage preference in *P. persimilis* feeding on *Tetranychus* prey. The objective of the study was to find suitable combinations of acaricides that will alter *T. urticae* population age structure and density to enhance the effectiveness of *P. persimilis*.

Materials and Methods:

### **Laboratory Assays**

Butterfly bush plants (*Buddleia x davidii* cv. 'White profusion') in plastic containers with a peat based soil-less medium and fertilized with 18-18-18 Osmocote slow release fertilizer. No pesticides were applied to stock plants.

Trials were conducted in glass canning jars. Fifteen-cm long cuttings were taken from *Buddleia x davidii* plants and the bottom leaves were removed leaving two or three nodes (four to six leaves). Cuttings were washed with water and rinsed under the faucet to remove any arthropods from foliage. A piece of copper screening with a small hole in it was placed in the bottom of the canning jar to prevent leaves from falling into the water. Three to six centimeters of water was added to the jars. The entire canning jar unit complete with cutting was placed inside a glass battery jar filled with five cm of water to reduce mite escape. Petroleum jelly was applied to the inner perimeter of the battery jar to prevent mite escape. The battery jar-canning jar system was placed in a large plastic tray, which had double sided sticky tape on the outside. The entire unit



was placed under HID (High Intensity Discharge) mercury vapor lighting with 14:10 L:D photoperiod.

One week before acaricide application *Buddleia x davidii* cuttings were infested with 10 *T. urticae*. On the day of the pesticide applications, the numbers of mite eggs, immatures and adults were counted. Abamectin, azadirachtin, bifenthrin, chlorfenapyr, Gowan 1725, hexythiazox, horticultural oil, neem oil, pyridaben and spinosad were mixed with tap water and applied to cuttings while under a fume hood using a hand held sprayer (Table 1). Control plants were sprayed with tap water. Cuttings were sprayed until run off, returned to their jar with water and allowed to dry in a fume hood for approximately ½ hour. The number of eggs, immatures and adults were counted three, seven and 14 days after application. Two trials of five cuttings for each trial were conducted for each chemical. Temperatures during the experiment averaged 28 °C with a range from 21.8 – 33.0 °C. Relative humidity averaged 45% with a range of 32-55%. Age structure data were analyzed by contingency tables and ANOVA and means were separated using Tukeys test. *Tetranychus urticae* densities analyzed by ANOVA and means were separated with Duncans test.

**Table 1: Acaricides tested**

Common Name	Trade Name + Formulation	Mix Rate
Abamectin	Avid 0.15 EC (Novartis, Greensboro, NC)	4 oz/ 100 gallons
Azadirachtin	Nimbecidine (Distributed by PBI Int. Potomac, MD)	50 oz/ 100 gallons
Bifenthrin	Talstar GH 0.67F (FMC, Philadelphia, PA)	40 oz/ 100 gallons
Chlorfenapyr	Pylon 2SC (American Cyanimid, Parsippany, NJ)	5.2 oz/ 100 gallons
Gowan 1725	Gowan 1725 0.1% EC (Gowan, Yuma, AZ)	20 oz / 100 gallons
Hexythiazox	Hexygon 50 WP (Gowan, Yuma, AZ)	1.5 oz / 100 gallons
Horticultural oil	Sunspray Ultra-Fine (Sun Company, Inc., Philadelphia, PA)	250 oz / 100 gallons
Neem oil	Triact 70 EC (Thermotriology Corporation, Columbia, MD)	250 oz/ 100 gallons
Pyridaben	Sanmite 75 WP (BASF Corporation, Research Triangle Park, NC)	4 oz/ 100 gallons
Spinosad	Conserve SC (Dow AgroSciences, Indianapolis, IN)	600 ml/ 100 gallons

## **Greenhouse Trials**

### Trial One

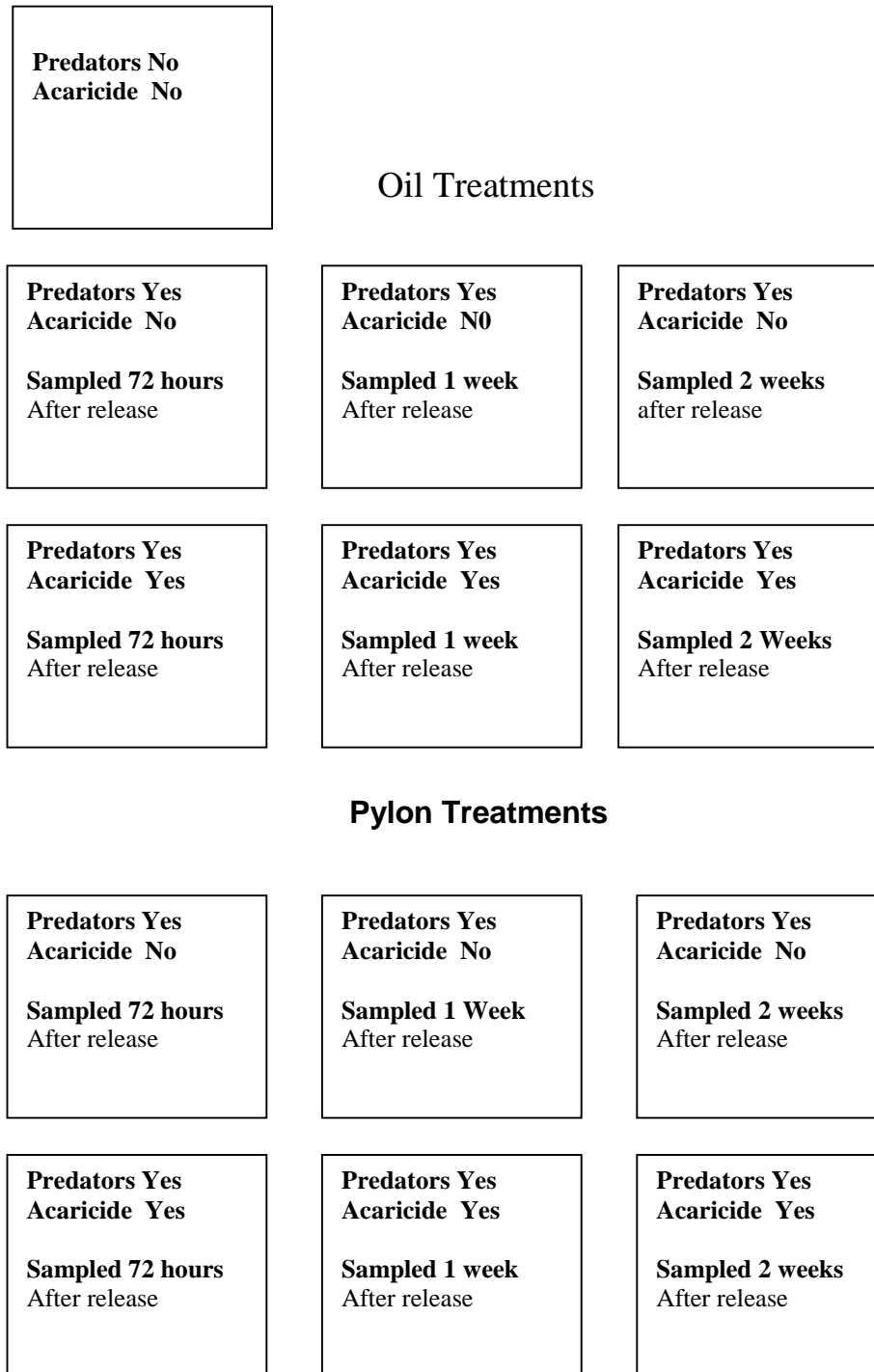
*Buddleia* plants were propagated from cuttings taken from stock plants. Once cuttings had rooted, they were grown in a greenhouse in 4-inch pots with a peat based soil-less media. Plants were fertilized with 18-18-18 slow release Osmocote fertilizer that was applied two weeks after transplanting. Plants were grown under natural light conditions until they reached a height of 30-45 cm. Granular imidacloprid was applied to the soil surface to suppress whitefly and aphid populations.

Plants were arranged as shown in Figure 1. There were thirteen blocks with 5 plants in each block. Blocks of plants were separated from each other to prevent plants touching and mites moving between blocks. Overhead watering was avoided to prevent mites from being washed off of plants. The experiment was conducted twice.

Each block of plants was manually infested with *T. urticae* until a minimum density was established on the plants. Maximum and minimum daily temperatures and relative humidity levels were recorded after plants were infested. The temperature in the greenhouse was increased and the plants were given 50 fc of continuous light with warm white, fluorescent lights to promote mite infestation. Daily visual plant inspections were conducted for damage. Pesticide applications were performed when visual damage was easily seen during inspection.

Chlorfenapyr and horticultural oil were chosen for the greenhouse trials because data from previous experiments demonstrated that the toxicity of their residues to *P. persimilis* were at opposite ends of the spectrum. Ten leaves (2 from each plant) with moderate damage were sampled from each block immediately prior to acaricide applications. Leaf samples were placed in sandwich bags and kept on ice for transport to the lab. In the lab, leaves were immersed in Mop and Glow ® floor wax and allowed to dry before being placed in the refrigerator for storage less than two weeks. The total number of *T. urticae* eggs, immatures, adults was counted on each leaf sampled from all treatments Figure 1.

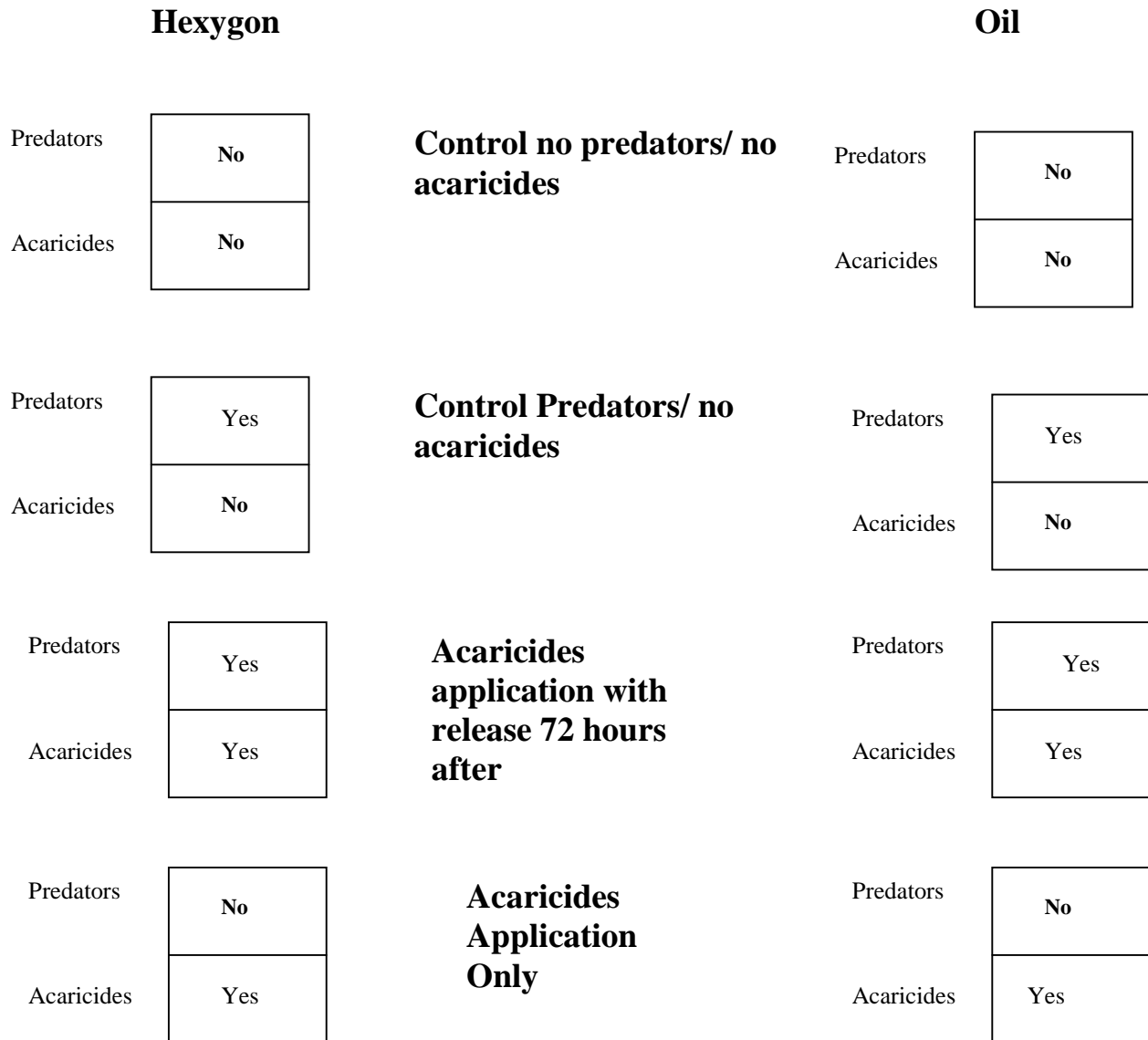
*Phytoseiulus persimilis* were released 72 hours after oil applications and two weeks after chlorfenapyr applications at a rate of 100 per block (Figure 1). Leaf samples were taken from all blocks immediately before predator release and the number of *T. urticae* eggs, immatures and adults were counted. Additional samples were taken at 3, 7, and 14 days after release on selected blocks of plants (Figure 1). Temperatures during the trial averaged 24.1 °C with a range of 14.7-35.2 °C. The average relative humidity was 54.8% with a range of 42- 70%. Data were analyzed with ANOVA and means were separated using Duncan's Test.



**Figure 1: Greenhouse Trial One: schematic diagram of experimental design.**

## Greenhouse Trial Two

*Buddleia* plants were grown in the same manner as the first field trial except plants were given natural light conditions with summer photoperiods. Plants were not treated with imidacloprid because aphid and thrips pressure was low. Five plants were used in each block and arranged as shown in Figure 2. Plants were infested with *T. urticae* by placing two infested bean leaves in each block. Infestations were allowed to progress until threshold levels were reached or visual damage began to appear. Hexythiazox and oil were applied to the appropriate blocks. *Phytoseiulus persimilis* were introduced to oil and hexythiazox groups at a rate of 100 per block three days after application. Samples were taken immediately before acaricide application, 3 days after application (immediately before predator release), and 3, 6, 9, 12 and 15 days after predator release. Ten leaves were taken from each block (2 from each plant) and dipped in floor wax. The total number of eggs, immatures and adults was recorded for each leaf. Temperatures during the experiment averaged 28 °C with a range of 12-43° C. Relative humidity levels averaged 46% with a range of 28-65%. Data were analyzed by repeated measures ANOVA, ANOVA and means were separated using Duncan's test.



**Figure 2** Second greenhouse trial: Schematic diagram of experimental design

## Results

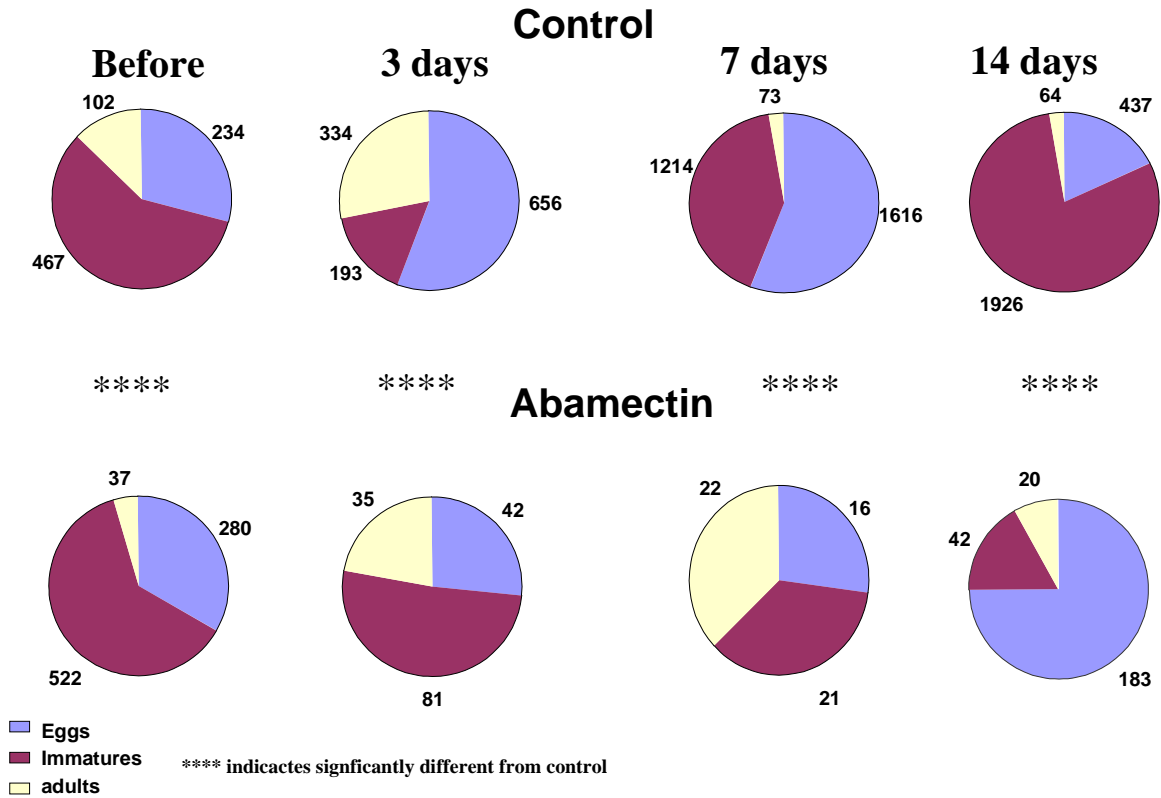
### Age Structure Manipulation

The age structure of *T. urticae* on cuttings treated with abamectin, azadirachtin, hexythiazox, horticultural oil, neem oil, pyridaben and spinosad were significantly different from their respective controls throughout the experiment. Cuttings treated with bifenthrin had age structures that were significantly different than the control before treatment (7 days after infestation), 3 and 14 days after application. However, the age structure 7 days after application of bifenthrin was not significantly different than the control. The age structure of cuttings treated with chlorfenapyr was not significantly different from the respective control before application, but the age structures at 3, 7 and 14 days after application were significantly different. Cuttings treated with Gowan 1725 had age structures that were significantly different from the control before, 3, and 7 days after application (Figure 3, A-J).

Further analysis was conducted to determine if treatments with specific acaricides significantly increased the proportion of eggs in a given population of *T. urticae* (Table 2). Bifenthrin, chlorfenapyr and Gowan 1725 had a significantly higher proportion of eggs compared to the combined controls 3 days after application. This trend continued in chlorfenapyr treatments 7 days after application. There was not a significant difference in the proportion of eggs in any treatments at any other times measured.

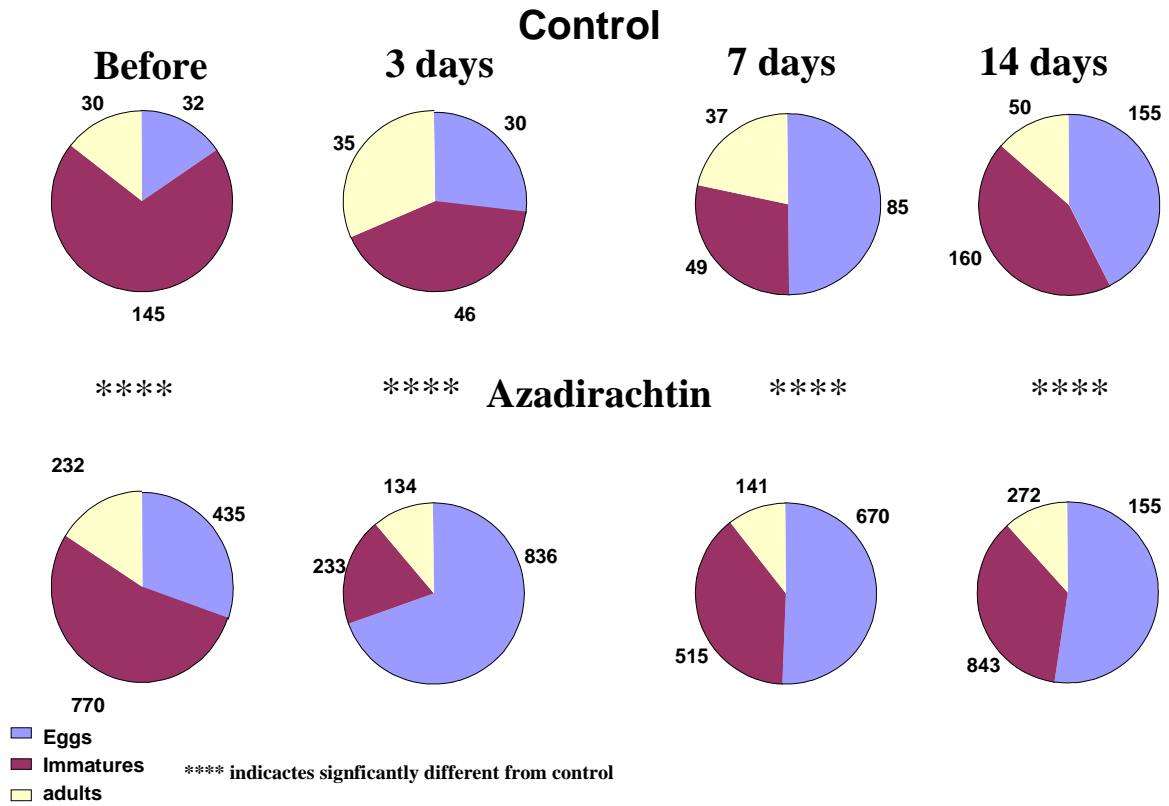


## Age Structures



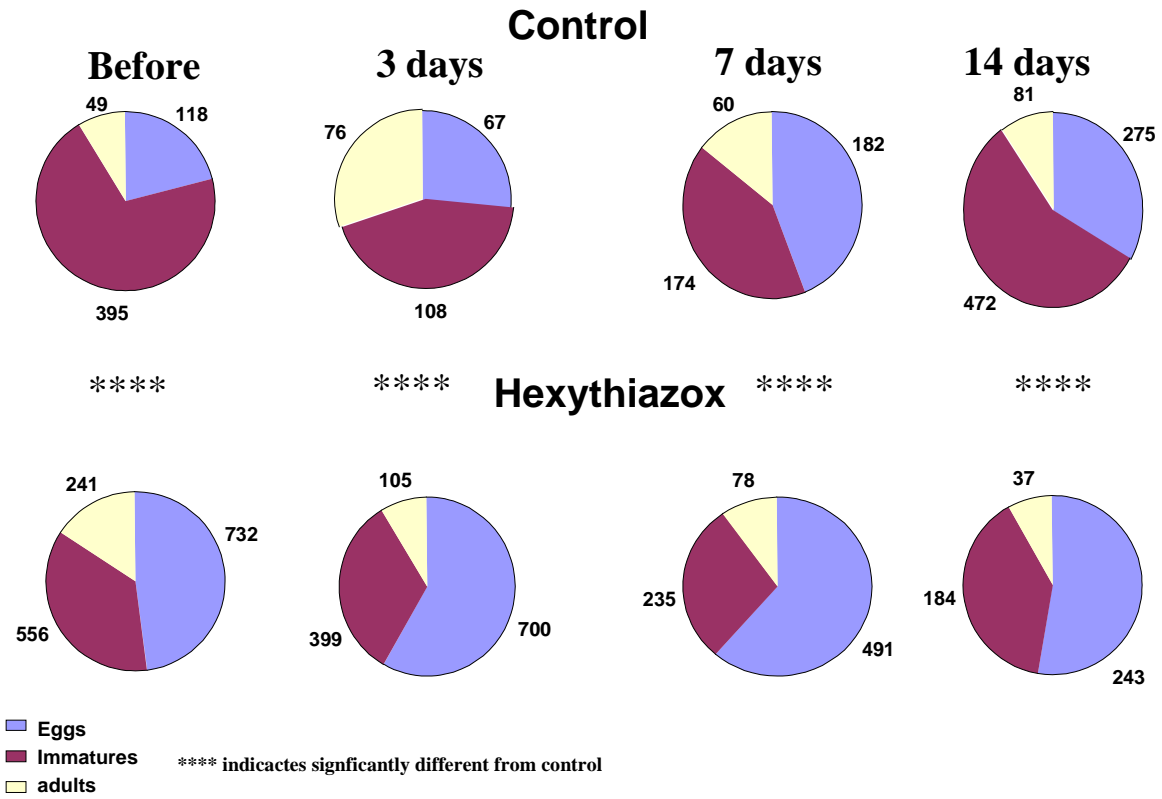
**Fig 3A:** The age structure of *T. urticae* at selected sampling times, before acaricide application and 3, 7, and 14 days after application

## Age Structures



**Fig 3 B:** The age structure of *T. urticae* at selected sampling times, before acaricide application and 3, 7, and 14 days after application.

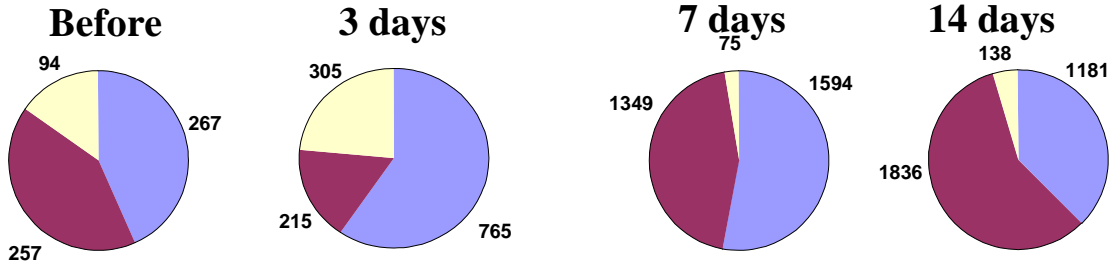
# Age Structures



**Fig 3 C:** The age structure of *T. urticae* at selected sampling times, before acaricide application and 3, 7, and 14 days after application.

# Age Structures

## Control



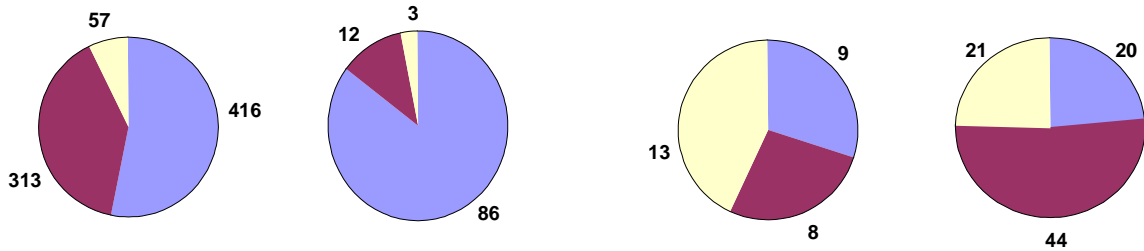
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## Oil

\*\*\*\*

\*\*\*\*

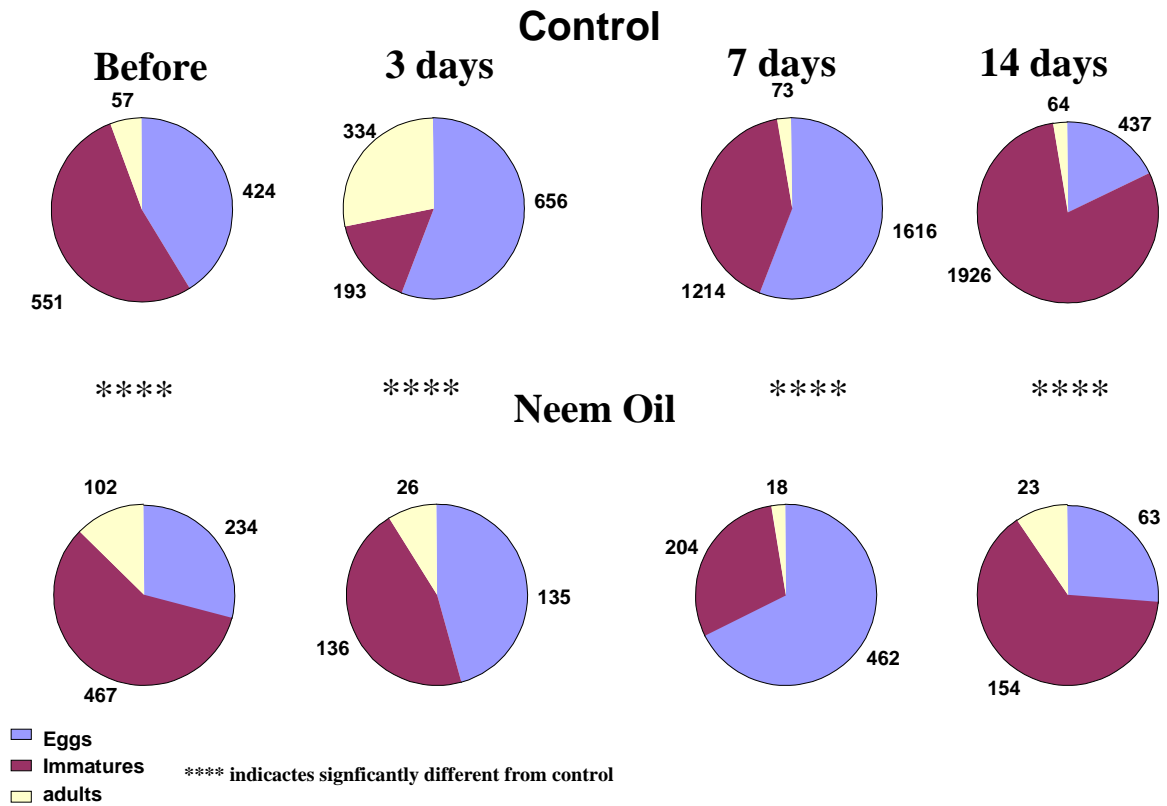


■ Eggs  
■ Immatures  
■ adults

\*\*\*\* indicates significantly different from control

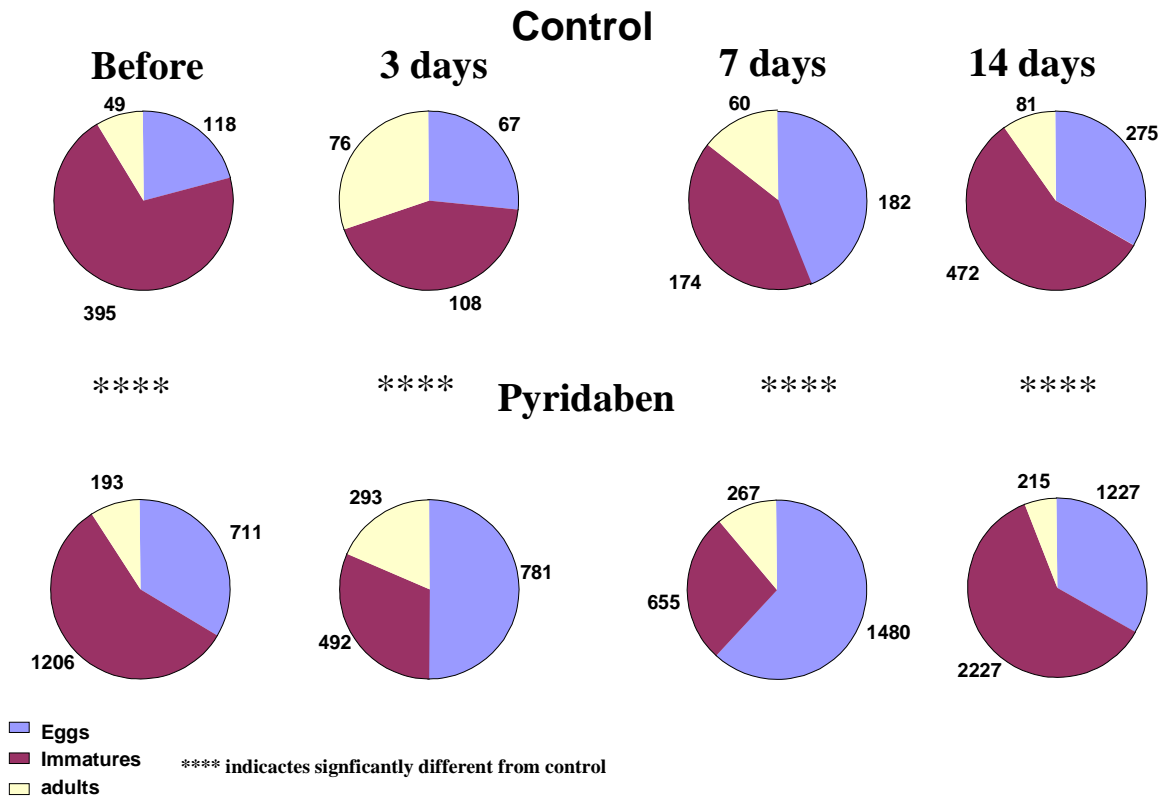
**Fig 3 D:** The age structure of *T. urticae* at selected sampling times, before acaricide application and 3, 7, and 14 days after application.

## Age Structures



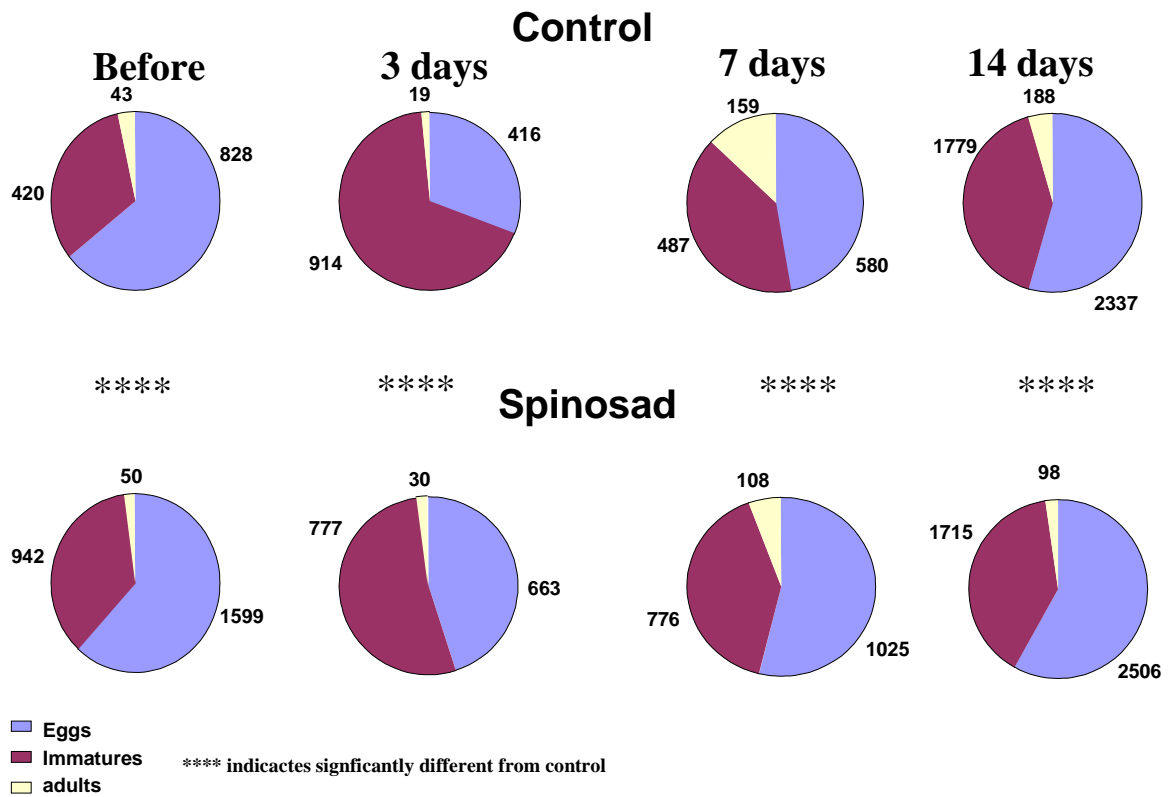
**Fig 3 E:** The age structure of *T. urticae* at selected sampling times, before acaricide application and 3, 7, and 14 days after application.

## Age Structures



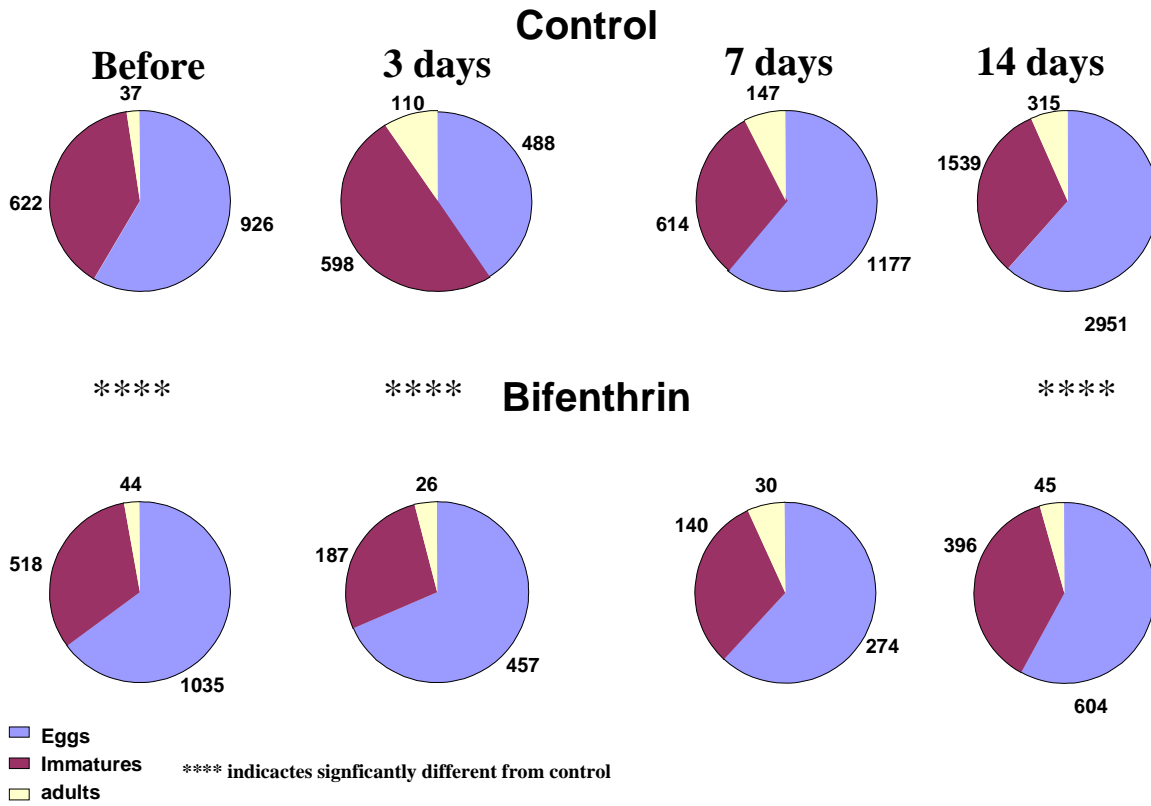
**Fig 3 F:** The age structure of *T. urticae* at selected sampling times, before acaricide application and 3, 7, and 14 days after application.

## Age Structures



**Fig 3 G:** The age structure of *T. urticae* at selected sampling times, before acaricide application and 3, 7, and 14 days after application.

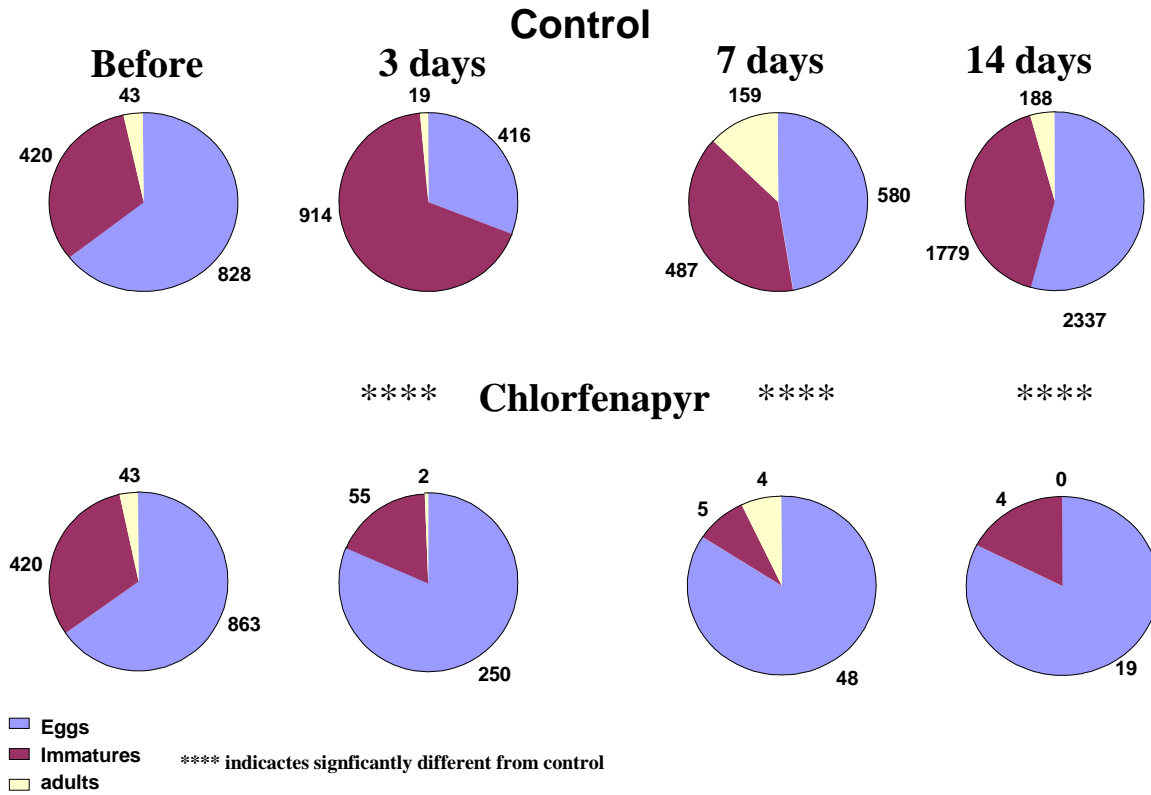
## Age Structures



**Fig 3 H:** The age structure of *T. urticae* at selected sampling times, before acaricide application and 3, 7, and 14 days after application.



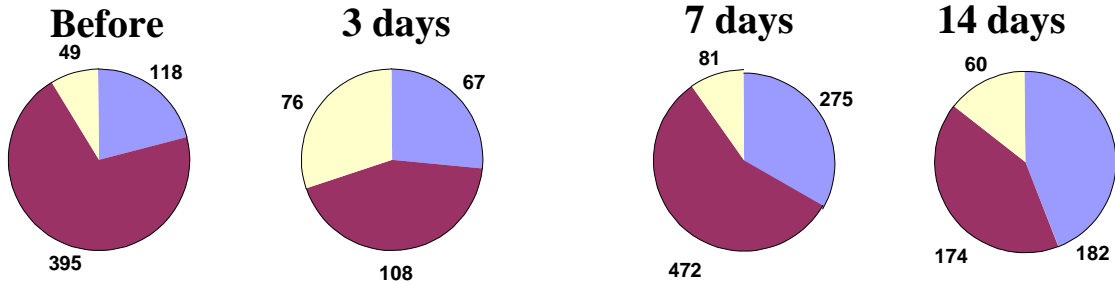
## Age Structures



**Fig 3 I:** The age structure of *T. urticae* at selected sampling times, before acaricide application and 3, 7, and 14 days after application.

## Age Structures

### Control

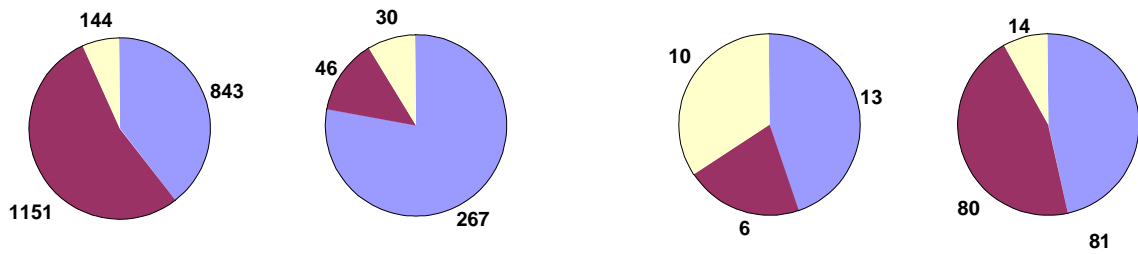


\*\*\*\*

\*\*\*\*

### Gowan 1725

\*\*\*\*



■ Eggs  
■ Immatures  
■ adults  
 \*\*\*\* indicates significantly different from control

**Fig 3 J:** The age structure of *T. urticae* at selected sampling times, before acaricide application and 3, 7, and 14 days after application.

**Table 2:** Mean proportion of eggs for each treatment at given time **Bold**

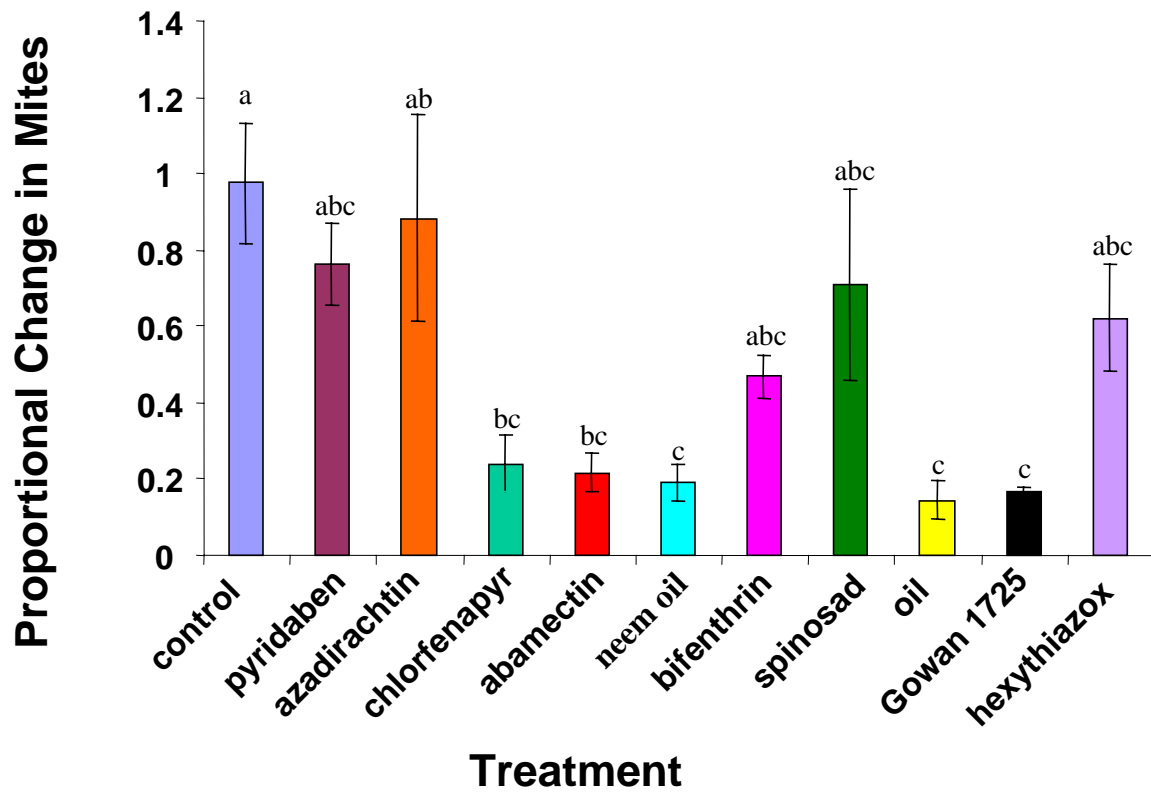
numbers display the mean proportion of eggs on each cutting. The regular font displays the SEM. Capital letters indicate significant differences in means across rows. \*\*\*\*\* indicates mean is significantly different than control.

<b>Treatment</b>	<b>Before</b>	<b>3 days</b>	<b>7 days</b>	<b>14 days</b>
Control	0.491 0.030 A	0.519 0.024 AB	0.274 0.027 B	0.451 0.027 B
Abamectin	0.359 0.082 A	0.227 0.090 A	0.096 0.056 A	0.282 0.123 A
Bifenthrin	0.703 0.051 A, *****	0.731 0.044 A, *****	0.409 0.097 AB	0.567 0.068 B
Chlorfenapyr	0.582 0.035 A	0.673 0.113 A, *****	0.599 0.147 A, *****	0.292 0.132 A
Gowan 1725	0.320 0.054 A	0.586 0.106 A, *****	0.506 0.146 A	0.182 0.101 A
Hexythiazox	0.421 0.066 A	0.450 0.080 A	0.575 0.049 A	0.356 0.083 A
Horticultural oil	0.604 0.090 A	0.528 0.141 A	0.223 0.129 A	0.287 0.133 A
Neem oil	0.474 0.072 A	0.382 0.104 A	0.441 0.124 A	0.264 0.118 A
Pyridaben	0.240 0.042 A	0.433 0.073 AB	0.368 0.081 AB	0.260 0.061 A
Spinosad	0.613 0.019 A	0.427 0.072 A	0.522 0.047 A	0.512 0.088 A

## Density Manipulation

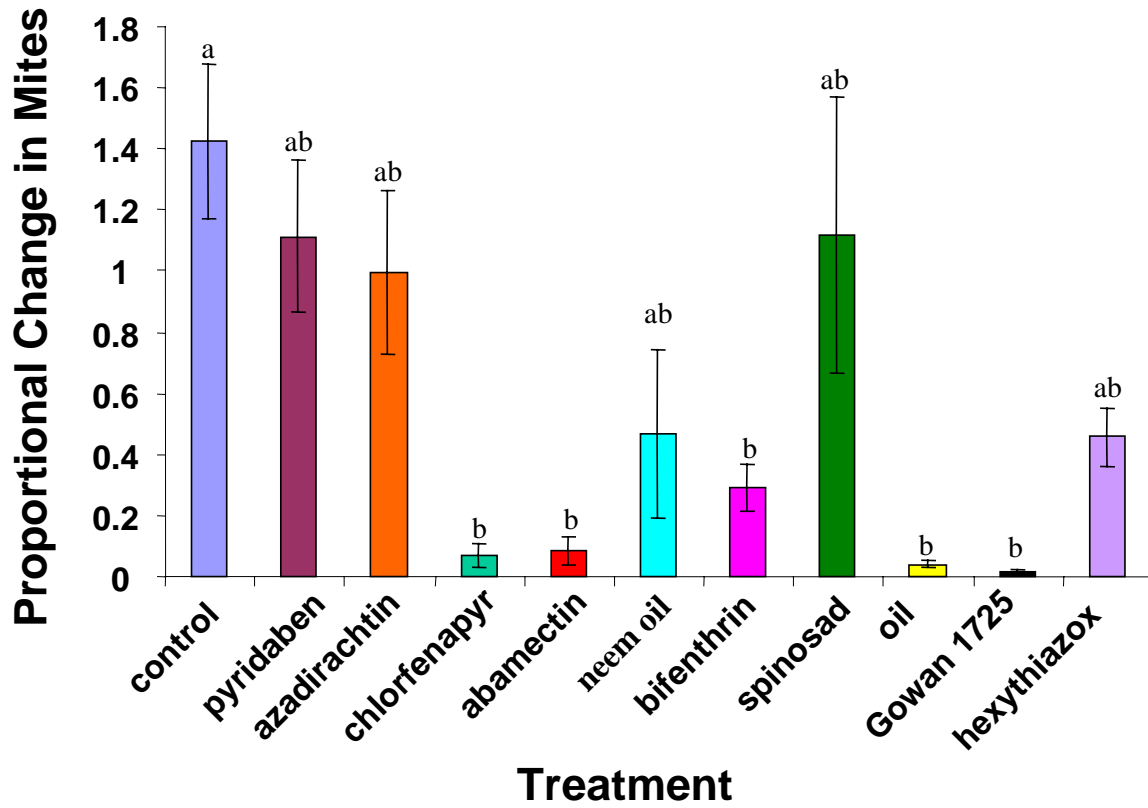
The mean numbers of *T. urticae* on cuttings treated with azadirachtin, bifenthrin, hexythiazox, pyridaben and spinosad were not significantly different from the control. The mean numbers of *T. urticae* on cuttings treated with abamectin, chlorfenapyr, Gowan 1725, neem oil and oil were significantly less than the control {(F= 3.36, P>F=0.0006) Figure 4 A}. One week after treatment with abamectin, bifenthrin, chlorfenapyr, Gowan 1725 and oil resulted in *T. urticae* populations that were significantly less than the control {(F=3.59,P>F=0.0003) Figure 4 B}. Two weeks after application, the mean number of *T. urticae* remained significantly lower than the control in cuttings treated with abamectin, bifenthrin, chlorfenapyr, Gowan 1725, hexythiazox, neem oil and oil {(F=9.32,P>F=<0.001) Figure 4 C}.

## Acaricide Efficacy 3 Days

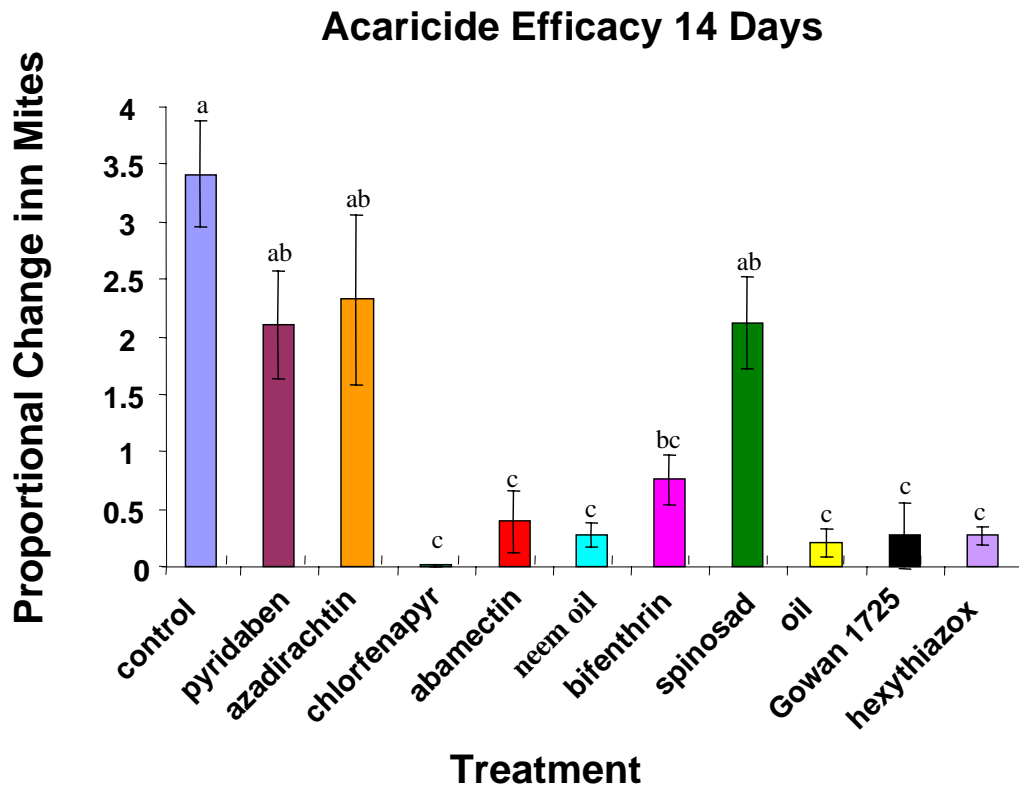


**Fig 4 A:** Effects of acaricides on the population density of *T. urticae* on infested *Buddleia* cuttings.

## Acaricide Efficacy 7 Days



**Fig 4 B:** Effects of acaricides on the population density of *T. urticae* on infested *Buddleia* cuttings



**Fig 4 C:** Effects of acaricides on the population density of *T. urticae* on infested *Buddleia* cuttings.

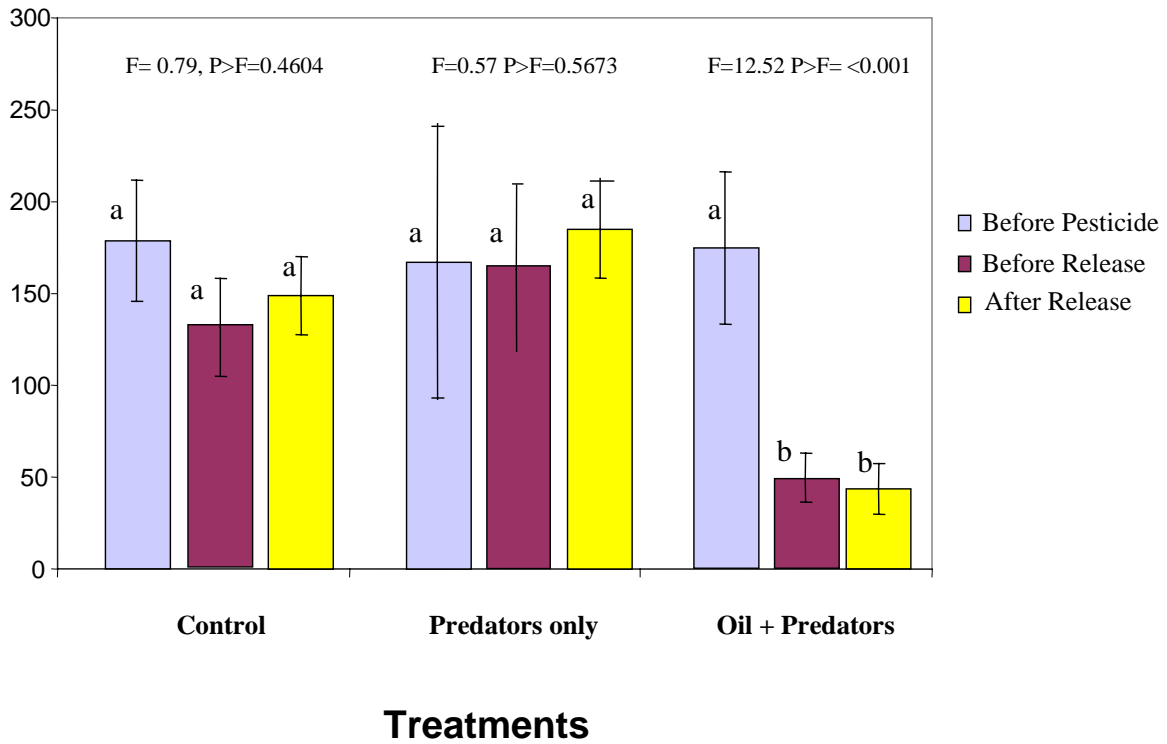
## Greenhouse Trials:

### Trial One

Plants inoculated with predators only in the oil portion of the trial had a significant reduction in the mean number of *T. urticae* 14 days after introduction of *P. persimilis*  $\{(F=4.97, P>F=0.0103)\}$  Fig. 5 C}. Severe damage occurred to test plants in the greenhouse despite the significant reduction in *T. urticae* after release of predators. There was not a significant reduction in the mean number of mites 3 days after predator release on plants without applications of oil  $\{(F=0.57, P>F=0.5673)\}$  Fig. 5 A}. In the chlorfenapyr portion of the experiment, there was not a significant reduction in the mean number of *T. urticae* compared to the mean number before release at all times after the release. These plants had severe damage from mite feeding. The combination of oil followed by release of predators 3 days after application may have provided some suppression of mite populations. Seven days after release, the mean number of *T. urticae* in plants with this treatment was significantly less than before release  $\{(F=10.84, P>F=0.001)\}$  Fig. 5 B}.

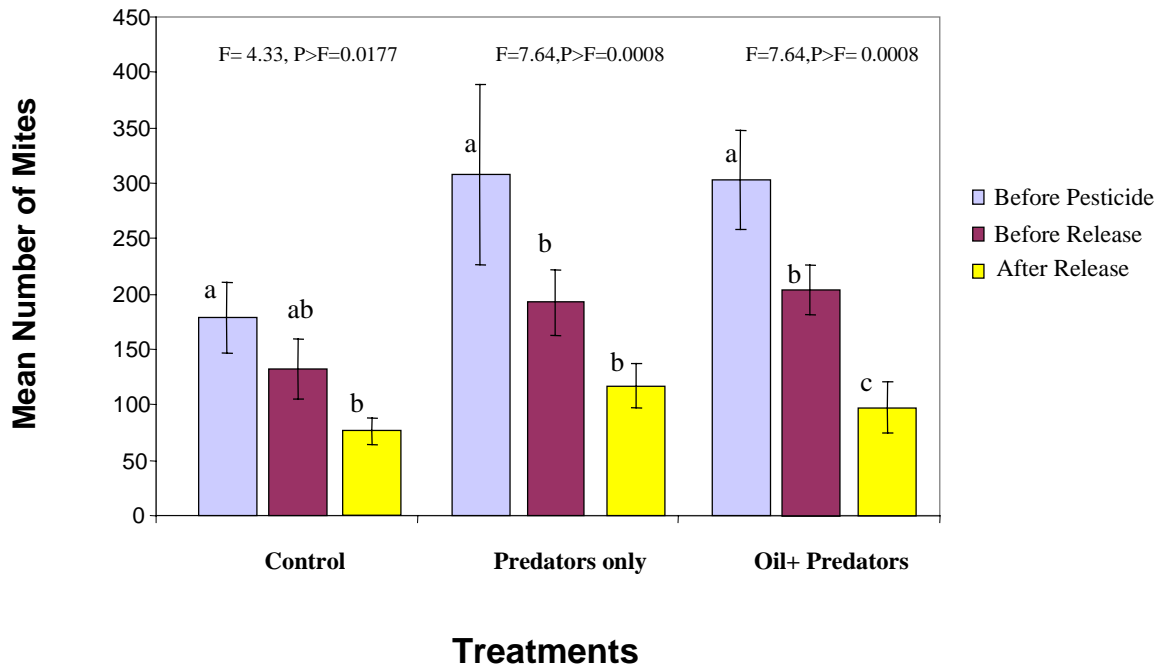


### Oil 3 Days After Release



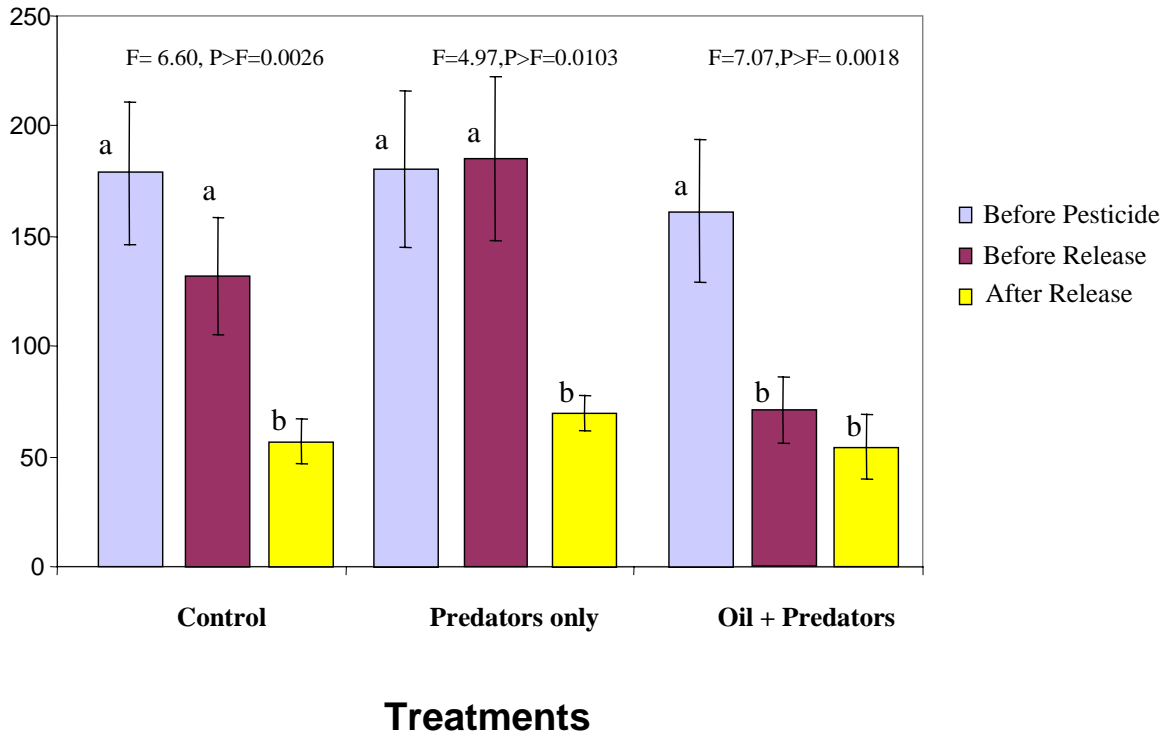
**Fig 5 A::** Results of first greenhouse trial using combinations of acaricides followed by release of *P. persimilis* to suppress *T. urticae* populations. Samples were taken from the oil portion of the experiment before acaricide application, before release and 3 days after introduction of *P. persimilis*

### Oil 7 Days After Release



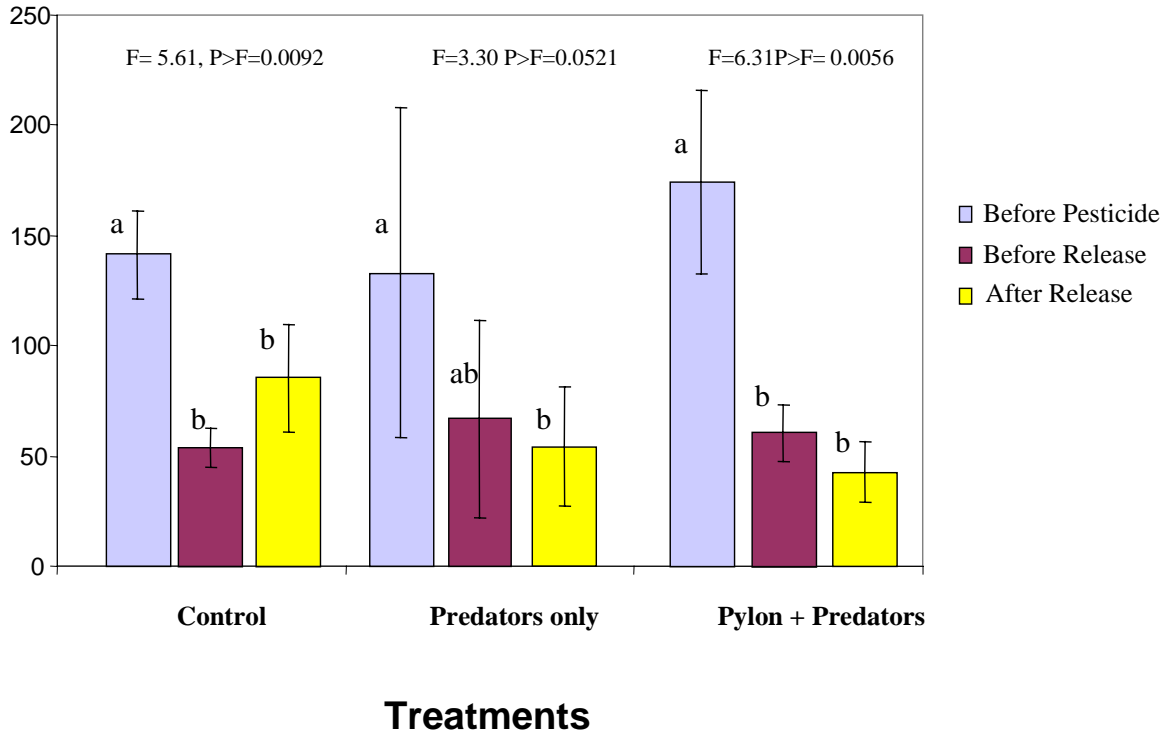
**Fig 5 B:** Results of first greenhouse trial using combinations of acaricides followed by release of *P. persimilis* to suppress *T. urticae* populations. Samples were taken from the oil portion of the experiment before acaricide application, before release and 7 days after introduction of *P. persimilis*.

### Oil 14 Days After Release



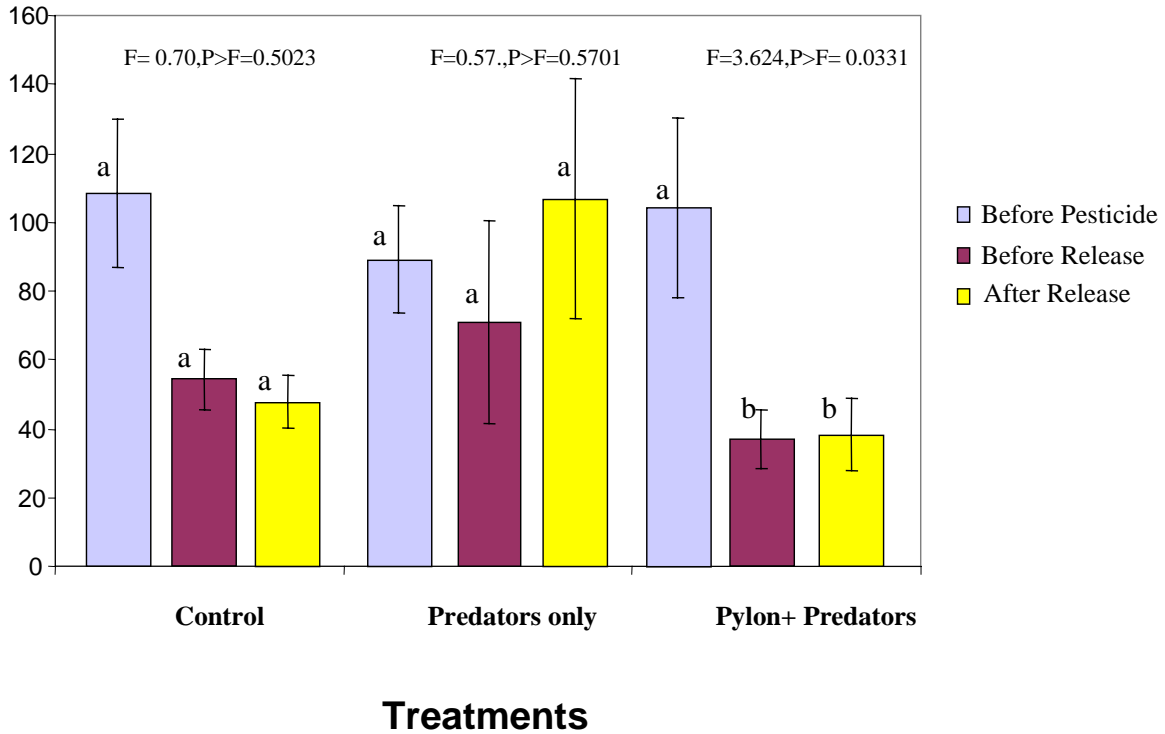
**Fig 5 C:** Results of first greenhouse trial using combinations of acaricides followed by release of *P. persimilis* to suppress *T. urticae* populations. Samples were taken from the oil portion of the experiment before acaricide application, before release and 14 days after introduction of *P. persimilis*.

### Chlorfenapyr 3 Days After Release



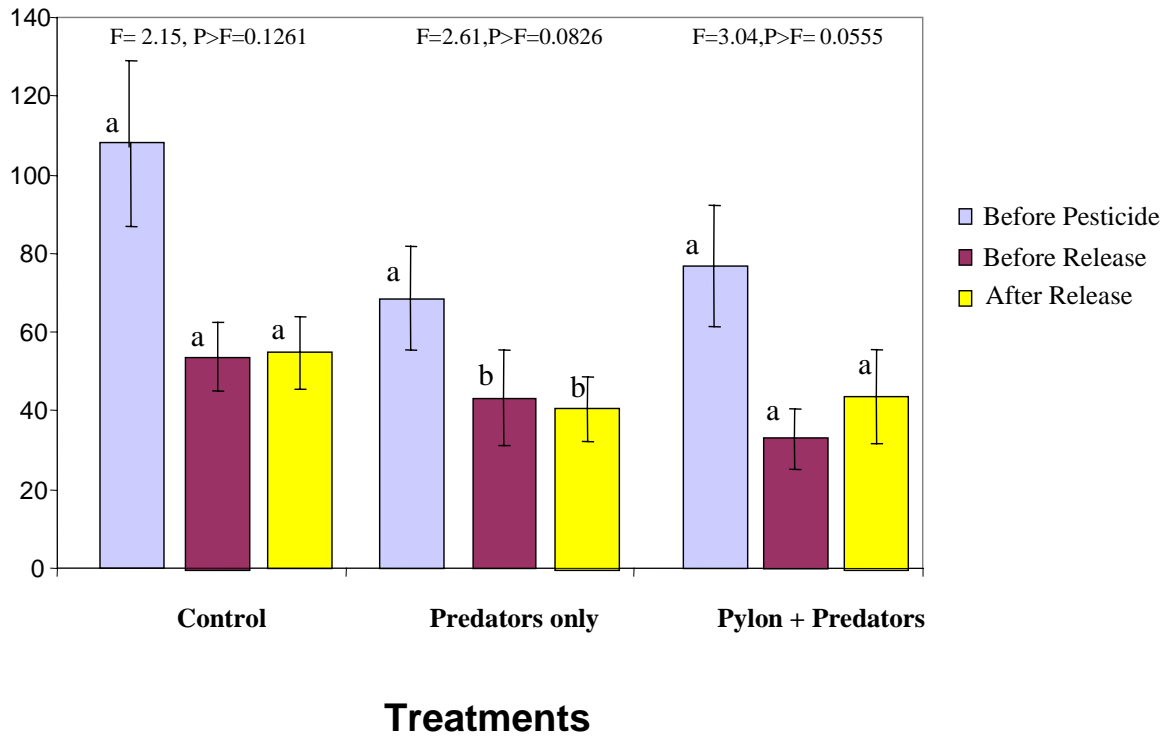
**Fig 5 D:** Results of first greenhouse trial using combinations of acaricides followed by release of *P. persimilis* to suppress *T. urticae* populations. Samples were taken from the chlorfenapyr portion of the experiment before acaricide application, before release and 3 days after introduction of *P. persimilis*.

### Chlorfenapyr 7 Days After Release



**Fig 5 E:** Results of first greenhouse trial using combinations of acaricides followed by release of *P. persimilis* to suppress *T. urticae* populations. Samples were taken from the chlorfenapyr portion of the experiment before application of acaricide, before release and 7 days after introduction of *P. persimilis*.

### Chlorfenapyr 14 Days After Release



**Fig 5 F:** Results of first greenhouse trial using combinations of acaricides followed by release of *P. persimilis* to suppress *T. urticae* populations. Samples were taken from the chlorfenapyr portion of the experiment before acaricide application, before release and 14 days after introduction of *P. persimilis*.

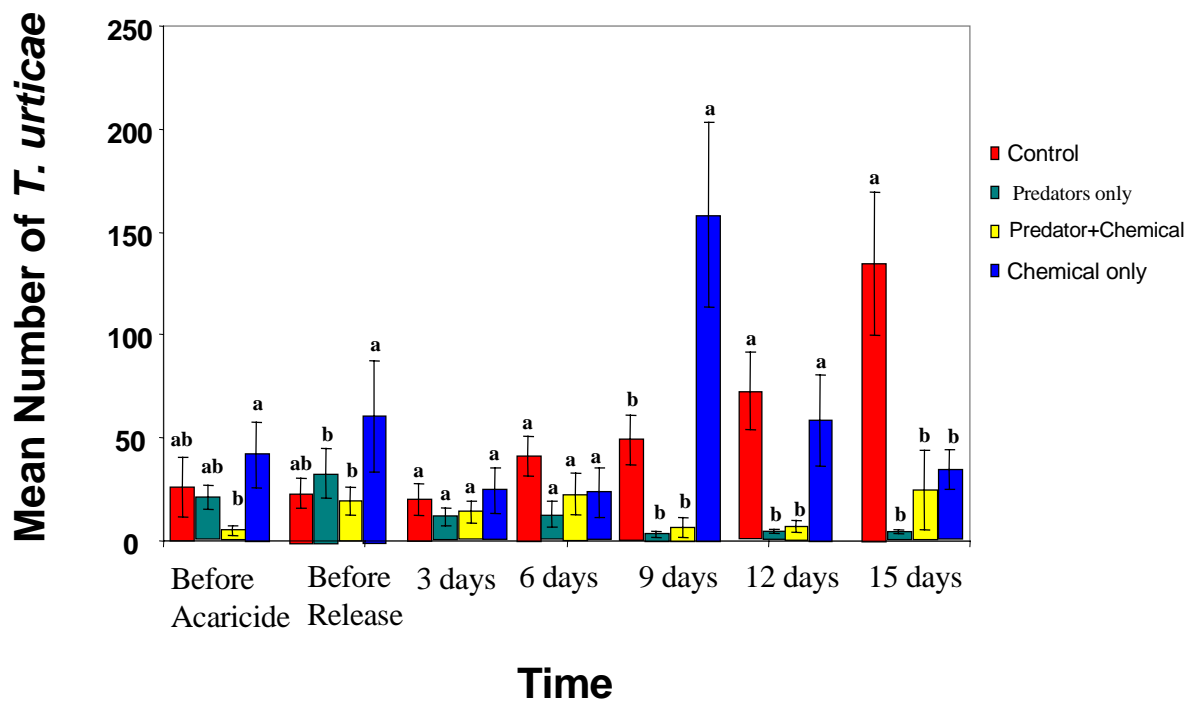
## Second Greenhouse Trial

Release of predators reduced *T. urticae* populations. The mean number of *T. urticae* in treatments with predators only was significantly lower than the control 9, 12 and 15 days after release in the oil portion of the experiment {(F=10.76, P>F=,0.0001; F=6.35, P>F=0.0014; F=9.24, P>F=0.0001) Figure 6 A}. In the hexythiazox portion of the trial, the mean number of *T. urticae* in treatments with predators only was significantly less than the control 6, 12 and 15 days after release {(F=7.08, P>F=0.0007; F=3.70, P>F=0.0204; F=9.57, P>F=<0.0001) Figure 6 B}. Although not statistically significant, the mean number of *T. urticae* in treatments with only predators in the oil portion of the experiment were less than the mean number of *T. urticae* in treatments with a combination of oil and predators at all times observed after release. The same was true in the hexythiazox portion of the experiment except for observations taken 3 and 6 days after release. At these times, the mean number of *T. urticae* was not different from predator only treatments compared to treatments with both predators and hexythiazox. The mean number of mites in treatments with oil alone was significantly greater than treatments with predators and predators+ acaricides; 9 and 12 days after release, (F=10.76, P>F=<0.0001; F=6.35, P>F= 0.0014), and 6, 12 and 15 days after release in plants treated with hexythiazox (F=7.08, P>F=0.0007; F=3.70, P<F=0.0204; F=9.57, P>F=<0.0001). The combination of *P. persimilis* release with compatible acaricide applications did not suppress *T. urticae* populations in a manner that was significantly greater than the acaricide application alone.

Repeated measures ANOVA of the data revealed significant differences in the response of *T. urticae* populations over time among treatments. Populations treated with oil + *P. persimilis* had trends that were significantly different from the control ( $T = 3.30, P \leq 0.00$ ). However, population trends of *T. urticae* treated with oil alone were not different from the control ( $T = -0.40, P = 0.68$ ). The same was not true for hexythiazox + *P. persimilis* combinations and hexythiazox. The populations trends of *T. urticae* in both treatments were significantly different from the control ( $T = -1.99, P = 0.04, T = 2.39, P = 0.02$ ). Populations treated with oil+ *P. persimilis* had trends that were significantly different from those treated with oil alone ( $T = -3.71, P = 0.0002$ ). *Tetranychus urticae* populations treated with hexythiazox + *P. persimilis* had trends that were significantly different from those treated with hexythiazox alone ( $T = -2.77, P = 0.006$ ). Populations of *T. urticae* treated with oil+ *P. persimilis* had trends that were significantly different from those treated with hexythiazox + *P. Persimilis* ( $T = 4.29, P \leq 0.0001$ ).

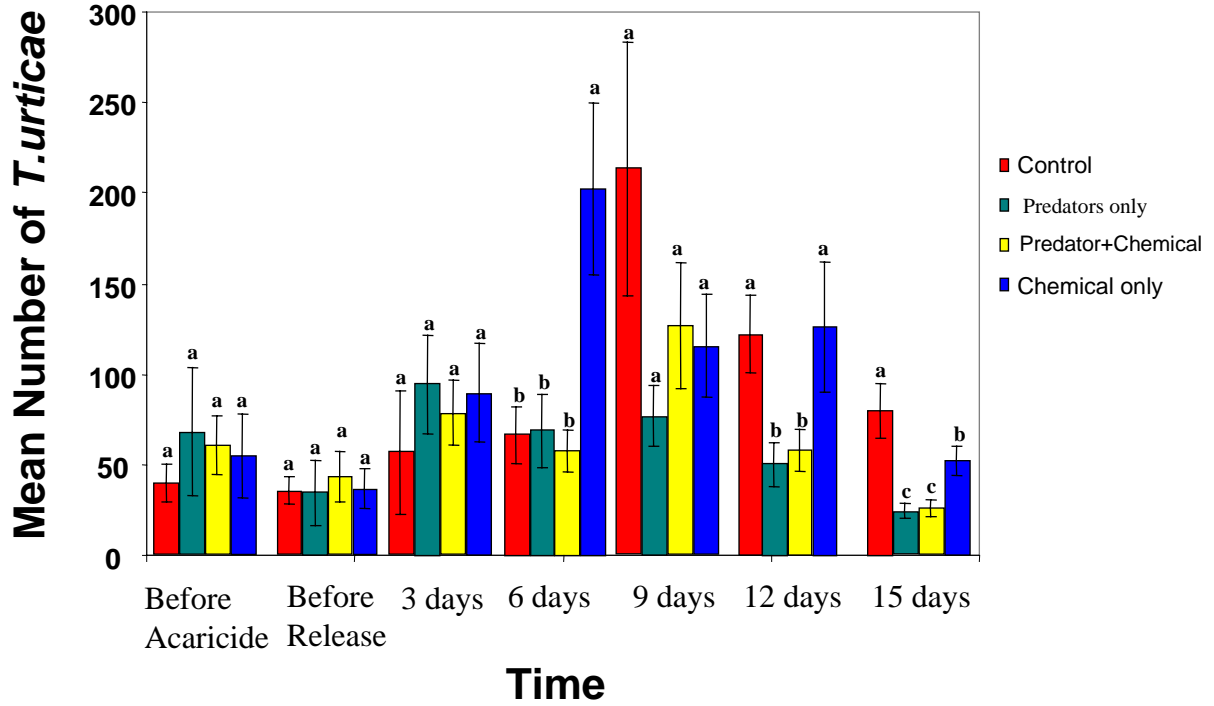


## Greenhouse Trial (Oil)



**Fig 6 A:** The results of acaricide applications, releases of *P. persimilis* and the combination of acaricide followed by introduction of *P. persimilis* on *T. urticae* populations.

## Greenhouse Trial (Hexythiazox)



**Fig 6 B:** The results of acaricide applications, releases of *P. persimilis* and the combination of acaricide followed by introduction of *P. persimilis* on *T. urticae* populations.

## Discussion

### Population Age Structure and Density

There were significant differences in the age structure of *T. urticae* populations in treatments compared to their respective controls. However, these differences were not distinguishable from natural fluctuations in the age structure. We hypothesized that because cuttings were infested at the same time, the age structure of the respective control and the acaricide treatment would have age structures that were not significantly different from each other before the acaricides are applied. However, this situation was not true for many of the treatments.

Plants treated with abamectin, azadirachtin, hexythiazox, horticultural oil, neem oil, pyridaben and spinosad had *T. urticae* age structures that were significantly different from their respective controls before and at all times measured after application. It is difficult to conclude if the differences are a result of the acaricide application or just asynchronous natural fluctuations in the age structure. Bifenthrin and Gowan 1725 also had age structures that were significantly different from their controls prior to application. Mite populations were allowed to grow for one week after infestation until initial measurements were taken immediately prior to application. Although all cuttings were treated in the same manner, uncontrollable differences in each individual cutting could have attributed to differences in age structure before acaricide application. . Chlorfenapyr may have to significantly change *T. urticae* population age structure over time. Chlorfenapyr treated cuttings had *T. urticae* populations with age

structures that were not significantly different from their respective control before application, but the age structures in the treated cuttings were significantly different from the control 3, 7, and 14 days after application. Chlorfenapyr is highly toxic and has a long residual period, which may have resulted in the change in age structure. The other acaricides tested may shape the age structure, but my experiments were not designed to measure this effect.

The proportion of eggs in cuttings treated with bifenthrin, chlorfenapyr and Gowan 1725 was significantly greater than the control 3 days after application. These acaricides may shape the age structure towards one that has more eggs. This may be important because populations that have higher proportions of eggs tend to have slower growth compared those which contain mixed aged populations because newly emerging individuals are more susceptible to mortality from pesticide residues and other factors (Stark and Banken, 1998)

*Tetranychus urticae* suppression provided by chemicals varied greatly. Abamectin, bifenthrin, chlorfenapyr, Gowan 1725, and oil provided excellent suppression of *T. urticae* populations 7 days after application in our laboratory trials. Azadirachtin, pyridaben and spinosad had did not suppress populations of *T. urticae*. Hexythiazox and neem oil did not provide good suppression of *T. urticae* populations 3 and 7 days after application, but populations began to decline 14 days after application.

Abamectin has been reported to provide excellent suppression of *T. urticae* at low concentrations (Zhang and Sanderson, 1990). Abamectin residues can kill *T. urticae* adults up to two weeks after application (Wright et al., 1984).

Our results agree with the findings of Wright et al. (1984). Two weeks after application, cuttings treated with abamectin in our trial showed little damage from mite feeding and had population densities that were significantly lower than the control.

Certain pyrethroids may suppress *T. urticae* populations while others may stimulate outbreaks by causing an increase in the density of a *T. urticae* population. Bifenthrin is a pyrethroid, which provides excellent suppression of mites. Many pyrethroids stimulate mites by increasing respiration which can increase mite feeding and egg laying (Mckee and Knowles, 1984). However, there are differences in the structure activity relationships of pyrethroids and the pharmacokinetics among individual pyrethroids (Mckee and Knowels, 1985).

Chlorfenapyr provided quick and long term suppression of twospotted spider mite populations. There was not any resurgence in the number of mites during the two week period of the trial. Other studies have found no short term resurgence in mite populations after application in the field (Allen and Kharboutli, 1999). Chlorfenapyr is a mitochondrial toxicant that affects the production of ATP by acting as an uncoupler at the site of ATP synthase. Pyridaben is also a mitochondrial toxicant that is a site 1 inhibitor on the electron transport system. However, pyridaben did not produce the positive results that chlorfenapyr did. Experiments conducted by Sekulic (1995) demonstrated that pyridaben has an LC 50 of 0.33 ug/ml for larval *T. urticae* and an LC 50 of 2.96 ug/ml for deutonymphs. This suggests that pyridaben can control younger stages of *T. urticae* more easily than older stages. In our experiments, we had a mixed

population, which may have caused the poor level of suppression that was observed.

Gowan 1725 and oil provided rapid and long-term suppression in our laboratory studies. Currently, little research has been conducted on Gowan 1725, but it demonstrated good results in our trials. The oil formulation used in our experiments provided a level of control that was rapid and long lasting. Haitas et al (1997) reported that concentrations of 2% refined horticultural oils significantly reduced both egg and mobile stages of *T. urticae*. Complete coverage was achieved in our experimental system and overhead watering was avoided on rooted cuttings. The same level of control may not be achieved under field conditions because of the inability to provide full coverage. The oil residue on the leaves may have had an antifeeding effect, but we were not able to determine this from our data. Oil residue on leaf surfaces may act as an antifeedant in the laboratory, but it would most likely not occur for long periods in outdoor environments because of rainfall and photodegradation.

Neem oil provided suppression with a slight resurgence in spider mite populations seven days after application. The 2 % application rate may have had a mechanical mode of action and physically suffocated the mites. Many neem products are known to have antifeedant effects on arthropod pests (Govindachari et al., 2000). The formulation of azadirachtin tested did not produce the same results as the neem oil formulation used in this study. Consistently poor suppression occurred at all times after application. Information about the type of azadirachtin in the formulation provided to us by the distributor was not available.

Azadirachtin A has been shown to have better antifeedant activity compared to azadirachtin B (Govindachari et al., 2000). The results in this study demonstrate a clear difference among different neem products in their ability to suppress mites.

Spinosad did not suppress *T. urticae* populations, even at two times the higher than the recommend concentration (Cote, unpublished data). The rate tested in our study may not have been high enough to provide suppression of *T. urticae* populations. Applying spinosad at a rate of 181 ppm produced the results shown here. Other research has demonstrated that spinosyns A and spinosyns D, the primary components of spinosad, applied at 400 ppm cause 100 % mortality to test populations of *T. urticae* (De Amicis et al., 1997).

Hexythiazox provided good control, but it seemed to be slow acting. Populations were suppressed after a period of two weeks. The mode of action of hexythiazox is not completely understood. It is known to have ovicidal action, but provide poor control of adult forms of *T. urticae* (Chapman and Marris, 1986). Hexythiazox causes female *T. urticae* to lay fewer viable eggs when treated (Chapman and Marris, 1986). This effect combined with the long residual toxicity of hexythiazox may suppress mite populations over time.

### **Greenhouse Trials**

Severe damage occurred in the first greenhouse trial conducted despite a reduction in the mean number of mites in certain treatments. Although imidacloprid was applied to the soil, it was probably not the cause of severe mite damage. Granular applications of imidacloprid to the soil have been

reported to cause an increase in damage from *T. urticae* populations in the landscape environment (Sclar et al, 1998). However, the increase in *T. urticae* damage is believed to be caused by toxicity to spider mite predators such as *Orius* species which occasionally feed on plant leaves (Sclar et al., 1998). Trials conducted in greenhouses with imidacloprid did not result in an increase in spider mite damage, therefore the dramatic damage seen in our trials was probably not caused by imidacloprid applications, but I have not way of being certain. The reduction in the mean number of mites that occurred in the oil+ predator treatments 7 days after release could have resulted from dispersal of mite populations and not predation, but it is difficult to determine whether this was the case. According to the laboratory trials we conducted, the proportion of eggs in *T. urticae* populations 3 days after oil applications is not significantly different from the proportion of eggs found 14 days after *T. urticae* populations were treated with chlorfenapyr. However, we did not determine if the same was true in the greenhouse trial. *Phytoseiulus persimilis* feeding on *T. urticae* eggs had shorter handling time for eggs compared to adults. We hypothesized that *P. persimilis* may be able to provide better biological control of *T. urticae* populations with higher proportions of eggs.

The second greenhouse trial produced much better results. Plants treated with predators and predators + acaricides suffered the least damage. It was obvious that the predators reduced the number of *T. urticae* and visual differences were observed in the quality of the plants. We did not observe a predator and chemical interaction that provided enhanced biological control.



*Phytoseiulus persimilis* were released at a rate of 20 per plant to determine if the predators would have an effect on the population of *T. urticae*. It may be difficult to detect the presence of predator and chemical synergism with such a large introduction of predators.

Results indicate that predators had an effect on population trends of *T. urticae* over time. Trends in *T. urticae* populations in plants treated with oil alone were not different from the control while oil+ *P. persimilis* were different from the control over time. Plants treated with a combination of predators and acaricides had *T. urticae* population trends that were different from those treated with acaricides alone. Treatments with oil+ *P. persimilis* and hexythiazox + *P. persimilis* also had trends that were different from each other. This may indicate that the type of pesticide used with predator release may effect *T. urticae* population trends.

Further testing of these chemical/predator combinations should be conducted with fewer predators introduced. It is likely that there is a point at which the predators cannot suppress *T. urticae* populations without the aid of compatible acaricides. Numerous field studies using *P. persimilis* and *P. persimilis* with compatible acaricides demonstrate that adequate control can be achieved with the correct combination of acaricides and *P. persimilis* (Cashion et al. 1994; Osborne and Petitt, 1985).

## Conclusion

The age structure of *T. urticae* populations treated with selective acaricides is not likely to persist over a long duration because of the mite's rapid

population growth and short life cycle. We did not measure this occurrence from our observations. It is possible that a significant change in age structure occurred during the times between our observations. Stable age structures may never be reached because *T. urticae* populations often kill the host plants before it can occur (Hance and Van Impe, 1999). Once plant quality decreases, dispersal often follows to allow for the creation of new colonies (Helle and Sabelis, 1985). These factors contribute to natural, rapid changes in age structure, which may make manipulation of *T. urticae* age structure unachievable.

Results of the field trial suggest that the number of prey mites that are present at the time of release is very important for achieving satisfactory biological control. The second greenhouse trial demonstrated better *T. urticae* suppression than the first. Combinations of oil and release of *P. persimilis* suppressed twospotted spider mites. Some ecologists suggest that predation disturbs the prey age structure which maintains the prey growth potential at a high rate (Hance and Impe, 1999). Others have found that the combination of predators and pesticides provides enhanced biological control of spider mite populations (Osborne and Pettitt, 1985). Additional combinations of predators and compatible acaricide must be investigated to measure their ability to enhance biological control.

## **Chapter 5 Summary**

There were significant differences in the age structure of all treatments compared to their respective controls. However, these differences were probably

not different from natural fluctuations in the age structure. We hypothesized that because cuttings were infested at the same time, the age structure of the respective control and the acaricide treatment would have age structures that were not significantly different from each other before the acaricides are applied. However, this situation was not true for many of the treatments. This made it difficult to conclude whether the differences were a result of the acaricide application or just asynchronous natural fluctuations in the age structure.

Cuttings treated with abamectin, azadirachtin, hexythiazox, horticultural oil, neem oil, pyridaben and spinosad had age structures that were significantly different from their respective controls before and at all times measured after application. Bifenthrin and Gowan 1725 also had age structures that were significantly different from their controls prior to application. Chlorfenapyr may have changed the age structure of *T. urticae*. Chlorfenapyr treating cuttings had *T. urticae* with a population age structure that was not significantly different from its respective control before application, but it was significantly different from the control 3, 7, and 14 days after application. The other acaricides tested may shape the age structure, but we may have not measured it during our observations.

There were significant differences in the efficacy of direct application of the acaricides tested. Azadirachtin, pyridaben and spinosad did not suppress *T. urticae* populations at the rates and formulations tested in the trial. Hexythiazox eventually suppressed *T. urticae* populations but it was slow acting. Abamectin,

bifenthrin, chlorfenapyr, Gowan 1725, oil and neem oil suppressed *T. urticae* populations.

Bifenthrin and chlorfenapyr residues were toxic to *P. persimilis* whereas, abamectin, Gowan 1725, hexythiazox, horticultural oil, neem oil, pyridaben and spinosad were not toxic to *P. persimilis*. Mortality of *P. persimilis* from exposure to residues of bifenthrin and chlorfenapyr was significantly greater than observed on the controls 1, 3, 7, and 14 days after application. *Phytoseiulus persimilis* mortality on leaf disks treated with abamectin, Gowan 1725, hexythiazox, horticultural oil, neem oil, pyridaben and spinosad was not significantly greater than on untreated leaf disks at any time after application.

The response of *T. urticae* to residue exposures was more variable than that of *P. persimilis*. *Tetranychus urticae* mortality from chlorfenapyr residues was significantly greater than the control 1, 3, 7 and 14 days after application. Even after two weeks, chlorfenapyr residues caused 55% mortality to adult *T. urticae* compared to 6% mortality in the control. *Tetranychus urticae* mortality from bifenthrin and abamectin residues was not significantly greater than the control 1 day after application. However, *T. urticae* mortality for both bifenthrin and abamectin residues was significantly greater than the control 3, 7, and 14 days after application. *Tetranychus urticae* mortality caused by Gowan 1725, horticultural oil, and neem oil residues was significantly greater than the control 24 hours after application but not at the other times tested. *Tetranychus urticae* mortality from hexythiazox and spinosad residues was not significantly greater than the control at any time tested.

The results of feeding preference tests demonstrated a preference for adults over eggs in only a few instances and consumption of one life stage over another did not occur consistently throughout the study. A persistent preference for a single life stage over a long duration in changing age structures is not likely to occur. Predatory mites appear to feed on the stage that is most abundant at the time of feeding (Helle and Sabelis, 1985). *Phytoseiulus persimilis* does not develop a search image when feeding on a prey monoculture for short time periods tested in our study. We found the functional response to be a type II response for both eggs and adults. The handling time was 0.079 hours for eggs and 3.39 hours for adults.

The effects of a combination of acaricides followed by release of *P. persimilis* on *T. urticae* populations were tested using greenhouse studies conducted on infested *Buddleia* plants. Severe damage occurred in the first greenhouse trial conducted despite the reduction in the mean number of mites in certain treatments. The reduction in the mean number of mites that occurred in the oil+ predator treatments 7 days after release could have resulted from dispersal of mite populations and not predation, but it is difficult to determine whether this happened. Previous studies we conducted on the functional response of *P. persimilis* feeding on *T. urticae* eggs demonstrated a shorter handling time for eggs compared to adults. The proportion of eggs in cuttings treated with bifenthrin, chlorfenapyr and Gowan 1725 was significantly greater than the control 3 days after application. These acaricides may shape the age structure towards one that has more eggs. *Phytoseiulus persimilis* may be able

to provide better biological control of *T. urticae* populations with higher proportions of eggs, but we cannot make the conclusion from these data. The second greenhouse trial demonstrated better *T. urticae* suppression than the first. Combinations of oil and release of *P. persimilis* may provide good suppression of *T. urticae*.

*Tetranychus urticae* population density may be the most important factor for affecting the success of biological control provided by *P. persimilis*. The results of our field trials demonstrate that. If pest densities are too great, adequate biological control will not be achieved. *Phytoseiulus persimilis* do not have a prey-stage preference when feeding on *T. urticae*, but will feed on all prey items that are available. Populations comprised of predominately eggs may be easier to control by predators because of shorter handling times compared to mobile forms of *T. urticae*. However, the greenhouse trials did not test this question. Further research must be conducted to examine this hypothesis because *Phytoseiulus persimilis* feeding on adult *T. urticae* may suppress *T. urticae* populations below threshold levels by a reducing the number of adults will lead to a reduction in the number of *T. urticae* eggs deposited on a plant. In certain situations, the combination of predators with compatible acaricides can provide enhanced control (Osborne and Pettitt, 1985), but enhanced control does not always occur. Fagan et al. (1998) reported that the combination of insecticides and releases of wolf spiders in rice cropping systems resulted in an interaction which caused pest densities to remain high. In this situation, an increase in wolf spiders which were released and the pesticide application, led to

a decrease in the number of mesovellids which feed on many pest species in rice cropping systems (Fagan et al.,1998). Our system is different from this system because we utilized the specialist predator, *P. persimilis* and the diversity of predators in the system was limited. The combination and oil and *P. persimilis* may provide enhanced suppression of twospotted spider mites. Our research suggests that abamectin and oil are two acaricides that would be less detrimental to the survival of *P. persimilis*. Their use in an integrated program on ornamental crops should be considered. Additional greenhouse trials with compatible acaricides and different release rates of *P. persimilis* should be conducted as well as research on the threshold density of *T. urticae* that will allow *P. persimilis* to provide adequate control. Reduction of acaricide applications may be possible with the selected use of acaricides and properly timed releases of predators.

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## **Vita**

Gardening began as a hobby for me while growing up in Reading, Pennsylvania, but became my profession after I completed my B. S. in horticulture at Penn State University in 1993. I worked for a large nursery in Pennsylvania, and then became the IPM coordinator for Hillwood Museum and Gardens in Washington, D.C. While working at Hillwood Museum, I became increasingly interested in entomology and decided to pursue a Master's degree in entomology at Virginia Tech. I intend to continue my career in public horticulture by providing educational programs in the areas of entomology and horticulture.