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ABSTRACT

IMPACTS OF INSECTICIDES ON PREDATORY MITE, NEOSEIULUS FALLACIS (ACARI: PHYTOSEIDAE) AND MITE FLARING OF EUROPEAN RED MITES, PANONYCHUS ULMII (ACARI: TETRANYCHIDAE)

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*Panonychus ulmi*, the European red mite, is a major agricultural pest found in most deciduous fruit growing areas. It is the most important mite species attacking tree fruits in humid regions of North America. Bristle-like mouthparts of this mite species pierce the leaf cell wall and ingestion of their contents including chlorophyll causes bronzing injury to leaves. Heavy mite feeding early in the season (late Jun and July) reduce tree growth and yield as well as the fruit bud formation, thereby reduce yields the following year. Biological control of this pest species by predators has been a cornerstone of IPM. Phytoseiid mite, *Neoseiulus fallacis* (Garman) is the most effective predator mite in Michigan apple orchards and provides mid- and late-season biological control of European red mites. Achieving full potential of biological control in tree fruit has been challenging due to the periodic sprays of broad-spectrum insecticides.

There have been cases of mite flaring reported by farmers in relation to the reduced-risk (RR) insecticides that were registered in commercial apple production in the past ten years. These insecticides are often used in fruit trees to control key direct pests such as the codling moth. Serious outbreaks of phytophagous mites occurring after insecticide applications indicate an imbalance of pest population dynamics. Several RR insecticides were used in this study to determine potential to flare ERM when applied on apple trees, which includes acetamiprid, chlorantraniliprole, spinetoram and novaluron. Two conventional insecticides esfenvalerate and
carbaryl were incorporated into this study based on their traditional use in codling moth management programs or as an apple thinning agent. This research utilized field studies, laboratory bioassays and residue profiling of apple leaves. Season long field evaluations of ERM were made to observe the incidence of mite flaring and reduction of predator mites. Field-based bioassays and residue profile analysis were used to determine the temporal toxicity of insecticides to *N. fallacis* after exposure to field-aged residues.

The most cases of mite flaring as evidence in this study were associated with esfenvalerate and carbaryl followed by acetamiprid, and limited cases in chlorantraniliprole, spinetoram and novaluron. Carbaryl in combination with other insecticides caused consistent reductions of predator mites populations. In addition, carbaryl caused the shortest lethal times and high mortality levels to *N. fallacis* from both topical spray and dry residue exposure. Esfenvalerate did not pose risk to *N. fallacis* under dry residue exposure, however exposure to wet concentration is very harmful. Spinetoram as dry residue showed moderate negative effects on *N. fallacis* and the likelihood of exposure is through grooming behaviour as this compound is active through ingestion. Acetamiprid, chlorantraniliprole and novaluron were not directly harmful to the predator mite *N. fallacis* through topical spray or exposure to dry residues.
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CHAPTER 1: INTRODUCTION

Apple IPM and Biological Control

Successful Insect Pest Management (IPM) in apple orchards depends on a balance of biological organisms in the agroecosystem. *Panonychus ulmi* (Koch) (Howitt 1993) is a serious indirect pest in apples, and without biological control acaricides are often needed to prevent economic injury (Cranham & Helle 1985). *Neoseiulus fallacis* (Acari: Phytoseiidae) is an important predator of the European Red Mite, *P. ulmi*. The role of phytoseiids in controlling phytophagous mites on various kinds of crops is well documented (Huffaker et al. 1970, Hardman et al. 1988). *Typhlodromus pyri* Scheuten, one of the reported phytoseiid mites, is an effective biological control agent because it has acquired resistance to certain organophosphate and carbamate insecticides, and can survive for extended periods on alternative sources of food such as pollen, mildew, rust mites and tydeid mites when spider mites are scarce (Overmeer 1985).

Biology and life cycle of ERM

European red mite (ERM) (Acari: Tetranychidae) was reported in North America in the 20th century (Howitt 1993) and its outbreak was mainly attributed to increased use of broad spectrum insecticides (Huffaker et al. 1970). The pest status first occurred in early 1930 in Connecticut as the result of a dry summer and cold winter as well as the transition in growing practices (Garman and Townsand 1938). In Michigan, the European red mite is considered the species of greatest economic importance among the phytophagous mites.
The ERM overwinters as a fertilized egg. It is oval, bright red, and has a small stalk arising from the top, approximately the length of the diameter of the egg (Howitt 1993). Overwintering eggs are deposited in groups on the roughened bark area, especially around buds and fruit spurs (Pfeiffer 1996). The summer eggs are lenticular, somewhat flattened at the poles, almost white to greenish amber when first deposited, but slowly change to a reddish-orange just before hatch. Immediately before hatching they become translucent like an empty eggshell. Six-legged larvae hatch from the eggs which are initially a pale orange, darkening to a pale green as they feed, depending on the amount of chlorophyll taken with their food.

The larvae molt to eight-legged protonymphs, which are variable in color from a pale green to reddish brown with the dark green predominating (Howitt 1993, Pfeiffer 1996). The second instar mite, protonymphs are somewhat larger varying according to the length of time elapsed after transforming from larval stage. The deutonymphs are variable in color from an amber color to a dark green, with a dark-green color predominating. Pale spots are distinct at the bases of the bristles. The eight-legged adult female mite has a globular body and is approximately 0.4 mm long, bright red to velvety brown in color, and has four rows of white hairs on its back. The adult male mite is smaller, has a pointed abdomen and more slender than the female (Pfeiffer 1996) with an average of 0.26 mm long. A newly molted adult usually is velvety green to brownish green, but changes to brownish red after a day or more (Howitt 1993). European red mites crawl within the trees and disperse between trees through the action of wind (Garman and Townsand 1938).

According to Pfeiffer and Schultz (1986), *P. ulmi* cause injury to the plant in multiple ways. They usually pierce the top layer of leaf and extract the content out of the epidermal cells, resulting in bronzing effect and necrosis (Pfeiffer and Schultz 1986). This leads to leaf abscission
and poor quality of the fruits (Garman and Townsand 1938). Croft and McGroarty (1977) reported that \textit{P. ulmi} was the most destructive mite in Michigan apple orchards where it had significant negative effect on fruit size, fruit coloration and the number of fruit the following season. Cagle (1946) reported that mated females produced female or male brood whereas unmated female tend to produce males. In addition, many generations of \textit{P. ulmi} occur during the apple growing season. These factors contribute to the frequent development of resistance in \textit{P. ulmi} to pesticides.

\textbf{The Ecology and Biology of \textit{Neoseiulus fallacis}}

Phytoseiids live on plants and in upper soil layers. They are the best known and most studied group of predatory mites, owing to their success in controlling spider mites, other mites and Thrips (Thysanoptera). Phytoseiids, as biological control agents of phytophagous mites, are effective in many agricultural systems (Hamlen & Lindquist 1981, Mizel & Schiffhauer 1991). Phytoseiids locate their prey through chemical cues known as ‘herbivore induced plant volatiles’ (van Wijk et al. 2008), emitted from host plants as a result of spider mite feeding activity (Takabayashi and Dicke 1992, Takabayashi et al. 1994, van Wijk et al. 2008). The perception of kairomones increases the probability of prey finding by phytoseiids (Dicke et al. 1990, Takabayashi et al. 1994, Wijk et al. 2008). Rosen and Huffaker (1982) regard searching ability as the most important attribute of an effective predator. Some phytoseiids also feed on non prey foods, such as pollen, honeydew, and plant juices (McMurty 1982), which may help to sustain them through periods when prey are at low densities (Huffaker & Flaherty 1966).

In the humid central and eastern regions of North America \textit{N. fallacis} occurs on trees and lower vegetation, whereas in the drier western parts, \textit{N. fallacis} usually inhabits low and
sprawling plants, unless living on irrigated tree crops (Morris et al. 1996). *N. fallacis* is the most common phytoseiid on apple trees in many mid western and eastern commercial apple orchards (Welty 1995) and is an efficient predator of the ERM, TSSM and other phytophagous mites, such as eriophyoids. The availability of the apple rust mite, *Aculus schlechtendali*, as a suitable alternate food is a major factor in maintaining *N. fallacis* populations on apple trees during lack of other prey. *Neoseiulus fallacis* spends the winter in the orchard ground cover, where it feeds upon overwintering two spotted spider mite and other mites. During the spring (May and June) mites disperse upward into tree canopies, where they provide mid- and late-season biological control of European red mites. Research in Michigan has revealed that the three most important factors influencing dispersal of *Neoseiulus* into the canopy are degree-day accumulation, initial density of predators in the ground cover, and prey density in the tree. When spider mites or rust mites were present in adequate numbers, this predator appeared in the trees 600±100 DD54°F after January 1. Spring frosts or freezing rains may suppress activity of this predator while it is still in the ground cover.

A generation of *N. fallacis* feeding on TSSM eggs and adults is completed in 4-5 days at 24-27°C. Females represent 66-75% of the adult population. Each female deposits 30-40 eggs within 2 weeks under conditions of near saturation (Ahlstrom & Rock 1973, Ball 1980, Boyne & Hain 1983). At 21.1°C, the life span is somewhat longer, 24-80 (average 62) days. The period from egg deposition until emergence of adults is 7.3 and 3.3 days at 21.1°C and 32.2°C, respectively. Higher egg production is achieved with greater prey availability. When offered an abundance of spider mites at 26.7°C, females devoured 10.6 eggs/day or 4.8 females/day. Consumption rose to 12.8 eggs/day as predators were subjected to a diurnal cycle of L10/D14h, indicating that these were the conditions under which *N. fallacis* would be the most efficient
natural enemy. McMurtry and Croft (1997) classified *N. fallacis* as a Type II-selective predator, feeding primarily within the spider mite family. Based on the feeding specialization classification, Type I consist of selective predators of *Tetranychus* spp. whereas Types III and IV are considered more generalist predators (McMurtry and Croft 1997, Croft et al. 1998). Pratt et al. (1999) showed in the event of starvation *N. fallacis* would reproduce on other prey. However, *N. fallacis* that fed and reproduced on other mites, on juveniles of various insects, and even on pollen, survival, activity, fecundity and the development of the second generation (F1) on these preys were reduced (Ahlstrom & Rock 1973, Pratt et al. 1999).

In Michigan, Croft & McGroarty (1977) devised guidelines for predicting the probability of ERM control on apples (cv. ‘Delicious’). They were based on an economic threshold of 15 ERM/leaf. As the effects of feeding damage are cumulative over time, mite damage is often expressed as a combination of the population level (intensity) plus an indication of length of time of feeding (duration). The unit is called "mite days" and defined as the product of the number of mites per leaf multiplied by the time they are present. Thus, 25 mite days could be the product of 5 mites per leaf for 5 days equals 25 mite days per leaf (Beers and Hoyt 1993). Continuous tallying of pest-predator ratios, in 100-leaf samples, provided guidelines for their future relationships. High ERM populations, in relative to predator number indicated a low likelihood of control by *A. fallacis*, necessitating acaricide sprays. When *N. fallacis* numbers were sufficiently high, relative to the pest, the chances for successful biocontrol were above 90%.

In regard to commercial apple production, the maintenance of ERM below economic threshold through biological control alone is not 100% guaranteed. Hence the successful management of mite prey must integrate the holistic approach of Integrated Pest Management (IPM). One tool of IPM, the use of pesticides, plays an important role in achieving a marketable
crop. Pesticides, however, must be used cautiously, not to interfere with predaceous mites and other beneficial arthropods’ behaviour or survival.

Insecticides such as organophosphates and carbamates have long been used in apple production, dating back over 40 years ago. Both of these pesticide classes are broad spectrum in activity. The US Environmental Protection Agency (EPA) has placed new restrictions on the use of the organophosphates and carbamates insecticides through the Food Quality Protection Act (FQPA) (USEPA 1996). FQPA requires that all registrations for pesticides used in food production be reconsidered for continued registration. This has resulted in the elimination or restriction of many of the pesticides important for apple production. Many new reduced-risk insecticides (USEPA 1997) have been introduced, but preliminary evidence has shown that these new insecticides may be harsher on beneficials than previously expected (Wise and Whalon 2009, Agnello et al 2009). In the last ten years a large number of new reduced-risk (RR) insecticides have been registered for use in commercial apple production. There have been reports from farmers of mite-flaring events in association with the use of some of the new RR compounds (Irish Brown CAT-Alert 2009). It is not clear, however, what mechanisms are responsible for the observed mite-flaring.

Insecticide applications are sometimes followed by serious outbreaks, not of the pest against which they were applied, but of other phytophagous insects and mites which, prior to the treatment were in numbers too low to be of economic importance (Abivardi 2008). Pest resurgence after application of pesticides using different modes of action and under different climatic conditions indicates that chemical control in these cases upsets the population dynamics of the pests in question. Four hypotheses have been suggested to further explaining this mite-flaring phenomenon: (I) The reduction of natural enemies by the pesticides; (II) pesticide-
induced reproductive stimulation of phytophagous arthropods; (III) the removal of competitive species; and (IV) the removal of alternative food sources that sustain the predators. Although sporadic literature confirms the validity of the second hypothesis—often called hormoligosis (i.e., pesticide-induced reproductive stimulation of phytophagous arthropods) (Ako et al. 2004), and its importance in pest resurgences, most information on the subject refers chiefly to the destructive effects of pesticides on the natural enemies of the phytophagous species and, to a lesser extent, to the removal of competitive fauna (Abivardi 2008).

Possible mechanisms responsible for mite flaring

1. Hormoligosis

Luckey (1968) defined hormoligosis as a term applied to the phenomenon in which harmful quantities of many stress agents may be helpful when presented to organisms in suboptimal environments. The hormoligant that may include chemicals, antibiotics, hormones, temperature, radiation, and minor wounds are stimulatory to an organism by providing increased efficiency to develop new or better systems to cope in a suboptimum environment. The occurrence of pesticide hormoligosis in agriculture is well documented and may be a common phenomenon, but it is rarely monitored so there is uncertainty of its importance in fostering outbreaks of certain pests or in accounting for failures in pest control programs. The pesticide-induced reproductive stimulation of phytophagous arthropods comprises the backbone of pesticide hormoligosis. Ako et al (2004) reported that application of imidacloriprid, a neonicotinoid insecticide, can lead to population build up of two spotted spider mite, *Tetranychus urticae* Koch in the field. Laboratory studies showed enhanced fecundity of *T. urticae* after an imidacloriprid treatment. Similar results observed by James and Price (2002), showed an increase of 20% of
egg production when two spotted spider mite *T. urticae* Koch was exposed systemically to imidacloprid.

2. Reduction of the natural enemies by direct toxicity of pesticides

Van de Vrie et al. (1972) reported that the outbreaks of *P. ulmi* (Koch) in apple orchards can be attributed to the use of pesticides that suppress their natural enemies. The use of synthetic pyrethroid insecticides for example, has been implicated in the reduction of phytoseiid mite species on a number of fruit tree crops in different parts of the world (AliNiazee 1984). Synthetic pyrethroids such as permethrin and fluvalinate caused 100% mortality of females *Phytoseiulus macropilis* after 72 hours exposure in the laboratory bioassay (Amin et al. 2009). As a result, synthetic pyrethroids have been eliminated from most IPM recommendations and are avoided in most apple production systems (Agnello et al. 2009, Wise et al. 2011). Organophosphate insecticides when first introduced were toxic to predaceous mites, but most species are now resistant to the OPs used in apple production. Laboratory studies have shown varying levels of direct toxicity of some reduced risk insecticides, including imidacloprid, acetamiprid, and spinosad to predacious mites (Bostonian et al 2010). Acetamiprid, for example, caused high mortality in *N. fallacis* adults but no significant reduction in oviposition was observed (Villanueva and Walgenbach 2007).

Research also suggests that the physico-chemical interactions between the plant, insect, and chemical are important for understanding the toxicity attributes of a given insecticide (Chowdhury et al. 2005, Wise et al. 2006). Residue profile analysis of insecticides reveals that the extent and duration of insecticide penetration in apple leaf tissues serves to regulate activity on the target pest (Wise and Whalon 2009). Other studies (Nauen et al. 1998, Isaacs et al. 1999)
have also documented behavioral effects on arthropods after exposure to or detection of internal plant residues. Documenting the duration and extent of insecticide bio-availability to predator mites under field conditions provides the necessary information for determining a compounds overall threat to biological control and IPM.

3. Reduction of natural enemies by sublethal effects

Sublethal effects are defined as effects (either physiological or behavioral) on individuals that survive exposure to a pesticide (the pesticide dose/concentration can be sublethal or lethal) (Desneux et al. 2007). The sublethal effects of some pesticides can be detrimental to nontarget organisms (Cabrera et al. 2004). For example, a drastic increase in the population of the fruit tree red spider mite, *Metatetranychus ulmi* (Koch) [*Panonychus ulmi* Koch], following the application of DDT was demonstrated in The Netherlands in the late 1940s. Studies on the sublethal effect of DDT on oviposition of this mite revealed stimulation of egg production at a DDT concentration much lower than the recommended rates (Abivardi 2008). Bowie et al (2001) reported that esfenvalerate residues reduced the number of eggs laid by female predatory mite *T. pyri* Scheuten (Acari: Phytoseiidae) by 50%. In a study testing the effects of fungicides on a parasitoid, Hafez et al. (1999) demonstrated that under laboratory conditions, the fungicides thiram, mancozeb, tolyfluamid and netzchwefel reduced the parasitism rate of *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae) by 100%.

Recently, growing literatures on sublethal effects are largely associated with the Insect Growth Regulator (IGRs) class of compounds (Medina et al. 2002, Sun et al 2003, Mommaerts 2006). Chitin synthesis inhibitor (CSIs), for example, have been reported to contribute largely to the sublethal effects observed in pollinators, such as honeybees and bumblebees (Thompson and
Hunt 1999) and other beneficial insect such as predatory lacewings (Medina et al 2002). For most of the CSIs tested, a reduction in egg hatching was observed (Mommaerts 2005). Diflubenzuron (DFB) penetrated into insect cuticle at a faster rate and later inhibited the chitin synthesis in insects resulting in a disruption of the molting process (Ishaaya and Horowitz 1998). On the mechanism of chitin inhibition by benzoylphenyl ureas (BPUs), recent assays using *Blatella germanica* (L.) and *Drosophila melanogaster* Meigen showed that such compounds work through the sulfonylurea receptor during chitin biosynthesis, which confirmed the embryocidal activity of CSIs (Abo elghar et al. 2004). The other class of IGRs, ecdysone agonist has also been reported to decrease mean fecundity and fertility of important pest species from several different insect orders (Aller and Ramsay 1988, Sun and Barrett 1999). Tebufenozide, for example, is renowned for its larvicidal properties (Wing et al. 1988). Smagghe et al. (1996) reported that exposure to the ecdysone agonist caused the ovaries to degenerate, while Swevers and Iatrou (1999) showed that the developmental arrest on *Bombyx mori* ovarian follicles was due to the alteration of some gene expression during mid late vitellogenesis and choriogenesis, due to ecdysone agonist effects. Juvenile hormone analog, such as fenoxycarb disturb molting at the pupae stage and may also have an ovicidal effect on isolated eggs of tortricids (Dorn et al. 1981).

Plum curculio larval exhibited reduced survival following mated female exposure to substrates treated with novaluron (Rimon®), a benzoylurea chitin synthesis inhibitor insecticide widely used in apple production (Wise et al. 2007). A similar result was recorded by Kostyukovsky and Trostanetsky (2006) following exposure of *Tribolium castaneum* (Herbst) to novaluron. Studies conducted by Gokce et al. (2009) showed significant sublethal activity of Novaluron by reducing codling moth egg viability subsequent to adult exposure.
Insecticides evaluated for mite-flaring potential in apple

Synthetic pyrethroids are a class of neurotoxins registered for insect control in apple orchards. Their application is known to disrupt integrated mite management programs by killing or repelling key predators of pest mites (Rock 1979, Hull et al. 1997) and causing spider mites population build up (Gerson and Cohen 1989). Esfenvalerate (Asana®) is one of the most commonly used pyrethroids in apple. It is composed of mixture of 4 stereoisomers, enriched with S-isomer, the most insecticidally active isomer. Esfenvalerate and other pyrethroids are typically fast acting, when binding to the nerve axon they inhibit the voltage gated sodium channel from closing properly causing hyperexcitation, convulsions, followed by lethargy and paralysis (Kelly 2003). Their mode of activity is direct mortality by contact or through ingestion. Direct contact with spray droplets provides the most effective method of insect pest controls. Consumption of spray droplets or residues present on treated foliage following esfenvalerate application provides a second mode of entry. This compound is extremely hydrophobic with high log Octanol-Water Partition Coefficient (Kow) (5.6 to 6.2) (Kelly 2003, Adelsbach and Tjeerdema 2003), indicative to its strong tendency to bind to soil particles, thus bioavailability reduces.

Carbamates are another class of neurotoxins registered for use in apple orchards. These compounds are esters of carbamic acid (Casida 1963). Carbaryl (Sevin®) is a commonly applied carbamate for insect control and it also is utilized as a plant growth regulator (PGRs) in apple chemical thinning programs to improve fruit size and overall apple production (Lang et al. 2011). As an insecticide, this compound inhibits enzyme cholinesterase, causing the neurotransmitter to continue to sends its electrical charge. This leads to overstimulation of the nervous system, and the insect dies (Relyea & Mills 2001). Its mode of activity is direct mortality by contact and foliar penetration is limited. Carbaryl is known to contribute to tetranychid mites population
build–up (Dittrich 1974, Walker and Aitken 1996) and it is highly toxic to predatory mites (Thistlewood and Elfving 1992).

Neonicotinoids are relatively new insecticides used in apple orchards to fill the gap left by the elimination of older insecticides, especially organophosphates. Neonicotinoids are generally effective on multiple life stages of insects, depending on the target species. Their low hydrophobicity attributes to excellent systemic and translaminar activity. Foliar penetration is translaminar and the compound is translocated inside plant cell acropetally (Stein-Dönecke et al. 1992, Westwood et al. 1998). Acetamiprid (Assail®) is a neonicotinoid insecticide used for control of aphids, leafhoppers, leafminers, thrips, and whiteflies, codling moth and apple maggot in apple (Natwick 2001, Parrish et al. 2001, Wise et al. 2011). This compound is a nicotinic acetylcholine receptor agonists, causing overstimulation of the nervous system that leads to poisoning and death. Its mode of activity is lethal mortality by contact and as a stomach poison. It also displays properties of antifeeding and curative activity (Liu and Casida 1993, Zhang et al. 2000). Acetamiprid has long residual inside the plant and less as a surface residue. Thus, its impacts on beneficial insects on the plant surface are presumed to be less important. However, a few studies have shown its contribution to mite-flaring (Ako et al. 2004) and toxicity to predator mites (Bostanian et al. 2009, 2010).

Chlorantraniliprole (Altacor®) is a new insecticide developed by DuPont for use against codling moth and other direct apple pests. It belongs to the diamide chemical class. Anthranilic diamide insecticides are derivative of anthranil with a novel mode of action (group 28 in the IRAC classification), and thus highly valuable options for IRM (Insecticide Resistance Management) strategies (Bassi et al. 2009). Diamides are known to be effective mainly on eggs and larval stages of target pests. Chlorantraniliprole is a ryanodine receptor modulator (Dinter et
This chemical has been shown to disrupt the developmental process where ryanodine locks the RyR partially open, like a doorstop, leaving the Ca ions trapped in between of the RyR (Cordova et al. 2006). This action leads to the muscle contraction and in most cases, larvae contracted to half size. Chlorantraniliprole is directly lethal to insects by ingestion, displaying ovi-larvicidal and larvicidal properties (Mertesdorf 2009). Foliar penetration is translaminar. Chlorantraniliprole is relatively safe to beneficial insects (Wise et al. 2011) and limited effects on phytoseiid mites have been observed in the laboratory (Amarasekare and Shearer 2013).

Spinetoram (Delegate®) is a spinosyn insecticide currently use for management of codling moth and other direct insect pests in apple IPM. Spinetoram is a nicotinic acetylcholine receptor agonist derived from the soil actinomycete *S. spinosa* under aerobic fermentation conditions. This compound alters function of nicotinic and GABA gated ion channels (Watson 2001). Mode of activity is a direct mortality by ingestion, the insecticidal action of spinetoram is from two spinosyns, factor J, XDE-175-J and factor L, XDE-175-L, which causes anomaly to insect nAChR receptors. When ingested, these factors are broadly distributed in tissue, however, the highest concentrations are found in the gastrointestinal tract (Bulletin Agro Dow chemical 2006). Foliar penetration is translaminar. This compound appears to be safe to many beneficial insects however a few cases of high toxicity to insect predators were reported to be associated with direct ingestion (Nasreen et al. 2003, Nakahira et al. 2010) and from phytoseiid mite exposure to dried residue (Lefebvre et al. 2011).

The objective of this dissertation research is to demonstrate the compounds or combination of compounds that will elicit flaring of ERM in Michigan apples, and document the subsequent impact on predacious mites in the system. In addition, research studies utilizing a combination of field and laboratory bioassays and residue profile analysis will be used to identify
the mechanisms or combinations of mechanisms responsible for the observed mite flaring. Of importance will be capturing the spatial and temporal dimensions of pesticide exposure and the subsequent lethal and sublethal effects on ERM and *N. fallacis*.
CHAPTER 2

EPISODES OF MITE FLARING IN ASSOCIATION WITH SEVERAL INSECTICIDES

Abstract

European Red Mite (ERM), *Panonychus ulmi* is the principal phytophagous mite pest in Michigan apples. ERM can cause severe bronzing and lead to decrease quality of apple fruits and become a major pest in the absence of its key predators, such as the phytoseiid mite, *Neoseiulus fallacis* (Garman) and *Zetzellia mali* (Ewing). Increased incidences of mite flaring have been observed in association with some new reduced risk (RR) compounds. Experiments were conducted for two consecutive years to document episodes of mite flaring in association with specific insecticides. Season long field evaluations of ERM and predator mite populations were made following treatments of esfenvalerate, carbaryl, acetamiprid, spinetoram, chlorantraniliprole, novaluron and combinations of insecticides with carbaryl on apples trees (*Malus domestica* Borkhausen cv. 'Red Delicious). The synthetic pyrethroid esfenvalerate and carbamate carbaryl showed highest incidence of mite flaring, in terms of magnitude of ERM populations. Acetamiprid treatments showed evidence of mite flaring however did not reduce populations of predator mites *N. fallacis* and *Z. mali*. Limited cases of mite flaring were seen in chlorantraniliprole, spinetoram and novaluron. Spinetoram treatments reduced predator mite populations while chlorantraniliprole and novaluron did not. Treatment combination of treatment insecticides with carbaryl yielded reductions of *N. fallacis* and *Z. mali* populations, consistently. Mite flaring and predator mites reduction in association with insecticides or combination of insecticides commonly used in apple IPM programmes is discussed.
Introduction

European red mite (ERM), *Panonychus ulmi* (Koch) is a species of great economic importance among the plant feeding mites. It was first reported in eastern North America early in the 20th century (Howitt 1993). The ERM overwinters as a fertilized egg. Overwintering eggs are deposited in groups on the roughened bark area, especially around buds and fruit spurs (Pfeiffer 1996). The summer eggs are lenticular, somewhat flattened at the poles, almost white to greenish amber when first deposited, but slowly change to a reddish-orange just before hatch. Pale spots are distinct at the bases of the bristles. The eight-legged adult female mite has a globular body and is approximately 0.4 mm long, bright red to velvety brown in color, and has four rows of white hairs on its back. The adult male mite is smaller, has a pointed abdomen and more slender than the female (Pfeiffer 1996) with an average of 0.26 mm long. A newly molted adult usually velvety green to brownish green and changes to brownish red after a day or more (Howitt 1993).

The predator mite, *Neoseiulus fallacis* (Garman) (Acari: Phytoseiidae) is the principal mite predator on apple trees in mid western and eastern commercial apple orchards (Welty 1995). *Neoseiulus fallacis* is an important predator of the ERM, *P. ulmi* (Koch) (Howitt 1993). Adult *N. fallacis* overwinters in orchard ground cover and emerges on tree in May and June of the subsequent year. *Neoseiulus fallacis* is a type II specialist according to the classification of the life style of phytoseiid mites (Croft et al. 2004). *Neoseiulus* spends the winter in the orchard ground cover, where it feeds upon overwintering two spotted spider mite (TSSM), *Tetranychus urticae* Koch and other mites. When spider mites or rust mites are present in adequate numbers, this predator appears in the trees after 600±100 DD54 post January 1(Ahlstrom & Rock 1973, Ball 1980, Boyne & Hain 1983).
A generation of *A. fallacis* feeding on TSSM eggs and adults is completed in 4-5 days at 24-27°C. Females represent 66-75% of the adult population. Each female deposits 30-40 eggs within 2 weeks under conditions of near saturation (Ahlstrom & Rock 1973, Ball 1980, Boyne & Hain 1983). Higher egg production is achieved with greater prey availability. Consumption may rise to 12.8 eggs/day as predators were subjected to a diurnal cycle of L10/D14h, indicating that these were the conditions under which *N. fallacis* would be a more efficient natural enemy (Ahlstrom & Rock 1973, Pratt et al. 1999).

High ERM populations, in relative to predator number indicated a low likelihood of control by *N. fallacis*, necessitating acaricide sprays. When *N. fallacis* numbers are sufficiently high, relative to the pest, the chances for successful biocontrol are above 90% (Beers and Hoyt 1993).

Organophosphates and carbamates have long been used in apple production, dated back over 40 years ago. The US Environmental Protection Agency (EPA) placed new restrictions on the use of the organophosphates and carbamates insecticides, and as the result of the Food Quality Protection Act (FQPA) (USEPA 1996). FQPA requires that all registrations for pesticides used in food production be reconsidered for continued registration. This has resulted in the elimination or restricted of many of that pesticides important for apple production. Many new reduced-risk insecticides (USEPA 1997) have been introduced, but preliminary evident has shown that these new insecticides maybe more harsh on beneficials than previously expected (Wise and Whalon 2009, Agnello et al 2009). In the last ten years a large number of new reduced-risk (RR) insecticides have become registered for use in commercial apple production. There have been reports from farmers of mite flaring events in association with the use of some
of the new RR compounds (Irish Brown CAT-Alert 2009). Therefore it is important to evaluate these insecticides for use in IPM for their potential for causing mite flaring.

Four hypotheses have been suggested to further explaining this mite-flaring phenomenon: (I) The reduction of natural enemies by the pesticides (Villanueva and Walgenbach 2007, Bostonian et al. 2010); (II) pesticide-induced reproductive stimulation of phytophagous arthropods (James and Price 2002, Ako et al. 2004); (III) the removal of competitive species; and (IV) the removal of alternative food sources that sustain the predators. Although sporadic literature confirms the validity of the second hypothesis—often called hormoligosis (i.e., pesticide-induced reproductive stimulation of phytophagous arthropods) (Ako et al. 2004), and its importance in pest resurgences, most information on the subject refers chiefly to the destructive effects of pesticides on the natural enemies of the phytophagous species and, to a lesser extent, to the removal of competitive fauna (Abivardi 2008).

The objective of this study was to document mite flaring events in association with several insecticides and insecticide combinations and their effects on predator mite *N. fallacis* and *Z. mali* abundance.

**Methods and Materials**

**Study location**

This study was conducted at the Michigan State University Trevor Nichols Research Center (TNRC) in Fennville, Michigan (42.5951°N, -86.1561°W) during the summers of 2010 through 2011.
Treatment applications

Treatment solutions were applied at times relevant to first generation codling moth, *Cydia pomonella* (L.), during summers 2010 and 2011. In 2010, treatments were sprayed on 25 May and 8 June with an FMC 1029 airblast sprayer, applied with 935 liter/ha (100 gal per acre) water diluents. Sampling began mid June and ended in mid August of the same year. In 2011, treatments were applied on 13 June and 27 June. Mites were sampled beginning early June throughout mid August of 2011. Data were collected on an interval of every two weeks.

Chemical compounds

The treatment insecticides used were esfenvalerate 55.46g [AI]/ha (Asana .66 EC 9.6 oz/acre) (DuPont, Wilmington, DE), spinetoram 91.03g [AI]/ha (Delegate 25 WG 5.2 oz/acre) (Dow AgroSciences LLC, Indianapolis, IN), novaluron 145.30g [AI]/ha (Rimon .83 EC 20 oz/acre) (Chemtura U.S.A. Corporation, Middlebury, CT), chlorantroniliprole 73.52g [AI]/ha (Altacor 35 WG 3 oz/acre) (DuPont, Wilmington, DE), acetamiprid 126.04 g [AI]/ha (Assail 30 SG 6 oz/acre) (UPI, King of Prussia, PA) and carbaryl 1120.37g [AI]/ha (Sevin 4 EC XLR 1 qt/acre) (Bayer Corporation, Kansas City, MO).

Field application

Field plots consisted of 15 year old semi-dwarf apple trees, *Malus domestica* Borkhausen cv. 'Red Delicious', at the MSU TNRC. The experimental design was a randomized complete block design with 11 treatments and 4 replicates. The blocking criterion was based on the pattern of ERM populations in the orchard in recent years; trees located on the west side of the block were more infested with red mites than the trees on the east side. Between each single tree
replicate, a row of trees was left untreated as buffer. Treatment were randomly applied to one of thirteen plots within a block and replicated across the blocks. The evaluation of all plots was conducted by picking 50 random leaves from each replicate for a total of 200 leaves per treatment. Apple trees were divided into four quadrants (North, South, East and West) and 12 to 13 leaves were collected from each quadrant. Once collected, apple leaves were placed in brown paper bags, with each paper bag marked according to the different treatments, then transferred into a cooler to maintain the integrity of the samples. Disposable rubber gloves were changed between treatments both in the field and laboratory to avoid potential contamination. Paper bags in the cooler were transported to the laboratory for further processing.

**Laboratory**

In the lab, mite counting was done with a mite brushing machine (Leedom Enterprises, Mi-Wuk Village, California). The device is equipped with rollers covered with soft bristles that dislodge mites from the leaf onto a revolving plate. A light film of glycerin was applied to the plates to prevent mites from escaping. Mites deposited on the plate placed below the bottom opening of the tubing were then counted under a stereo microscope (Nikon SMZ1000). The microscope was used to identify and distinguish individual stages of the ERM, phytoseiid predatory mite, *N. fallacis*, and stigmaeid mite, *Z. mali* Ewing.

**Statistical analysis**

The ERM and predatory mite motiles and eggs were counted approximately every two weeks throughout the field season for a total of 3 months duration. A repeated measure ANOVA was utilized to analyze the data using PROC GLIMMIX in software SAS Systems for Windows
version 9.3. (SAS Institute Inc. 2010). For repeated measures, the response variable was the log-transformed mean proportion of live mites. The class variable was the post evaluation days that were treated as repeated measures. Significant treatment or day or interactions were explored using LSMEAN statements. Data were log transformed (log x + 1) to reduce heterogeneity of variance and a linear mixed model was fitted considering treatment, day, and block effects. An AR (1) type covariance matrix was used to model repeated measures for each tree. A sliced F test (simple effect test) was used to evaluate the global comparison of treatment means within each day. When a significance difference was noted, a mean separation was carried out with Tukey-Kramer honestly significance difference (HSD) test.

Results

2010 Motile: There was a significant effect of time in which post treatment-day evaluations were conducted on the mean number of motiles (\( F=52.7, \) df=127.3, \( P=<0.0001 \)), but there was no significant effect of treatment applied and there was not a significant interaction between these two main effects (Table 2.1). Evidence of mite flaring was observed through partitioning of the data by date. Among the five post-treatment evaluations, 36 DAT showed a significant increase in ERM numbers after exposure to insecticide residues in the field (\( F=2.43, \) df= 147.6, \( P=0.0106 \)) (Figure 2.1). At this date, chlorantraniliprole and acetamiprid treatments yielded significantly higher numbers of ERM motiles than the untreated control. For ERM motiles counted 57 DAT, the single applied compounds acetamiprid, esfenvalerate, spinetoram, novaluron and carbaryl all caused significantly higher numbers of ERM. Treatment combinations
chlorantroniliprole-carbaryl, spinetoram-carbaryl and novaluron-carbaryl also caused mite flaring at 57 DAT.

2010 Egg: There was a significant effect of time in which post treatment day evaluations were conducted on the mean number of eggs ($F=29.99$, $df= 123.7$, $P=<0.0001$,) and there was a significant interaction between treatment and day main effects ($F=1.49$, $df= 12.8$, $P= 0.0501$) (Table 2.1). Treatment with chlorantraniliprole and acetamiprid resulted in slightly significant increases in the number of ERM eggs observed in the field at 36 DAT (Figure 2.2). Partitioning the repeated measure analysis data, there was a significant effect of day main effect observed at 72 DAT ($F= 2.36$, $df= 118.7$, $P=0.0141$).

Table 2.1  Statistical main effects and interactions for repeated measure analysis of motile and egg stages of European red mite after exposure to field aged residues. In 2010, mites were counted 8, 21, 36, 57 and 72 DAT. In 2011, mites were counted 7, 18, 32, 47 and 64 DAT.

<table>
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<th>Effect</th>
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<th>Denominator DF</th>
<th>F value</th>
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<tr>
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<td></td>
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<tr>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
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Figure 2.1 Mean number of European red mite motiles per 50 leaves in 2010 evaluation within five post evaluations days (8, 21, 36, 57 and 72 days). Line graph with * above them designate significant difference (α <0.05) from untreated control at the same post treatment evaluation day. Line graph with * above them represents significant difference (α <0.05) for the single compound versus control at the same post treatment evaluation day. ∆ represents significant difference (α <0.05) for the combination compounds versus untreated control. Analysis performed on log transformed (log x +1) data; untransformed means are shown.
Figure 2.2  Mean number of european red mite eggs per 50 leaves in 2010 evaluation within five post evaluations days (8, 21, 36, 57 and 72 days). Line graph with * above them designate significant difference ($\alpha <0.05$) from untreated control at the same post treatment evaluation day. Line graph with * above them represents significant difference ($\alpha <0.05$) for the single compound versus control at the same post treatment evaluation day. $\Delta$ represents significant difference ($\alpha <0.05$) for the combination compounds versus untreated control. Analysis performed on log transformed (log x +1) data; untransformed means are shown.
2011 Motile: There was a significant effect of time when post treatment day evaluations were conducted on the mean number of motiles \((F= 57.75, \text{df}= 156.1, \text{P}= <.0001)\). There was no significant treatment main effect or treatment \(\times\) day interaction (Table 2.1). ERM motile densities were significantly higher in acetamiprid treatment plots than in the controls at 47 DAT (Figure 2.3). There was a significant effect on 64 DAT \((F= 1.91, \text{df}= 183.3, \text{P}= 0.0462)\), with the mean number of ERM motiles in the esfenvalerate treatment significantly higher compared to the untreated control (Figure 2.3).

2011 Egg: There was a significant effect of time when post treatment day evaluations were conducted on the mean number of eggs \((F=41.42, \text{df}= 162.3, \text{P}=<.0001)\) in the trials (Table 2.1). The treatment applied also had a significant effect \((F=2.40, \text{df}= 55.84, \text{P}= 0.0189)\). However there was not a significant interaction effect between these two main effects. On evaluation dates 7 DAT, 18 DAT and 32 DAT, egg numbers in treatments were not significantly different than the untreated control (Figure 2.4). The mean numbers of ERM eggs were significantly higher on 32 DAT \((F=1.96, \text{df}= 159.7, \text{P}= 0.0399)\) and 47 DAT \((F=3.0, \text{df}=159.7, \text{P}=0.0017)\). Esfenvalerate and acetamiprid flared mites as compared to the untreated control on 32 DAT (Figure 2.4). The mean number of ERM egg was significantly higher for the chlorantraniliprole - carbaryl, chlorantraniliprole and esfenvalerate treatments on 47 DAT. Esfenvalerate had the highest mean value of ERM eggs on the 64 DAT.

Overall, there were a total of 20 cases of mite flaring observed for egg and motile counts across the sample dates in 2010 and 2011. Esfenvalerate had the highest incidence of mite flaring with the total of 5 cases, followed by acetamiprid with 5 cases, chlorantraniliprole had 3 cases, and one case each contributed by spinetoram, novaluron and carbaryl. There were a total
Figure 2.3  Mean number of European red mite adult in 2011 evaluation after exposure to field residue within five post evaluation days (7, 18, 32, 47 and 64 days). Evaluations were made after the first of 2 field treatments. The 6 June evaluation data were included in repeated measure analysis, but excluded from graphs because no significant effects. Line graph with * above them represents significant difference (α <0.05) for the single compound versus control at the same post treatment evaluation day. ∆ represents significant difference (α <0.05) for the combination compounds versus untreated control. Analysis performed on log transformed (log x +1) data; untransformed means are shown.
Figure 2.4  Mean number of european red mite eggs in 2011 evaluation after exposure to field residue within five post evaluations days (7, 18, 32, 47 and 64 days). Evaluations were made after the first of 2 field treatments. The 6 June evaluation data were included in repeated measure analysis, but excluded from graphs because no significant effects. Line graph with * above them represents significant difference ($\alpha <0.05$) for the single compound versus control at the same post treatment evaluation day. $\Delta$ represents significant difference ($\alpha <0.05$) for the combination compounds versus untreated control. Analysis performed on log transformed (log x +1) data; untransformed means are shown.
of four cases for treatment combinations, chlorantraniliprole-carbaryl having two cases and one case reported each from spinetoram-carbaryl and novaluron-carbaryl.

Predator mites 2011

*Neoseiulus fallacis*: The overall mean numbers of phytoseiid mites, *N. fallacis*, was significantly different across treatments ($F=2.77$, df= 33, $P=0.0133$) (Figure 2.5). There was a significant effect of days post-treatment on the number of observed live predator mites ($F=38.68$, df= 132, $P=<.0001$) as well as significant interaction between the two main effects ($F=1.42$, df=132, $P=0.0707$). Esfenvalerate, spinetoram, novaluron- carbaryl, spinetoram- carbaryl, acetamiprid-carbaryl and chlorantraniliprole- carbaryl significantly reduced the number of predator mites *N. fallacis* observed in the field at 32 DAT. On 64 DAT, the treatment combination acetamiprid-carbaryl and single treatment esfenvalerate produced significantly high number of *N. fallacis*. Treatment with chlorantraniliprole did not have a significant effect, but did lead to numerically higher numbers of predator mites on this date.

*Zetzellia mali*: The overall mean numbers of stigmaeid mites, *Z. mali*, was not significantly different across treatments. There was a significant effect of time in post evaluation days ($F=3.65$, df= 132, $P=0.0075$) (Figure 2.6) but no significant treatment and time interaction. Examining treatment effects by day revealed that the combination chlorantraniliprole-carbaryl resulted in significantly lower number of *Z. mali* on 32 DAT. The mean number of *Z. mali* also was significantly reduced 64 DAT following treatment with esfenvalerate, novaluron-carbaryl and acetamiprid-carbaryl.

Overall, there were ten cases of predator mite reductions associated with insecticide treatments in 2011. The impact of treatments on predator mites was not assessed in 2010 due to
Table 2.2  Statistical main effects and interactions for repeated measure analysis of predatory mites *N. fallacis* and *Z. mali* after exposure to field aged residues. Predator mites were counted 7, 18, 32, 47 and 64 DAT.

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<tr>
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</tbody>
</table>
Figure 2.5  Mean number of predatory mites *N. fallacis* in 2011 evaluation after exposure to field residue within five post evaluations days (7, 18, 32, 47 and 64 days). Evaluations were made after the first of 2 field treatments. Line graph with * above them represents significant difference (α <0.05) for the single compound versus control at the same post treatment evaluation day. Δ represents significant difference (α <0.05) for the combination compounds versus untreated control. Analysis performed on log transformed (log x +1) data; untransformed means are shown.
Figure 2.6  Mean number of predatory mites Z. mali in 2011 evaluation after exposure to field residue within five post evaluations days (7, 18, 32, 47 and 64 days). Evaluations were made after the first of 2 field treatments. Line graph with * above them represents significant difference (α <0.05) for the single compound versus control at the same post treatment evaluation day.  ∆ represents significant difference (α <0.05) for the combination compounds versus untreated control. Analysis performed on log transformed (log x +1) data; untransformed means are shown.
very low abundances of *N. fallacis* and *Z. mali* throughout the study period. Treatment with esfenvalerate, spinetoram, chlorantraniliprole- carbaryl, spinetoram- carbaryl, acetamiprid- carbaryl and novaluron- carbaryl led to reduced densities of predator mites on at least one sampling date in 2011. On a single sample date, the esfenvalerate, chlorantraniliprole and acetamiprid- carbaryl treatments also resulted in an increase in densities of *N. fallacis*.

**Discussion**

Our study expands the understanding of mite flaring impacts of insecticides on the tetranychid mite, *P. ulmi* and population reduction effects on phytoseiids mites, *N. fallacis* and *Z. mali*. Results showed a total 19 cases of mite flaring and 10 cases of reduced numbers of predator mites associated with insecticides or combinations of insecticides commonly used in apple IPM programs.

The synthetic pyrethroid esfenvalerate dominantly contributes to the highest incidence of mite flaring, which appears to be associated with toxicity to predator mites. Much lower densities of both phytoseiid and stigmaeid mites were observed from esfenvalerate exposure in both years of the study. AliNiazee (1984) reported a similar finding of predator mite species on a number of fruit tree crops after exposure to synthetic pyrethroids. However, our study also showed that *N. fallacis* populations were able to recover later in the season. Although present in abundance, this late occurrence of *N. fallacis* is likely insufficient to control the peak number of *P. ulmi* observed in mid-July in the season. A study by Gerson and Cohen (1989) indicated that spider mite fecundity increased with less time taken between developmental stages after
exposure to synthetic pyrethroids. More females were produced where it favored the faster reproduction thus contributed to the mite resurgence or hormoligosis.

The carbamate carbaryl alone contributed to a limited number of mite flaring cases, but high magnitude ERM numbers, likely through compound toxicity to predator mites. Carbaryl caused the lowest occurrence of *Z. mali* during the mid-July and low numbers continued toward the end of the season, whereas *N. fallacis* were negatively affected by carbaryl through the middle of the study season. A study by Dittrich (1974) found an increase in female proportion and higher egg production in female *Tetranychus urticae* Koch after exposure to carbaryl residue. Hormoligosis is assumed as the cause for the enhanced *T. urticae* female reproduction. Walker and Aitken (1996) similarly reported a high incidence of tetranychid mite, *P. citri* in California citrus after exposure to carbaryl. Hormoligosis through the stimulation of carbaryl on ERM females reproduction is likely the possible cause for the high number of ERM motiles and egg observed in the study.

The neonicotinoid acetamiprid showed evidence for causing mite flaring, however it appeared to have little or no toxicity to predator mites of both species. Ako et al (2004) reported that application of imidacloprid, a neonicotinoid insecticide can lead to population buildup of two-spotted spider mite, *Tetranychus urticae* Koch, in the field. Laboratory studies showed enhanced fecundity of *T. urticae* after an imidacloprid treatment. Similar result showed by James and Price (2002), an increase of 20% of egg production was observed when imidacloprid was exposed systemically to two spotted spider mite T. urticae Koch.

The diamide chlorantraniliprole showed limited evidence of mite flaring (in terms of magnitude of ERM motiles) when applied alone and exposure of both predator mites to this compound was harmless. Similarly, previous studies have reported chlorantraniliprole to be non-
harmful to phytoseiid mites. For instance, Gradish et al. (2010) reported *Amblyseius swirskii* (Athias-Henriot) were negatively affected by chlorantraniliprole in both laboratory and greenhouse trials. Another study by Reis et al. (2011) showed that chlorantraniliprole was selective for the studied phytoseiid mite species, *Iphiseiodes zuluagai* Denmark & Muma, *Amblyseius herbicolus* (Chant) and *Euseius citrifolius* Denmark & Muma. They concluded that chlorantraniliprole was not harmful, from the observation of overall low mortality on phytoseiid mites.

Chlorantraniliprole, the new anthranilic diamide is active on chewing pests and its mode of entry is primarily by ingestion followed by contact. Chlorantraniliprole stimulates the release of calcium stores from the sarcoplasmic reticulum of muscle causing paralysis and death (Dinter et al. 2008). Previous study reported insects with high sensitivity are more susceptible to chlorantraniliprole (Dinter et al. 2008, Reis et al 2011). Thus, observed negative effect of this insecticide to predator mites were likely due to the high selectivity toxicity of chlorantraniliprole. Whereas, the present of insecticide resistant genes in *P. ulmi* could potentially contributed to the low ERM population build ups.

The spinosyn spinetoram showed limited evidence for mite flaring (in terms of magnitude of ERM motiles) when applied alone, although it appeared to be moderately toxic to predator mites. It may be that its negative effects are more short lived than other materials. The IGR novaluron showed limited evidence of mite flaring and was harmless to predator mites *N. fallacis* and *Z. mali*. The non-toxic effect of novaluron on phytoseiid mites, *Galendromus occidentalis* (Nesbitt) was also reported by Lefebvre et al. (2011). The apple rust mite, *Aculus schlechtendali*, is an important alternate food in maintaining *N. fallacis* populations on apple trees during lack of other prey (Gerson et al. 2008). Although apple rust mites were not measured in this study,
novaluron is known to be active on them. Therefore the non-target effect of novaluron might have eliminated *A. schlechtendali* and thus indirectly limiting on *N. fallacis population dynamics*.

Novaluron is a benzoylurea chitin synthesis inhibitor insecticide widely used in apple production. Previous studies showed that novaluron caused sub-lethal effects to the insect predators and pests. Wise et al. (2007) reported a reduction in Plum curculio larval survival following mated female exposure to treated substrate (Wise et al. 2007). Kostyukovsky and Trostanetsky (2006) recorded a similar result for exposure of *Tribolium castaneum* (Herbst) to novaluron. Non harmful effect of novaluron observed on phytoseiids mites is likely the result of predator mite reduction by sublethal effects rather than direct toxicity.

The treatment combinations provided interesting results in that carbaryl alone only showed weak negative impact on predators, but when in combination with any of the other insecticides consistently reduced *N. fallacis* and *Z. mali* populations. Why this phenomena occurred is unclear, but is worthy of further investigation, since many apple growers use tank-mixes of similar combinations.

Further investigation is needed to confirm the mechanisms responsible for mite flaring associated with these compounds, and the duration and conditions of toxicity that are most harmful. Insecticides toxicity varies with exposure route. Populations of predator mites were exposed to direct foliar sprays during field application as well as dry residues, hence it would be valuable to determine effects of these insecticides from each form of exposure on the principal phytoseiid mite in the apple orchard, *N. fallacis*. Furthermore, study on the potential sub-lethal effects is also important as it is known to affect the physiology, population dynamic and behaviour of non-target species. In conclusion, the new reduced risk insecticides
chlorantraniliprole, novaluron and spinetoram, when applied alone, were found to be relatively
innocuous to predator mite *N. fallacis* and *Z. mali* and can safely incorporated into apple IPM
programs. When the fruit thinning agent, carbaryl, is used in apple production limited mite
flaring can be anticipated. Acetamiprid caused mite outbreaks and its application should be made
with caution so as not to mitigate a disruptive effect on IPM. Conventional insecticides such
carbamates and esfenvalerate caused high ERM population build-ups as well as high toxicity to
predator mites, thus, their application should be limited and/or eliminated to avoid disrupting
effective IPM programs.
CHAPTER 3

TOPICAL TOXICITY OF INSECTICIDES ON *NEOSEIULUS FALLACIS*

Abstract

*Neoseiulus fallacis* (Garman) (Acari: Phytoseiidae) is an efficient predator of the European red mite (ERM) in Michigan apple orchards and an important part of integrated pest management (IPM). Four reduced-risk insecticides (acetamiprid, spinetoram, chlorantraniliprole and novaluron) and two conventional insecticides (esfenvalerate and carbaryl) were tested against *N. fallacis* for topical toxicity effects. Bioassays using a Potter Spray Tower were conducted to measure the topical toxicity of compounds when applied at field rate concentrations to *N. fallacis*. Lethal time was measured for adult *N. fallacis* at 6, 24, 36 and 48 hours after treatment. Carbaryl and esfenvalerate showed the highest levels of toxicity to *N. fallacis* with shortest lethal time values ($LT_{50}$) whereas the reduced risk insecticides novaluron, acetamiprid, spinetoram and chlorantraniliprole were non toxic. This study provides important information to apple growers regarding direct lethal effect of insecticides on predator mites and its implications to integrated mite management.
Introduction

*Neoseiulus fallacis* (Garman) is an important predacious phytoseiid mite in North American apple orchards and an efficient predator of the European red mite (ERM), *Panonychus ulmi*, two-spotted spider mites (TSSM), *Tetranychus urticae* and other phytophagous mites. In the humid central and eastern regions of North America *N. fallacis* occurs on trees and lower vegetation, whereas in the drier western parts, *N. fallacis* usually inhabits low and sprawling plants, unless living on irrigated tree crops (Morris et al. 1996). Winter in the orchard ground cover, *N. fallacis* feeds upon overwintering two spotted spider mite (Croft 1990), apple rust mite *Aculus schlectendali*, cyclamen mite *Steneotarsonemus pallidus* and tomato russet mite, *Aculus lycopersici* (Rincon Vitova 2008). Efficacy of *N. fallacis* for biological control has been reported extensively. In Western and Eastern North America, *N. fallacis* is effective on pest-mite populations on variety of crops including apple, strawberry, corn, soybean, sorghum and hops (Croft & McGroarty 1977, Croft 1990, Morris et al. 1996).

High population of *P. ulmi* and *T. urticae* negatively affect plant health, reducing fruit size, increasing the number of premature fruit and causing leaf bronzing (van de Vrie 1985). High ERM populations, relative to predator numbers indicate a low likelihood of control by *N. fallacis*, necessitating acaricide sprays. However, acaricide usage leads to lower farm profits along with development of resistance, thus is unfavorable to integrated mite management (Villanueva 1997). In regard to commercial apple production, the maintenance of ERM below economic thresholds is not 100% guaranteed with biological control alone. Successful management of mite pests must utilize the holistic approach of Integrated Pest Management (IPM). One tool of IPM, the use of pesticides, plays an important role in achieving a marketable crop. Pesticides, however, must be used cautiously, not to interfere with beneficial arthropods’
behaviour or survival. Insecticides used to control arthropod pests may be toxic to *N. fallacis*, causing disruption to the successful application of integrated mite management through biological control.

Apple orchards in the western and eastern United States have historically relied heavily on broad spectrum synthetic insecticides such as organophosphates and carbamates (Jones et al. 2009). Enactment of Food Quality Protection Act (FQPA) led to the restrictions on the use of organophosphate and carbamate insecticides thus eliminating or restricting many of those insecticides important for apple production (USEPA 1996). As a result, newer pesticides with novel modes of action and fewer environmental effects have been introduced (Whalon et al. 1999, Agnello et al. 2009). However, growing evident has shown that these newer pesticides are not always compatible with the beneficial insects (Wise and Whalon 2009, Agnello et al 2009) and predator mites (Villanueva & Walgenbach 2005, Bostanian et al. 2009, Lefebvre et al. 2012).

Insecticide applications are sometimes followed by serious outbreaks, not of the pest against which they were applied, but of other phytophagous insects and mites which, prior to the treatment were in very small numbers too low to be of economic importance (Abivardi 2008). Pest resurgence occurrence after application of pesticides exhibiting different modes of action and under different climatic conditions indicates that chemical control in many cases upsets the population dynamics of the pests in question. Van de Vrie et al. (1972) reported that the outbreaks of *P. ulmi* (Koch) in apple orchards have been attributed to the use of pesticides that suppress their natural enemies. The use of synthetic pyrethroid insecticides for example, has been implicated in the reduction of phytoseiid mite species on a number of fruit tree crops in different parts of the world (AliNiazee 1984). Synthetic pyrethroids such as permethrin and
fluvalinate caused 100% mortality of females *Phytoseiulus macropilis* after 72 hours exposure in the laboratory bioassay (Amin et al. 2009). As a result, synthetic pyrethroids have been eliminated from most IPM recommendations and are avoided in most apple production systems (Agnello et al. 2009, Wise et al. 2011). Organophosphate insecticides when first introduced were toxic to predaceous mites, but most species are now resistant to the OPs used in apple production. Laboratory “worse case” studies have shown varying levels of direct toxicity of new reduced risk insecticides, including imidacloprid, acetamiprid, and spinosad to predacious mites (Bostonian et al. 2010).

Despite past efforts to determine the effects of insecticides on predator mites, there is a gap in understanding the toxicity of several new insecticide classes, including diamides, insect growth regulators and spinosyns, to predaceous mites in apple production systems. Bioassays that address toxicity, as well as the “lethal time” of new compounds, is needed to fully understand the potential impact of modern IPM programs on predator mite populations. The purpose of this study was to measure the toxicity effects of *N. fallacis* exposure to foliar applied insecticides belonging to several newer insecticide classes through direct exposure to spray droplets.
Methods and Materials

Field sampling

*Neoseiulus fallacis* were collected in an apple orchard, *Malus domestica* Borkhausen cv. 'Red Delicious', maintained free from insecticides within the Michigan State University Trevor Nichols Research Center (TNRC) in Fennville, Michigan (42.5951°N, -86.1561°W). Field observations to determine the distribution of *N. fallacis* in the apple orchard were done prior to the beginning of the experiment. A high abundance of predator mites was observed in blocks that contained relatively high levels of grassy groundcover. Collections were done in the morning to ensure a high yield of healthy predator mites. Sixty to seventy apple leaves were collected at random from the tree canopy. Individual leaves were placed in a brown paper bag and transported to the laboratory in a portable ice chest.

Laboratory preparation

Apple leaves were removed from the paper bag and held at room temperature for 10 minutes. Following this, leaves were passed through a mite brush machine and *N. fallacis* were collected in large size petri dishes (6 cm diameter). They were immediately transferred to small petri dishes (3 cm) that were sealed with a thin layer of Arabic gum to prevent predator mite escapes. Ten adults were placed in each petri dish, with a total of 7 replicates of each treatment. The petri dishes were held overnight in a refrigerator at 5 degree Celsius prior to conducted bioassays the following day.
Bioassay

The interior of the top and bottom of each Petri dish were treated with 2 ml insecticide solution (field rate equivalent solution) at 20 psi using a Potter Spray Tower® (Potter 1952) and allowed to dry prior to the bioassay. The predator mites were held in the treated petri dishes for 6, 24, 36 and 48 hours. The control consisted of untreated predator mites sprayed with distilled water. Following exposure to the insecticides or control treatment, *N. fallacis* were hand transferred to clean petri dish using a camel paint brush and stored at a constant temperature and light intensity in an incubator. After 6, 24, 36 and 48 h of exposure the bioassays were taken apart and evaluated. The numbers of living predator mites were recorded with mortality defined as the mites that were immobile after prodding with a camel hair brush.

Chemical compounds

Insecticides tested in the bioassay were esfenvalerate at 59.31 ppm (Asana .66 EC 9.6 oz/acre)(DuPont, Wilmington, DE)), spinetoram at 97.35 ppm (Delegate 25 WG 5.2 oz/acre in 100 gallon per acre diluent equivalent) (Dow AgroSciences LLC, Indianapolis, IN), novaluron at 155.39 ppm (Rimon .83 EC 20 oz/acre) (Chemtura U.S.A. Corporation, Middlebury, CT), chlorantroniliprole at 78.63 ppm (Altacor 35 WG 3 oz/acre) (DuPont, Wilmington, DE), acetamiprid at 134.80 ppm (Assail 30 SG 6 oz/acre) (UPI, King of Prussia, PA) and carbaryl at 1198.25 ppm (Sevin 4 EC XLR 1 qt/acre)(Bayer Corporation, Kansas City, MO).

Statistical analysis

Adult mortality data were corrected for the check mortality using Abbott’s formula (Abbott 1925). The percentage mortality was arcsine transformed before analysis of variance.
using ANOVA procedures. Treatments means were separated using LSD test at \( P = 0.05 \) (PROC GLM SAS Institute 2002). Tukey’s honestly significant differences (HSD) test was used for multiple comparisons. The probit analysis was performed to calculate the time in hours at which 50\% of the mites were killed (LT\(_{50}\)), slope of the log time-probit line (± SE), the confidence interval and the fiducial limits.

**Results**

The results from the topical toxicity assay for six insecticides are presented in Fig. 3.1 and Table 3.1. Predator mite adults exposed to 96-h topical assays showed significantly higher levels of mortality from the carbaryl and esfenvalerate treatments as compared to the untreated control, whereas other compounds did not cause mortality different than the untreated control (\( F=10.50, \text{df} = 6, P= <.0001 \)) (Figure 3.1).

**Table 3.1** \( LT_{50} \) values for *N. fallacis* mortality after 96 h of topical exposure to a given treatment insecticide.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>n</th>
<th>Slope (+SE)</th>
<th>( LT_{50} ) (h)</th>
<th>95% CI</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTC</td>
<td>10</td>
<td>4.143 (+0.8134)</td>
<td>107.38</td>
<td>(87.62, 156.22)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>10</td>
<td>1.311 (+0.2636)</td>
<td>4.14</td>
<td>(1.19, 7.81)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Esfenvalerate</td>
<td>10</td>
<td>1.676 (+0.2709)</td>
<td>18.71</td>
<td>(11.76, 26.46)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Novaluron</td>
<td>10</td>
<td>1.616 (+0.3299)</td>
<td>89.75</td>
<td>(61.40, 172.32)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>10</td>
<td>1.912 (+0.3709)</td>
<td>92.96</td>
<td>(66.56, 163)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Chlorantraniliprole</td>
<td>10</td>
<td>0.688 (+0.2559)</td>
<td>128.71</td>
<td>(55.08, 5687)</td>
<td>0.0071</td>
</tr>
<tr>
<td>Spinetoram</td>
<td>10</td>
<td>0.662 (+0.2549)</td>
<td>135.56</td>
<td>(55.85, 11849)</td>
<td>0.0094</td>
</tr>
</tbody>
</table>
The time in hours at which 50% of the predator mites were killed by insecticides ($LT_{50}$) was calculated as a measure of toxicity from insecticides as exposure time increased. Carbaryl was significantly more toxic to adults of $N. fallacis$ and corrected mortality (Abbott 1925) reached 88% within 96 hours from topical application (Figure 3.1). The $LT_{50}$ of carbaryl was significantly lower as compared to the untreated with the most rapid lethal time $LT_{50}$ value of 4.14 h ($\chi^2 = <.0001$) (Table 3.1). Esfenvalerate was significantly toxic to $N. fallacis$ adults with mortality rate 84% after 96 hours exposure to topical application. The $LT_{50}$ of esfenvalerate showed significantly rapid lethal time as compared to the untreated control with calculated value of 18.71 hours ($\chi^2 = <.0001$). Novaluron and acetamiprid were less toxic to predator mites with 13% and 28% of mortality within 96 hours. The lethal time of novaluron was calculated as $LT_{50}$ value of 89.75 hours ($\chi^2 = <.0001$), while acetamiprid had lethal activity at the calculated $LT_{50}$ value of 92.96 hours ($\chi^2 = <.0001$) with overlapped 95% confidence interval. Chlorantraniliprole and spinetoram were the least toxic to $N. fallacis$ adult with 31% and 16% of mortality within 96 hours, and the calculated lethal time showed the slowest lethal activity with $LT_{50}$ value of 128.71 ($\chi^2 = .0071$) and 135.56 hours ($\chi^2 = .0094$) respectively (Table 3.1).
Figure 3.1 Mean (±SEM) survival of *Neoseiulus fallacis* on apple leaf discs following 96 h exposure to different insecticides. Means with the same letter are not significantly different at $\alpha \leq 0.05$. Mean separation calculated using the Tukey’s honestly significant differences (HSD) test. Data shown are non-transformed means.

Discussion

This study provides important new information about the direct lethal effects of insecticides to *N. fallacis* following topical sprays. Results varied quite dramatically from compounds to compound, with some showing relatively little effect while others having high lethality.

Carbaryl was highly toxic to *N. fallacis* as a result of direct exposure to topical sprays. The LT$_{50}$ value of this carbamate insecticide showed the least time (in hours) needed to cause *N.*
fallacis mortality. These results agree with those of Edward & Hodgson (1973) showing that carbamates were highly toxic to an important mite predator in citrus, Stethorus nigripes.

Similarly, a two year field study by Walker & Aitken (1996) validated that carbaryl elevated populations of citrus red mite, Panonychus citri, in California citrus. The toxicity of carbaryl against N. fallacis through direct sprays therefore is likely a primary contributor to any mite flaring associated with the application of this compound.

The pyrethroid esfenvalerate also was highly toxic to the predator mite N. fallacis. From the LT$_{50}$ value, this compound showed the time it takes to kill 50% of the N. fallacis adult was four fold that of carbaryl, but fast-acting none-the-less. This result is in concordance with Rock (1979) and AliNiazee (1984) who documented mortality to predator mites, N. fallacis and Typhlodromus arboreus, after exposure to synthetic pyrethroids. Similarly, Cho et al. (1997) found high toxicity in a test of esfenvalerate against the insect predator, Harmonia axyridis.

Esfenvalerate, a voltage-dependent sodium-channel agonist is generally is highly active on the nervous system of insects or arthropods (Royal Soc. Chem. 1994, E.I. DuPont de Nemours 2002). Direct contact through exposure to spray droplets provide the most effective route of entry followed by consumption of spray droplets or dried residue (Kelly 2003). As evident in our study, topical spray exposure demonstrated high mortality to N. fallacis. Since this synthetic pyrethroid is toxic to the predator mite N. fallacis, the application of esfenvalerate would likely reduce the conservation of N. fallacis thus causing pest mite outbreaks.

The insect growth regulator novaluron was relatively non-toxic to N. fallacis. The observed lethality time, LT$_{50}$ value of this compound was slightly shorter than that of the untreated control, but with substantial over-lapping of the confidence intervals. The non-toxic effect of novaluron on phytoseiid mites, Galendromus occidentalis (Nesbitt) was also reported
by Lefebvre et al. (2011). In addition, Amarasekare and Shearer (2013) observed low toxicity in the adult predatory mired bug, *Deraeocoris brevis* (Uhler) after exposure in topical bioassays. The neonicotinoid acetamiprid was not toxic to *N. fallacis*. The observed lethality time, \( LT_{50} \) value of this compound was slightly shorter than that of untreated control, but with substantial over-lapping of the confidence intervals. Similar result was shown by Pozzebon et al. (2011) who studied the toxicity of thiamethoxam to *Phytoseiulus persimilis* (Athias-Henriot) through multiple routes of exposure, with topical exposure resulted in low mortality of predatory mites. They concluded that topical spray might have provided less amount of thiamethoxam that mites took up, and suggested that predatory mites use prominent role of tarsal uptake as compared to integument absorption. In addition, the body surfaces of mites were relatively small as compared to the treated leaf and this also suggested the role of residual exposure was superior to the topical exposure (Pozzebon et al. 2011). In contrast, Bostanian et al. (2009, 2010) reported that acetamiprid was moderately to highly toxic to *G. occidentalis* and *N. fallacis* in “worst-case” exposure scenario.

Acetamiprid, a first generation neonicotinoid developed by Aventis CropScience is more effective systematically after foliar application (Horowitz et al. 1998). Its low hydrophobicity contributes to its excellent systemic and translaminar activity. Studies by Stein-Dönecke et al. 1992 and Westwood et al. 1998 demonstrated that neonicotinoids are mainly acropetally transported in the xylem. Systemic insecticides can contaminate floral nectar when systemically distributed throughout the plant (Lord et al. 1968) and cause detrimental effects to parasitoids (Cate et al. 1972). In our study, the low incidence of *N. fallacis* mortality may be due to lack of exposure to treated plant material or through feeding on treated prey.
The diamide chlorantraniliprole was relatively non-toxic to the predator mite *N. fallacis*. The observed lethality time, LT_{50} value of this compound was similar to that of the untreated control, and with substantial over-lapping of the confidence intervals. Acute mortality from chlorantraniliprole in topical assays has given diverging results. Amarasekare and Shearer (2013) found that chlorantraniliprole were highly toxic to two insect predators, green lacewings *Chrysoperla carnea* and *C. johnsoni*, whereas slight toxicity was observed for *G. occidentalis* (Nesbitt) in a worst case exposure scenario in the laboratory.

A macrocyclic lactone spinosyn insecticide, spinetoram, was the least toxic to the predator mite *N. fallacis* when applied topically. There was no significant mortality in predator mite adults and the time taken to kill 50% of the adult population took substantially longer than that of chlorantraniliprole. Nasreen et al. (2003) reported that topical treatments had no significant lethal effect on the insect predator, *Chrysoperla carnea*, but 100% mortality occurred when adults ingested treated prey. Likewise, no effect on the predation index was observed following exposure of the predatory mite, *Euseius tularensis* Congdon, to spinosad (Khan and Morse 2006). In contrast, high mortality was observed for the predatory mirid bug, *Pilophorus typicus* Distant, following the exposure to low spinosad concentrations (Nakahira et al. 2010).

Active through ingestion, the insecticidal action of spinetoram is from two spinosyns, factor J, XDE-175-J and factor L, XDE-175-L, which causes anomalies to insect nAChR receptors. When ingested, these factors were broadly distributed in tissue, however, the highest concentrations were found in the gastrointestinal tract (reference bulletin Agro Dow chemical). Acute mortality from spinosad and spinosyn on predatory mites from exposure to dried residue on leaves has been extensively reported in the past (Villanueva & Walgenbach 2005, Van Driesch et al. 2006, Olszak & Sekrecka 2008, Rahman et al. 2011 and Lefebvre et al. 2011).
Thus, topical exposure of spinetoram from direct sprays is not anticipated as the route contributing to toxicity of this predator or to subsequent mite flaring.

In this study, effects attained by single exposure route, topical spray, varied depending on the insecticide. Conventional insecticides such carbamates and esfenvalerate were highly toxic and their applications in the field need to be carefully considered as to limit interference with the conservation of *N. fallacis*. The reduced risk insecticides novaluron, acetamiprid, spinetoram and chlorantraniliprole were relatively harmless to predator mites from direct sprays, but impacts of residual exposure on sprayed plant materials must be considered before full integrated mite management recommendations can be made.
CHAPTER 4

RESIDUAL TOXICITY OF INSECTICIDES ON *NEOSEIULUS FALLACIS*

Abstract

Field-based bioassays and residue profile analysis were used to determine the temporal patterns of esfenvalerate, carbaryl, acetamiprid, chlorantraniliprole, spinetoram and novaluron toxicity to the phytoseiid predator mite, *Neoseiulus fallacis* (Garman) (Acari: Phytoseiidae) in apples. *N. fallacis* adults were exposed to field-aged residues of acetamiprid, spinetoram, chlorantraniliprole, esfenvalerate and carbaryl (1, 7, 14 and 21 d post application), and predator mite mortality and lethal time were measured over 48 hours of exposure. Residue analysis conducted at 1, 7, 14 and 21 d post-application was to assess temporal dimension of insecticide activity. The carbamate carbaryl caused high mortality of *N. fallacis* after exposure to dry residues and the shortest lethal time values (LT<sub>50</sub>), followed by the spinosyn spinetoram, with moderate lethal time values. Esfenvalerate showed no direct toxicity effects to *N. fallacis* although high proportions of residue were detected throughout the 14 days of study. Acetamiprid, chlorantraniliprole and novaluron showed no observed reduction in *N. fallacis* mortality. Potential impacts of new insecticide alternatives on IPM programs and predator mite populations are enhanced with greater understanding of temporal toxicity of insecticides under field aging and estimation of lethal time.
Introduction

*Neoseiulus fallacis* (Garman) is a predatory mite that is common in apple orchards and distributed throughout North America, with greater densities in the humid mid-western and eastern regions (Croft and McGroarty 1977, Welty 1995). In the drier western parts, *N. fallacis* usually inhabits low and sprawling plants, whereas more *N. fallacis* have been found on trees and lower vegetation in the more humid regions of central and east of North America (Morris et al. 1996). *Neoseiulus fallacis* is an efficient predator of the European red mite (ERM), Two-spotted spider mite (TSSM) and other phytophagous mites, such as eriophyoids.

McMurtry and Croft (1997) classified *N. fallacis* as a Type II- selective predator of the spider mite family. Based on the feeding specialization classification, Type I consist of selective predators of *Tetranychus* spp. whereas Types III and IV were considered more generalist predators (McMurtry and Croft 1997, Croft et al. 1998). Pratt et al. (1999) showed that *N. fallacis* reproduced on other prey in the event of starvation. According to Ball (1980), temperatures plays a role in the life cycles length of phytoseiid mite *N. fallacis* with an average of 3.5 days at 26.4°C and 12.3 days at 13.3°C for immatures. Oviposition in *N. fallacis* required mating, unlike Tetranychidae, and females were observed to have more stages of instar as compared to the male (Ballard 1954, Smith and Newsom 1970). Each female deposits an average of 3.5 eggs/day at 26.4 °C and 0.9 eggs/day at 13.3°C (Ball 1980). The period from egg deposition until emergence of adults is 7.3 and 3.3 days at 21.1°C and 32.2°C, respectively. Higher egg production is achieved with greater prey availability. When offered an abundance of spider mites at 26.7°C, females devoured 10.6 eggs/day or 4.8 females/day. The rate of egg production varied depending on the amount of prey consumed (McMurtry et al. 1970).
In apple orchards, *N. fallacis* spends the winter in the ground cover, where it feeds upon overwintering two spotted spider mite and other mites. It migrates upward into the canopy in the spring and spends the summer in the tree potentially providing mid- and late-season biological control of European red mites, *Panonychus ulmi* (Koch) (McGroarty and Croft 1978, McMurtry and Croft 1997, Bostanian et al. 1998).

Croft and McGroarty (1977) reported that *P. ulmi* was the most destructive mite in Michigan apple orchards where it has significant negative effect on fruit size, fruit coloration and the number of fruit the following season. Although acaricides has been used to control high population of *P. ulmi* in the past, evidence of resistance in tetranychids has reduced the desirability of acaricides for mite management (Herron et al. 1994, Villanueva 1997). As an alternative, predatory mite were introduced as biological control agents in the apple orchards, however the maintenance of *P. ulmi* below economic threshold is not 100% guaranteed with biological control alone. Pesticides, as one tool of integrated pest management (IPM), play an important role in achieving a marketable crop. Pesticides toxicity, however, may have disruptive effects on predatory mites population hence a major hinderance to *N. fallacis* survival in the orchards.

Synthetic pyrethroids have been eliminated from most IPM recommendations and are avoided in most apple production systems (Agnello et al. 2009, Wise et al. 2011). A study by Hull and Starner (1983) found that the use of pyrethroids in Pennsylvania orchards caused a decline in phytoseiid populations. The use of carbaryl was found to be harmful to predator mite survival. Cone et al. (1990) reported that the use of carbaryl in 1960’s and 1970’s contributed to spider mite outbreaks. In laboratory studies, carbaryl was shown to cause nearly 100% mortality to *N. fallacis* (Thistlewood and Elfving 1992). Organophosphate insecticides when first
introduced were toxic to predaceous mites, but most species are now resistant to the OPs used in apple production.

The implementation of the Food Quality Protection Act (FQPA) led to restrictions on the use of organophosphates and carbamates insecticides (USEPA 1996). FQPA requires that all registrations for pesticides used in food production be reconsidered for continued registration. As a result, newer pesticides with novel mode of action and fewer environmental effects have been introduced (Whalon et al. 1999, Agnello et al. 2009). With the use of these new materials, however, there have been reports of some detrimental effects on beneficial insects (Wise and Whalon 2009, Agnello et al 2009), including predators of P. ulmi (Villanueva & Walgenbach 2005, Bostanian et al. 2009, Lefebvre et al. 2012).

Different toxicity levels of new reduced-risk insecticides to predator mites have been shown in the laboratory. Imidacloprid, for example, was highly toxic to predator mite Galendromus occidentalis (Nesbitt) and N. fallacis (James 2003) but harmless to Agistemus fleshneri summers (Bostanian and Larocque 2001). Studies on the risk assessment on predatory mites showed that species belonging to the genera Hypoaspis, Neoseiulus, Typhlodromus and Thyphlodromips was negatively affected after exposure to dried spinosad residue (Biondi et al. 2012).

Residue profile analysis of the insecticides is an effective method for showing the temporal dimension of insecticide activity on the target pest under field conditions (Wise and Whalon 2009). Recent studies have shown that persistence and bioavailability of organophosphate, neonicotinoid, spinosyn, and insect growth regulator (IGRs) on apple fruit and foliage vary widely (Wise et al. 2006). Bioavailability of insecticides varies according to their interaction with leaf tissue, and performance behaviour of spray droplets may greatly be
influenced by the physics and chemistry properties of the cuticle. Hydration of the cuticle, for e.g., may increase the sorption of hydrophilic compounds (low log $K_{ow}$) (Kirkwood 1999).

Despite information on how insecticides affect predator mites under laboratory conditions, there is limited understanding on the temporal toxicity of new insecticides under field-aging conditions. Bioassays that address toxicity, as well as the “lethal time” of new compounds are needed to fully understand the potential impact of modern IPM programs on predator mite populations. The objective of this study was to determine the temporal toxicity patterns of insecticides to *N. fallacis* using field-aged dry residue bioassays.
Methods and Materials

Field treatment and sampling

Residual activity bioassays were used to determine the toxic effects of selected registered pesticides on *N. fallacis* as they age in the field. Insecticides were applied to apple trees in small field plots and predatory mites were subsequently exposed to treated leaves in a laboratory bioassay. Insecticides were applied to ‘Delicious’ apple trees at the Trevor Nichols Research Center in Fennville, Michigan (42.5951°N, -86.1561°W) with an FMC 1029 airblast sprayer set at delivering 100 gpa at 2.5 mph. The experimental design was a randomized complete block. Each block consisted of two standard mature apple trees (cultivar Red Delicious) in a row and treatments were replicated 5 times. Between each replicate, a row of trees were left untreated as buffer. Samples of 50 leaves per replicate were collected randomly around the tree canopy, placed in a portable ice chest and brought to the laboratory. *N. fallacis* was collected from apple leaves obtained from an unsprayed apple block, and in the laboratory were removed using the mite brush machine and held in large size petri dishes prior to their use in bioassays.

Chemical compounds

The treatment pesticides used were esfenvalerate 55.46g [AI]/ha (Asana .66 EC 9.6 0z/acre)(DuPont, Wilmington, DE)), spinetoram 91.03g [AI]/ha (Delegate 25 WG 5.2 oz/acre) (Dow AgroSciences LLC, Indianapolis, IN), novaluron 145.30g [AI]/ha (Rimon .83 EC 20 oz/acre) (Chemburo U.S.A. Corporation, Middlebury, CT), chlorantroniliprole 73.52g [AI]/ha (Altacor 35 WG 3 oz/acre) (DuPont, Wilmington, DE), acetamiprid 126.04 g [AI]/ha (Assail 30
Laboratory bioassays

For each of the 7 replicates per treatment, 8 leaf punches were taken from 2 leaves (high, low) on each side (N,S,E,W) of the tree. Moist filter paper (5.5 cm) was pressed into a 3 cm wide petri dish and the leaf punches (2.4 cm) placed into the dish on top of the filter paper. The leaf puncher was dipped in acetone between each treatment for sterilization. Ten *N. fallacis* were placed into each bioassay chamber. The dishes were sealed with a thin layer of Arabic gum to prevent predator mite escapes, labeled by treatment, and stored at a constant temperature and light intensity in an incubator. After 6, 24, 36 and 48 h of exposure the bioassays were taken apart and evaluated. The numbers of living predator mites were recorded with mortality defined as the mites that were immobile after prodding with a camel hair brush. Observation of behavior inhibition was recorded. New bioassays were set at 1, 7, 14 and 21 d after a single field application of each treatment insecticide or combination of chemicals.

Residue Profile Analysis

Residue profiles were generated for each of the insecticides and at each of the four post-application intervals. Foliage sample were taken from apple field plots at 1 d, 7, 14 and 21 d post-treatment and stored in 100 ml volume of high performance liquid chromatography (HPLC) grade acetonitrile. Composite samples of 40 leaves each were transported to the Michigan State University pesticide laboratory in East Lansing, Mi. Samples were then grounded and decanted through 10-25 g of reagent-grade anhydrous sodium sulfate (EMD Chemicals, Inc) to remove
water and collected in a round bottom flask. Samples were then dried via rotary evaporation and 2ml of acetonitrile (solvent) was added to the remaining particles. Samples were passed through 0.45 µm Acrodisc 13 mm syringe filter (Pall, East Hills, NY) to remove any particulates from the sample and placed in 2.5 ml gas chromatography vial for HPLC analysis. Insecticides were determined using Water 2695 separator module HPLC equipped with a Water ZQ Mass Spectrum. The column was a C18 reversed phase column with 3.0 mm bore and 50 mm column.

**Statistical Analysis**

To determine the relationship between lethal effects on predator mite and pesticide residues, repeated measure anova were performed on 96 h adult mortality data from the five post-application residual activity bioassays and the pesticide residue data for each sample date (PROC GLM, SAS Institute 2002). Regression analysis (PROC GLM, SAS Institute 2002) was used to determine the relationship between the amount of surface residues of leaves and *N. fallacis* mortality. Probit analysis was performed to calculate the time in hours at which 50% of the mites were killed (LT₅₀), slope of the log time-probit line (± SE), the confidence interval and the fiducial limits.
Results

In comparing the relative toxicity of six compounds to *N. fallacis*, differences were seen within each of the four field-aged residual experiments (Table 4.1). In the 1 day field-aged bioassay experiment, carbaryl and spinetoram showed significantly higher levels of mortality to *N. fallacis* adults after 96-h of exposure, whereas acetamiprid, novaluron, esfenvalerate and chlorantraniliprole were not significantly different from the untreated control (*F*=20.06, df=6, *P*=<.0001) (Table 4.1). In the 7 day field-aged residue bioassay experiment, only carbaryl caused significantly higher mortality to *N. fallacis* than the UTC, however spinetoram, acetamiprid and chlorantraniliprole were not statistically different from carbaryl (*F*=5.56, df=6, *P*=.0007). In the 14 day field-aged bioassay, carbaryl caused significant mortality to *N. fallacis* as compared to all other treatments (*F*=4.63, df=6, *P*=0.0022), whereas acetamiprid, esfenvalerate and novaluron were not statistically different from the carbaryl treatment.

Table 4.1  Mean number (± SE) of *Neoseiulus fallacis* adult at five post application dates after 96-h exposure to insecticides.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>8.0 (0.5) a</td>
<td>9.6 (0.2) a</td>
<td>8.8 (0.5) a</td>
<td>9.0 (0.4) a</td>
</tr>
<tr>
<td>Esfenvalerate</td>
<td>6.4 (1.0) a</td>
<td>8.4 (0.5) a</td>
<td>7.8 (0.7) ab</td>
<td>*</td>
</tr>
<tr>
<td>Spinetoram</td>
<td>2.8 (1.2) b</td>
<td>4.6 (1.0) ab</td>
<td>8.2 (0.2) a</td>
<td>7.0 (0.5) ab</td>
</tr>
<tr>
<td>Novaluron</td>
<td>6.4 (0.9)a</td>
<td>7.4 (1.1) a</td>
<td>7.8 (0.2) ab</td>
<td>*</td>
</tr>
<tr>
<td>Chlorantranilprole</td>
<td>6.2 (1.1) a</td>
<td>6.2 (1.1) ab</td>
<td>9.2 (0.2) a</td>
<td>*</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>8.6 (0.6) a</td>
<td>4.6 (0.7) ab</td>
<td>7.4 (0.8) ab</td>
<td>7.2 (0.6) ab</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>0.2 (0.2) c</td>
<td>2.6 (1.0) b</td>
<td>5.4 (0.9) b</td>
<td>6.4 (0.2) b</td>
</tr>
</tbody>
</table>

* asterisk, assays were not conducted for the specific insecticide on that sampling date.  
* Observed data were log transformed [log( X + 1)] before ANOVA. Untransformed mean showed for comparison.  
* Treatment with different letters are significantly different at α=0.05 (Tukey HSD). Mean separation calculated using Bonferroni (Dunn).
For day 21, *N. fallacis* exposed to carbaryl showed significantly higher mortality as compared to other treatments \((F=4.49, \text{df}=6, P=0.018)\), however spinetoram and acetamiprid were not statistically significant from carbaryl.

Focusing on residual toxicity patterns of carbaryl, using a student t-test analysis, there was significantly higher mortality of *N. fallacis* in the 1 day, 7 day, 14 day and 21 day field-aged bioassays as compared to the untreated control \((t=-13.65, \text{df}=8, P=<.0001; t=-3.76, \text{df}=4.03, P=0.0195; t=-2.97, \text{df}=8, P=0.018; t=-5.39, \text{df}=8, P=0.0007\), respectively) (Figure 4.1 A). The probit analysis estimation for Lethal Time (LT\(_{50}\)) value indicated that lethal time for *N. fallacis* was shortest when exposed to 1 day field-ages residues, followed by 7 day, and slower rates of mortality after exposure to 14 day and 21 day field-aged residues (Table 4.2). Also, the slope log time–probit line for day 21 (95% CI 2.18 - 7.43, \(P= 0.0003\)) was relatively steeper than the other treatment days (Table 4.2).

For spinetoram, there were a significance difference in *N. fallacis* mortality for 1 day, 7 day and 21 day field-aged residues as compared to the untreated control \((t=-3, \text{df}=4, P=0.03; t=-2.98, \text{df}=4, P=0.04; t=-2.65, \text{df}=8, P=0.03\), respectively) (Figure 4.1 B). Probit analysis estimation indicated that spinetoram showed a moderate rate of toxicity in terms of calculated LT\(_{50}\) value with 66.92 LT hours (95% FL 54 – 90) for 1 day field-aged residue bioassay, 99.65 LT hours (95% FL 66 – 447) on 7 day bioassay, and 161.3 LT hours (95% FL 106 -677) on day 21 (Table 4.2). Slope log time-probit line of day 21 (95% CI 1.4 – 5.6, \(P= 0.0011\)) was steeper than the other treatment days (Table 4.2).

For esfenvalerate, there were no significance differences between treatment days and the corresponding untreated controls (Figure 4.1 C). In probit analysis, esfenvalerate showed low toxicity to *N. fallacis*, with calculated lethal times ranging from 138.16 hours (1 day; 95% FL
Figure 4.1 (A-F) Mean (±SEM) number of live predator mites, *Neoseiulus Fallacis*, observed after 96 h within four post application intervals (1, 7, 14, 21 d). Bar with * above them designate significance difference ($\alpha < 0.05$) from untreated control at the same post-treatment day.
100 – 333) to 432 h (7 day; 95% FL 155 – 3.27258E72), but all essentially representing little to no toxicity. However 14 day exposure showed a much steeper slope than 1 day and 7 day (95% CI 1.8 – 7.9, P= 0.0017) (Table 4.2).

For acetamiprid, the level of *N. fallacis* mortality was not significantly different across field aged residue days and controls except for 7 day (*t*=-4.47, df=4.1954, *P*=0.0099) (Figure 4.1 D). The calculated LT<sub>50</sub> also indicated that day 7 had the shortest lethal time with 78.45 hours (95% FL 56 – 181) as compared to the other days (Table 4.2).

For chlorantraniliprole, only 7 day field-aged residue bioassays had a significantly different level of mortality than the untreated control (*t*=-3.04, df=4.228, *P*=0.0358) (Figure 4.1 E). However, data from probit analysis showed that 1 day field aged residue had shorter lethal time compared to 7 day and 14 day residues. Steeper slope log time - probit was observed on 14 day (95% CI (-1) - 7) than on day 1 and day 7 (Table 4.2).

For novaluron, none of the mortality rates of *N. fallacis* were different from untreated controls (Figure 4.1 F). The calculated LT<sub>50</sub> indicated that novaluron had the lowest lethal time effect on *N. fallacis* among the compounds tested, with 181.39 hours (95% FL 98 - 14590) on 1 day with increasing LT<sub>50</sub> value on 7 day and 14 day. Slope log time-probit was steeper on 7 day (95% CI 1 – 6) than any other field-aged residue day (Table 4.2).

**Residue profile**

Initial, day 1, foliar residue recovery levels were highest for esfenvalerate, carbaryl and novaluron and lowest for chlorantraniliprole, acetamiprid and spinetoram (Figure 4.2). Esfenvalerate had the highest proportion of foliar residue recovery levels on the apple leaf surfaces 1 day after field application, with a fairly linear decline over the next 14 days. Carbaryl
Table 4.2  LT$_{50}$ values for *N. fallacis* mortality after residual spray exposure to a given treatment insecticide.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>day</th>
<th>Slope (+SE)</th>
<th>LT50</th>
<th>95% CI</th>
<th>x2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaryl</td>
<td>1</td>
<td>4.8089 (+ 1.3397)</td>
<td>16.12494</td>
<td>(6.62939, 21.99067)</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>3.3048 (+0.7484)</td>
<td>49.04972</td>
<td>(37.63665, 64.41407)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1.8872 (+0.7447)</td>
<td>126.214</td>
<td>(75.68369, 2833)</td>
<td>0.0113</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>5.2417 (+ 1.3474)</td>
<td>128.0648</td>
<td>(98.83762, 242.51382)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Spinetoram</td>
<td>1</td>
<td>-3.9162 (+</td>
<td>66.91621</td>
<td>(54.08024, 89.99798)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.1296 (+ 0.7375)</td>
<td>99.6488</td>
<td>(66.34737, 446.88339)</td>
<td>0.0039</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2.2148 (+ 1.1732)</td>
<td>492.3343</td>
<td>*</td>
<td>0.0591</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>3.5102 (+1.0717)</td>
<td>161.3021</td>
<td>(106.27007, 676.917)</td>
<td>0.0011</td>
</tr>
<tr>
<td>Esfenvalerate</td>
<td>1</td>
<td>4.2544 (+ 1.156)</td>
<td>138.1586</td>
<td>(100.2389, 332.89062)</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.5416 (+ 1.2814)</td>
<td>432.8419</td>
<td>(155.1865, 3.27258E+72)</td>
<td>0.0473</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>4.8485 (+ 1.5444)</td>
<td>167.5155</td>
<td>(115.11758, 686.54187)</td>
<td>0.0017</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>1</td>
<td>2.6644 (+ 1.4524)</td>
<td>500.5379</td>
<td>*</td>
<td>0.0666</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.3374 (+ 0.7269)</td>
<td>78.44589</td>
<td>(55.86589, 180.55314)</td>
<td>0.0013</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>4.1184 (+ 1.3341)</td>
<td>179.189</td>
<td>(116.47093, 976.45051)</td>
<td>0.002</td>
</tr>
<tr>
<td>Chlorantraniliprole</td>
<td>1</td>
<td>2.2942 (+ 0.8369)</td>
<td>165.6978</td>
<td>(96.26248, 2713)</td>
<td>0.0061</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1.8996 (+0.7822)</td>
<td>166.6586</td>
<td>(90.5977, 20630)</td>
<td>0.0152</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>2.8866 (+ 1.993)</td>
<td>721.9359</td>
<td>*</td>
<td>0.1475</td>
</tr>
<tr>
<td>Novaluron</td>
<td>1</td>
<td>2.0435 (+ 0.8166)</td>
<td>181.3898</td>
<td>(97.76102, 14590)</td>
<td>0.0123</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>3.6221 (+1.2016)</td>
<td>188.0581</td>
<td>(116.804, 1371)</td>
<td>0.0026</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.9361 (+ 0.89)</td>
<td>2336</td>
<td>*</td>
<td>0.2929</td>
</tr>
</tbody>
</table>

* asterisk, fiducial limits could not be calculated
had high foliar surface residues 1 day after field application, however the level decreased rapidly from 280 ppm to 2.324 ppm by 7 days and remained low for the 14 and 21d field aged residue experiments. Novaluron residues were relatively uniform over the three sampling periods (1, 7, 14 d), averaging 25.2% of total residue recovery on 1 day with an increment of 8.4% to 33.33% on 7 and 14 day. Surface residue of spinetoram were highest at 1 day, then declined across three field-aged sample periods, with the lowest residue level detected at 0.617 ppm on 14 day. Chlorantraniliprole had a persistent residue profile on the leaf surface although it was recovered at a very low rate (below 2 ppm). Acetamiprid recoveries were highest 1 day after field application, declining about 50% by day 7 and declining gradually after 14 and 21 days of field-aging.

Figure 4.2  Mean residue recovery (SE) from leaf residue samples at four sampling interval. Carbaryl, esfenvalerate and novaluron were represented on y-axis while chlorantraniliprole, acetamiprid and spinetoram were represented on y-axis.
Discussion

Our study provides important new information on the temporal patterns of insecticide toxicity to *N. fallacis* and its relation to surface residue levels on foliage. Carbaryl insecticide was the most toxic to *N. fallacis* with high mortality of *N. fallacis* observed after exposure to dry residues. The high lethality of carbaryl compared to other insecticides was also evident in that LT$_{50}$ values were the lowest needed to cause *N. fallacis* mortality. Meyerdirk et al. (1982) reported highly residual toxicity of carbaryl up to 30 days post treatment against the natural enemies of the citrus mealy bug, *Planococcus cirri* (Risso). The high toxicity of carbaryl to *N. fallacis* is not surprising given the high leaf residue levels detected 1d after treatment. Residues declined substantially beginning 7 days after application, but the toxicity effect on *N. fallacis* remained high. This suggests that bioavailability of carbaryl residues on apple leaves remains high for long periods, even as environmental degradation occurs, and is likely responsible for the high incidence of mite flaring reported herein and elsewhere.

The spinosyn insecticide, spinetoram, caused overall high to moderate toxicity to *N. fallacis*. The highest mortality was observed within 96 hour of exposure to dried residue but decreased seven day after the treatment, along with residue on the leaf surface. The evidence of moderate residual toxicity as indicated by LT$_{50}$ values showed that longer exposure was needed to kill *N. fallacis* as compared to carbaryl. Lefebvre et al. (2011) reported similar finding for the western predatory mite *Galendromus occidentalis* (Nesbitt), with 100% mortality of females after 72 h of exposure to spinosyn dried residues on leaves.

As a member of spinosyn class, spinetoram is a highly active compound when ingested and causes rapid death when applied to various insect pests (Biondi et al. 2012). Phytoseiids
mites are known for efficient self-grooming behavior (Wekesa et al. 2007). Therefore, in our study, exposure of *N. fallacis* to dried spinetoram residues likely resulted in ingestion of active ingredient via grooming behaviour. Olszak and Sekrecka (2008) also demonstrated that high mortality of the phytoseiid mite *Typhlodromus pyri* Scheuten were observed after spinosad was applied to apple orchards. On the other hand, Jones *et al.* (2005) found that no acute toxicity to predatory mite *Amblyseius cucumeris* (Oudemans) when exposed 48-h to spinosad residues on cucumber leaves.

Chlorantraniliprole, a diamide insecticide, was overall less toxic than carbaryl or spinetoram to the predator mite *N. fallacis*. Our study showed there was no observed reduction in *N. fallacis* mortality except in one case. From the LT$_{50}$ value chlorantraniliprole takes substantially longer time for mortality. Although chlorantraniliprole has extended residual activity, it was largely non-lethal to *N. fallacis*.

Acetamiprid showed generally low toxicity to *N. fallacis*, with observed residual toxicity similar to that of chlorantraniliprole. Exposure to dried acetamiprid residue caused minimal toxicity as evident by large LT$_{50}$ values. However in the case of minimal toxicity, the time it takes to kill 50% of the *N. fallacis* adult was half time shorter than that of chlorantraniliprole. Ako *et al.* (2004) reported that application of imidacloprid, a neonicotinoid insecticide led to population buildup of two-spotted spider mite, *Tetranychus urticae* Koch, in the field. Although this compound has received reports of causing mite flaring, our study suggests that this is not associated with direct toxicity to *N. fallacis*.

Acetamiprid has excellent efficacy through systemic and translaminar activity (Horowitz *et al.* 1998) and is mainly acropetally transported in the xylem (Stein-Dönecke *et al.* 1992 and Westwood *et al.* 1998). Our study demonstrated low surface residue values detected and this was
likely due to the high systemic activity of acetamiprid. Systemic activity is known to increase over the period of time thus more residues were likely present inside the plant tissue. A study by Pozzebon et al. (2010) shown that *T. urticae* Koch had increased exposure to neonicotinoid when leaf cells were punctured by cheliceral stylets.

The pyrethroid esfenvalerate has no acute toxicity to the predator mite *N. fallacis*. This compound showed little to no direct toxicity effects when *N. fallacis* were exposed to dry leaf residues, although high proportion of residue were detected throughout the 14 days of study. This suggests that surface residues have high affinity to the plant cuticle and thus are less bioavailable to the predator. The time taken to kill 50% of the *N. fallacis* population (LT₅₀) were 20 fold as that of carbaryl suggesting exposure to dried exposure was harmless to *N. fallacis*.

Novaluron, an IGR insecticide, was non toxic to *N. fallacis* and we did not find significant effect of exposure to dried residue on the mortality of *N. fallacis*, even as persistence of novaluron residues appear to be substantial in duration. However our study suggested that novaluron likely provides longer residual control.

Carbaryl and spinetoram are a broad spectrum insecticides used against a wide range of orchard pests. From these result, carbaryl and spinetoram caused significant mortality to *N. fallacis* fostering the outbreak of spider mites such as ERM. Carbaryl may not be compatible with a phytoseiid mite, *N. fallacis* and it’s use in apple IPM should be done with caution and limitation. As a thinning agent on apple trees, repeat treatments of carbaryl should be reduced to avoid its disruptive effect on predatory mites. In addition, carbaryl should be applied at early bloom and late in the season to avoid direct exposure to *N. fallacis*, which is known to seek shelter in ground cover during winter and early spring. Spinetoram is a fast acting insecticide through its translaminar activity, however its short-lived residual might lessen long term negative
impact on predator mite *N. fallacis*. In this study, the bioassays only tested direct toxicity effects whereas insecticides found to be nontoxic, such as novaluron, acetamiprid and chlorantraniliprole could have effects on fecundity, reproduction or longevity. Further evaluation on the sublethal effects could be important to an integrated pest management program and help improve the survival rate of *N. fallacis* in the apple orchards.
CHAPTER 5

SUBLETHAL EFFECTS OF NOVALURON ON NEOSEIULUS FALLACIS’S ADULT FECUNDITY AND EGG VIABILITY

Abstract

Laboratory bioassays were conducted to determine sublethal effects of the insect growth regulators, novaluron, on Neoseiulus fallacis (Garman), a phytoseiid predator mite. Novaluron was applied at 2 mL solution with a Potter Spray Tower and bioassays were set up at seven oviposition periods to assess effects of direct spray and residual contact on the adult fecundity and egg viability. Novaluron showed no harmful effect on egg production whereas slightly reduced egg viability was observed from residual exposure. However, novaluron was harmless to N. fallacis as an overall effect. Evaluation on the sublethal effects of novaluron is important to determine the likely cause of mite flaring in apple orchards.
Introduction

The phytoseiid *Neoseiulus fallacis* (Garman) is a natural enemy residing on apple trees in temperate humid areas of North America (Ballard 1954). Adult *N. fallacis* overwinters in orchard ground cover and migrates to apple trees in May and June of the subsequent year. In orchards across the eastern US and Canada, *N. fallacis* is known to cause high mortality to *Panonychus ulmi*, the European red mite (ERM), with successful rates above 90% as a biological control agent (McMurtry and Croft 1997).

A generation of *N. fallacis* is completed between 4-5 days at 24-27°C. The degree-day requirement for *N. fallacis* dispersal on tree canopies is 600±100 DD54 °F. Adult females usually have three stages of instars which consist of six-legged larvae, eight-legged protonymphs and deutonymphs whereas no deutonymphal stage is observed in males (Ballard 1954). *N. fallacis* deposits eggs within two weeks in batches of 30-40. The period from egg deposition until emergence of adults is 7.3 and 3.3 days at 21.1°C and 32.2°C, respectively (Ahlstrom & Rock 1973, Ball 1980, Boyne & Hain 1983, Pratt et al. 1999). Ball 1980 reported that an average of 3.5 eggs/day were laid at 26.4 °C, with a total of 55 eggs laid/female. A greater number of eggs were produced in correlation to the high number of prey (pest mites) for e.g. with an abundance of spider mites, *N. fallacis* devoured 11 eggs/day. The life span of adult *N. fallacis* ranges between 20 to 30 days (Kain and Nyrop 1995).

*N. fallacis* has been utilized as a biological control agent on various crops to control spider mites. Biological control is a standard technique utilized under integrated pest management (IPM) in apple orchards, as an alternative to sole reliance on acaricides. In commercial apple production, pesticides are also utilized to help maintain ERM below economic
threshold levels. The implementation of the Food Quality Protection Act (FQPA) by United States Environmental Protection Agency (US EPA) in 1996 resulted in the elimination and restriction of many pesticides important for apple production. This led to the introduction of newer reduced-risk insecticides to reduce toxicity impacts on humans, mammals and other wildlife (US EPA 2006). However, growing evidence has revealed that these new insecticides maybe more harsh on natural enemies than previously expected (Wise and Whalon 2009, Agnello et al 2009). Although the new reduced-risk insecticides provide expanded modes of action for apple IPM (IRAC 2012), there are recent indications of negative effects on non-target organisms (Beers et al. 2014). Over time, insect control requires new alternative and more ecologically based insecticides, which provides opportunities for the recently developed of ‘biorational’ insecticides. These compounds are characterized by selective toxicity, different modes of action, non biochemical sites in vertebrates, lack of cross-resistance with broad spectrum insecticides and safety for natural enemies including predators, parasites and disease (Cutler and Scott-Dupree 2007). One of the two distinct groups of biorational insecticides contain products which consist of insect pheromones and insect growth regulators (IGRs). Pheromones are not lethal to insects, while IGRs cause negative effect on the developmental process of target insects and lead to the arrested growth in the specific insects (Ishaaya and Horowitz 1997).

Sublethal effects of IGR insecticides have been studied extensively for insect pest control (Medina et al. 2002, Sun et al 2003, Mommaerts 2006, Wise et al 2007, Gocke et al 2009, Kim et al. 2013). Chitin synthesis inhibitors (CSIs), for example, have been reported to contribute largely to the sublethal effects observed in pollinators, such as honeybees and bumblebees (Thompson and Hunt 1999) and other beneficial insect such as predatory lacewings (Medina et al
For most of the CSIs tested, a reduction in egg hatching was observed (Mommaerts et al. 2006). Diflubenzuron (DFB) penetrates the insect cuticle at a fast rate and subsequently inhibits chitin synthesis in insects that results in a disruption of the molting process (Ishaaya and Horowitz 1998). On the mechanism of chitin inhibition by benzoylephenyl ureas (BPUs), recent assay in Blatella germanica (L.) and Drosophila melanogaster Meigen showed that such compounds work through the sulfonylurea receptor during chitin biosynthesis, which confirmed the embryocidal activity of CSIs (Abo elghar et al. 2004). The other class of IGRs, ecdysone agonist has also been reported to decrease mean fecundity and fertility of important pest species from several different insect orders (Aller and Ramsay 1988, Sun and Barrett 1999). Tebufenozide, for example, is renowned for its larvicidal properties (Wing et al. 1988). Smagghe et al. (1996) reported that this ecdysone agonist caused the ovaries to degenerate, while Swevers and Iatrou (1999) showed that the developmental arrest on Bombyx mori ovarian follicles was due to the alteration of some gene expression during mid late vitellogenesis and choriogenesis, due to the ecdysone agonist effect. Juvenile hormone analogs, such as fenoxycarb, disturb molting at the pupae stage and may also have an ovicidal effect on isolated eggs of tortricids (Dorn et al. 1981).

Novaluron, a benzoyleurea CSI insecticide is widely used in apple orchards where N. fallacis is the principal predatory mite. This compound interferes with the molting process of insect, prevents chitin production resulting in unhatched eggs (Medina et al. 2002). A recent study by Gokce et al. (2009) showed significant sublethal activity of Novaluron by reducing codling moth egg viability subsequent to adult exposure. Novaluron also was reported to reduce plum curculio larval survival following mated female exposure to treated substrate (Wise et al. 2007). Similar results were recorded by Kostyukovsky and Trostanetsky (2006) with
novaluron on *Tribolium castaneum* (Herbst). A study by Ishaaya et al. (2001) found that phytoseiid mite populations in a sprayed cotton fields were not significantly affected after exposure to novaluron. Similarly, Cabrera et al. (2005) reported that novaluron had no mortality effect on the protonymphs and the developmental process of soil dwelling predatory mites *Stratiolaelaps scimitus* (Womersley) in the laboratory study.

This study examined the sublethal effects of novaluron on the fecundity and viability of *N. fallacis* eggs resulting from direct spray versus exposure to dry residues. The interaction between compound and mite reproduction was evaluated through effects on egg deposition and hatching.

**Methods and Materials**

**Prey**

Two-spotted spider mites, *Tetranychus urticae* Koch were used as prey for *N. fallacis*. Beans, *Phaseolus vulgaris* were planted in plastic pots (15 cm diameter) in a soil mixture. Plants were fertilized and watered as required. *T. urticae* were collected from a greenhouse on the campus of Michigan State University and placed on 10 to 14-d-old bean plants. Mites colonized the plants for 14-21 days.

**Predator Mites**

*N. fallacis* were purchased from a commercial biological supplies company, IPM Laboratories (Locke, NY). Adults were maintained in 3 × 3 cm plastic petri dishes, with 30 -50
mites per dish. Colonies were held in the laboratory at ambient room temperature and relative humidity (24 ± 2°C, 75 % RH) and photoperiod of 16:8 (L:D) h. Adults were more than 1 week old when tested.

**Novaluron**

Formulated insecticide, novaluron (Rimon. 83 EC 20 oz/100 gal equivalent) (Chemtura U.S.A. Corporation, Middlebury, CT), was used to evaluate the effects on adult’s oviposition and egg fecundity and viability. The labeled novaluron rate for codling moth control of 20 fl/oz per acre using 100 gal/water per acre was used. This dilution, using distilled water, was equal to a novaluron solution of 155.39 ppm.

**Spraying**

Adult *N. fallacis* were treated with insecticide using a Potter Spray Tower (Potter 1952)(Burkard, Ricksmanworth, UK). The sprayer was calibrated to deliver 20 psi with a 2.0 ml spray aliquot. The evenness of the spray deposit was checked with water sensitive paper prior to conducting the bioassays.

**Bioassays**

**Direct Spray Test**

Two non-sprayed apple leaf disks were placed in each of 7 petri dishes (3 cm diameter), and 10 – 15 prey mites, *T. urticae*, were placed on each disk. Predator mites, *N. fallacis* were sprayed topically with 2 mL of novaluron solution using the Potter Spray Tower. After 1 hour of drying at room temperature, five *N. fallacis* females and five males were placed on the apple leaf
disks containing prey mites in each of the 7 petri dishes. The petri dishes were placed in the incubator at 24 ± 2°C, 75% RH, and a photoperiod of 16:8 (L:D) h. The leaf disks were changed every three days for the total of 21 days to allow oviposition and observation of hatched and unhatched eggs. Treatments were replicated seven times, as described above.

**Residual contact**

Residual contact bioassays were carried out using unsprayed apple leaf disks and five females and five males adult predator mites, *N. fallacis*. A 2 mL solution of novaluron was sprayed through a Potter Spray Tower on apple leaf disks (approximately 1.5 cm diameter). The sprayed leaf disks (including water sprayed controls) were let to air dry for 1 h before five adult females and five males were placed on them. Adult *N. fallacis* were exposed to the treated leaves for 4 hours. After this period, 10 adult predatory mites (5 female and 5 male) were transferred to a clean petri dish that contained two leaf disks infested with 10 two-spotted spider mites (*T. urticae*) as a food source. The treatments were replicated seven times, with one petri dish equivalent to one replicate. Seven consecutive 3 day oviposition periods were set and after each interval, adults were transferred into new petri dishes with two leaf disks infested with two-spotted spider mites. The eggs laid on the leaf disks were counted and allowed to incubate for the total of seven days, the numbers of eggs hatched and unhatched were recorded.

**Statistical Analysis**

Data were evaluated using SAS Systems for Windows, version 9.3. Egg fecundity was determined from the total number of egg deposited for the seven consecutive 3 day oviposition periods. The egg viability was determined by dividing the number of hatched egg by the total
number of egg deposited for each cohort. A repeated measure ANOVA was utilized to perform analysis on egg fecundity and viability using PROC GLIMMIX in software SAS Systems for Windows version 9.3. (SAS Institute Inc. 2010). The variables fecundity, percentage eggs hatched were treated as a binomial (live/dead, hatched/unhatched), with the binomial distribution specified in the model statement. For repeated measures, the response variable was the mean proportion of the egg deposited/hatched. The class variable was the cohorts treated as repeated measures. Significant treatment or cohort or interactions were explored using LSMEAN statements.

**Results**

**Egg fecundity**

Novaluron topical- treated mites: In the number of eggs laid per cohort per mite, there was no significant effect of treatment applied as compared to control ($F= 0.44$, $df= 12$, $P=0.5215$) but there was a significant effect of cohort ($F=12.51$, $df= 72$, $P=0.0001$). There was not a significant interaction between these two main effects ($F=0.81$, $df= 72$, $P=0.5672$) (Figure 5.1). By slicing cohort main effect, there was a significant effect of cohort 7 as compared to control ($F=3.1372$, $df=72$, $P=0.0810$).

Novaluron residual -treated mites: In the number of eggs laid per cohort per mite, there was no significant effect of treatment applied as compared to the control ($F= 1.76$, $df= 12$, $P=0.2093$) but there was a significant effect of cohort ($F=3.10$, $df= 71$, $P=0.0094$). There was not
significant interaction between treatment x cohort interaction ($F=0.75$, df$=71$, $P=0.6105$) (Figure 5.2).

**Egg viability**

Novaluron topical- treated mites: In the proportion of eggs hatching, there was no significant difference of treatment from control ($F=0.06$, df$=12$, $P=0.8175$). There was a significant effect of cohort ($F=2.19$, df$=72$, $P=0.05$) but no significant interaction between these two main effects ($F=0.46$, df$=72$, $P=0.8360$) (Figure 5.3).

Novaluron residual-treated mites: In the proportion of eggs hatching, there was no significant effect of treatment applied as compared to the control ($F=0.91$, df$=12$, $P=0.3587$) and there was no significant effect of cohort ($F=0.93$, df$=72$, $P=0.4802$) or treatment x day interaction ($F=1.51$, df$=72$, $P=0.1860$). By slicing cohort main effect, there were significant effect of cohort 1 ($F=3.38$, df$=72$, $p=0.0700$) and cohort 7 ($F=3.18$, df$=72$, $P=0.0789$) (Figure 5.4).

![Figure 5.1](image-url)

**Figure 5.1.** Egg fecundity of *Neoseiulus fallacis* in novaluron topical-treated mites assay exposed 0.156 g Al L$^{-1}$ of novaluron.
Figure 5.2. Egg fecundity of *Neoseiulus fallacis* in novaluron residual- treated mites assay exposed for 4 hours to 0.156 g Al L$^{-1}$ of novaluron.

Figure 5.3. Egg viability of *Neoseiulus fallacis* in novaluron topical-treated mites assay exposed to 0.156 g Al L$^{-1}$ of novaluron.
Figure 5.4. Egg viability of *Neoseiulus fallacis* in novaluron residual-treated mites assay exposed for 4 hours to 0.156 g Al L$^{-1}$ of novaluron.

**Discussion**

This study provides new information of the sublethal activity of novaluron on the fecundity and egg viability of *N. fallacis*. Although the results of this study did not demonstrate dramatic and highly significant effects, there were cases of evidence that exposure to novaluron can trigger mild effects on the egg production and viability.

Most other sublethal studies testing novaluron show little to no effect on fecundity (Kim et al. 2011, Gocke et al. 2009, Alyokhin et al. 2007, Kostyukovsky & Trostanetsky 2004). Our present results are also consistent with those findings, which showed little effect, except the one case which reflects slightly depressed egg production by *N. fallacis* exposed to novaluron. Therefore we do not expect this to be a major contributor to any reported mite flaring.
Previous laboratory studies on insects like codling moth, colorado potato beetle and the red flour beetle suggested dramatic negative sublethal effects of novaluron on egg viability (Gocke et al. 2009, Alyokhin et al. 2007, Cutler et al. 2006, Kostyukovsky & Trostanetsky 2004), whereas our study demonstrated only limited effects. Although not highly significant, there were two cohort cases in the residual exposure that showed reduced egg viability. It is also important to note that if our exposure time was longer, then, greater effects on the egg viability may have been seen. This could be attributed to the activity of novaluron that requires longer time to penetrate and affects the reproduction of insects (Kostyukovsky & Trostanetsky 2004).

In laboratory experiments with the soil dwelling predatory mite Stratiolaelaps scimitus (Womersley)(Cabrera et al. 2005), like in our study, no effect was observed, even with 72 hour of exposure. Protonymphs were exposed to novaluron for 72 hours and monitored for their subsequent development. Although molt was slightly delayed, mortality of protonymphs and effect on subsequent developmental stages was similar to that among the controls (Cabrera et al. 2005).

In Kim et al. (2011), Gocke et al. (2009), and Cutler et al. (2006), sublethal effects were observed for topical, ingestion and residual exposure. In our study, it was established that only residual exposure showed effects on egg viability. When adults of N. fallacis were exposed to treated leaves, mite grooming behaviour was observed to induce entry of chemical via ingestion. Self-grooming behaviour among phytoseiids mites, for e.g. Phytoseiulus longipes was observed to be highly efficient (Wekesa et al. 2007). This possibly is an important factor that contributes to the observed reduced egg viability from residual exposure.

Although a limited case can be made from our study, it is unlikely that sublethal activity of novaluron will lead to dramatic mite flaring in apple orchards. Other mechanisms for how
novaluron may disrupt predator-prey balance in apple agroecosystems should continue to be explored.
CHAPTER 6

Conclusion

Over the past 30 years, apple growers generally have kept codling moth in check using organophosphate insecticides. However, due to recent restriction on use of organophosphates, more interest has developed towards incorporating new reduced-risk insecticides such as neonicotinoids, insect growth regulators, spinosyns and diamides, into their IPM programs. Use of pyrethroids have also been on the rise nation-wide in response to the presence of invasive species such as the Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* (Stål), and Spotted Wing Drosophila, *Drosophila suzukii*. Successful biological control programs in tree fruits depend on the conservation of *N. fallacis* and other mite predators to control European red mite (ERM) and two-spotted spider mite (TSSM). The reports of mite flaring by Michigan apple farmers in recent years prompted this research to identify the compounds or combinations of compounds responsible for the imbalances in their apple agroecosystems. Secondarily the intent was to determine the mechanisms contributing to the mite flaring events, so as to provide practical insights for correcting the problems. Because of the importance of spinosyn, diamide, neonicotinoid and IGR insecticides for apple IPM, as tools for controlling key insect pests and also as rotational options for resistance management, I have developed recommendations for each compound to avoid mite flaring.
Final Assessment and Recommendations for Each Insecticide Class

**Pyrethroids – Esfenvalerate**

The use of this compound caused strong mite flaring, and was observed to be highly toxic to predator mites in topical spray exposure. If this compound must be used in apple IPM, some tactics that would reduce the negative effect on predator mites should be utilized. The use esfenvalerate is generally not recommended in IPM as it can cause secondary pest outbreaks. However, recent heavy infestations and fruit injury in several states from Pennsylvania to Maryland by the invasive species BMSB, has forced apple growers to shift to older and broad spectrum insecticides such as pyrethroids. If apple growers must use pyrethroids to control BMSB, late season applications will minimize the degree of mite flaring since ERM will have already entered diapause and deposited overwintering eggs in the tree.

Although dry residues of esfenvalerate did not cause lethal effects on *N. fallacis*, the high surface residues observed in this study likely contributed to mite flaring via hormoligosis. A compatible adjuvant such as plant penetrants applied to tank mixes might enhance penetration of esfenvalerate into apple leaves, and thus reduce bioavailability to ERM on the surface.

**Carbamates – Carbaryl**

Carbaryl had the most overall negative effects on both mite flaring and predator mites. Its application in the field should be done with caution as it has the highest toxicity compared with other compounds. Carbaryl, however, is an important apple thinning agent and its use on apple trees improves the size of apple fruit and overall production. Other chemical apple thinning such as naphthaleneacetic acid (NAA), naphaleneacetamide (NAD) and benzyladenine (BA) are available, but carbaryl has the most advantages in term of affordable cost and overall
effectiveness. The application of carbaryl should be limited, therefore, to one application as much as possible, since toxic effects persist for long duration even as residues diminish.

**Benzoylureas – Novaluron**

The benzoylurea novaluron demonstrated few cases of mite flaring, generally only in combination with carbaryl. Previous reports have shown novaluron does have potent sublethal effects on insect eggs and larvae. Although few sublethal effects of novaluron to the predator mite *N. fallacis* were found in my study, this compound is known to be highly toxic to apple rust mites, *Aculus schlechtendali*. The apple rust mite is an alternate food to ERM and a major factor in maintaining *N. fallacis* populations on apple trees during lack of other prey. Thus high toxicity of novaluron to apple rust mites will deplete the food source, causing removal of *N. fallacis* from the tritrophic interaction between prey-plant-predator. Novaluron can be applied at an earlier timing such as petal fall to avoid negative effects on rust mites and minimize disruptive effects on *N. fallacis*.

**Spinosyn – Spinetoram**

The spinosyn spinetoram is an important tool for controlling codling moth in tree fruit, where it is being applied to control first and second generation of this major pest. It is also a key rotation material in resistance management therefore complete avoidance of this compound is unacceptable to apple farmers. Although exposure to predator mites caused reduced survivality, this compound has demonstrated short-lived residual thus has lesser long term impact on predator mites. Therefore timing applications of this compound for second generation of codling
moth is most suitable, since late season applications will minimize the degree of mite flaring and be least disruptive of the biological control from predator mite populations.

**Neonicotinoid – Acetamiprid**

The neonicotinoid acetamiprid was non toxic to the predator mite, *N. fallacis*, although it use still shows moderate cases of mite flaring. Although my study did not provide evidence of direct toxicity or negative effects through contact with dry residues, research suggest hormoligosis is the main factor associated to mite flaring, in which ERM was stimulated hormonally to produce more overwintering eggs and increase number of generations per season. This compound is highly systemic and high residue levels were recovered from inside the plant. This likely increases the exposure of tetranychid mites to neonicotinoids, as it is known that they punctured the top layer of leaf to extract the content of epidermal cells. Neonicotinoids are important tools for resistance management and thus complete avoidance of their use in apple IPM is not practical. Therefore timing applications of this compound for second generation codling moth is most suitable, since later season applications will minimize the degree of mite flaring due to ERM nearing diapause at that time.

**Diamide - Chlorantraniliprole**

The diamide chlorantraniliprole is the least impactful on predator mites with the fewest incidences of mite flaring in my study. This compound is important tool for control of first generation of codling moth, since it is least likely to cause disruption to apple agroecosystems.
Conserving Predators for Optimal Apple IPM

Predatory mites are an important component of biological control, thus conservation of predatory mites is crucial for successful implementation of apple IPM. Maintenance of predator mites in apple orchards is the most cost-effective means of sustaining low pest mite populations. Apple growers need to utilize best tactics to allow survival of predatory mites in apple agrosystems including the use of selective insecticides, insecticide rotation, use of cover crops or weeds and maintenance of alternate food supply, such as apple rust mites. The presence of sufficient apple rust mites in spring provides valuable food resources and maintains high number of predator mite populations on fruit trees. Movement of predator mites from the ground cover into the trees is important and this can be optimized through strategic pruning practices. Pruning of apple limbs at the lower portion of the tress should be minimized. This provides more area for migration of predator mites into the apple trees. A long term mite control and reduced development of ERM populations can be achieved through these conservation practices.
APPENDIX
APPENDIX 1

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2014-07


Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

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<th>Life Stage</th>
<th>Quantity</th>
<th>Preservation</th>
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BIBLIOGRAPHY


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