MANAGING PESTICIDE RESIDUE LEVELS OF MICHIGAN APPLES AND CHERRIES TO MEET GLOBAL MRLS

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Entomology—Doctor of Philosophy

2017

ABSTRACT

MANAGING PESTICIDE RESIDUE LEVELS OF
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Profitability in global food markets requires meeting high food quality sanitary and phytosanitary standards, often through the judicious use of crop protection materials, including pesticides. At the same time, many export market targets for US fruit crops set standards for maximum pesticide residue limits (MRLs) that are often lower than the domestic tolerances held by the USEPA. Meeting this challenge is especially difficult with the recent prevalence of late season invasive pests, like the Brown Marmorated Stink Bug (*Halyomorpha Halys*) (Stal) (BMSB) and Spotted Wing Drosophila (*Drosophila suzukii*) (SWD). Fruit growers need more data to determine which compounds hold the highest risks of rejection for export-bound crops. These data will also support establishment of “Export PHIs” guides that growers can use to avoid load rejections from export-target countries. Treatment regimens with minimum and maximum seasonal applications, addition of adjuvants, and the use of post-harvest water rinsing were tested for their effects on residue levels at harvest.

Dedicated to my wife Rachel and daughter Charlotte VanWoerkom

ACKNOWLEDGEMENTS

There are many people I would like to thank for the completion of my dissertation and Doctoral Degree. I would first like to thank my advisor, John Wise for the exciting opportunity and encouragement. I thank my committee members, Mark Whalon, Dan Kunkel, and Larry Gut for their advice and guidance. I would also like to thank Chris Vandervoort and Tom Garavaglia for their hard work analyzing the residue samples. Thank you to my lab mates Charlie and Jen Coslor for their field, lab, and statistical analysis assistance. Thanks to the staff of Trevor Nichols Research Center in Fennville Michigan for the great maintenance of the facility. A big thanks to my colleague Celeste Wheeler, Ignatius Andika, and the Mark Whalon lab for the residue sample collecting and processing assistance. Thanks to the USDA Specialty Crop Block Grant – Strategic Growth initiative, and the Michigan Apple Committee & Michigan Cherry Committee in which funding was provided for this research. A special thanks to my wife Rachel for her patience and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

DAT – days after treatment

df – degrees of freedom

FIFRA – federal insecticide fungicide and rodenticide act

FQPA – food quality protection act

MRL – maximum residue limit

OECD – organization of economic cooperation and development

PHI – pre harvest interval

PPB – parts per billion

PPM – parts per million

RCBD – randomized complete block design

CHAPTER 1: LITERATURE REVIEW

Introduction

The United States is a major apple (second worldwide) and cherry (fifth worldwide) producing country, with apples being the third most valuable fruit crop grown (Devadoss et al. 2009). According to the USDA National Agriculture Statistics Service (NASS), cherries are also valuable, especially to the state of Michigan with 75% of the nation's production (NASS 2015), (www.nass.usda.gov). Many states produce apples, but the states of Washington, Michigan, New York, and California dominate the market with 75% of the US production (Krissoff et al. 1997). Most US apple and cherry production relies upon pesticides as an important tool of Integrated Pest Management (IPM) programs to protect fruit from insects, disease, and weed pests.

While meeting market standards for blemish-free fruit depends on precise spray timings and a wide array of active ingredients, achieving this while maintaining chemical residues to meet standards for export markets is a challenge, especially concerning new invasive pests that are a late season problem such as the brown marmorated stink bug (*Halyomorpha Halys*) (Stal) (BMSB) on apple and the spotted wing drosophila (*Drosophila suzukii*) (SWD) on cherry. The Food Quality Protection Act of 1996 (Schierow 1999) and the Green Movement are also creating a difficult environment for maintaining sufficient insecticide options on the market for apple and cherry growers. Maximum residue limits (MRLs) are standards for the maximum level of pesticide residues allowed to remain on or in food and feed products at harvest (Christova-Bagdassarian et al. 2014). MRL is the term used in much of the world while the US

uses the term tolerance (Winter and Jara 2015). While pre-harvest intervals (PHIs) are set with residue studies, the US Environmental Protection Agency (EPA) performs a risk assessment to assure there is no unacceptable risk with residue levels that may occur at the requested PHI.

With the importance of late season insecticide-use, and the risk of residue levels exceeding global MRL standards, there is need to understand the factors that influence pesticide persistence and residues at harvest. Pesticide degradation is the breakdown of the active ingredient mainly due to abiotic environmental exposures such as ultra violet (UV) light, rainfall, oxidation, dilution, or biotic factors such as metabolism within the plant tissue (Van Eerd et al. 2003). Ultra violet degradation or photo degradation of insecticides is the breakdown of the pesticide from exposure to sunlight. Photo degradation can break down the active ingredient of the pesticide on the foliage, surface of the soil, and the air (Burrows et al. 2002). Pesticide loss from rainfall is when precipitation washes the pesticide residues off of the plant material after an application. Seasonal precipitation in Michigan for apples (April-September) is 500+ mm (20.45 in.) and cherry (April-July) is 380+ mm (15.06 in.) (www.enviroweather.msu.edu). The effect of rain on the fate of pesticides has important implications and varies widely between the classes of compounds and fruit crop (Wise et al. 2017, Hulbert et al. 2011, Hulbert et al. 2012).

Adjuvants are an ingredient in the pesticide's prescription or maybe added to spray tanks, which assists or modifies the action of the active ingredient (Foy 1987). The adjuvants enhance the pesticides solubility, adsorption, penetration, and translocation of the active ingredient to the target. They can also increase rain fastness,

and change the selectivity of the active ingredient (Krogh et al. 2003). Growers often benefit from insecticide delivery tools such as adjuvants to protect their crops from pests. The choice of the adjuvant in agrochemicals is crucial (Castro 2014). One reason why some say adjuvants effect pesticide residues is because of the change in permeability of the plant cuticle and increased penetration of the active ingredient (Ryckaert et al. 2007). It has been shown that degradation curves may be influenced by tank-mix adjuvants and side effects of adjuvants may cause higher residue and a decrease in degradation rate (Ryckaert et al. 2007). These results would likely increase the efficacy, but also increase the risk of MRL exceedance.

Pesticide residues will remain after an agricultural treatment and may penetrate plant tissues (Christia et al. 2015). When pesticides penetrate the fruit tissue there may be a greater chance of pesticide exposure when the ability to wash any residues off of the surface are gone. Pesticides have varying penetration attributes in plant tissues, depending on class of compound and crop type (Bostanian et al 2012, Wise et al. 2009). Pesticide penetration has been detected on many crops including apples and cherries (Balinova et al. 2006, Wise et al. 2006, Wise et al. 2009, Hoffman et al. 2009). Research has also shown that pesticides are evident not only in the external part of the fruit, but also in the fruit flesh (Christia et al 2015).

To meet the challenge of global MRL standards, growers need decision support tools to indicate which insecticides and adjuvants to use, at what rate to use them, and when to reduce use to lower the risk of MRL violations in the domestic and international markets. Moreover, decision tools developed for growers should be easy to use, very accessible, and safe for consumers. Disparity index is a term developed to measure

MRL differences, which is the US MRL divided by the lowest foreign MRL, which reveals the differences in all MRLs, whether high or low, in domestic or international markets that test for MRLs on food products (JC Wise, unpublished). This calculated value provides a simple way to identify which compounds exhibit the highest and lowest risk for growers targeting national and global export markets.

Importance of Global MRLs to Specialty Crop Industries

Consumers and retailers around the world expect fresh vegetables and fruits all year long. Moreover, the increasing global demand that farmers, packers, and processors all meet this worldwide global maximum residue limit (MRL) demand. International trade allows producers to utilize various advantages of different growing conditions across the globe, therefore, diversification of food supply, and global stabilization of year-round supplies of fresh fruits and vegetables (Ambrus 2016). Therefore, assures consistent markets and better returns. Fruit crops have drawn increased attention and residue globally in monitoring programs around the world since more fruits are consumed raw, further, fruits have become a year-round source of food. Therefore, today fresh and processed fruits are a major part of the human diet, and because of this massive production, storage, and transport; it is expected that some fruit may contain higher pesticide residues compared to other food groups of plant origin (Lozowicka 2013).

To assure fruit quality and protected human health domestically, export barriers and standards have resulted in increased demands for global harmonization of MRLs. Global MRL harmonization is the outcome of international political agreement to control

pesticide residue limits, thereby limiting trade interruption. The earth's increasing global population has led to expanding markets, which pressure markets to expand, increasing costs to produce, processing, and shipping quality foods. Governments, pesticide registrants, and international organizations like CODEX, together with farmers and government programs such as the USA's Interregional Project Four (IR-4) are critical contributors to the overall goal of global MRL harmonization.

Residue data from Good Laboratory Practice (GLP) programs which supervise residue studies from pesticide registrants together with effective programs such as IR-4 (http://ir4.rutgers.edu/), are today's basis for setting MRLs for pesticide residue limits and safety in food systems (MacLachlan, Hamilton 2010).

Good Laboratory Practice standards are a quality system that intends to ensure: through daily, documentation, the quality, trustworthy, integrity, and continuous safety data (Jiang 2005) for regulatory authorities.

Harmonization, a term that describes global cooperation and agreed-upon standards, generates efficiencies in international trade since producers have to focus on one set of regulations instead of many independent country by country standards which slow down trade, transport, and market opportunities (Engler et al. 2012). In summary, MRLs are needed to regulate pesticide use and safe foods and these systems have been adopted as global standards for food in world-wide trade (MacLachlan, Hamilton 2010).

Typically, MRLs are measured internationally in milligram(s) of pesticide active ingredient per kilogram(s) of harvested plant material. Milligrams are also known as parts per million (ppm) and/ or micrograms per gram. The purpose and/ or function for

MRLs is to informally regulate the pesticide residues domestically and internationally whether on food or feed products, in order to enforce proper use of pesticides domestically, and to set a reliable standard for trade.

Therefore, MRLs improve trade (Drogue, DeMaria 2012), preserves the public health (Nasreddine, Parent-Massin 2002), and are set according to Good Agricultural Practices (GAP) (MacLachlan, Hamilton 2010).

MRLs were originally recommended for international trade in 1966 by the Joint FAO/ WHO Meeting on Pesticide Residues (JMPR) (MacLachlan, Hamilton 2010). The establishment of MRLs follows the regulatory step of “public health risk assessment” (MacLachlan, Hamilton 2010). Good Agricultural Practices (GAP), to use according to the label is a standard that government agencies, like the US EPA, use to enforce the code of conduct addressing human health, safety, working conditions, and environmental management on US farmland (Amekawa 2009).

Pesticide Degradation and MRL Calculation

In order to set an MRL, various application rates and treatment timings are needed to determine pesticide breakdown and degradation estimations for the GAP. Determination of degradation curves can and do prevent accidental MRL exceedance (Ryckaert et al. 2007). There is generally a trend of linear or exponential degradation of most pesticides, which often makes pesticide residue levels at a known PHI (pre-harvest interval) relatively predictable. The more robust residue data sets are, the more accurate residue predictions may become.

Programs such as IR-4, which is a USDA and land grant University funded program that provides pest management solutions for specialty crop growers. ensures specialty crop growers that they receive registered uses for crop protection chemicals. IR-4 collects very large pesticide datasets for many crops in different environments (locations) over many years in order to arrive at reliable and accurate data. They are a large collection of small data sets (typically available for estimating MRLs presence) to obtain consistent and repetitive values (MacLachlan, Hamilton 2010). To obtain consistent values, large datasets and accurate calculation methods are required. The values may become more consistent, yet there are still many sources of variability, which makes the setting of an MRL difficult. Even residues from trials conducted with the same PHI are inherently variable under similar conditions (MacLachlan, Hamilton 2010).

Therefore, inconsistent values may cause overestimation of a residue which can lead to regulators not permitting a particular use. This may unnecessarily restrict producers to specific pesticides (MacLachlan, Hamilton 2010). Obviously, such inconsistency requires more than a simple calculation to set a reliable MRL.

Apple and cherry producers must also be aware of global MRLs standards if they intend to target export markets. Many of the export countries use their own pesticide residue calculation system(s) to obtain their MRLs and many use the OECD (Organization of Economic Cooperation and Development) MRL calculator or just MRL calculator (Handford et al. 2015). Such episodes can cause delays in the process for a global pesticide registration as many calculation methods differ. When countries use their own method, the outcomes often result in un-harmonized global MRLs, which are a significant

risk to specialty crop growers who desire access to export markets. Therefore, the calculator computes pesticide tolerances or MRLs, which the United States, Canada, and other countries use.

The guiding principles of the calculation procedure must be practical with sound statistics, simple to use without extensive statistical knowledge, and produce a clear and unambiguous MRL proposal for most datasets. These MRLs can harmonize the EU and NAFTA procedures as much as possible. The OECD calculator user-guide, with background information, can be found at (http://www.oecd.org, series on Pesticides, No 56, 2011). Even though there is a standard and effective calculator used by many, the OECD still states that to date there is no definitive analysis that would allow trials with widely varying application rates or PHIs to be combined (MacLachlan, Hamilton 2010). Obviously, global MRL calculation differences are just one of the many challenges of global MRL harmonization.

Influences and Challenges of Risk Assessment with MRL Establishments

The process of setting an MRL involves assessing the pesticide's risk to consumers (MacLachlan, Hamilton 2010). Risk assessment is a scientifically based procedure used for hazard identification, hazard characterization, intake assessment, and risk characterization (Renwick 2002). This is the basis on which World Trade Organizations make their agreements with the application of Sanitary and Phytosanitary Measures, which is an agreement with governments through the World Trade Organization (WTO) on animal, plant health, and food safety measures. Hazard identification is defining the potential adverse effects of the compound (Renwick 2002).

This can be done with bacterial and mammalian exposure experiments. There are three outcomes to pesticide hazard identification: Acceptable daily intake (ADI), Acute reference dose (ARfD), and Acceptable operator exposure level (AOEL). Intake assessment is the potential intake of a pesticide prior to approval in order to ensure that the exposure levels would not exceed the ADI, ARfD, or AOEL (Renwick 2002). Risk characterization is defines as the comparison of the potential pesticide intakes due to residues from the pesticide's use according to Good Agricultural Practices (GAP) with the ADI, or other health based exposure limits, such as the ARfD (Renwick 2002).

Therefore, when pesticide residues are recovered and analyzed from field studies, the potential intakes from these residues are calculated and compared with the ADI and ARfD. If the results of the field study do not produce pesticide residues higher than the ADI or ARfD, the pesticide could be approved based upon those conditions (Renwick 2002).

From the European Crop Protection Association and Crop Life America (http://croplife.org), the proposal for US-EU regulatory Cooperation states in March of 2014: "A harmonized risk assessment system for pesticide regulation is necessary to ensure the highest level of consumer and environmental protection, while promoting international trade, creating jobs, and enhancing social and economic viability of the EU and the US".

The EU is the largest importer of agricultural products, whereas the US is the largest exporter. Together, they are the two largest pesticide regulatory systems in the world. MRL harmonization should be a realistic future global goal, making trade simpler

worldwide once the US and EU can agree to harmonize their risk assessment procedures cooperatively.

Managing MRL Risks for Apples and Cherries

In the United States fenpropathrin, cyantraniliprole, phosmet, and spinetoram are among the most effective insecticides registered for use in apples and cherries. They are recommended for late season control of insect pests in Michigan (Wise et al, 2016). These compounds are commonly used because they have demonstrated efficacy against late season apple and cherry pests. On apple, these insecticides are registered as late season tools to control the damaging insect pests, such as the apple maggot, *Rhagoletis pomonella*, codling moth, *Cydia pomonella*, and BMSB. On cherries, these insecticides are registered as late season tools to control the direct insect pests, such as the cherry fruit fly, *Rhagoletis cingulata*, SWD, plum curculio (PC), *Conotrachelus nenuphar* (Herbst), and obliquebanded leafroller (OBLR), *Choristoneura rosaceana* (Harris).

Therefore, near-harvest pest control is challenging for apple and cherry growers because the ripening fruit is highly susceptible to injury from the above mentioned insect pests, while the late season sprays must be applied within the labeled PHIs.

The next topics will describe how the number of pesticide applications, water washing the fruit, and the addition of adjuvants to the tank mix may influence the overall pesticide residue level at harvest and have impact on global MRL harmonization.

Influences of Multiple Applications on Residues at Harvest

With fruit farmers facing dual pressures of detaining blemish-free fruit, while meeting MRL standards in foreign markets, critical research is needed to inform late season decisions on which pesticides to use, and how adjusting pesticide use-patterns may reduce the risks of load rejections. It has been shown that application techniques like uniform spray deposition of pesticides, crop varieties, crop architecture (tree and orchard structure), application or synchronizing timing, rainfall, and the growth stage of the crop can all play an important role in affecting pesticide residues (Poulsen 2012). There is also the potential for buildup of residues from multiple applications of the same compound (Haviland, Beers 2012). Sequential applications of the same pesticide may also increase residues at harvest depending on the compound's persistence and penetration attributes (Mota-Sanchez et al, 2012). As the number of applications increases and the later in the season the applications are made, the higher pesticide residue may be on the fruit at harvest (USDA 1931). Moreover, it is not clear, what role multiple applications of a single compound are compared to a single application. Growers need to know just how spray behaviors such as different applications frequency, affect final residue levels on tart cherries and apples at harvest.

The Influences of Fruit Washing on Pesticide Residues Post Harvest

Fruit washing is an important and costly post-harvest operation in both industries. Obviously, washing can influence the quality and safety of the treated fruit (Pao et al. 2012, Al-Taher 2013). Therefore, washing can improve the cleanliness and reduce chemical contaminants while maintaining profits and critical public health protection

(Pao et al. 2012). Therefore, Food and Drug Administration (FDA) guidelines exist for all produce to be washed prior to transporting from the field. Obviously, fruit washing can be used as a control point to reduce residues on the produce (Pao et al. 2012).

Fruit have been commercially washed for decades before they are consumed. Washing has been shown to improve appearance, make sorting and grading easier as well as maintaining quality while not injuring the fruit (USDA 1931). Therefore, washing the fruit should be left to a planned governmental program to ensure the fruit is cleaned properly, instead of leaving it up to each farmer (USDA 1931) to ensure consistent uniformity.

Apple processing and packing also includes additional washing processes for the purposes of cleaning, sorting and grading fruit quality. The apples are floated in water down to a sorting line conveyer belt to remove the damaged apples for discard or processing. Apples undergo a cooling process after they reach the packing facility, unlike cherries which are harvested into water tanks, some fruit are held under cold storage and some are treated with controlled atmosphere (CA) storage. Before marketing, apples are brought out of storage and sorted (into various quality levels) before sale (USDA 1931). The apples are then cleaned in water and disinfectants such as chlorine, then air-dried (USDA 1931) before being packed for transportation to the market.

Tart cherries are a soft, thin-skinned, delicate fruit susceptible to damage when handled, thus calling for additional post-harvest procedures. Tart cherries are kept cool through the entire processing and packing procedures. Tart cherries undergo a cooling or water floating/sorting process to prevent fruit cracking and maintain fruit quality,

which includes keeping the fruit firm for packing and shipping which reduces damage and discoloration during handling and transportation (Cargill 1969).

As soon as cherries are harvested, their quality begins to decline. Tart cherries have been shown to decrease their firmness after harvest by 35 percent (Timm and Guyer 1998). Tart cherry cooling processes slow the degradation and cracking processes. Tart cherries usually will initiate hydro-cooling processes on the farm where they were harvested (Pao et al. 2012). To prevent cracking, tart cherries are held in large water tanks for six to eight hours, while cold water 10-12ºC is continually being circulated through the tanks (Mitchel and Levin 1969). Running cherries though cold water quickly cool the cherries to maintain their quality, with circulating water, cherry bruising is minimized while en-route to the processing plant (Timm and Guyer 1998). The industry generally uses 250 gallon tanks which run water at around 8-10 gallons per minute for a short period of time (Mitchel and Levin 1969) to cool the cherries for transport. Then the flow rate slows to 2-3 gallons per minute while the cherries sit for about 2 hours (Mitchel and Levin 1969). The cherries are drained and ready for shipping.

Once they reach the packing facility, they are placed in a cold storage facility. When they are ready to be packed they are brought out of storage and floated down flumes of water so debris and leaves can be removed. The cherries then go through a cutter which removes the stems before they are sorted for pitting. After pitting, the cherries are packaged and go to market.

There is solid evidence that the standard water cooling processes in the cherry fruit industry can affect the amount of pesticide residue remaining on fruit as they enter

the market. It has been demonstrated that the industry's washing methods are sufficient to remove residues, as Al-Taher 2012 determined. The process includes rinsing for 30 seconds with cold tap water and this process reduced many pesticide residues including endosulfan, permethrin, diazinon, dichlorodiphenyldichloroethyhlene, methoxychlor, malathion, captan, iprodione, and chorothalonil. Not only is wash time efficiency demonstrated, but so is water temperature, which prevents cracking.

Washing tomatoes in 5 ºC water showed reductions similar to those obtained when washing in 10 ºC water, demonstrating a slight temperature variation would not affect efficiency of washing off residues. Another study shows that 13-34% can be reduced on Okra by washing and 67-76% reduction has been observed in recent studies demonstrating the consistency of such washing data (Samriti 2011).

In terms of the market, the reduction in residues below the US MRL has also been shown from washing Okra with water for either a double dose or a single dose of chlorpyrifos. Therefore, fruit washing is a worthwhile procedure for consumer's protection (Samitri 2011). Therefore, non-toxic washing treatments to reduce pesticides from fruit can facilitate the commercialization and reduce consumer health impacts (Pugliese et al. 2004). Lastly, a study has shown that simple tap water resulted in 12%, 14%, and 21% reduction of endosulfan, bifenthrin, and cypermethrin residues on cauliflower (Abdullah 2016). Therefore, there is specific classes of insecticides that have been shown to be effectively washed off. Organophosphorus compounds such as phosmet, which is considered a surface material, are also susceptible to washoff (Wise et al. 2017). Finally, it is now known that the removal of pesticides by washing is more influenced by the compound's penetrative attributes than by water solubility (Sung et al.

2011). Cherries and other crops rinsed with water decreases the amount of certain pesticide residues, but very little has been done to demonstrate that the industry specific cherry cooling process based on Cargill et al (1969) can play a role in pesticide residue mitigation.

Influence of Adjuvants on Residues at Harvest

Adjuvants date back to the eighteenth and nineteenth centuries when additives such as pitch, resins, flour, molasses, and sugar that were used to improve sticking, which in turn influences the biological performance of certain active ingredients by modifying the physical and chemical characteristic of the compounds (Castro 2014). Today, we know that adjuvants can act as stickers, spreaders, pH buffers, extenders, compatibility agents, plant penetrants, accelerators, drift controllers, crop oils, silicone-based-de-foaming agents, and thickeners.

Adjuvants that are known as accelerators can change the viscosity of wax on the cuticle and allow the pesticide to penetrate more rapidly. Adjuvants such as oils can increase the rate of penetration with changes in polarity. Therefore, differently designed experiments with more adjuvant oil/ polarity types can help determine where various risks lie. Understanding these details, would help determine whether to use lower doses of active ingredient with tank mixes, since the adjuvant may yield a prolonged outcome (Ryckaert et al. 2007).

There are two categories of adjuvants; spray adjuvants and formulation adjuvants. Formulation adjuvants are already part of the pesticide formulation while spray adjuvants are added to the spray tank before a pesticide application (Krogh et al.

2003). Spray adjuvants can also be called ‘tank mixing additives’ or ‘adjuvants’ while ‘formulation adjuvants’ are called additives or inerts (Krogh et al. 2003).

The environmental fate of the pesticide after application is thought to be independent of the type of adjuvant (Krogh et al. 2003). Higher propiconazole and diclofopmethyl residues have been found when a nonylphenol ethoxylate or a polymer was used (Ryckaert et al. 2007).

Higher herbicide residues have also been found with the addition of adjuvants in soil and roots of sugar beets (Kucharski and Sadowski 2006). These increased residues may be due to some adjuvants (surfactants) that cause a spray droplet to spread on the leaf, which will lower the mass of the active ingredient per unit area without any change in concentration until the spray solution evaporates (Castro 2014).

A specific type of surfactant called a “nonionic surfactant” are good dispersing agents, which have low toxicity to plants and animals while exhibiting stability in cold water (Yoon et al. 2011). While sticker adjuvants such as NuFilm 17 are film-forming-polymers which encapsulate the pesticide and protect the active ingredient from weather. This reaction increases the duration of the active ingredient of the pesticide or bio-pesticide by 50-100% (Rajkovic and Markovic 2012). There are limited adjuvant studies demonstrating their mechanism and effect on pesticide residues on apples and cherries at harvest, yet there are many studies showing their modes of activity. Yet, the major route or mode of activity that adjuvants bring is plant tissue penetration, which in turn, allows producers a wide variety and means to manipulate plant growth, maturity, uniformity, as well as other effects.

Influences of Insecticide Penetration on Residues at Harvest

Pesticides can penetrate the plant through the foliage, fruit, stems, bark, or roots, which then may support of entry through the stomata cells, lenticels in the cuticle, or the mesophyll cells (Bostanian et al. 2012). There are several factors that influence the various penetrations of insecticides into the leaf or fruit surface. The characteristics of the particular plant in which an insecticide was applied, such as permeability, and the properties of the insecticide are the initial factors to consider in understanding rates, delivery, and activity rate (Bukovac and Petracek 1993). Permeability of the plant leaf or fruit surface are influenced by many factors. Different plant species and plant ages have different amounts of waxy cuticle, different densities of surface hairs, and varying cuticle thickness, which all influence the penetration rate of the insecticide (Bukovac and Petracek 1993).

Fruit have a thicker cuticular membrane than leaves, but the fruit have higher permeability (Mota-Sanchez et al. 2012). Different cultivars can also vary in cuticular thickness. Apple varieties such as “Red Delicious and ‘Golden Delicious’ fruit also vary in cuticular thickness (Mota-Sanchez et al. 2012). Environmental factors can also increase and decrease the rate of penetration.

For example, if there is high temperature during or shortly after the application, the insecticide may penetrate faster into the fruit surface (Bukovac and Petracek 1993). The active ingredients chemical structures ability to move, as well the physical formulation of the compound influence movement as well. Different formulations may also have different degrees of solubility in water. The increased volumes of water in plants help break down certain pesticides into metabolites (Van Eer 2003).

Pesticide or active ingredient penetration depends on the stability of the compound in the lipoid-like layer or waxy layer on top of the cuticle, which signifies that the more waxy or oily the skin is, the greater pesticide penetration there will be making it harder to wash off (Sung et al. 2011).

The following examples are transportation mechanisms through foliar plant tissue. Insecticides can be translaminar, when the insecticide penetrates the leaf surface and enters the mesophyll cells, which then form a reservoir (Jansson and Dybas 1996, Bostanian et al. 2012). This allows for the active ingredient to avoid the environmental break-down factors, and remain more persistent for protection of the plant from foliar feeders (Wise et al. 2017).

Another type of insecticidal movement through the plant tissue is acropetal, which occurs when the insecticide penetrates the leaf surface and enters the xylem before moving from the central part of the leaf out to the marginal ends (McCann 1982, Bostanian et al. 2012).

A third type of movement is basipetal, which occurs when the insecticide penetrates the leaf surface and enters the phloem, then moves downward into the leaf tissue (McCann 1982, Bostanian et al. 2012). Insecticides which are non-polar tend to travel through the fat loving, lipophilic pathway such as wax. Ionic insecticides tend to take the polar path into the cuticle (Bostanian et al. 2012). The insecticide penetration into the fruit cuticle involves sorption into the lipids, diffusion across the membrane, and desorption into the epidermal cells of the fruit tissue (Mota-Sanchez et al. 2012, Bostanian et al. 2012). The partition coefficient of the insecticide, the surface concentration, and the physical characteristics of the fruit cuticle are all major factors

influencing the rate of penetration of the insecticide into the fruit surface (Mota-Sanchez et al. 2012, Bostanian et al. 2012).

Peeling fruit has been shown to decrease some pesticide residue. In other words, the bulk of the residues are removed with the peel of the fruit (Balinova et al. 2006). Results have also shown that pesticide contamination is evident not only in the external part of the fruit, as well as in the fruit flesh (Christia et al 2015). There has been extensive penetration observed in peach and pears where there was almost equal residues recovered in the peel and flesh. Apple residues were mainly located in the peel (Christia et al 2015). Another study found that there was 3.00 parts per million (ppm) of acetamiprid found in the interior of the cherry and 1.22 ppm found at the surface of the cherry (Hoffman et al. 2010). A similar study shows that 0.02 ppm of acetamiprid was found at the surface and 0.66 ppm was found at the subsurface (Hoffman et al. 2009).

Conclusion

The number of applications, water washing, and adjuvants have all shown to make an impact on pesticide residue. Chemical penetration into the plant tissue has shown to be the significant factor, or mode of transportation which affects the pesticide's residue level. Thus penetration abilities of both the insecticides and adjuvants play a major, and complex role in MRLs presence and activity, along with residue levels in apples and cherries at harvest.

The next few chapters will discuss the impacts of multiple pesticide applications, water washing, and adjuvant's contribution to insecticide residues at harvest for several key insecticides on apple and cherry. First, is comparison of single versus multiple applications on apple. Second, is comparison of single versus multiple applications as

well as water-washing versus no-water-washing on cherry. Lastly, the comparison of adding adjuvants to the tank mix versus no adjuvants on apple and cherry will be addressed.

CHAPTER 2: INFLUENCE OF SINGLE AND MULTIPLE INSECTICIDE APPLICATIONS ON RESIDUES AT HARVEST AND ASSOCIATED RISK FOR APPLE EXPORTS

Abstract

Residue decline profiling was used to determine the degradation curves of five key insecticides registered for apple. Single and multiple application treatment regimens with minimum and maximum seasonal applications were tested for their effects on residue levels at harvest. Fenpropathrin (Danitol 2.4 EC®), cyantraniliprole (Exirel 0.83 SE™), phosmet (Imidan 70 WP®), and spinetoram (Delegate 25 WG®). were foliar direct applied onto semi dwarf Red Delicious apples trees (*Malus domestica* Borkhausen) at the Michigan State University (MSU) Trevor Nichols Research Center (TNRC). The residue profiling suggests that fenpropathrin and spinetoram would be low risk for international export for most prospective markets. Cyantraniliprole was found to exceed the majority of international prospective markets for one season, making cyantraniliprole a high risk for international export at harvest for a single and multiple applications. Phosmet was found to exceed Taiwan's MRL at harvest for one season making phosmet a moderate risk for a majority of the international market. Potential mitigation strategies are discussed. Fruit growers need more data to determine which compounds hold the highest risks of rejection for export-bound crops. These data will support establishment of export pre-harvest intervals (PHIs) guides that growers can use to avoid rejections from targeted export countries with lower maximum residue limits (MRLs).

Introduction

The United States is a major apple producing country, with apples being the third most valuable fruit crop grown (Devadoss et al. 2009). Every state produces apples, but the states of Washington, Michigan, New York, and California dominate the market with 75% of the US production (Krissoff et al. 1997). The production of apples is important to the state of Michigan. According to the USDA National Agriculture Statistics Service (NASS), the state of Michigan is a national production leader of apples behind Washington and New York with 39,000 bearing acres (www.nass.usda.gov). Most US apple production relies upon pesticides as an important tool of Integrated Pest Management (IPM) programs to keep fruit clean from diseases and insect pests.

While meeting market standards for blemish-free fruit depends on precise spray timings and a wide array of active ingredients, achieving this while maintaining chemical residues at acceptable levels for export markets is a challenge, especially concerning new invasive pests such as the late season brown marmorated stink bug (*Halyomorpha Halys*) (Stal) (BMSB) on apple. The Food Quality Protection Act of 1996 (Schierow 1999) and the Green Movement (Lehman 1993) are also creating a challenging environment for maintaining a sufficient quantity of insecticide options on the market for apple growers. Maximum residue limits (MRLs) are the maximum level of pesticide residues allowed to remain on or in food and feed products (Christova-Bagdassarian, et al. 2014). MRL is the term used to set such standards in much of the world and the US uses the term pesticide tolerance (Winter and Jara 2015). While pre-harvest intervals (PHIs) are set with residue studies, the US Environmental Protection Agency (EPA)

performs the risk assessment to assure there is no unacceptable risk with the GAP, to assure residues at harvest do not exceed label tolerances.

Apple producers must also be aware of global standards for MRLs if they intend to target export markets. Many of the export countries use their own pesticide residue calculation system(s) to obtain their MRLs and many use the OECD (Organization of Economic Cooperation and Development) MRL calculator or just MRL calculator (Handford et al. 2015). Such episodes can cause delays in the process for a global pesticide registration as many calculation methods differ. This often results in un-harmonized global MRLs, which is a risk to specialty crop growers who desire access to export markets.

In the United States fenpropathrin, cyantraniliprole, phosmet, and spinetoram are among the most effective insecticides registered for use in apples, that are available for late season control of insect pests in Michigan (Wise et al, 2016). These compounds are commonly used, and have demonstrated efficacy as late season options to control direct insect pests, such as the apple maggot, *Rhagoletis pomonella*, codling moth, *Cydia pomonella*, and BMSB. Near-harvest pest control is particularly challenging for apple growers because the ripening fruit is highly susceptible to injury from insect pests, while the final sprays must be applied within the labeled PHIs.

Cyantraniliprole is a novel cross-spectrum anthranilic diamide insecticide which selectively activates the ryanodine receptors in the insect muscles (Ammar 2015) causing paralysis. Cyantraniliprole is systemic insecticide which is effective through ingestion and contact routes. This reduced risk insecticide is effective on a wide range of insects including lepidoptera, hemiptera, and diptera (Wise et al. 2016, Van Steenwyk

et al. 2008). The label rate on apple is 0.06-0.15 kg ai/ ha with a 3 day PHI, 12 hour REI (re-entry interval), and a minimum of 7 day application interval. The primary late season target pest for cyantraniliprole is apple maggot and BMSB.

Phosmet is a broad spectrum organophosphate insecticide which is a cholinesterase inhibitor causing nerves to continue sending signals. It is a conventional insecticide traditionally most relied upon for codling moth, *Cydia pomonella*, control in Michigan (Mota-Sanchez et al. 2008), but more recently used as late season control of BMSB. The label rate on apple is 1.67-4.51 kg ai/ ha with a 7 day PHI, and 7 day REI. Phosmet is most effective on lepidopteran such as fruitworms, leafrollers, codling moth, and Oriental fruit moth, but is also used for a range of coleopteran and dipteran insect pests (Wise et al. 2016, Mouzin and Reed 1979).

Fenpropathrin is a broad spectrum type II synthetic pyrethroid with insecticidal and acaricidal activity (Saryazdi et al. 2014), which is a voltage-gated sodium channel inhibitor which create more ways for the sodium ions to pass through the membrane and propagate the action potential. This keeps the sodium channels in the open position causing paralysis. The label rate on apple is 0.23-0.46 kg ai/ ha with a 3 day PHI, 24 hour REI, and a minimum of 10 day application interval. Fenpropathrin is most effective on leafhoppers, aphids, fruitworms, leafminers, mites, leafrollers (Wise et al. 2016, Walgenbach and Palmer 2003), and recently relied upon for late season control of leafrollers and BMSB.

Spinetoram is a second generation spinosyn insecticide which targets lepidopteran larvae and thrips, it also works broadly on diptera, coleoptera, hemiptera, hymenoptera, isoptera, orthoptera, siphonaptera, thysanoptera, mites (Sagato 1998,

Hogmire 2008), and more recently relied upon for late season control of BMSB. Spinosyns are a novel insecticide effective by contact and ingestion and target the binding site of the nicotinic acetylcholine receptor in the nervous system which causes hyper excitation of the nervous system and paralysis (Salgato 1998). The Label rate on apple is 0.08-0.12 kg ai/ ha with a 7 day PHI, 4 hour REI, and a minimum of 7 day application interval.

With the importance of late season insecticide-use, and the risk of residue levels exceeding global MRLs, there is need to understand the factors that influence pesticide persistence and residues at harvest. Pesticide degradation is the breakdown of the active ingredient mainly due to abiotic environmental exposures such as ultra violet (UV) light, rainfall, oxidation, dilution, or a biotic factor such as metabolism within the plant tissue (Van Eerd et al. 2003). Ultra violet degradation or photo degradation of insecticides is the active ingredient breakdown of the pesticide from exposure to sunlight. Photo degradation can break down the active ingredient of the pesticide on the foliage, surface of the soil, and the air (Burrows et al. 2002). Pesticide loss from rainfall is when precipitation washes the pesticide residue off of the plant material after an application. Rain has important implications for the fate of pesticides that are sprayed on apples (Wise et al. 2017). With seasonal (April-September) precipitation in Michigan approximating 500+ mm (20.45 in.) (www.enviroweather.msu.edu), growers often need additional applications to protect their crops from pests. Sequential applications of the same pesticide may also increase residues at harvest depending on the compound's persistence and penetrative attributes (Mota-Sanchez et al, 2012)

To address the challenge of meeting global MRL standards, growers need a decision support tool to indicate which insecticides to use, at what rate to use, and when to use them with a low risk of MRL violation for their specific market destinations. This decision tool should be easy to use and very accessible by the grower. Disparity index is a term developed to measure MRL differences, which is the US MRL divided by the lowest foreign MRL, which equals the greatest difference in the US MRL and lowest foreign MRL (JC Wise, unpublished). This calculated value provides a simple way to identify which compounds are the highest risk for growers targeting global export markets. It allows a single value for difference instead of multiple values trying to explain how the difference affects the market.

The objectives of this study is to determine for several key insecticides the residue concentrations on apple fruit at harvest resulting from a single application versus a multiple application treatment regime. The aim is to inform a support tool for growers to use for decisions on insecticide sprays targeting late season insect pests, including the new invasive BMSB, while avoiding export MRL risks.

Materials and Methods

Field Plots-2014 and 2015 Seasons

Field work was conducted at the MSU Trevor Nichols Research Center (TNRC) in Fennville, MI, USA (latitude 42.5951°: longitude -86.1561°). Plots were established in a 3.30 m tall, 27 year-old 'Red Delicious' (Indigo) apple (*Malus* Miller; Rosaceae) planting at TNRC with one row buffers. The plot size was three consecutive trees with a 6.10 m row width and 3.05 m tree spacing or total plot dimensions of 6.10 m wide and

9.14 m long, with a total area of 55.74 square m. Treatment plots were replicated three times and set up in a randomized complete block (RCB) design.

Applications-2014 Season

Single application treatments were made at maximum label rates prior to fruit harvest, on 16 September. Each of the four insecticides were selected from currently registered materials used late season in pome and stone fruits: cyantraniliprole (Exirel 0.83 SE, DuPont Crop Protection, Wilmington, DE) at 0.15 kg active ingredient (AI)/ ha (20.5 fl oz formulated product per acre), phosmet (Imidan 70 WP, Gowan Company, Yuma, AZ) at 2.35 kg active ingredient (AI)/ ha (3 lb formulated product per acre), and fenpropathrin (Danitol 2.4 EC, Valent U.S.A., Walnut Creek, CA) at 0.06 kg active ingredient (AI)/ ha (21.3 fl oz formulated product per acre). A water pH buffering agent was used with Imidan 70 WP. This pH buffer is aliphatic polycarboxylate (TriFol L®, Wilbur-Ellis Company, Fresno, CA) at 0.24 L per 378.54 L (0.5 pt/ 100 gal). Test materials were applied with an FMC 1029 airblast sprayer calibrated to deliver material for full coverage at 935 l/ ha (100 gallons per acre), 1.12 m per second (2.5 miles per hour), and a 26.50 L tank mix (7.0 gallons). Regular maintenance foliar applications were applied to all treatments and included the fungicides mancozeb (Manzate®), penthiopyrad (Fontelis®), oxytetracycline hydrochloride (Fireline™), difenoconazole (Inspire Super®), copper sulfate (Copper Sulfate), and captan (Captan®). The single insecticide applied in all plots was thiacloprid (Calypso®) for leafhopper control, Glyphosate (Gly Star Plus®), indaziflam (Alion®), carfentrazone (Aim®), and simazine (Princep®) were banded below the trees for weed control.

Applications-2015 Season

Treatment applications were made at maximum label rates prior to fruit harvest. Applications were made for each of five insecticides according to the maximum allowed on current label for pome fruits: cyantraniliprole (Exirel 0.83 SE) at 0.15 kg active ingredient (AI)/ ha (20.5 fl oz formulated product per acre), and phosmet (Imidan 70 WP) at 2.35 kg AI/ ha (3 lb formulated product per acre), and fenpropathrin (Danitol 2.4 EC) at 0.06 kg active ingredient (AI)/ ha (21.3 fl oz formulated product per acre), and spinetoram (Delegate 25 WG, DOW AgroSciences, Indianapolis, IN) at 0.12 kg active ingredient (AI)/ ha (7.0 oz formulated product per acre) (Table 1). A water pH buffering agent was used with Imidan 70 WP. This pH buffer is aliphatic polycarboxylate (TriFol L) at 0.24 liters per 378.54 liters (0.5 pt/ 100 gal) (Table 1). Applications were performed on alternate days to ensure all samples could be collected at the proper timing. Test materials were applied with an FMC 1029 airblast sprayer calibrated to deliver diluent at 935 liters/ ha (100 gallons per acre), 1.12 meters per second (2.5 miles per hour), and a 26.50 liter tank mix (7.0 gallons). Regular maintenance foliar applications were applied to all treatments and included the fungicides mancozeb (Manzate), difenoconazole (Inspire Super), mancozeb (Dithane), and triflumizole (Procure®). A single insecticide thiacloprid (Calypso), was applied to all plots for leafhopper control. Norflurazon (Solicam®), glyphosate (Round Up®), simazine (Princep), carfentrazone (Aim), and paraquat dichloride (Gramoxone®) were banded below the trees for weed control.

Table 1. 2015 apple application dates

Treatment/ Formulation	Application Dates
UTC	
Exirel 0.83 SE, 1 Appl.	28-Sep
Exirel 0.83 SE, 3 Appl.	14-Sep, 21-Sep, 28-Sep
Imidan 70 WP, 1 Appl. TriFol L	28-Sep
Imidan 70 WP, 3 Appl. TriFol L	14-Sep, 21-Sep, 28-Sep
Danitol 2.4 EC, 1 Appl.	28-Sep
Danitol 2.4 EC, 2 Appl.	18-Sep, 28-Sep
Delegate 25 WG, 1 Appl.	28-Sep
Delegate 25 WG, 3 Appl.	14-Sep, 21-Sep, 28-Sep

Sample Collection-2014 and 2015 Seasons

Residue samples were collected, prepared, and recovered using methods based on US EPA standards for GLP field residue studies (USEPA 40 CFR 160). These methods are also known as the QuEChERS method or “gold standard” for multiple pesticide residue analysis for a variety of different sample types (Kong et al. 2016). QuEChERS is an abbreviation for quick, easy, cheap, effective, rugged, and safe. Three labeled gallon Ziploc bags were used to collect 24 total fruit with 8 apples per bag for each replicate sample. The apples were selected randomly from the N, S, E, W cardinal direction sides of the tree, low/ middle/ high, and shielded/ exposed portions of the tree crown. Shielded was any fruit at least 60.96 cm (24 in) inside of the tree crown and exposed was the outer 60.96 cm 24 in. Low was the bottom 1.22 m (four ft), middle was the center 1.22 m (four feet), and high was the upper 1.22 m (four ft) of the tree crown. The 2014 season samples were collected on the specific day after treatment (DAT) or

day after last application and ± 1 day for the 3, 7, 14, and 21 DAT samples. Samples from all treatments were collected at 1 DAT (17 Sept), 3 DAT (19 Sept), 7 DAT (23 Sept), 14 DAT (30 Sept), and 21 DAT (7 Oct). The 2015 season sampling was extended to 28 days and the collection dates are listed in table 2.

Table 2. 2015 apple sample collection dates and timings

Treatment/ Formulation	Sample DAT Number					
	1	3	7	14	21	28
UTC	29-Sep	1-Oct	5-Oct	12-Oct	19-Oct	26-Oct
Exirel 0.83 SE, 1 Appl.	29-Sep	1-Oct	5-Oct	12-Oct	19-Oct	26-Oct
Exirel 0.83 SE, 3 Appl.	29-Sep	1-Oct	5-Oct	12-Oct	19-Oct	26-Oct
Imidan 70 WP, 1 Appl. TriFol L	29-Sep	1-Oct	5-Oct	12-Oct	19-Oct	26-Oct
Imidan 70 WP, 3 Appl. TriFol L	29-Sep	1-Oct	5-Oct	12-Oct	19-Oct	26-Oct
Danitol 2.4 EC, 1 Appl.	29-Sep	1-Oct	5-Oct	12-Oct	19-Oct	26-Oct
Danitol 2.4 EC, 2 Appl.	29-Sep	1-Oct	5-Oct	12-Oct	19-Oct	26-Oct
Delegate 25 WG, 1 Appl.	29-Sep	1-Oct	5-Oct	12-Oct	19-Oct	26-Oct
Delegate 25 WG, 3 Appl.	29-Sep	1-Oct	5-Oct	12-Oct	19-Oct	26-Oct

Sample Processing-2014 and 2015 Seasons

Once all the apples were picked for that specific DAT, they were brought back to the lab to be processed. Apples were taken out of their labeled bags, one repetition and treatment at a time to prevent cross contamination. Apples were each cut into quarters with a clean kitchen knife and cutting board while wearing Nitrile gloves. All equipment was sanitized with acetone and gloves changed between each repetition and treatment to prevent cross contamination. Once each apple was cut, opposite quarters were placed back into one of the three original sample bags representing the sample. The

remaining quarters were discarded. All of the final quarters representing the sample in the Ziploc® bag were weighed and equaled a minimum of 1.81 kg (4 lbs) and a minimum of 28 fruit. The apple samples were then put into a -20° C chest freezer (Kenmore®, Hoffman Estates, Ill.) and monitored to assure temperature ranges did not rise above -5 ° C for storage until homogenization procedures.

Homogenization Procedures-2014 and 2015 Seasons

Once all samples were collected for the harvest season, they were ground using a commercial Hobart® food processor (Hobart Corporation, Troy, OH) beginning with the latest sample date (21 and 28 DAT) and working towards earliest DAT. Six hundred g of dry ice were added to each sample to prevent softening of the fruit while processing. Each sample was ground for 5 min. Samples were taken with a clean sanitized spoon from all four quadrants of the homogenous ground sample to fill clean labeled sample 120 ml jars (Qorpak Bottle Beakers®, Berlin Packaging, Chicago IL). Sample jars were then placed back into the freezer to keep frozen until the next step. The food processor was dissembled and all parts and tools were sanitized with acetone to prevent cross contamination between each treatment. Twenty four to thirty six h later, the samples were taken out of the freezer and ten gram samples were removed and placed into clean labeled jars. Next, four grams of magnesium sulfate, one gram of sodium chloride, and 15 ml of HPLC grade dichloromethane were added to the new jars. The samples were placed into the refrigerator for two days to separate fruit tissue from compound. The samples were first shaken then decanted through 12 g of reagent-grade anhydrous sodium sulfate (EMD Chemicals, Inc.) to remove water for one hour.

The samples were then dried by evaporation under a chemical hood at ambient temperature and the remaining particles were brought back up with two ml of acetonitrile. The final two ml were transferred to a two ml vial (Agilent Technologies, Santa Clara, CA) for HPLC analysis.

Sample Extraction-2014 and 2015 Seasons

Levels of parent compound were quantified using a waters 2695 separator module High Profile Liquid Chromatography (HPLC) equipped with a Waters MicroMass ZQ mass spectrometer detector (Waters, Milford, MA), and a C_{18} reversed phase column (50 by 3.0mm bore, 3.5 µm particle size, (Waters, Milford, MA). The mobile phase, solvent A was with water and 0.1% formic acid. Solvent B was with acetonitrile with 0.1% formic acid (Table 3). Solvent A began at 80% and solvent B at 20% with a gradient and the column temperature of 20 degrees Celsius (Table 4). A standard for each insecticide was developed to compare the experimental concentrations. The standards of the insecticides were massed and diluted into solution with acetonitrile. The serial dilutions were made from the stock solution. The concentrations used were 7.57 g/ml, 0.155 g/ml, 0.0757 g/ml, 0.00155 g/ml, 0.000757 g/ml, and 0.0000155 g/ml.

Table 3. The mobile phase for each insecticide used for HPLC residue analysis 2014 and 2015.

Chemical	Solvent A	Solvent B	Flow Rate (ml/min)
Fenpropathrin	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.3
Cyantraniliprole	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.3
Phosmet	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.3
Spinetoram	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.3

Table 4. The gradient mobile phase flow used for each insecticide for HPLC residue analysis 2014 and 2015.

Active Ingredient	Time (min)	Solvent A (%)	Solvent B (%)
Fenpropathrin	0	80	20
	4	10	90
	4.5	10	90
	4.6	80	20
	10	80	20
Cyantraniliprole	0	80	20
	1	80	20
	4	20	80
	6	20	80
	6.1	80	20
	11	80	20
Phosmet	0	80	20
	1	80	20
	3	50	50
	3.1	20	80
	4	20	80
	4.1	80	20
	8	80	20
Spinetoram	0	80	20
	1	80	20
	3	50	50
	3.1	20	80
	4	20	80
	4.1	80	20
	8	80	20

Table 5. The ions (m/z) monitored, detector dwell time, and cone voltages for detection of the insecticides in HPLC residue analysis 2014 and 2015.

Chemical	Channel 1	Channel 2	Dwell (s)	Cone1 (V)	Cone2 (V)
Fenpropathrin	265	181	0.5	30	45
Cyantraniliprole	284	484	0.5	50	25
Phosmet	209	175	0.5	55	55
Spinetoram	872.2	886	0.5	55	55

The first step was to determine the range of concentrations, and highest concentration in range. The next step was to make desired concentrations with distilled acetonitrile based off a compounds molecular weight. Then the stock solution was used to make the next dilution, and this solution was used to make the next dilution, etc. Every other dilution was 100 fold diluted and the dilutions in between were 50 fold. The HPLC level of quantification was 0.08 µg/g parts per million (ppm) of active ingredient, and level of detection was 0.038 ppm.

The residue data for each compound were analyzed with mixed models using the MIXED procedure in SAS 9.4 (SAS Institute, Cary, NC, 2013). The fruit residues were analyzed with repeated measures best adjusted using an unstructured and a first-order heterogeneous autoregressive covariance structure. Repetition and treatment were used as subjects of repeated measurements. When the main effects or their interactions were statistically significant ($P < 0.05$), examination i.e. slicing of interactions within main effects was performed, *F*-tests (Acimovic et al. 2014) were conducted and pairwise or specific time or treatment comparisons were conducted using *t*-tests ($\alpha = 0.05$).

Results

Residue Profiles-2014 Single Application

Fenpropathrin was detected throughout the 21 day decline residue profile with a general decrease in concentration (Figure 1). Fenpropathrin was detected at 1 d after application with a maximum mean concentration of 0.30 ppm. At 3 d after application the concentration decreased to 0.28 ppm, decreased to 0.22 ppm at 7 days, decreased to 0.11 ppm at 14 days, and decreased to a minimum of 0.07 ppm for the final 21 day after application sample (Figure 1). Mean residue values following a single application were below the US MRL for all sample dates. Internationally, a single application and 14 day PHI would be low risk for export to most prospective countries, with some exceptions. Fenpropathrin concentrations fell below Canada, Mexico, China, Taiwan, and Vietnam's MRL and would be accepted for export to those countries for the single application with a 14 day PHI. Since there is not a CODEX MRL for fenpropathrin, there would be significant risk for export to Colombia, Guatemala, India, Israel, Jordan, Nicaragua, Philippines, and Singapore who do not have default MRLs, thus must assume no detectable residues.

Cyantraniliprole was detected throughout the 21 day decline residue profile with a general decrease in concentration and a large decrease in concentration from 14 to 21 days after application (Figure 1). Cyantraniliprole was detected at 1 day after application with a mean concentration of 0.06 ppm. From 3 days to 14 days after application the concentration remained fairly steady from 0.06 ppm to 0.08 ppm, then decreased to a minimum of 0.02 ppm for the final 21 day after application sample (Figure 1). Mean residue values following a single application were below the US MRL

for all sample dates. Internationally, cyantraniliprole with a single application and 3 day PHI would be low risk for export to most prospective countries. Cyantraniliprole concentrations also fell below the CODEX MRL of 0.8, thus being acceptable for, Saudi Arabia, India, Thailand, and Vietnam. There are many other prospective locations in which the trade would be compatible.

Phosmet was detected throughout the 21 day decline residue profile with a general decrease in concentration from 7 to 21 days after application (Figure 1). Phosmet was detected at 1 day after application with a mean concentration of 3.09 ppm. At 3 days after application the concentration decreased to 2.53 ppm, increased to 2.92 ppm at 7 days, decreased to 2.20 ppm at 14 days, and decreased to a minimum of 1.80 ppm for the final 21 day after application sample (Figure 1). Mean residue values following a single application were below the US MRL for all sample dates. Internationally, phosmet with a single application and 7 day PHI would be low risk for export to most prospective countries, but with several exceptions. Phosmet residues exceeded Taiwan's MRL at the 7 day PHI, making it unacceptable for export to Taiwan, while mean residues at 21 day PHI were very close to Taiwan's 2.0 ppm standard. Phosmet concentrations fell below China, Israel, Brazil, Thailand, and Vietnam's MRL and would be accepted for export to those countries for the single application with a 7 day PHI. There are many other prospective locations in which the trade would be compatible.

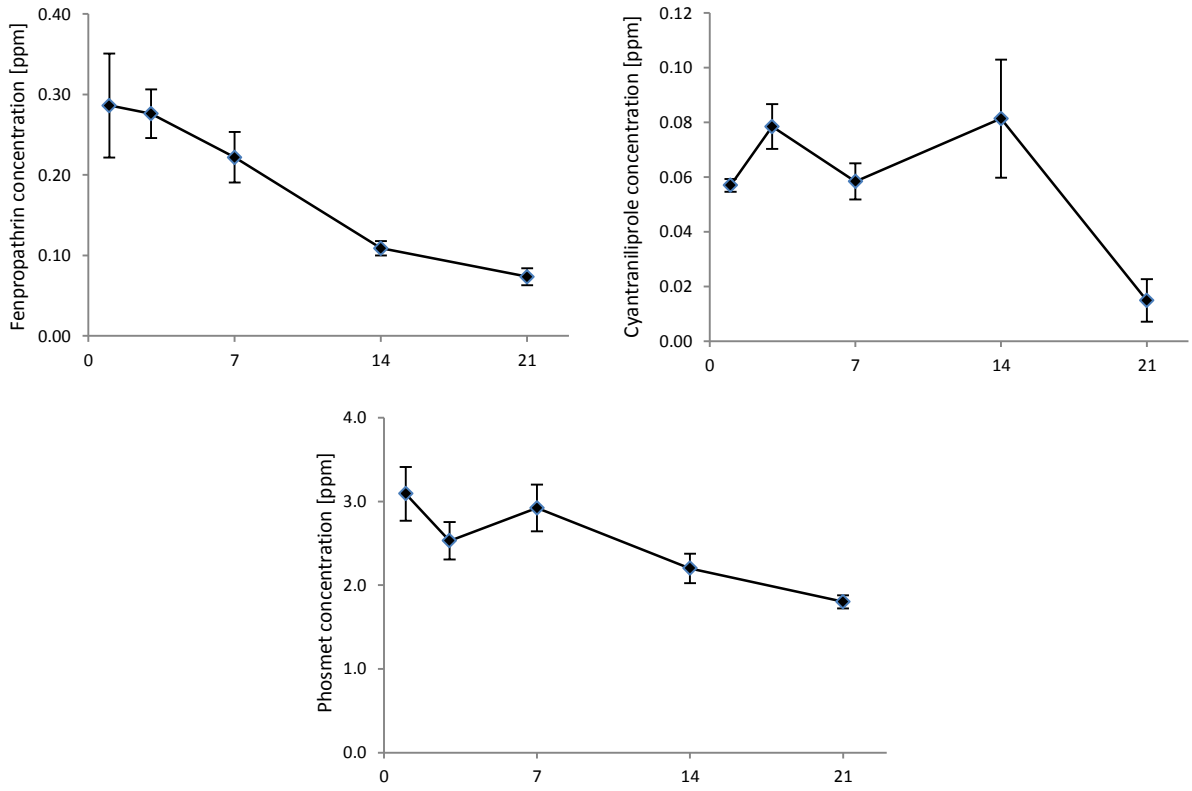

Figure 1. 2014 Fenpropathrin, cyantraniliprole, and phosmet 1, 3, 7, 14, and 21 day decline residue profiles in 'Red Delicious' apple fruit. Concentration means within one date followed by different letters are significantly different (*t-tests p<0.05*). Error bars represent standard error of the mean (SEM). Fenpropathrin has a United States MRL of 5 ppm, while the lowest international MRL at a perspective market is 0.5 ppm in Taiwan. Cyantraniliprole has a United States MRL of 1.5 ppm, while the lowest international MRL at a perspective market is 0.8 ppm in Saudi Arabia. Phosmet has a United States MRL of 10 ppm, while the lowest international MRL at a perspective market is 2 ppm in Taiwan.

Residue Profiles-2015 Single and Multiple Applications

Fenpropathrin was detected throughout the 28 day decline residue profile with relatively flat degradation curves for the single and multiple application treatments (Figure 2). The multiple spray regime resulted in significantly higher residues than the single spray treatment for the overall sample set (F= 3.12, df= 17.5, P=0.0342). Partitioning the repeated measure analysis, there was a significant effect of day main effect observed at 1 DAT with multiple spray greater than single spray (F= 57.67, df= 22.24, P=0.0001), 3 DAT with multiple spray greater than single spray (F= 12.87, df= 22.24, P=0.0022), 7 DAT with multiple spray greater than single spray (F= 50.17, df= 22.24, P=0.0001), 14 DAT with multiple spray greater than single spray (F= 64.97, df= 22.24, P=0.0001), 21 DAT with multiple spray greater than single spray (F= 57.55, df= 22.24, P=0.0001), and 28 DAT with multiple spray greater than single spray (F= 66.98, df= 22.24, P=0.0001). Two applications of fenpropathrin with a 10 day application interval resulted in significantly higher fenpropathrin concentrations than a single application of fenpropathrin at harvest, 14 days after the last application (PHI). Even so, mean residue values for single and multiple application regimes were below the US MRL for all sample dates. Internationally, fenpropathrin with single or multiple application concentrations and 14 day PHI would be low risk for export to most prospective countries. Fenpropathrin concentrations fell below Canada, China, Taiwan, and Vietnam's MRL and would be accepted for export to those countries for the single or multiple applications with a 14 day PHI.

Cyantraniliprole was detected throughout the 28 day decline residue profile with general decrease in concentrations for the single and multiple application treatments

(Figure 2). There were no significant differences in detectable residues between the single and multiple spray treatments for the overall sample set (F= 2.3, df= 18.7, P=0.0861). Partitioning the repeated measure analysis, there was a significant effect of day main effect observed at 1 DAT with multiple spray greater than single spray (F= 23.92, df= 19.96, P=0.0001), 3 DAT with multiple spray greater than single spray (F= 12.43, df= 19.96, P=0.0023), 14 DAT with multiple spray greater than single spray (F= 12.5, df= 19.96, P=0.0023), 21 DAT with multiple spray greater than single spray (F= 17.24, df= 19.96, P=0.0006), and 28 DAT with multiple spray greater than single spray (F= 9.43, df= 19.96, P=0.0064). There were no significant differences between the single and multiple applications at 7 DAT (F= 0.71, df= 19.96, P=0.4095). Three applications of cyantraniliprole with a 7 day application interval results in significantly higher cyantraniliprole concentrations than a single application of cyantraniliprole at harvest, 3 days after the last application (PHI). Mean residue levels following single and multiple application regimes exceeded the US MRL for the 3 day PHI. Internationally, cyantraniliprole with single and multiple application regimes would be low risk for export to most prospective countries, with some exceptions. Cyantraniliprole exceeded Saudi Arabia's MRL of 0.8 ppm, a perspective market with the lowest MRL for the 3 day PHI, making it unacceptable for export to Saudi Arabia. Cyantraniliprole concentrations also exceeded Canada's MRL of 1.5 ppm, Mexico at 1.5 ppm, Brazil's at 0.8 ppm, Thailand at 0.8 ppm, India at no detectable residues, and Vietnam at of 0.8 ppm and was unacceptable for export to those countries for a single or multiple applications with a 3 d PHI.

Phosmet was detected throughout the 28 d decline residue profile with general decrease in concentrations for the single and multiple application treatments (Figure 2). There were no significant differences in detectable residues between the single and multiple spray treatments for the overall sample set (F= 0.33, df= 11.6, P=0.8824). Partitioning the repeated measure analysis, there was no significant effect of day main effect observed at 1 DAT (F= 0.73, df= 17.8, P=0.4105), 3 DAT (F= 3.22, df= 17.8, P=0.0989), 7 DAT (F= 3.86, df= 17.8, P=0.0738), 14 DAT (F= 3.54, df= 17.8, P=0.0851), 21 DAT (F= 0.73, df= 17.8, P=0.4107), and 28 DAT (F= 2.23, df= 17.8, P=0.1623). While three applications of phosmet with a 7 day application interval results in numerically higher residue profile, it was not significantly different than the single application of phosmet at a 7 days PHI. Mean residue values following a single or multiple application regimes were below the US MRL for all samples dates. Internationally, phosmet residues following single or multiple applications would be low risk for export to most prospective countries. Phosmet residues were under Saudi Arabia's MRL of 10 ppm, a perspective market with the lowest MRL for the 7 day PHI, making it acceptable for export to Saudi Arabia. Phosmet concentrations also fell below Mexico's MRL of 10 ppm, Canada at 10 ppm, China at 3 ppm, Taiwan at 2 ppm, and Vietnam's MRL of 10 ppm and would be accepted for export to those countries for the single and multiple applications with a 7 day PHI.

Spinetoram was detected throughout the 28 day decline residue profile with rapid declines in concentrations for the single and multiple application treatments (Figure 2). The multiple spray regime resulted in significantly higher residues than the single spray treatment for the overall sample set (F= 8.36, df= 14.3, P=0.0007). Partitioning the

repeated measure analysis, there was a significant effect of day main effect observed at 1 DAT with single spray greater than multiple spray (F= 54, df= 17.45, P=0.0001) and 3 DAT with single spray greater than multiple spray (F= 6, df= 17.45, P=0.0277). There were no significant differences between the single and multiple applications at 7 DAT (F= 0, df= 17.45, P=1), 14 DAT (F= 0, df= 17.45, P=1), 21 DAT (F= 0, df= 17.45, P=1), and 28 DAT (F= 0, df= 17.45, P=1). Three applications of spinetoram with a 7 day application interval results in numerically, not significantly higher spinetoram concentrations than a single application of spinetoram at the 7 day PHI. Mean residue values following a single or multiple applications were below the US MRL for all sample dates. Internationally, spinetoram with single or multiple applications and 7 day PHI would be low risk for export to most prospective countries. Spinetoram residues fell below Saudi Arabia's MRL of 0.05 ppm and was acceptable for export to Saudi Arabia. Spinetoram concentrations also fell below Canada's MRL of 0.2 ppm, Taiwan at 0.2 ppm, and Vietnam at 0.05 ppm and would be accepted for export to those countries for the single or multiple applications with a 7 day PHI.

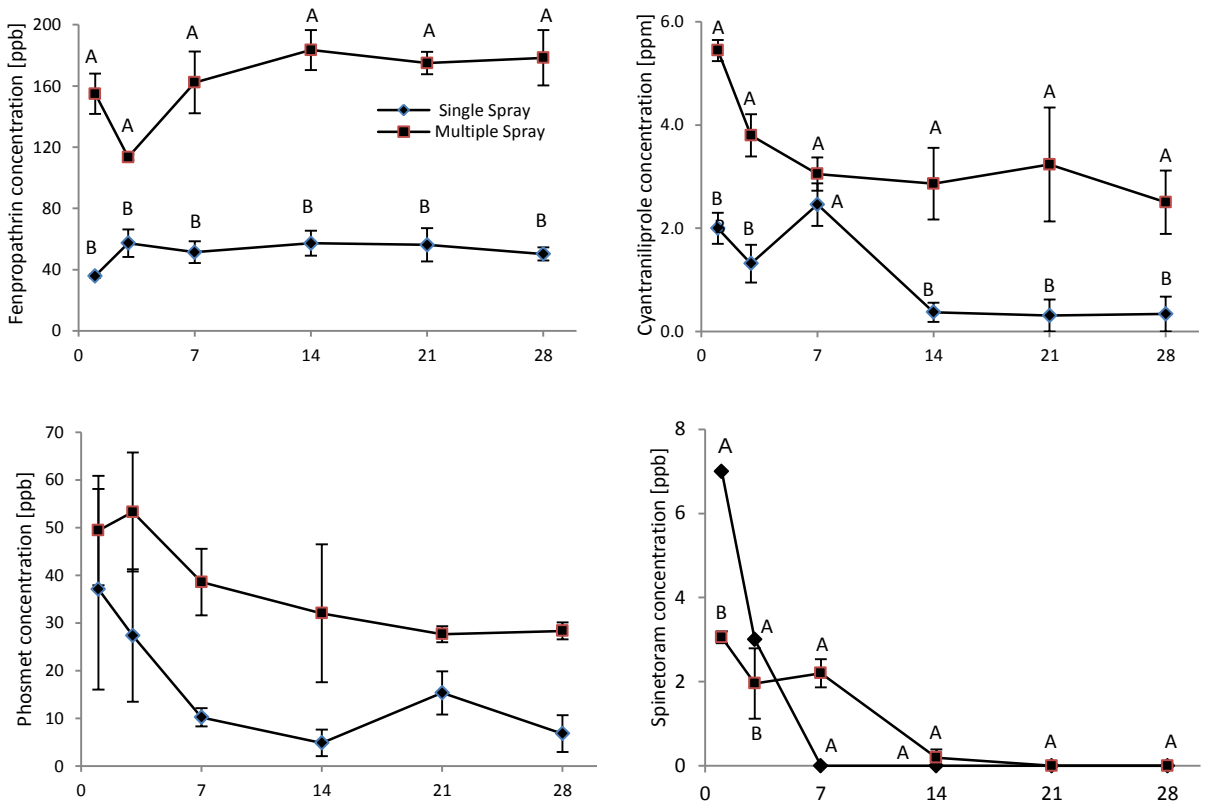

Figure 2. 2015 Fenpropathrin, cyantraniliprole, phosmet, and spinetoram, 1, 3, 7, 14, 21, and 28 day decline residue profiles in 'Red Delicious' apple fruit. Concentration means within one date followed by different letters are significantly different (*t-tests* $p<0.05$). Error bars represent standard error of the mean (SEM). Fenpropathrin has a United States MRL of 5 ppm, while the lowest international MRL at a perspective market is 0.5 ppm in Taiwan. Cyantraniliprole has a United States MRL of 1.5 ppm, while the lowest international MRL at a perspective market is 0.8 ppm in Saudi Arabia. Phosmet has a United States MRL of 10 ppm, while the lowest international MRL at a perspective market is 2 ppm in Taiwan. Spinetoram has a United States MRL of 0.2 ppm, while the lowest international MRL at a perspective market is 0.05 ppm in Saudi Arabia.

Table 6. Weather data for 2014 and 2015 seasons at Trevor Nichols Research Center in Fennville, MI.

2014 Apple Fennville TNRC				2015 Apple Fennville TNRC			
Date	Max. Air Temp. (°F)	Precip. (mm.)	Action	Date	Max. Air Temp. (°F)	Precip. (mm)	Action
09/16/2014	61.6		Application	09/14/2015	79.3		Application 1
09/17/2014	65.3		Sample 1	09/15/2015	81.5		
09/18/2014	62.2			09/16/2015	81.5		
09/19/2014	69.8		Sample 2	09/17/2015	85		
09/20/2014	72.1	5.59		09/18/2015	76.4	17.53	Application 2
09/21/2014	60.7	15.75		09/19/2015	69.4	1.27	
09/22/2014	62.3			09/20/2015	71.5		
09/23/2014	71.8		Sample 3	09/21/2015	77.2		Application 3
09/24/2014	75.7			09/22/2015	78.8		
09/25/2014	73.9			09/23/2015	81.7		
09/26/2014	78.2			09/24/2015	79.5		
09/27/2014	78.7			09/25/2015	79.9		
09/28/2014	75.6			09/26/2015	76.3		Application 4
09/29/2014	65.8	1.27		09/27/2015	72.4		
09/30/2014	53.9	0.25	Sample 4	09/28/2015	78.1		Application 5
10/01/2014	66.3			09/29/2015	65.8	8.13	Sample 1
10/02/2014	61.7	12.45		09/30/2015	65.8		
10/03/2014	63.1	26.92		10/01/2015	64.5		Sample 2
10/04/2014	45.6	8.89		10/02/2015	63.6		
10/05/2014	46.5			10/03/2015	53.8	3.81	
10/06/2014	54.2			10/04/2015	58.6		
10/07/2014	58.6		Sample 5	10/05/2015	60.7		Sample 3
				10/06/2015	63.6		
				10/07/2015	66.8		Application 6
				10/08/2015	76.6	3.81	
				10/09/2015	60.8		Sample 1
				10/10/2015	60.3		
				10/11/2015	72.6		Sample 2
				10/12/2015	68.2		Sample 4
				10/13/2015	56.5		
				10/14/2015	58.3		
				10/15/2015	60.7		Sample 3
				10/16/2015	53.8		
				10/17/2015	48.8		
				10/18/2015	55.3		
				10/19/2015	69.3		Sample 5
				10/20/2015	67.6	8.64	
				10/21/2015	73.7	6.35	
				10/22/2015	62.1		Sample 4

Table 6 (cont'd)

10/23/2015	59.6	1.02	
10/24/2015	67.5	6.60	
10/25/2015	58.4		
10/26/2015	62.7		Sample 6
10/27/2015	60.5	0.25	
10/28/2015	57.5	15.24	
10/29/2015	48.1	1.78	Sample 5
10/30/2015	54.7	0.25	
10/31/2015	53.2	15.75	
11/01/2015	56.4	0.25	
11/02/2015	71.1		
11/03/2015	74.4		
11/04/2015	72.8		
11/05/2015	72.8		Sample 6

Discussion

This research contributes important information to the insecticide residue profiling database for domestic and international apple markets. The results show how multiple applications of certain compounds can result in significantly higher residue concentrations at harvest.

It is noteworthy to see the range of insecticide concentrations from year to year while some concentration differences may be explained by weather and some may not. This study demonstrates the importance of abiotic factors such as weather and biotic factors such as tree growth (canopy size, density, and structure).

The influence of precipitation was demonstrated with this study where the 21 day study period of 2014 there was a total of 71.12 mm (2.8 in) of rain, in parallel study periods in 2015 there was less, with no single precipitation event in 2015 exceeding 17.78 mm (0.7 in) (Table 6). It is expected that with increased rainfall, the insecticide residue concentrations would decrease, and with decreased rainfall, the residues would remain at higher concentrations without exposure to rain wash off. What actually

happened was that generally, there was greater total rainfall in 2014 with greater residue than in 2015 which had less rainfall.

The other factor influencing residue concentration is tree growth. The same tree variety and age was used for 2014 and 2015 studies, but the tree canopy size, density, and structure was changed due to pruning for the 2014 season. The 2014 apple trees were pruned and the 2015 trees were not pruned prior to the study initiation. Pruning decreases the tree size and density of the canopy, which causes greater spray deposition to all portions of the tree canopy, most importantly the shielded portion. Shielded refers to the fruit being less exposed, mainly due to extra coverage by foliage and branches. In this study, the defined location of shielded is 60.96 cm (24 in) inside of the crown. It has been shown that horizontal spray distribution is influenced by canopy density (Wise et al. 2010). This indicates that for the 2014 study the greater spray coverage contributed to higher overall residue concentrations at harvest. Growers need to be mindful of variability from year to year when managing MRLs.

The results also show how the multiple application treatments resulted in higher residue concentrations at harvest than the single application treatments for some compounds, but not others. In our study, compounds of the organophosphate and pyrethroid classes showed the greatest disparity of residue levels between single and multiple applications, and secondarily the diamides. There are several factors that could influence this phenomenon. One factor may be the differing plant penetration attributes of the compounds. Neonicotinoids, diamides and spinosyns have plant penetrative attributes allowing mobility into and beneath the plant cuticle (Wise et al 2017), which would decrease the susceptibility to rain wash off. Organophosphates and pyrethroids

remain largely on the plant surface; with limited cuticle penetration allowing increased susceptibility to rain wash off. Another factor is the longevity of the compound. In our study the compounds known to have longer half-lives were more likely to show greatest disparity of residue levels between single and multiple applications (Wise and Whalon 2009). These factors influence the persistence of the compounds on the plant surface over multiple applications, limiting rain wash off and other environmental degradation events. Growers need to be mindful of variability from year to year when managing MRLs.

Fenpropathrin, like most pyrethroids, have limited cuticular penetration, but as lipophilic compounds have natural affinity to cuticular waxes. This is likely one of the factors responsible for the moderate rainfastness seen for pyrethroids in other studies (Hulbert et al. 2011). Fenpropathrin is relatively unstable and degrades rapidly in normal environmental conditions (Akhtar 2004). This may indicate that with a single application, fenpropathrin will begin to degrade quickly resulting in lower concentrations, but with a second application, the residue levels may be maintained at a higher concentration, resulting in concentration differences between the single and multiple application regimes. Regardless, fenpropathrin after single or multiple applications and 14 day PHI would be low risk for export to most prospective countries.

Cyantraniliprole, like most diamides, has translaminar penetration in plant tissues, thus forming a reservoir from direct environmental exposure. Wise et al. (2017) demonstrated a similar diamide, chlorantraniliprole, to be moderately rainfast, which may explain why the residue profiles for cyantraniliprole in this study showed lower concentrations in 2014 than in 2015. Cyantraniliprole is susceptible to rain washoff,

indicating that the increased total rainfall may have played a factor in decreasing the cyantraniliprole concentrations in 2014. Cyantraniliprole is moderately persistent in normal environmental conditions (Dong et al. 2011). This may indicate that with a single application, cyantraniliprole will begin to degrade resulting in somewhat lower concentrations, but with a second and third application, the residue levels may be maintained at higher concentrations, resulting in concentration differences between the single and multiple application regimes. While this study suggests cyantraniliprole with single and multiple application regimes and 3 day PHI would be low risk for export to many prospective countries, there were several cautions worth noting. First, the residue profile trial in 2014 showed cyantraniliprole residues at the 3 d PHI well below the US MRL of 1.5 ppm, but in 2015 both the single and multiple application treatment regimens exceeded the US MRL. The single spray residue profile curve includes an increase of residue at 7 d, which does not fit the overall decline curve well. This may have been from a random “hot spot” not well balanced-out by the sampling procedures. None-the-less, apple growers should be cautious if targeting countries with MRLs harmonized with USA if using more than a single application near harvest. Of greater concern is that our study showed cyantraniliprole residues to exceed Saudi Arabia, Thailand, Brazil and Vietnam’s MRLs of 0.8 ppm, making it high risk to export to these countries. India not allowing any detectable residues would also be a serious concern for export. To safely target international trade with these high risk countries, one mitigation strategy could be to artificially extend the PHI to 14 days for a single application only. According to the degradation curves in this study, this would likely reduce the cyantraniliprole residues at harvest below the MRL of 0.8.

Phosmet, like most organophosphates, has limited cuticular penetration. Phosmet is known to be very susceptible to rain wash off (Wise et al 2017) as a majority of the active ingredient has been shown to stay on the surface of the plant material. This may explain why the residue profiles for phosmet in this study showed lower concentrations in 2014 than in 2015. The results in this study indicate that there are no statistically significant differences between the single and multiple application treatments, however the multiple application regime resulted in a consistent pattern of higher mean residue levels throughout the duration of the study. This study suggests phosmet with a single or multiple application regimes and 7 day PHI would be low risk for export to many prospective countries, as most are well harmonized with US MRLs. Based on the 2014 results, however, Saudi Arabia, Israel, Taiwan, Brazil and China would be considered a moderate risk even if the PHI was artificially extended to 21 days.

Spinetoram, like most spinosyns, has translaminar penetration in plant tissues, thus forming a sort of reservoir from direct environmental exposure (Bostanian et al. 2012). This likely contributes to the moderate rainfastness documented for this compound (Wise et al. 2017). While this may contribute to the significant differences between the single and multiple application regimes in the first few days of harvest, the effect was quickly lost as residue profiles rapidly declined. The rapid degradation rate of spinetoram in this study is similar to patterns documented in other studies (DOW 2014). The study results for spinetoram suggest that single or multiple applications and 7 day PHI would be low risk for export to most prospective countries, both those that are well harmonized with US MRLs, as well as those with lower MRL values. It has been shown

in previous studies that spinosyns such as spinetoram are useful products near harvest because the US MRL is similar to other markets and will not cause illegal issues with short persistence (Haviland and Beers 2012).

Summary: This MRL study presents valuable data pertaining to invasive species (BMSB) that are crucial to the Michigan apple industry. Growers typically stop spraying insecticides 3+ weeks before harvest, but with the late season BMSB, growers are making insecticide sprays nearer to harvest, which will increase the risk of MRL violations. This research will help inform decisions of apple growers during late season pest management and what possible tactics can be used to lower the risks of violations according to specific export targets.

This research provides important data to the residue profiling database to create application and harvest regimens to best suit the growers' needs. The data presented will assist in creating additional degradation curves for the commonly used late season insecticides. Adding additional insecticides to the database is a large factor in achieving the goals because of the different modes of action, penetrative attributes, and environmental persistence in which each compound possesses. This is just one of many data sets needed to achieve to the overall goal, but the goals of this project were achieved to add another chapter to the MRL database. This project brings us closer to setting more accurate PHI's for growers to use to avoid export rejections. The results show that insecticide residue levels can be predicted using specific spray rates and timings before the harvest of apples. With the advantage of making insecticide residue level forecasts, fruit can be sprayed at specific rates and timings to obtain residue levels legal for shipment to more locations increasing sales and benefitting the economy.

CHAPTER 3 : INFLUENCE OF POST HARVEST WATER WASHING AND MULTIPLE APPLICATIONS ON INSECTICIDE RESIDUES AT HARVEST AND ASSOCIATED RISK FOR CHERRY EXPORTS

Abstract

Decline residue profiling was used to determine the degradation curves of four key insecticides registered for tart cherry. Single and multiple application treatment regimens with minimum and maximum seasonal applications were tested for their effects on residue levels at harvest. The effects of a simulated industry post-harvest typical cherry washing procedure was also tested (Cargill et al. 1969). Fenpropathrin (Danitol 2.4 EC®), cyantraniliprole (Exirel 0.83 SE™), phosmet (Imidan 70 WP®), and spinetoram (Delegate 25 WG®) were foliar direct applied onto Montmorency and Balaton tart cherry trees (*Prunus cerasus* Borkhausen) at the Michigan State University (MSU) Trevor Nichols Research Center (TNRC) and at the MSU Northwest Michigan Horticultural Research Station (NWMHRS). The residue profiling suggests that fenpropathrin, cyantraniliprole, phosmet, and spinetoram would be relatively low risk for international export to most prospective markets. Fenpropathrin was found to exceed the EU's MRL at harvest for a single or multiple application and unwashed or washed treatment, making fenpropathrin a moderate risk for EU export. Potential mitigation strategies were also discussed below.

Fruit growers are in a significant need for more data to determine which insecticides hold the highest risks of rejection for exported cherry crops. Therefore, the following data will support establishment of export pre-harvest interval (PHIs) guidelines

that growers can use to effectively avoid exported cherry rejections in countries with maximum residue limits (MRLs) that are likely above export residue levels.

Introduction

The United States (US) is a major cherry producing country, especially in the state of Michigan. According to the USDA National Agriculture Statistics Service (NASS), the state of Michigan is a national production leader of tart cherries, producing 70% of the nation's cherries with 26,200 bearing acres (www.nass.usda.gov).

Meeting market standards for infestation-free fruit depends on the judicious use of pesticides (Wise and Whalon 2009), while maintaining chemical residues at acceptable levels for export markets. This is especially challenging, especially with new invasive pests such as the late season feeding behavior of spotted wing drosophila (*Drosophila suzukii*) (SWD). The Food Quality Protection Act (FQPA) of 1996 (Schierow 1999) and the Green Movement (Lehman 1993) have also created a difficult environment for maintaining sufficient pesticide options in the market for cherry growers. The EPA's Food Quality Protection Act (FQPA) sets a single standard for pesticide tolerances. Maximum residue limits (MRLs) are the legal maximum level of pesticide residues allowed to remain on or in food and feed products (Christova-Bagdassarian et al. 2014). "MRL" is the globally accepted term used to set such standards throughout the world to describe pesticide residues on harvested produce. The US characterizes the allowed pesticide residue with the term "tolerance" (Winter and Jara 2015). While pre-harvest intervals (PHIs) are determined through extensive residue studies. The US Environmental Protection Agency (EPA) performs the all risk assessment to assure

there are no unacceptable risks with the designated GAP, to assure that residues at harvest do not exceed domestic or international label tolerances.

Therefore, cherry Producers must be aware of global standards for MRLs if they intend to trade in export markets. Many of the export countries use their own pesticide residue calculation system(s) to obtain their MRLs and many use the OECD (Organization of Economic Cooperation and Development) MRL calculator or just MRL calculator (Handford et al. 2015). Such episodes can cause delays in the process for a global pesticide registration as many calculation methods differ. This often results in un-harmonized global MRLs, which is a significant risk to specialty crop growers who desire access to international export markets.

In the United States fenpropathrin, cyantraniliprole, phosmet, and spinetoram are among the most effective insecticides registered for use in cherries, and recommended for control of late season control of insect pest management in Michigan (Wise et al, 2016). These compounds are commonly used as late season options to control direct insect pests, such as the cherry fruit fly, Rhagoletis cingulata, SWD, plum curculio (PC), *Conotrachelus nenuphar* (Herbst), and the obliquebanded leafroller, *Choristoneura rosaceana* (Harris). Near-harvest pest control is particularly challenging for cherry growers because the ripening fruit is highly susceptible to injury from insect pests, while the final sprays must be applied within the labeled PHIs.

Cyantraniliprole is a novel cross-spectrum anthranilic diamide insecticide which selectively activates the ryanodine receptors in the insect muscles (Ammar 2015) causing paralysis. Cyantraniliprole is systemic insecticide which is effective through ingestion and contact routes. This reduced risk insecticide is effective on a wide range

of insects including lepidoptera, hemiptera, diptera (Wise et al. 2016), and more specifically used for late season control of SWD and leafrollers (Shearer and Brown 2013). The label rate on cherry is 0.10-0.15 kg ai/ ha with a 3 day PHI, 12 h REI (re-entry interval), and a minimum of 7 day application interval.

Phosmet is a broad spectrum organophosphate insecticide which is a cholinesterase inhibitor causing nerves to continue sending signals. It is a conventional insecticide traditionally most relied upon for PC and SWD, control in Michigan (Wise et al. 2015, Hoffman et al. 2010), and more recently relied upon for late season control of SWD. The label rate on cherry is 1.67-4.51 kg ai/ ha with a 7 day PHI, and 3 day REI. Phosmet is most effective on lepidopteran such as fruitworms, leafrollers, codling moth, and Oriental fruit moth, but is also used for a range of coleopteran and diptera insect pests (Wise et al. 2016).

Fenpropathrin is a broad spectrum type II synthetic pyrethroid with insecticidal and acaricidal activity (Saryazdi et al. 2014), which is a voltage-gated sodium channel inhibitor which create more ways for the sodium ions to pass through the membrane and propagate the action potential. This keeps the sodium channels in the open position causing paralysis. The label rate on cherry is 0.23-0.46 kg ai/ ha with a 3 day PHI, 24 h REI, and a minimum of 10 day application interval. Fenpropathrin is effective against leafhoppers, aphids, fruitworms, leafminers, mites, leafrollers (Wise et al. 2016), and it is relied upon for late season for control of OBLR and SWD (Shearer and Brown 2013).

Spinetoram is a second generation spinosyn insecticide which targets lepidopteran larvae and thrips, it also works broadly on diptera, coleoptera, hemiptera, hymenoptera, isoptera, orthoptera, siphonaptera, thysanoptera, and mites (Salgato

1998). It is primarily used late season for control of SWD and leafrollers (Shearer and Brown 2013). Spinosyns are a novel insecticide effective by contact and ingestion and target the binding site of the nicotinic acetylcholine receptor in the nervous system which causes hyper excitation of the nervous system and paralysis (Salgato 1998). The Label rate for cherry is 0.08-0.12 kg ai/ ha with a 7 day PHI, 4 h REI, and a minimum of 7 day application interval.

With the importance of late season insecticide-use, and the risk of residue levels exceeding global MRLs, there is need to understand the factors that influence pesticide persistence and residues at harvest. Pesticide degradation is the breakdown of the active ingredient mainly due to abiotic environmental exposures such as ultra violet (UV) light, rainfall, oxidation, dilution, or a biotic factor such as metabolism within the plant tissue (Van Eerd et al. 2003). Ultra violet degradation or photo degradation of insecticides is the active ingredient breakdown of the pesticide from exposure to sunlight. Photo degradation can break down the active ingredient of the pesticide on the foliage, surface of the soil, and the air (Burrows et al. 2002). Pesticide loss from rainfall is when precipitation washes the pesticide residue off of the plant material after an application. Rain has important implications for the fate of pesticides that are sprayed (Wise et al. 2017). With seasonal (April-July) precipitation in Michigan approximating 380+ mm (15.06 in.) (www.enviroweather.msu.edu), growers often need additional applications to protect their crops from pests. Sequential applications of the same pesticide may also increase residues at harvest depending on the compound's persistence and penetrative attributes (Mota-Sanchez et al, 2012).

To address the challenge of meeting global MRL standards, growers need a decision support tool to indicate which insecticides to use the material, at what rate to use, and when to use them under a low risk of MRL violation for specific market destinations. This decision tool should be easy to use and very accessible by the grower. Disparity index is a term developed to measure MRL differences, which is the US MRL divided by the lowest foreign MRL, which equals the greatest difference in the US MRL and lowest foreign MRL (JC Wise, unpublished). This calculated value provides a simple way to identify which compounds are the highest risk for growers targeting global export markets. It allows a single value instead of multiple values to explain how such differences affect the market place.

Therefore, the objectives of this study were to determine the residue concentrations of several key insecticides applied late season on cherry fruit at harvest resulting from a single application versus a multiple application treatment regime. A second objective was to determine residue concentrations of the insecticides on cherries at harvest following post-harvest water washing simulation or no water washing. The aim is to inform a support tool for growers to use for decisions on insecticide sprays targeting late season insect pests, including the new invasive SWD, while avoiding export MRL risks.

Materials and Methods

Field Plots-2014 Season

Field work was conducted at the MSU Northwest Michigan Horticultural Research Station (NWMHRS), in Traverse City, MI, USA (latitude 44.8831°: longitude -85.6777°). Plots were established in a 5.74 m tall 20 year-old Montmorency' (Sare Montmorency) cherry planting at NWMHRS with a six tree and one row buffer. The plot size was three consecutive trees with a 6.10 m row width and 4.57 m tree spacing or total plot dimensions of 6.10 m wide and 13.72 m long, with a total area of 83.61 sq. m. Treatment plots were replicated three times and set up in a randomized complete block (RCB) design.

Field Plots-2015 Season

Field work was conducted at the MSU Trevor Nichols Research Center (TNRC) in Fennville, MI, USA (latitude 42.5951° : longitude -86.1561°). Plots were established in a 2.74 m tall, six year-old 'Balaton' (Tart Cherry 5) cherry planting at TNRC. Treatment plots were replicated 3 times and set up in a design which alternated active ingredients so there would not be cross contamination of active ingredients. These alternations of rows were considered the buffer rows. The plot size was 10 consecutive trees with a 6.10 m row width and 4.57 m tree spacing or total plot dimensions of 6.10 m wide and 45.72 m long, with a total area of 279 sq. m.

Applications-2014 Season

Single application treatments were made at maximum label rates prior to fruit harvest, on 22 July. Four insecticides were selected from currently registered materials for stone fruits: cyantraniliprole (Exirel 0.83 SE, DuPont Crop Protection, Wilmington, DE) at 0.15 kg active ingredient (AI)/ ha (20.5 fl oz formulated product per acre), phosmet (Imidan 70 WP, Gowan Company, Yuma, AZ) at 1.67 kg AI/ ha (2.125 lb formulated product per acre), and fenpropathrin (Danitol 2.4 EC, Valent U.S.A., Walnut Creek, CA) at 0.06 kg active ingredient (AI)/ ha (21.3 fl oz formulated product per acre), and spinetoram (Delegate 25 WG, DOW AgroSciences, Indianapolic, IN) at 0.12 kg active ingredient (AI)/ ha (7.0 oz formulated product per acre). A water pH buffering agent was used with Imidan 70 WP as it is its intended use commercially. This pH buffer is aliphatic polycarboxylate (TriFol L®, Wilbur-Ellis Company, Fresno, CA) at 0.24 L per 378.54 L (0.5 pt/ 100 gal). Test materials were applied with an FMC 1229 airblast sprayer calibrated to deliver diluent at 561 L/ ha (60 gallons per acre), 1.34 m per second (3.0 miles per h), and a 37.85 L tank mix (10.0 gallons). Regular maintenance foliar applications were applied to all treatments and included the fungicides propiconazole Orbit®, sulfentrazone Elite®, captan (Captan®), myclobutanil (Rally®), tebuconazole/ trifloxystrobin (Adament®), fluopyram/ trifloxystrobin (Luna Sensation®), trifloxystrobin (Gem 500®), fenbuconazole (Indar®), chlorothalonil (Bravo Weather Stik®), and copper hydroxide (Kocide 3000®). The insecticide thiamethoxam (Actara®) was applied to the entire block for control of plum curculio (*Conotrachelus nenuphar*), Paraquat dichloride (Gramoxone®) was banded below the trees for weed control.

Applications-2015 Season

Treatment applications were made at the maximum label rates prior to fruit harvest. Applications were made for each of four insecticides according to the maximum allowed on current label for stone fruits: cyantraniliprole (Exirel 0.83 SE, DuPont Crop Protection, Wilmington, DE) at 0.15 kg active ingredient (AI)/ ha (20.5 fl oz formulated product per acre), phosmet (Imidan 70 WP, Gowan Company, Yuma, AZ) at 1.67 kg AI/ ha (2.125 lb formulated product per acre), fenpropathrin (Danitol 2.4 EC, Valent U.S.A., Walnut Creek, CA) at 0.06 kg active ingredient (AI)/ ha (21.3 fl oz formulated product per acre), and spinetoram (Delegate 25 WG, DOW AgroSciences, Indianapolic, IN) at 0.12 kg active ingredient (AI)/ ha (7.0 oz formulated product per acre) (Table 7). A water pH buffering agent was used with Imidan 70 WP as it is its intended use commercially. This pH buffer is aliphatic polycarboxylate (TriFol L, Wilbur-Ellis Company, Fresno, CA) at 0.24 L per 378.54 L (0.5 pt/ 100 gal) (Table 7). Applications were performed on alternate days to ensure all samples could be collected at the proper timing. Test materials were applied with an FMC 1029 airblast sprayer calibrated to deliver diluent at 935 L/ha (100 gallons per acre), 1.12 m per second (2.5 miles per h), and a 94.64 L (25.0 gallon) tank mix. Regular maintenance foliar applications were applied to all treatments and included the fungicides fenbuconazole (Indar), sulfentrazone Elite, chlorothalonil (Bravo Weather Stik), fluxapyroxad/ pyraclostrobin (Merivon®), propiconazole Orbit, propiconazole (Tilt®), fluopyram/ trifloxystrobin (Luna Sensation), trifloxystrobin (Gem 500), myclobutanil (Rally), and copper hydroxide (Kocide 3000). The Insecticide thiamethoxam (Actara) was applied to the entire block for control of plum curculio

(*Conotrachelus nenuphar*),. Paraquat dichloride (Gramoxone), rimsulfuron (Matrix 25®), and flumioxazin (Chateau 51®) were banded below the trees for weed control.

Table 7. 2015 cherry application dates

Treatment/ Formulation	Application Dates
UTC	
Exirel 0.83 SE, 1 Appl.	Jul-7
Exirel 0.83 SE, 3 Appl.	24-Jun, 29-Jun, Jul-6
Imidan 70 WP, 1 Appl. TriFol L	Jul-7
Imidan 70 WP, 3 Appl. TriFol L	24-Jun, 29-Jun, Jul-6
Danitol 2.4 EC, 1 Appl.	Jul-7
Danitol 2.4 EC, 2 Appl.	26-Jun, Jul-6
Delegate 25 WG, 1 Appl.	Jul-7
Delegate 25 WG, 3 Appl.	24-Jun, 29-Jun, Jul-6

Sample Collection-2014 and 2015 Seasons

Residue samples were collected, prepared, and parent AI were recovered using methods based on US EPA standards for GLP field residue studies (USEPA 40 CFR 160).. These methods are also known as the QuEChERS method for multiple pesticide residue analysis for a variety of different sample types (Kong et al. 2016). QuEChERS is an abbreviation for quick, easy, cheap, effective, rugged, and safe. One labeled gallon Ziploc® bag was used to collect 0.91 kg (2 lbs) total fruit per bag for each replicate sample. The cherries were selected randomly from the N, S, E, W cardinal direction sides of the tree, low/ middle/ high, and shielded/ exposed portions of the tree crown. The shielded location were fruit at least 60.96 cm (24 in) inside of the tree crown and

exposed was the outer 60.96 cm 24 in. Low was the bottom 1.22 m (four ft), middle was the center 1.22 m (four feet), and high was the upper 1.22 m (four ft) of the tree crown. Samples were collected on the specific day after treatment (DAT) or day after last application and ± 1 day for the 3, 7, 14, 21, and 28 DAT samples. The 2014 season samples were collected from all treatments at 1 DAT (23 July), 3 DAT (25 July), 7 DAT (29 July), 14 DAT (5 Aug), 21 DAT (12 Aug), and 28 DAT (19 Aug). The 2015 season sampling dates are listed in table 8.

Table 8. 2015 cherry sample collection dates and timings

Treatment/ Formulation	Sample DAT Number					
	1	3	7	14	21	28
UTC	7-Jul	9-Jul	13-Jul	20-Jul	27-Jul	3-Aug
Exirel 0.83 SE, 1 Appl.	8-Jul	10-Jul	14-Jul	21-Jul	28-Jul	4-Aug
Exirel 0.83 SE, 3 Appl.	7-Jul	9-Jul	13-Jul	20-Jul	27-Jul	3-Aug
Imidan 70 WP, 1 Appl. TriFol L	8-Jul	10-Jul	14-Jul	21-Jul	28-Jul	4-Aug
Imidan 70 WP, 3 Appl. TriFol L	7-Jul	9-Jul	13-Jul	20-Jul	27-Jul	3-Aug
Danitol 2.4 EC, 1 Appl.	8-Jul	10-Jul	14-Jul	21-Jul	28-Jul	4-Aug
Danitol 2.4 EC, 2 Appl.	7-Jul	9-Jul	13-Jul	20-Jul	27-Jul	3-Aug
Delegate 25 WG, 1 Appl.	8-Jul	10-Jul	14-Jul	21-Jul	28-Jul	4-Aug
Delegate 25 WG, 3 Appl.	7-Jul	9-Jul	13-Jul	20-Jul	27-Jul	3-Aug

Cherry Washing-2014 and 2015 Seasons

Cherries underwent a water washing procedure to simulate the standard industry methods for tart cherries modified from Cargill et al. (1969). The industry generally uses 946.35 L (250 gal) tanks which run water at around 30.28-37.85 L (8-10 gal) per minute for two hours. Then the rate slows to 15.14-22.71 L (4-6 gal) per minute and finally the

cherries sit in cooling tanks for approximately 2 h. Study methods closely resembled the industry protocol, but on a small scale. The research-scale washing method was calibrated 3 times using a stop watch and sprinkler valves before each use. The flow rate was timed to reach the graduated gallon marks on 18.93 L (5 gal) buckets. Cherries were picked for each prescribed DAT, then brought back to the water washing location. The water washing system was connected to a water tap. There was a single hose connected to the tap which had a four hose splitter manifold connected. There were four hoses connected to each manifold which were connected to and entered the side wall of four-18.93 L (5 gal) buckets. Each hose also had an in-line sprinkler valve with flow control so the flow rate could be regulated entering the 18.93 L (5 gal) buckets. Mesh screens were tied to the tops of each bucket so the cherries did not fall out of the buckets while the water ran into and out of the buckets. The cherries were placed into clean buckets and rinsed for 2 hrs ± 15 min at 18.93 L (5 gal) per minute in 2014 and 7.57 L (2 gal) per minute flow rate for the 2015 season. Variation was due to differing water pressure at the tap. After the cold water rinse, the cherries were placed back into their labeled Ziploc® (SC Johnson, Racine, Wisconsin) bags. The cherries were then taken out of their labeled bags one repetition at a time and one treatment at a time to prevent cross contamination. The cherries were each pitted with a sanitized Leifheit® single cherry pitter (Leifheit, Nassau, Germany), cutting board, and purple Nitrile gloves. All equipment was sanitized with acetone and gloves changed between each repetition and treatment to prevent cross contamination. Once the cherries were pitted they were placed back into the original sample bag representing the sample. The cherries in the Ziploc bag were weighed and should equal a minimum of 0.91 kg (2 lbs). These

cherries are put into a -20° C chest freezer (Kenmore®, Hoffman Estates, Ill.) and monitored to assure temperature ranges did not rise above -5 ° C for storage until homogenization procedures.

Homogenization Procedures-2014 and 2015 Seasons

Once all samples were collected for the harvest season, they were ground using a commercial Hobart® food processor (Hobart Corporation, Troy, OH) beginning with the latest sample date (28 DAT) and working towards the earliest DAT. Six hundred g of dry ice were added to each sample to prevent softening of the fruit while processing. Each sample was ground for 5 min. Samples were taken with a sanitized spoon from all four quadrants of the homogenous ground sample to fill clean labeled sample 120 ml jars (Qorpak Bottle Beakers®, Berlin Packaging, Chicago IL). Sample jars were then placed back into the freezer to store before the next step. The food processor was dissembled and all parts and tools were sanitized with acetone to prevent cross contamination between each treatment. Twenty four to thirty six h later, the samples were taken out of the freezer and ten gram samples were taken out and placed into new clean labeled jars. Next, four g of magnesium sulfate, one gram of sodium chloride, and 15 ml of dichloromethane were added to the new jars. The samples were placed into the refrigerator for two days to separate fruit tissue from the AI. The samples were shaken then decanted through 12 g of reagent-grade anhydrous sodium sulfate (EMD Chemicals, Inc.) to remove water for one hour. The samples were then dried by evaporation under a chemical hood and the remaining particles were brought back up

with two ml of acetonitrile. The final two ml were transferred to a two ml vial (Agilent Technologies, Santa Clara, CA) for HPLC analysis.

Sample Extraction-2014 and 2015 Seasons

Levels of parent compound were quantified using a waters 2695 separator module High Profile Liquid Chromatography (HPLC) equipped with a Waters MicroMass ZQ mass spectrometer detector (Waters, Milford, MA), and a C_{18} reversed phase column (50 by 3.0mm bore, 3.5 µm particle size, (Waters, Milford, MA). The mobile phase, solvent A was with water and 0.1% formic acid. Solvent B was with acetonitrile with 0.1% formic acid (Table 9). Solvent A began at 80% and solvent B at 20% with a gradient and the column temperature of 20 degrees Celsius (Table 10). A standard was developed for each insecticide to compare the experimental concentrations. The standards of the insecticides were massed and diluted into solution with acetonitrile. The serial dilutions were made from the stock solution. The concentrations used were 7.57 g/ml, 0.155 g/ml, 0.0757 g/ml, 0.00155 g/ml, 0.000757 g/ml, and 0.0000155 g/ml.

Table 9. The mobile phase for each insecticide used for HPLC residue analysis 2014 and 2015.

Chemical	Solvent A	Solvent B	Flow Rate (ml/min)
Fenpropathrin	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.3
Cyantraniliprole	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.3
Phosmet	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.3
Spinetoram	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.3

Table 10. The gradient used for each insecticide for HPLC residue analysis 2014 and 2015.

Active Ingredient	Time (min)	Solvent A (%)	Solvent B (%)
Fenpropathrin	0	80	20
	4	10	90
	4.5	10	90
	4.6	80	20
	10	80	20
Cyantraniliprole	0	80	20
	1	80	20
	4	20	80
	6	20	80
	6.1	80	20
	11	80	20
Phosmet	0	80	20
	1	80	20
	3	50	50
	3.1	20	80
	4	20	80
	4.1	80	20
	8	80	20
Spinetoram	0	80	20
	1	80	20
	3	50	50
	3.1	20	80
	4	20	80
	4.1	80	20
	8	80	20

Table 11. The ions (m/z) monitored, detector dwell time, and cone voltages for detection of the insecticides in HPLC residue analysis 2014 and 2015.

Chemical	Channel 1	Channel 2	Dwell (s)	Cone1 (V)	Cone2 (V)
Fenpropathrin	265	181	0.5	30	45
Cyantraniliprole	284	484.1	0.5	50	25
Phosmet	209	175	0.5	55	55
Spinetoram	872.2	886	0.5	55	55

The first step was to determine the range of concentrations, and highest concentration in range. The next step was to make desired concentrations with distilled acetonitrile based off the compound's molecular weight. The stock solution was used to make the next dilution, and this solution was used to make the next dilution, etc. Every other dilution was 100 fold and the dilutions in between were 50 fold. The HPLC level of quantification was 0.08 µg/g (ppm) of active ingredient, and the level of detection was 0.038 ppm.

The residue data for each compound were analyzed with mixed models using the MIXED procedure in SAS 9.4 (SAS Institute, Cary, NC, 2013). The fruit residues were analyzed with repeated measures best adjusted using an unstructured and a first-order heterogeneous autoregressive covariance structure. Repetition and treatment were used as subjects of repeated measurements. When the main effects or their interactions were statistically significant ($P < 0.05$), examination i.e. slicing of interactions within main effects was performed, *F*-tests (Acimovic et al. 2014) were conducted and pairwise or specific time or treatment comparisons were conducted using *t*-tests ($\alpha = 0.05$).

Results

Residue Profiles-2014 Washed and Unwashed Cherries

Fenpropathrin was detected throughout the 28 day decline residue profile with a relatively flat decline curve for the washed and unwashed treatments (Figure 3). There was no significant difference in detectable residues between the unwashed and washed treatments for the overall sample set ($F= 0.78$, df= 18.7, $P=0.5743$). After partitioning

the repeated measure analysis, there was no significant effect of a day main effect observed at 1 DAT (F= 0.1, df= 22.39, P=0.7599), 3 DAT (F= 0.01, df= 22.39, P=0.9335), 7 DAT (F= 0.06, df= 22.39, P=0.8114), 14 DAT (F= 2.09, df= 22.39, P=0.1652), and 28 DAT (F= 3.02, df= 22.39, P=0.0984). There was a significant effect of a day main effect observed at 21 DAT with unwashed greater than washed (F= 4.6, df= 22.39, P=0.0453). For the unwashed and washed treatments of fenpropathrin, mean residue values were below the US MRL of 5 ppm for all sample dates. Internationally, unwashed or washed treatments would be low risk for export to most prospective countries. Fenpropathrin residues exceeded EU's MRL of 0.01 ppm and thus would be unacceptable for export to the EU at the current 3 day PHI. Fenpropathrin concentrations fell below Mexico, Canada, China, Japan, Australia, Korea, and Taiwan's MRL, thus it would be accepted to those countries for the washed and unwashed treatments with a 3 day PHI. Since there is not a CODEX MRL for fenpropathrin, there would be significant risk for export to Colombia, Guatemala, India, Jordan, Nicaragua, Philippines, and Singapore because they do not have default MRLs, thus we must assume no detectable residues.

Cyantraniliprole was detected throughout the 28 day decline residue profile with a gradual decrease in concentrations for the unwashed and washed treatments (Figure 3). There was a significant difference in detectable residues between the unwashed and washed treatments for the overall sample set (F= 4.37, df= 18.2, P=0.0087). Partitioning the repeated measure analysis, there was a significant effect of day main effect observed at 1 DAT with unwashed greater than washed (F= 45.73, df= 23.99, P=0.0001), 3 DAT with unwashed greater than washed (F= 25.47, df= 23.99,

P=0.0001), 7 DAT with unwashed greater than washed (*F*= 4.89, df= 23.99, *P*=0.0401), 21 DAT with unwashed greater than washed (*F*= 19.1, df= 23.99, *P*=0.0004), and 28 DAT with unwashed greater than washed (*F*= 3.32, df= 23.99, *P*=0.0847). There were no significant differences between the unwashed and washed treatments at 14 DAT (*F*= 1.85, df= 23.99, *P*=0.1908). Mean residue levels following unwashed and washed regimes fell below the US MRL of 6 ppm for the 3 day PHI. Internationally, unwashed or washed treatments fell below Korea's MRL of 6 ppm, making it acceptable for export to Korea. Cyantraniliprole concentrations fell below the EU, Mexico, Canada, Japan, and Australia's MRL and would be accepted to those countries for the washed and unwashed treatments with a 3 day PHI.

Phosmet was detected throughout the 28 day decline residue profile with a rapid decrease in concentrations for the unwashed and washed treatments (Figure 3). There was a significant difference in detectable residues between the unwashed and washed treatments for the overall sample set (*F*= 7.78, df= 16.3, *P*=0.0007). Partitioning the repeated measure analysis, there was no significant effect of day main effect observed at 7 DAT (*F*= 1.12, df= 9.394, *P*=0.3063), 14 DAT (*F*= 0.21, df= 9.394, *P*=0.6561), 21 DAT (*F*= 0.01, df= 9.394, *P*=0.9302), and 28 DAT (*F*= 0.19, df= 9.394, *P*=0.665). There were significant differences between the unwashed and the washed treatments at 1 DAT with unwashed greater than washed (*F*= 52.88, df= 9.394, *P*=0.0001) and 3 DAT with unwashed greater than washed (*F*= 12.48, df= 9.394, *P*=0.0027). Mean residue values following the unwashed and washed regimes were below the US MRL of 10 ppm for all samples dates, making domestic trades acceptable. Internationally, residues following unwashed and washed treatments were also under Korea's MRL, making it

acceptable for export to Korea. Phosmet concentrations fell below the EU's MRL of 1 ppm, Mexico at 10 ppm, Canada at 7 ppm, Japan at 0.1 ppm, Australia at 1 ppm, and Taiwan at 2 ppm, and would be accepted to those countries for the washed and unwashed treatments with a 7 day PHI. Since there is not a CODEX MRL for phosmet, there would be significant risk for export to Colombia, Guatemala, India, Israel, Jordan, Nicaragua, Philippines, and Singapore who do not have default MRLs, thus must assume no detectable residues.

Spinetoram was detected throughout the 28 day decline residue profile with rapid declines in concentrations for the unwashed and washed treatments (Figure 3). There was no significant difference in detectable residues between the unwashed and washed treatments for the overall sample set (F= 0.05, df= 13.4, P=0.9984). There were no significant differences between the unwashed and washed treatments after the last application. Partitioning the repeated measure analysis, there was no significant effect of day main effect observed at 1 DAT (F= 0.09, df= 12.28, P=0.7731), 3 DAT (F= 0.01, df= 12.28, P=0.9427), 7 DAT (F= 0, df= 12.28, P=0.9978), 14 DAT (F= 0, df= 12.28, P=0.9999), 21 DAT (F= 0.19, df= 12.28, P=0.6693), and 28 DAT (F= 0.11, df= 12.28, P=0.7487). Mean residue values following an unwashed or washed treatment were below the US MRL of 0.3 ppm for all sample dates. Internationally, residues following an unwashed or washed spinetoram treatment were below the EU's MRL of 0.05 ppm, making it acceptable for export to the EU. Spinetoram concentrations fell below the Mexico, Canada, Korea, Japan, Australia, and Taiwan's MRL and would be accepted to those countries for the washed and unwashed treatments with a 7 day PHI. Since there is not a CODEX MRL for spinetoram, there would be significant risk for export to

Colombia, Guatemala, India, Israel, Jordan, Nicaragua, Philippines, and Singapore who do not have default MRLs, thus must assume no detectable residues.

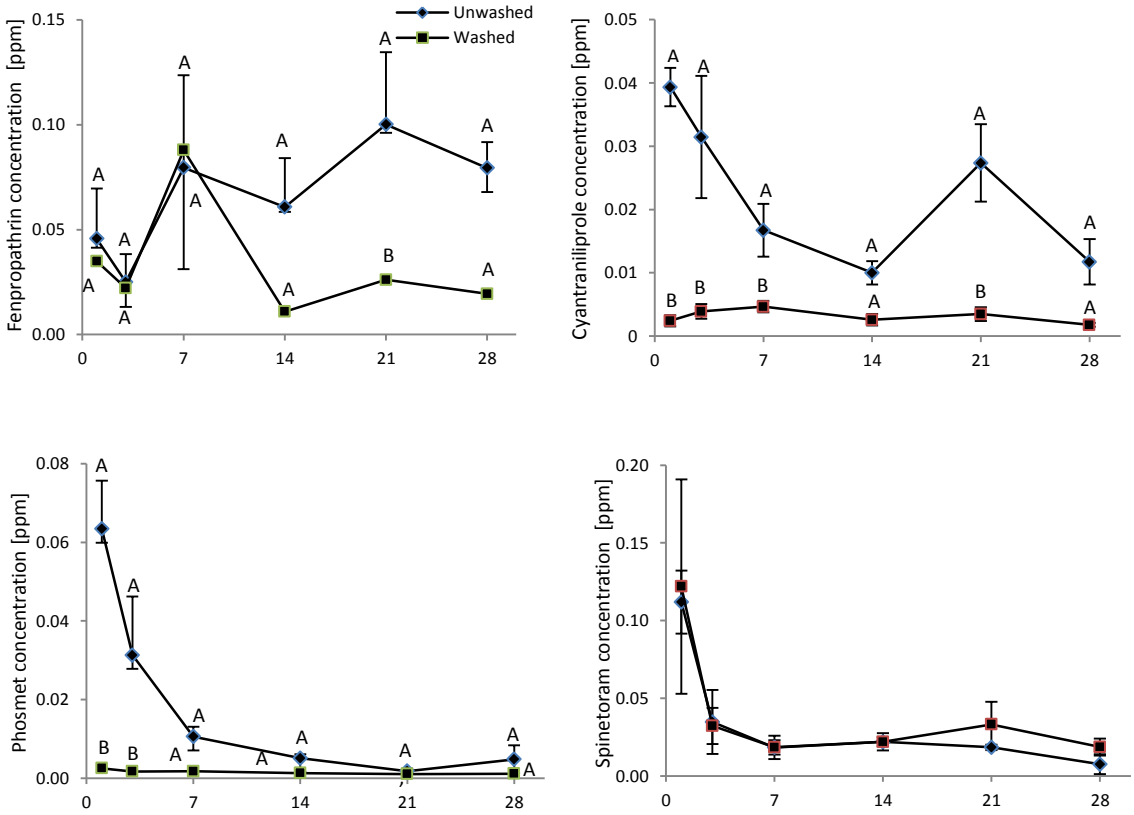

Figure 3. 2014 Fenpropathrin, cyantraniliprole, phosmet, and spinetoram 1, 3, 7, 14, 21, and 28 day decline residue profiles in 'Montmorency' cherry fruit comparing unwashed and washed treatments. Concentration means within one date followed by different letters are significantly different (*t-tests p<0.05*). Error bars represent standard error of the mean (SEM). Fenpropathrin has a United States MRL of 5 ppm, while the lowest international MRL at a perspective market is 0.01 ppm in the European Union. Cyantraniliprole has a United States MRL of 6 ppm, while the lowest international MRL at a perspective market is 6 ppm in Korea. Phosmet has a United States MRL of 10 ppm, while the lowest international MRL at a perspective market is 0.05 ppm in Korea.

Figure 3 (cont'd)

Spinetoram has a United States MRL of 0.3 ppm, while the lowest international MRL at a perspective market is 0.05 ppm in the EU and the highest MRL is 0.5 ppm in Japan.

Residue Profiles-2015 Single and Multiple Applications

Fenpropathrin was detected throughout the 28 day decline residue profile with a gradual decrease in concentrations for the single and multiple application treatments (Figure 4). The multiple spray regime resulted in significantly higher residues than the single spray treatment for the overall sample set (F= 8.91, df= 19.1, P=0.0002). Partitioning the repeated measure analysis, there was a significant effect of day main effect observed at 1 DAT with multiple spray greater than single spray (F= 129.89, df= 14.56, P=0.0001), 3 DAT with multiple spray greater than single spray (F= 54.49, df= 14.56, P=0.0001), 7 DAT with multiple spray greater than single spray (F= 58.15, df= 14.56, P=0.0001), 14 DAT with multiple spray greater than single spray (F= 17.54, df= 14.56, P=0.0005), 21 DAT with multiple spray greater than single spray (F= 22.5, df= 14.56, P=0.0001), and 28 DAT with multiple spray greater than single spray (F= 13.59, df= 14.56, P=0.0016). Mean residue values for single and multiple application regimes were below the US MRL of 5 ppm for all sample dates. Internationally, single or multiple applications would exceed EU's MRL of 0.01 ppm for all samples dates, making it unacceptable for export to the EU. Fenpropathrin concentrations fell below Mexico, Canada, China, Japan, Australia, Korea, and Taiwan's MRL and would be accepted to those countries for the single and multiple application treatments with a 3 day PHI.

Cyantraniliprole was detected throughout the 28 day decline residue profile with a rapid decrease in concentrations for the single and multiple application treatments in the first 3 days, followed by a gradual decline (Figure 4). There were no significant differences in detectable residues between the single and multiple spray treatments for the overall sample set (F= 0.15, df= 15.6, P=0.9757). There were no significant differences between the single and multiple spray concentrations for the 1, 3, 14, 21, and 28 days after the last application. Partitioning the repeated measure analysis, there was no significant effect of day main effect observed at 1 DAT (F= 0.63, df= 23.11, P=0.4375), 3 DAT (F= 0.01, df= 23.11, P=0.9049), 7 DAT (F= 0.13, df= 23.11, P=0.7221), 14 DAT (F= 0.02, df= 23.11, P=0.8799), 21 DAT (F= 0.03, df= 23.11, P=0.8624), and 28 DAT (F= 0, df= 23.11, P=1). Mean residue levels following single and multiple application regimes fall below the US MRL of 6 ppm for all samples dates. Internationally, single and multiple application treatments fall below Korea's MRL of 6 ppm for all samples dates, making it acceptable for export to Korea. Cyantraniliprole concentrations fell below the EU, Mexico, Canada, Japan, and Australia's MRL and would be accepted to those countries for the single and multiple application treatments with a 3 day PHI.

Phosmet was detected throughout the 28 day decline residue profile with gradual decrease in concentrations for the single and multiple application treatments (Figure 4). There was significant difference in detectable residues between the single and multiple spray treatments for the overall sample set (F=34.97, df= 4.18, P=0.0036). Partitioning the repeated measure analysis, there were significant effects of day main effect observed at 7 DAT with multiple spray greater than single spray (F= 6.73, df= 19.48,

P=0.0215), 14 DAT with multiple spray greater than single spray (*F*= 7.26, df= 19.48, *P*=0.0177), 21 DAT with multiple spray greater than single spray (*F*= 11.06, df= 19.48, *P*=0.0051), and 28 DAT with multiple spray greater than single spray (*F*= 7.62, df= 19.48, *P*=0.0155). There were no significant differences in concentrations at 1 DAT (*F*= 2.88, df= 19.48, *P*=0.1123) and 3 DAT (*F*= 1.6, df= 19.48, *P*=0.2273). Mean residue values following a single or multiple application regimes were below the US MRL of 10 ppm for all samples dates. Internationally, residues following single and multiple application treatments were also under Korea's MRL of 0.05 ppm for all samples dates, making it acceptable for export to Korea. Phosmet concentrations fell below the EU, Mexico, Canada, Japan, Australia, and Taiwan's MRL and would be accepted to those countries for the single and multiple application treatments with a 7 day PHI.

Spinetoram was detected throughout the 28 day decline residue profile with rapid declines in concentrations in the first seven days for the single and multiple application treatments (Figure 4). There was significant difference in detectable residues between the single and multiple spray treatments for the overall sample set (*F*= 8.66, df= 12.4, *P*=0.001). Partitioning the repeated measure analysis, there was a significant effect of day main effect observed at 1 DAT with multiple spray greater than single spray (*F*= 104.31, df= 18.88, *P*=0.0001) and 3 DAT with multiple spray greater than single spray (*F*= 35.73, df= 18.88, *P*=0.0001), 7 DAT with multiple spray greater than single spray (*F*= 9.11, df= 18.88, *P*=0.0104, 14 DAT with multiple spray greater than single spray (*F*= 6.82, df= 18.88, *P*=0.0222), 21 DAT with multiple spray greater than single spray (*F*= 5.39, df= 18.88, *P*=0.038), and 28 DAT with multiple spray greater than single spray (*F*= 5.39, df= 18.88, *P*=0.038). Mean residue values following a single or multiple

application regimes were below the US MRL of 0.3 ppm for all samples dates. Internationally, residues following a single or multiple application spinetoram treatment were below the EU's MRL of 0.05 ppm, making it low risk for export to the EU. Spinetoram concentrations fell below the Mexico, Canada, Korea, Japan, Australia, and Taiwan's MRL and would be accepted to those countries for the single and multiple application treatments with a 7 day PHI.

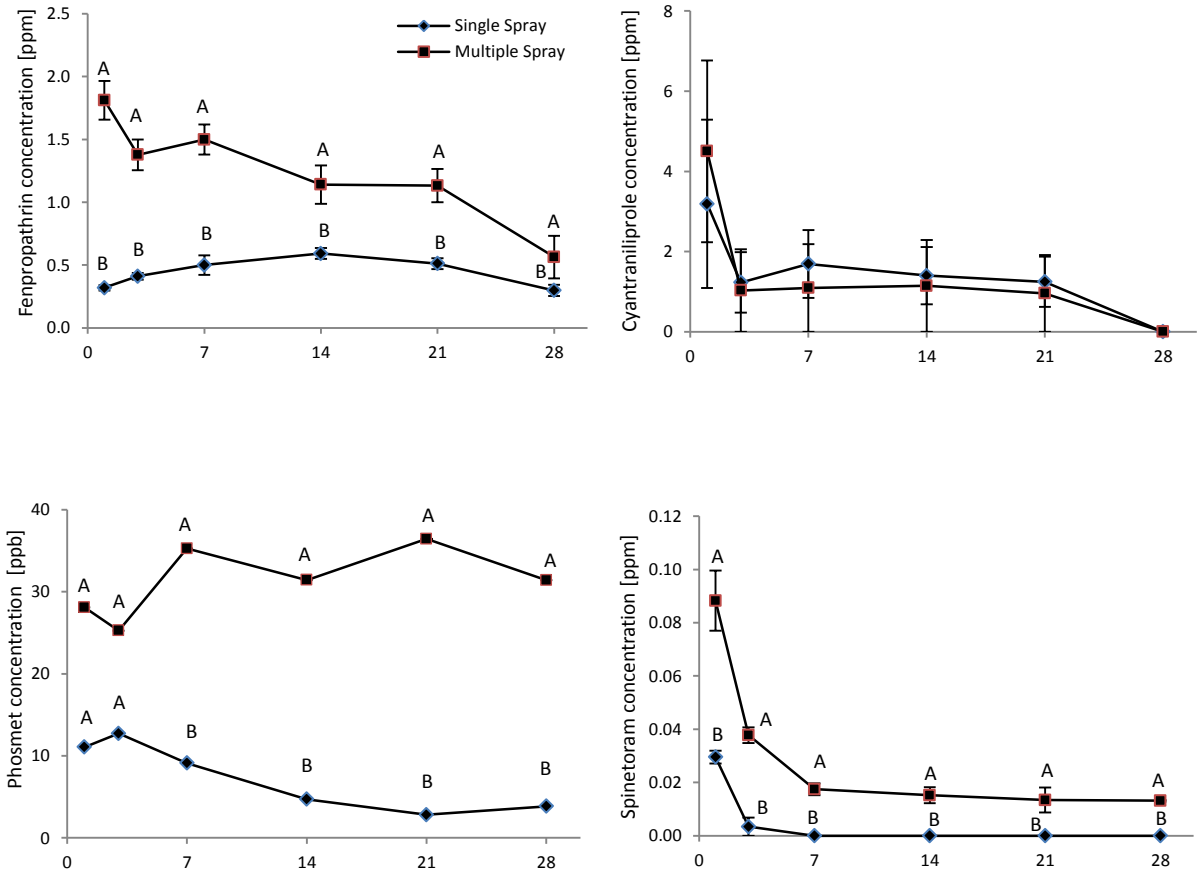

Figure 4. 2015 Fenpropathrin, cyantraniliprole, phosmet, and spinetoram 1, 3, 7, 14, 21, and 28 day decline residue profiles in 'Montmorency' cherry fruit comparing unwashed and washed treatments. Concentration means within one date followed by different letters are significantly different (*t-tests p<0.05*). Error bars represent standard error of

Figure 4 (cont'd)

the mean (SEM). Fenpropathrin has a United States MRL of 5 ppm, while the lowest international MRL at a perspective market is 0.01 ppm in the European Union.

Cyantraniliprole has a United States MRL of 6 ppm, while the lowest international MRL at a perspective market is 6 ppm in Korea. Phosmet has a United States MRL of 10 ppm, while the lowest international MRL at a perspective market is 0.05 ppm in Korea. Spinetoram has a United States MRL of 0.3 ppm, while the lowest international MRL at a perspective market is 0.05 ppm in the EU and the highest MRL is 0.5 ppm in Japan.

Table 12. Weather data for 2014 and 2015 seasons at the Northwest Michigan Horticultural Research Station in Traverse City, MI and Trevor Nichols Research Center in Fennville, MI.

2014 Cherry Northwest Station			2015 Cherry Fennville TNRC		
Date	Precip. (mm)	Action	Date	Precip. (mm)	Action
7/22/2014	0.51	Application	6/24/2015		Application 1
7/23/2014		Sample 1	6/25/2015	2.54	
7/24/2014			6/26/2015		Application 2
7/25/2014		Sample 2	6/27/2015		
7/26/2014	12.19		6/28/2015		
7/27/2014	1.02		6/29/2015	3.56	Application 3
7/28/2014			6/30/2015		
7/29/2014		Sample 3	7/1/2015		
7/30/2014			7/2/2015		
7/31/2014			7/3/2015		
8/1/2014			7/4/2015		
8/2/2014			7/5/2015		
8/3/2014			7/6/2015		Application 4
8/4/2014			7/7/2015	6.10	Application 5, Sample 1

Table 12 (cont'd)

8/5/2014	0.51	Sample 4	7/8/2015		Sample 1
8/6/2014			7/9/2015	3.30	Sample 2
8/7/2014			7/10/2015		Sample 2
8/8/2014			7/11/2015		
8/9/2014			7/12/2015		
8/10/2014			7/13/2015	37.85	Sample 3
8/11/2014			7/14/2015	5.59	Sample 3
8/12/2014		13.72	Sample 5	7/15/2015	
8/13/2014			7/16/2015	1.52	
8/14/2014			7/17/2015	11.18	
8/15/2014			7/18/2015	4.06	
8/16/2014	16.00		7/19/2015		
8/17/2014			7/20/2015		
8/18/2014	1.78		7/21/2015		Sample 4
8/19/2014	4.06	Sample 6	7/22/2015		Sample 4
			7/23/2015		
			7/24/2015		
			7/25/2015		
			7/26/2015		
			7/27/2015		
			7/28/2015		Sample 5
			7/29/2015		Sample 5
			7/30/2015		
			7/31/2015		
			8/1/2015		
			8/2/2015	21.84	
			8/3/2015		
			8/4/2015		Sample 6

Discussion

This research contributes important information to the insecticide residue profiling database for domestic and international cherry markets. The results show how post-harvest washing procedures and number of applications of certain compounds can

influence residue concentrations at harvest. This study also demonstrates the importance of biotic factors such as tree size and canopy density and abiotic factors such the weather and spray volumes. In the 29 day study period of 2014 there was a total of 49.78 mm (2.0 in) of rain, in parallel study periods in 2015 there was a total of 75.69 mm (3.0 in) (Table 12).

It is noteworthy to see the range of insecticide concentrations from year to year while some concentration differences may be explained by weather and some may not. As seen in this study, it is expected that increased rain would result in less residue. There was 25.4 mm (1.0 in) more rain in 2015 than 2014. There was generally higher residue concentrations in 2015 for all compounds, except spinetoram, which was very similar for both years.

In this study, weather does not fully explain the differences in residue from year to year, but as mentioned previously, there are several different factors which may influence these differences. Tree size (canopy size, structure, and density) and sprayer GPA plays an important role in residue differences in 2014 and 2015. The 2014 'Montmorency' cherry trees were older with very dense canopy structure and size standing 5.74 m tall. The 2015 'Balaton' cherry trees are approximately half the canopy size and an open canopy structure with decreased density standing 2.74 m tall, The sprayer GPA in 2014 was 60 and in 2015 was 100. It is expected that with increased tree size along with decreased GPA, pesticide deposition would decrease for the shielded portion of the canopy. This is especially true when comparing trees that have approximately half of the canopy size and density with approximately double GPA. In our study there was increased residue on the smaller tree size and increased GPA in

2015 compared to the larger tree size and decreased GPA in 2014. Larger mature trees would be more difficult to penetrate with more foliage and braches protecting the fruit on the shielded portion of the tree crown. Shielded refers to the fruit being less exposed mainly due to extra coverage by foliage and branches. In this study, the defined location of shielded is 60.96 cm (24 in) inside of the crown. It has been shown that horizontal spray distribution is influenced by canopy density (Wise et al. 2010). This indicates that for the 2015 study the greater spray coverage and spray volumes contributed to higher overall residue concentrations at harvest. This demonstrates the importance of biotic factors such as tree growth (canopy size and density) and abiotic factors such the weather and spray GPA Growers need to be mindful of variability from year to year when managing MRLs.

In this study, compounds of the organophosphate and pyrethroid classes showed the greatest disparity of residue levels between single and multiple applications, and secondarily the spinosyns. There are several factors that could influence this phenomenon. One factor may be the differing plant penetration attributes of the compounds. Diamides and spinosyns have plant penetrative attributes allowing mobility into and beneath the plant cuticle (Bostanion 2012, Wise et al 2017). Organophosphates and pyrethroids remain largely on the plant surface, with limited cuticle penetration. Another factor may be the persistence of the compound. In our study the compounds known to have longer half-lives were more likely to show greatest disparity of residue levels between single and multiple applications (Wise and Whalon 2009).

The results also showed the organophosphate and diamide compounds to be more sensitive to wash-off from the water washing procedure. These factors appear to have the greatest influence on the likelihood of higher or lower residue concentrations at harvest under our treatment regimes.

Fenpropathrin, like most pyrethroids, have limited cuticular penetration, but as lipophilic compounds have natural affinity to cuticular waxes. This is likely one of the factors responsible for the moderate rainfastness seen for pyrethroids in other studies (Hulbert et al. 2011). Fenpropathrin is relatively unstable and degrades rapidly in normal environmental conditions (Akhtar 2004). This may indicate that with a single application, fenpropathrin will begin to degrade quickly resulting in lower concentrations, but with a second application, the residue levels may be maintained at a higher concentration, resulting in concentration differences between the single and multiple application regimes. Residue levels for pyrethroids (fenpropathrin) were shown to be more variable and have greater persistence than spinosysns (spinetoram), with residue levels ranging from 0.89 to 2.93 at 3 DAT (Haviland and Beers 2012). The factors which may cause the difference between the Haviland/ Beers study and this study may include the rainfall levels, crop type, post-harvest washing procedures, and application timing. The average rainfall for the California cherry growing season (February-May) is approximately 136 mm (5.35 in.) (www.intellicast.com) and the average seasonal (April-July) precipitation in Michigan approximates 380+ mm (15.06 in.) (www.enviroweather.msu.edu). However, this is not the rainfall which occurred during the 2014 and 2015 seasons. This difference in rainfall levels for the two growing regions

may indicate that fenpropathrin residues in California may be more persistent with less rainfall potentially causing wash-off.

The next major difference between the studies is fruit type, relates to the use of tart cherries which have thinner skins. The Haviland/ Beers study uses sweet cherries which have thicker skins. Not only is the taxonomy of these fruits very different, but the tart cherries undergo the cold water washing and the sweet cherries do not. This may also indicate why fenpropathrin residue was higher in the Haviland/ Beers study with no residue water washing. While this study suggests that fenpropathrin (with unwashed or washed and single or multiple applications and a 3 day PHI) would be low risk in export markets in most prospective countries, as most of these markets are well harmonized with US MRLs. In this study, residues exceeded the EU's MRL of 0.01 ppm.

To safely target international trade with the EU, one mitigation strategy could be to artificially extend the PHI. According to the degradation curves in this study, this would likely reduce the fenpropathrin residues at harvest below the EU's MRL. The EU has an extreme difference of 500-fold lower MRL for fenpropathrin when compared with the US, therefore, export may always be an issue no matter when the material is applied or the persistence (Haviland and Beers 2012).

Cyantraniliprole is moderately persistent in normal environmental conditions (Dong et al. 2011), and like most diamides, it has translaminar penetration in plant tissues, thus forming a "reservoir" from direct environmental exposure (Bostanian et al. 2012). There were several rain events within the duration of the 2014 trial, the highest being near 12.7 mm (0.5 in.). While diamides are known to be moderately rainfast (Wise et al. 2017), the disparity shown in this study between washed and unwashed

treatments of cyantraniliprole suggests that post-harvest washing procedures can have high impact on residues at harvest. This study suggests cyantraniliprole with unwashed or washed and single or multiple application regimes and a 3 day PHI would be low risk for export to many prospective countries. Most countries are well harmonized with US MRLs. The single spray unwashed residue profile curve in 2014 includes a spike at 21 d, which does not fit the overall decline curve well. This may have been a result of the 0.5 in rainfall event, causing redistribution of canopy residues. Therefore, cherry growers should feel confident if exporting internationally to most prospective markets.

Phosmet, like most organophosphates, has limited cuticular penetration. Phosmet is also known to be highly susceptible to rain wash off (Wise et al 2017) as a majority of the active ingredient remains on the surface of the plant. This study shows similarly that post-harvest washing procedures have high impact on phosmet residues at harvest. This likely explains why residue profiles for phosmet in this study showed very similar concentrations in 2014 and 2015, even though there was more total rainfall in 2015 than in 2014. Phosmet being generally more persistent than other materials, explains the tendency to accumulate with multiple applications, leading to higher residues at harvest. This study suggests that unwashed or washed cherries and single or multiple application regimes and 7 day PHI would be low risk for export to many prospective countries.

Spinetoram, like most spinosyns, has translaminar penetration in plant tissues, thus forming a “reservoir” from direct environmental exposure (Bostanian et al. 2012). This behavior likely contributes to the moderate rainfastness documented for this compound (Wise et al. 2017). A similar pattern was seen in this study, as the washing

procedure had a minimal effect on residues. The effect of the treatment difference was not lost as residue profiles rapidly declined, yet it still resulted in significantly higher residues for the multiple applications treatment. The rapid degradation rate of spinetoram in this study is similar to patterns documented in other studies (DOW 2014). It has also been shown by Haviland and Beers (2012), that spinetoram residues on sweet cherries degrade quickly with concentrations ranging from nondetectable to 0.19 ppm at 0, 3, 7, and 21 DAT. These residues are acceptable for most prospective markets. Therefore, this study's results for spinetoram suggest that unwashed or washed and single or multiple applications and 7 day PHI would be low risk for export to most prospective countries, as well as the markets that are well harmonized with US MRLs. It has been shown in previous studies that spinosyns such as spinetoram are useful products near harvest because the US MRL is similar to other markets and will not cause issues with short persistence (Haviland and Beers 2012).

Summary: This MRL study contains valuable data pertaining to controlling the invasive species SWD, that is crucial to the Michigan cherry industry. With late season SWD, growers are making insecticide sprays nearer to harvest, which increases the risk of MRL violations. This research will help inform grower decisions during late season pest management. It will also contribute to which possible tactics can be used to lower the risks of MRL violations according to specific export targets.

This research provides important data for a residue profiling database to create applications and harvest regimens that best suit growers' needs. The data presented will assist in creating additional degradation curves for the commonly used late season insecticides. Adding additional insecticides to the database is a large factor in achieving

these goals because of different modes of action, penetrative attributes, and environmental persistence. This is just one of many data sets needed to achieve safe and reliable pest management. Yet the goal of this project was to add substantially to the MSU MRL database for tart cherry. Therefore, this project brings us closer to setting more accurate PHI's for growers to use in order to avoid export rejections. This research shows that insecticide residue levels and PHI's could be predicted using specific spray rates and timings by the time of harvest in cherries. With the advantage of making insecticide residue level predictions, fruit can be sprayed at specific rates and timings to obtain residue levels legal for shipment to more locations increasing sales and benefitting the economy.

CHAPTER 4: INFLUENCE OF ADJUVANTS ON INSECTICIDE RESIDUES AND SURFACE PENETRATION AT HARVEST AND ASSOCIATED RISK FOR APPLE AND CHERRY EXPORTS

Abstract

Declining residue profiling was used to determine the influences of adjuvants on the degradation curves of four key insecticides registered for apple and cherry. Surface and subsurface skin penetration residue profiling was also conducted to better understand the decline profile results. Multiple application treatment regimens based on the labeled maximum seasonal usage were tested, including and excluding adjuvants, for their effects on residue levels at harvest. The insecticides, fenpropathrin (Danitol 2.4 EC®), cyantraniliprole (Exirel 0.83 SE™), phosmet (Imidan 70 WP®), and spinetoram (Delegate 25 WG®), and the adjuvants pinene polymers, hydrocarbon resin, petrolatum omega-hydroxypoly (Nu Film 17®), and alkyl aryl polyoxyethylene, ethoxylated alcohols, aliphatic acid (SuperSpread 7000™) were foliar direct applied onto semi-dwarf Red Delicious apples trees (*Malus domestica* Borkhausen) and Montmorency and Balaton tart cherry trees (*Prunus cerasus* Borkhausen) at the Michigan State University (MSU) Trevor Nichols Research Center (TNRC). The residue profiling for apple suggests that fenpropathrin and cyantraniliprole with, and some cases without adjuvant tank mixes would be high risk for international export for most prospective markets. Phosmet with no adjuvant was found to be moderate risk for international export as concentrations fell below many markets, but with NuFilm and SuperSpread tank mixes were high risk for

international export, which will be brought down to acceptable levels without the use of NuFilm or SuperSpread.

The residue profiling for cherry suggests that cyantraniliprole and spinetoram with no adjuvant, and combined with NuFilm or SuperSpread would be low risk for export to most prospective markets.

The skin surface and subsurface studies results indicate a majority of the insecticide concentrations were found in the skin of the apple and cherry with the addition of the adjuvants, NuFilm or SuperSpread and showed significant increase of insecticide residue depending on the adjuvant type. On cherry, cyantraniliprole in combination with Superspread resulted in significantly higher residue located in the cherry skin compared to no adjuvant and also resulted in significantly higher residue in the whole fruit than no adjuvant at the 3 day PHI. SuperSpread combined with cyantraniliprole results in significantly higher cyantraniliprole residue because SuperSpread allows for increased penetration of cyantraniliprole subsurface of the cherry. On apple, phosmet and cyantraniliprole in combination with NuFilm and SuperSpread adjuvants results in higher phosmet and cyantraniliprole residue located in the apple skin compared to the no adjuvant and also resulted in significantly higher residue in the whole fruit than the no adjuvant. SuperSpread and NuFilm combined with phosmet or cyantraniliprole results in significantly higher residue because the adjuvants allow for increased fruit penetration and sticker ability. The increased insecticide residue is a risk for international export. Certainly, with these data, it is very clear that fruit growers need more data to determine which compounds hold the highest risks of rejection for export-bound crops. These data will also support establishment of export

pre-harvest intervals (PHIs) guides that growers can use to avoid load rejections from export-target countries with lower maximum residue limits (MRLs).

Introduction

The United States is a major apple and cherry producing country, with apples being the third most valuable fruit crop grown (Devadoss et al. 2009). Cherries are also valuable, especially to the state of Michigan with the production of 75% of the nation's cherries (NASS 2015). Every state produces apples, but the states of Washington, Michigan, New York, and California dominate the market with 75% of the US production (Krissoff et al. 1997). Most US apple and cherry production relies upon pesticides as an important tool of Integrated Pest Management (IPM) programs to keep fruit clean from insects and disease pests.

While meeting market standards for blemish-free fruit depends on precise spray timings and a wide array of active ingredients, achieving this while maintaining chemical residues at acceptable levels for export markets is a challenge, especially concerning new invasive pests such as the late season brown marmorated stink bug (*Halyomorpha Halys*) (Stal) (BMSB) on apple and the spotted wing drosophila (*Drosophila suzukii*) (SWD) on cherry. The Food Quality Protection Act of 1996 (Schierow 1999) and the Green Movement (Lehman 1993) have added additional challenges and difficulty of maintaining sufficient insecticide options for apple and cherry pest management.

Maximum residue limits (MRLs) are the maximum level of pesticide residues allowed to remain on or in food and feed products (Christova-Bagdassarian et al. 2014) as determined by the USEPA. "MRL" is the term used in much of the world while the

US uses the term tolerance (Winter and Jara 2015). While pre-harvest intervals (PHIs) are set with residue studies, the US Environmental Protection Agency (EPA) performs the risk assessment to assure there is no unacceptable risk with each PHI, to assure residues at harvest do not exceed label tolerances.

Apple and cherry producers must also be aware of global MRLs if they intend to target export markets. Many of the export countries use their own pesticide residue calculation system to obtain their MRLs otherwise identified as the OECD calculator or MRL calculator (Handford et al. 2015). Current national and international array of residue program can cause delays in the global pesticide registration process as many calculation methods differ. Too often this array of different MRL assessment systems and terminology results in un-harmonized global MRLs, which is a direct threat to specialty crop growers who seek access in export markets.

In the United States fenpropathrin, cyantraniliprole, phosmet, and spinetoram are among the most effective insecticides registered for use in apples and cherries. Generally, these materials are recommended for late season insect pests in Michigan (Wise et al, 2016). These compounds are commonly used materials, and have demonstrated efficacy against late season apple and cherry pests. In apple production,, these insecticides are registered as late season tools to control the direct insect pests, including the apple maggot, Rhagoletis pomonella, codling moth, *Cydia pomonella*, and BMSB. On cherry, these insecticides are registered as late season tools to control the direct insect pests, including the cherry fruit fly (CFF), Rhagoletis cingulata, SWD, plum curculio (PC), *Conotrachelus nenuphar* (Herbst), and obliquebanded leafroller (OBLR), *Choristoneura rosaceana* (Harris). Near-harvest pest control is challenging for apple

and cherry growers because the ripening fruit is highly susceptible to injury from insect pests, while the late season sprays must be applied within the labeled PHIs.

Fenpropathrin is a broad spectrum type II synthetic pyrethroid with insecticidal and acaricidal activity (Saryazdi et al. 2014). Fenpropathrin has limited plant cuticular penetration and translocation capabilities (Tucker et al. 1984). The label rate on apple is 0.23-0.46 kg ai/ ha with a 3 day PHI (24 hour REI), and a minimum application interval of 10 days. The label rate on cherry is 0.23-0.46 kg ai/ ha with a 3 day PHI, 24 h REI, and a minimum of 10 day post application interval. Fenpropathrin is most effective on leafhoppers, aphids, fruitworms, leafminers, mites, leafrollers (Wise et al. 2016, Walgenbach and Palmer 2003) and it is relied upon for late season control of BMSB and leafrollers on apple as well as OBLR and SWD on cherry.

Cyantraniliprole is a novel cross-spectrum anthranilic diamide insecticide which has translaminar (forms a reservoir under the plant surface) plant penetration capabilities (Kodandaram et al. 2010). Cyantraniliprole is a systemic insecticide which is effective through ingestion and contact routes. This reduced risk insecticide is effective on a wide range of insects including lepidoptera, hemiptera, and diptera (Wise et al. 2016, Van Steenwyk et al. 2008). The label rate on apple is 0.06-0.15 kg ai/ ha with a 3 day PHI, 12 hour REI (re-entry interval), and a minimum of 7 day application interval. The label rate on cherry is 0.10-0.15 kg ai/ ha with a 3 day PHI, 12 h REI (re-entry interval), and a minimum of 7 day application interval. The primary late season target pest on apple is apple maggot and BMSB and for cherry is SWD and leafrollers.

Phosmet is a broad spectrum organophosphate insecticide which has limited plant cuticular penetration capabilities and is considered a surface material (Wise et al.

2017). It is a conventional insecticide traditionally most relied upon for PC and SWD control in Michigan (Wise et al. 2015, Hoffman et al. 2010), but more recently used as late season control of BMSB and leafrollers on apple as well as SWD and leafrollers on cherry. The label rate on apple is 1.67-4.51 kg ai/ ha with a 7 day PHI, and 7 day REI. The label rate on tart cherry is 1.67-4.51 kg ai/ ha with a 7 day PHI, and 3 day REI. Phosmet is most effective on lepidopteran such as fruitworms, leafrollers, codling moth, and Oriental fruit moth, but is also used for a range of coleopteran and diptera insect pests (Wise et al. 2016, Mouzin and Reed 1979).

Spinetoram is a broad spectrum spinosad insecticide which targets lepidopteran larvae and thrips, it also works broadly on diptera, coleoptera, hemiptera, hymenoptera, isoptera, orthoptera, siphonaptera, thysanoptera, and mites (Salgato 1998, Hogmire 2008). Spinetoram is relied upon as a late season control for BMSB and leafrollers on apple and SWD, leafrollers, cherry fruit fly and PC on cherry. Spinosads are a novel insecticide effective by contact and ingestion and have translaminar plant cuticular penetration capabilities. The Label rate on apple is 0.08-0.12 kg ai/ ha with a 7 day PHI, 4 hour REI, and a minimum of 7 day application interval. The label rate on cherry is 0.08-0.12 kg ai/ ha with a 7 day PHI, 4 h REI, and a minimum of 7 day application interval.

Adjuvants provide a key ingredient in many pesticide prescriptions. Typically they assist or modify the action of the insecticidal active ingredient (Foy 1987). They enhance the pesticides solubility, adsorption, penetration, and translocation of various active ingredients to the target. They can also increase rain fastness, and change the selectivity of the active ingredient (Krogh et al. 2003). It also has been shown that many

pesticides can move into the fruit tissue, especially those which are of chemical classes that hold systemic attributes (Wise et al. 2009, Hoffman et al. 2009). Residues of systemic pesticides penetrate into the plant tissues after an agricultural treatment and have demonstrated to be evident on the surface and inner flesh of fruit (Balinova et al. 2006 and Christia et al. 2015). One way that adjuvants effect pesticide residues is changing the permeability of the plant cuticle and thus increasing penetration of the active ingredient (Ryckaert et al. 2007).

Pinene polymers, hydrocarbon resin, petrolatum omega-hydroxypoly is an adjuvant used as a sticker and extender. This adjuvant contains resin which was first used in the eighteenth century. Sticker adjuvants increase the adhesion of pesticide applications to plants and form a protective film over the target surface (Rajkovic and Markovic 2012). The adhesion and protective film increase the material's resistance to rain wash off. These adjuvants will auto-polymerase to form a film when exposed to air and UV light (Rajkovic and Markovic 2012). The label rate on apple and cherry is 0.18-0.24 liters/ 378.54 liters of water when applied dilute.

Alkyl aryl polyoxyethylene, ethoxylated alcohols, aliphatic acid is a nonionic surfactant adjuvant used as a spreader for more uniform coverage and a spray buffer for the solution. Spreaders used to enhance the bioactivity of pesticides by reducing the surface tension of water (Yoon et al. 2011). This adjuvant also will increase absorption into the leaf surfaces because it is oil based and has a sequestering agent (www.wilburellis.com). The label rate on apple and cherry is 0.12-0.47 L/ 378.54 L of spray solution.

With the importance of late season insecticide-use, and the risk of residue levels exceeding global MRLs, there is need to understand the factors that influence pesticide persistence and residues at harvest. Pesticide degradation is the breakdown of the active ingredient mainly due to abiotic environmental exposures such as ultra violet (UV) light, rainfall, oxidation, dilution, or a biotic factor such as metabolism within the plant tissue (Van Eerd et al. 2003). Ultra violet degradation or photo degradation of insecticides can effectively be in the active ingredient's breakdown from exposure to sunlight. Photo degradation can break down the pesticide's active ingredient on foliage, soil surface of the soil, or ambient air (Burrows et al. 2002).

Pesticide loss from rainfall occurs when precipitation washes the pesticide residue off of the plant material after an application. Rain has critical implications for the fate of pesticides that are sprayed in Eastern US states where precipitation is common throughout the growing season (Wise et al. 2017). With seasonal apple (April-September) precipitation in Michigan being 500+ mm (20.45 in.) and seasonal cherry (April-July) precipitation in Michigan being 380+ mm (15.06 in.) (www.enviroweather.msu.edu), growers often benefit from insecticide delivery tools such as adjuvants to protect their crops from pests. Such pesticide degradation curves may therefore be influenced by tank-mix adjuvants. In addition, side effects of adjuvants may cause higher residue and a decrease in degradation rate (Ryckaert et al. 2007) in certain circumstances.

To address the challenge of meeting global MRLs, growers need information and tools to assist in determining which insecticides and adjuvants to use, at what rate, and when to such use will yield a low risk of MRL violation for specific market destinations.

For producer's adoption, such a decision tool should be easy to use and very accessible for producers and their labors. Therefore, disparity Index is a term developed to measure MRL differences, which is the US MRL divided by the lowest foreign MRL. This calculation equals the greatest difference in the US MRL and lowest foreign MRL (JC Wise, unpublished). Such a calculated value provides a simple and direct way to identify which compounds are the highest risk for growers targeting global export markets. Further, this approach allows a single value for difference instead of multiple values which trying to explain how the difference affect various markets.

The objectives of the following study was 1) to determine several key insecticide residue concentrations on apple and cherry fruit at harvest from the addition of NuFilm and SuperSpread adjuvants and 2) to locate where the insecticide residues are in the fruit tissue and how these adjuvants affected the pesticide's localities with surface via subsurface penetrative studies. The overall aim was to inform a support tool for growers to use in deciding which insecticides to use against the late season insect pests, as well as, the new invasive BMSB and SWD. The essential outcome of this study was to avoid export MRL risks.

Materials and Methods

Field Plots-Apple

Field work was conducted at the MSU Trevor Nichols Research Center (TNRC) in Fennville, MI, USA (latitude 42.5951°: longitude -86.1561°). Plots were established in a 0.30 m tall, 27 year-old 'Red Delicious' (Indigo) apple (*Malus* Miller; Rosaceae) planting at TNRC with one row buffers. The plot size was three consecutive trees with a

6.10 m row width and 3.05 m tree spacing or total plot dimensions of 6.10 m wide and 9.14 m long, with a total area of 55.74 square m. Treatment plots were replicated three times and set up in a randomized complete block (RCB) design.

Field Plots-Cherry

Field work was conducted at the MSU Trevor Nichols Research Center (TNRC) in Fennville, MI, USA (latitude 42.5951° : longitude -86.1561°). Plots were established in a 0.30 m tall, six year-old 'Balaton' (Tart Cherry 5) cherry planting at TNRC. Treatment plots were replicated 3 times and set up in a design which alternated active ingredients so there would not be cross contamination of active ingredients. These alternations of rows were considered the buffer rows. The plot size was 10 consecutive trees with a 6.10 m row width and 4.57 m tree spacing or total plot dimensions of 6.10 m wide and 45.72 m long, with a total area of 279 sq. m.

Applications-Apple

Treatment applications were made at maximum label rates prior to fruit harvest. Applications were made for each of five insecticides according to the maximum allowed on the current label for pome fruits: fenpropathrin (Danitol 2.4 EC, Valent U.S.A., Walnut Creek, CA) at 0.06 kg active ingredient (AI)/ ha (21.3 fl oz formulated product per acre), cyantraniliprole (Exirel 0.83 SE, DuPont Crop Protection, Wilmington, DE) at 0.15 kg active ingredient (AI)/ ha (20.5 fl oz formulated product per acre), phosmet (Imidan 70 WP, Gowan Company, Yuma, AZ) at 2.35 kg active ingredient (AI)/ ha (3 lb formulated product per acre), spinetoram (Delegate 25 WG, DOW AgroSciences, Indianapolis, IN) at

0.12 kg active ingredient (AI)/ ha (7.0 oz formulated product per acre) (Table 13). A water pH buffering agent was used with Imidan 70 WP as it is its intended use commercially. This pH buffer is aliphatic polycarboxylate (TriFol L®, Wilbur-Ellis Company, Fresno, CA) at 0.24 L per 378.54 L (0.5 pt/ 100 gal). The adjuvants used were pinene polymers, hydrocarbon resin, petrolatum omega-hydroxypoly (Nu Film 17, Miller Chemical and Fertilizer Corporation, Hanover, PA) at 0.24 liters per 378.54 liters (8 fl oz/ 100 gal), and alkyl aryl polyoxyethylene, ethoxylated alcohols, aliphatic acid (SuperSpread 7000, Wilbur-Ellis Company, Fresno, CA) at 0.47 liters per 378.54 liters (1 pint/ 100 gal) (Table 13). The application dates for apple are listed in table 13.

Test materials were applied with an FMC 1029 airblast sprayer calibrated to deliver material for full coverage at 935 L/ha (100 gallons per acre), 1.12 meters per second (2.5 miles per hour), and an 30.28 liter (8.0 gallon) tank mix. Regular maintenance foliar applications were applied to all treatments and included the fungicides trifloxystrobin (Flint®), myclobutanil (Rally®), manganese ethylenebisdithiocarbamate (Penncozeb®), difenoconazole (Inspire Super®), penthiopyrad (Fontelis®), pyrimethanil (Scala®), captan (Captan®), and dodine (Syllit®). The insecticides esfenvalerate (Asana®), and methomyl (Lannate®) were applied to all plots for control of foliar feeding pests. Diuron (Parrot™) and 2, 4-D (Weedar®) were banded below the trees for weed control.

Table 13. Apple compounds and application dates.

Treatment/ Formulation	Application Dates
UTC	
Danitol 2.4 EC	20-Sep, 29-Sep
Danitol 2.4 EC NuFilm L	20-Sep, 29-Sep
Danitol 2.4 EC SuperSpread 7000 L	20-Sep, 29-Sep
Exirel 0.83 SE	13-Sep, 20-Sep, 27-Sep
Exirel 0.83 SE NuFilm L	13-Sep, 20-Sep, 27-Sep
Exirel 0.83 SE SuperSpread 7000	13-Sep, 20-Sep, 27-Sep
Imidan 70 WP TriFol L	13-Sep, 20-Sep, 27-Sep
Imidan 70 WP TriFol L NuFilm L	13-Sep, 20-Sep, 27-Sep
Imidan 70 WP TriFol L SuperSpread 7000	13-Sep, 20-Sep, 27-Sep
Delegate 25 WG	13-Sep, 20-Sep, 27-Sep
Delegate 25 WG NuFilm L	13-Sep, 20-Sep, 27-Sep
Delegate 25 WG SuperSpread 7000	13-Sep, 20-Sep, 27-Sep

Applications-Cherry

Treatment applications were made at maximum label rates prior to fruit harvest. Applications were made for each of two insecticides according to the maximum allowed (current label) for stone fruits: cyantraniliprole (Exirel 0.83 SE, DuPont Crop Protection, Wilmington, DE) at 0.15 kg active ingredient (AI)/ ha (20.5 fl oz formulated product per acre), spinetoram (Delegate 25 WG, DOW AgroSciences, Indianapolis, IN) at 0.12 kg

active ingredient (AI)/ ha (7.0 oz formulated product per acre) (Table 14). A water pH buffering agent was used with Imidan 70 WP as it is its intended use commercially. This pH buffer is aliphatic polycarboxylate (TriFol L, Wilbur-Ellis Company, Fresno, CA) at 0.24 L per 378.54 L (0.5 pt/ 100 gal) The adjuvants used were pinene polymers, hydrocarbon resin, petrolatum omega-hydroxypoly (Nu Film 17, Miller Chemical and Fertilizer Corporation, Hanover, PA) at 0.24 liters per 378.54 liters (8 fl oz/ 100 gal), and alkyl aryl polyoxyethylene, ethoxylated alcohols, aliphatic acid (SuperSpread 7000, Wilbur-Ellis Company, Fresno, CA) at 0.47 liters per 378.54 liters (1 pint/ 100 gal) (Table 14). The Delegate treatments were applied on 14-Jun for the first application, 21-Jun for the second application, and 27-Jun for the last application. The Exirel treatments were applied on 14-Jun for the first application, 21-Jun for the second application, and 28-Jun for the last application. Test materials were applied with an FMC 1029 airblast sprayer calibrated to deliver material for full coverage at 935 L/ha (100 gallons per acre), 1.12 meters per second (2.5 miles per hour), and a 94.64 liter (25.0 gallon) tank mix. Regular cherry maintenance foliar applications were applied to all treatments and included the fungicides copper hydroxide (Kocide 3000®), chlorothalonil (Bravo Weather Stik®), sulfentrazone Elite®, myclobutanil (Rally®), fenbuconazole (Indar®), trifloxystrobin (Gem 500®), thiophanate-methyl (Topsin®), copper oxychloride/ copper hydroxide (Badge®), and basic copper sulfate (Cuprofix Ultra®). The insecticides phosmet (Imidan®), and chlorpyrifos (Lorsban®) were applied to all plots for control of PC and leafrollers. Fluazifop (Fusilade DX®), and 2, 4-D (Weedar®) were banded below the trees for weed control.

Table 14. Cherry compounds and application dates

Treatment/ Formulation	Application Dates
UTC	
Delegate 25 WG	14-Jun, 21-Jun, 27-Jun
Delegate 25 WG NuFilm L	14-Jun, 21-Jun, 27-Jun
Delegate 25 WG SuperSpread 7000 70 EC	14-Jun, 21-Jun, 27-Jun
Exirel 0.83 SE	14-Jun, 21-Jun, 28-Jun
Exirel 0.83 SE NuFilm L	14-Jun, 21-Jun, 28-Jun
Exirel 0.83 SE SuperSpread 7000 70 EC	14-Jun, 21-Jun, 28-Jun

Sample Collection-Apple

Residue samples were collected, prepared, and recovered using methods based on US EPA standards for GLP field residue studies (Jiang 2005). These methods are also known as the QuEChERS method or “gold standard” for pesticide residue analysis for a variety of different sample types (Kong et al. 2016). QuEChERS is an abbreviation for quick, easy, cheap, effective, rugged, and safe analysis. Three (labeled) gallon Ziploc bags were used to collect 24 total fruit with 8 apples per bag for each replicate sample. The apples were selected randomly from the N, S, E, W cardinal direction sides of the tree, low/ middle/ high, and shielded/ exposed portions of the tree crown. Shielded was any fruit at least 60.96 cm (24 in) inside of the tree crown and exposed was the outer 60.96 cm 24 in. Low was the bottom 1.22 m (four ft), middle was the center 1.22 m (four feet), and high was the upper 1.22 m (four ft) of the tree crown. The

samples were collected on the specific day after treatment (DAT) or day after last application and ± 1 day for the 3, 7, 14, 21, and 28 DAT samples. The dates for all of the samples collections are listed in table 15.

Table 15. Apple sampling timing and dates

Treatment/ Formulation	Sample DAT Number					
	1	3	7	14	21	28
UTC	30-Sep	3-Oct	6-Oct	13-Oct	20-Oct	27-Oct
Danitol 2.4 EC	30-Sep	3-Oct	6-Oct	13-Oct	20-Oct	27-Oct
Danitol 2.4 EC NuFilm L	30-Sep	3-Oct	6-Oct	13-Oct	20-Oct	27-Oct
Danitol 2.4 EC SuperSpread 7000 L	30-Sep	3-Oct	6-Oct	13-Oct	20-Oct	27-Oct
Exirel 0.83 SE	28-Sep	30-Sep	5-Oct	12-Oct	19-Oct	26-Oct
Exirel 0.83 SE NuFilm L	28-Sep	30-Sep	5-Oct	12-Oct	19-Oct	26-Oct
Exirel 0.83 SE SuperSpread 7000	28-Sep	30-Sep	5-Oct	12-Oct	19-Oct	26-Oct
Imidan 70 WP TriFol L	28-Sep	30-Sep	5-Oct	12-Oct	19-Oct	26-Oct
Imidan 70 WP TriFol L NuFilm L	28-Sep	30-Sep	5-Oct	12-Oct	19-Oct	26-Oct
Imidan 70 WP TriFol L SuperSpread 7000	28-Sep	30-Sep	5-Oct	12-Oct	19-Oct	26-Oct
Delegate 25 WG	28-Sep	30-Sep	5-Oct	12-Oct	19-Oct	26-Oct
Delegate 25 WG NuFilm L	28-Sep	30-Sep	5-Oct	12-Oct	19-Oct	26-Oct
Delegate 25 WG SuperSpread 7000	28-Sep	30-Sep	5-Oct	12-Oct	19-Oct	26-Oct

Sample Collection-Cherry

Residue samples were collected, prepared, and recovered using the QuEChERS method. One labeled gallon Ziploc bag was used to collect 0.91 kg (2 lbs) total fruit per bag for each replicate sample. The cherries were collected from the same tree locations and days after treatment as the apple study. The sampling dates and timings are listed in table 16.

Table 16. Cherry sampling timing and dates

Treatment/ Formulation	Sample DAT Number					
	1	3	7	14	21	28
UTC	28-Jun	30-Jun	5-Jul	11-Jul	18-Jul	25-Jul
Delegate 25 WG	28-Jun	30-Jun	5-Jul	11-Jul	18-Jul	25-Jul
Delegate 25 WG NuFilm L	28-Jun	30-Jun	5-Jul	11-Jul	18-Jul	25-Jul
Delegate 25 WG SuperSpread 7000 70 EC	28-Jun	30-Jun	5-Jul	11-Jul	18-Jul	25-Jul
Exirel 0.83 SE	29-Jun	1-Jul	5-Jul	12-Jul	19-Jul	26-Jul
Exirel 0.83 SE NuFilm L	29-Jun	1-Jul	5-Jul	12-Jul	19-Jul	26-Jul
Exirel 0.83 SE SuperSpread 7000 70 EC	29-Jun	1-Jul	5-Jul	12-Jul	19-Jul	26-Jul

Cherry Washing

Cherries were washed using a water washing or cooling method to simulate the industry methods modified from Cargill (1969). The industry generally uses 946.35 L (250 gal) tanks which run water at around 30.28-37.85 L (8-10 gal) per minute for a short period of time. The rate slows to 15.14-22.71 L (4-6 gal) per minute and finally the cherries sit in cooling tanks for approximately 2 h. Study methods closely resemble the

industry on a small scale. The research scale washing method was calibrated 3 times using a stop watch and sprinkler valves before each use. The flow rate was timed to reach the graduated gallon marks on 18.93 L (5 gal) buckets.

Cherries were picked for each prescribed DAT, then brought back to the water washing location. The water washing system was connected to a water tap. There was a single hose connected to the tap which had a four hose splitter manifold connected. There were four hoses connected to each manifold which were connected to and entered the side wall of four-18.93 L (5 gal) buckets. Each hose also had an in-line sprinkler valve with flow control so the flow rate could be regulated entering the 18.93 L (5 gal) buckets. Mesh screens were tied to the tops of each bucket so the cherries did not fall out of the buckets while the water ran into and out of the buckets. The cherries were placed into clean buckets and rinsed for 2 hrs ± 15 min at 18.93 L (5 gal) per minute in 2014 and 7.57 L (2 gal) per minute flow rate for the 2015 season. Variation was due to differing water pressure at the tap. After the cold water rinse, the cherries were placed back into their labeled Ziploc® (SC Johnson, Racine, Wisconsin) bags. The cherries were then taken out of their labeled bags one repetition at a time and one treatment at a time to prevent cross contamination. The cherries were each pitted with a sanitized Leifheit® single cherry pitter (Leifheit, Nassau, Germany), cutting board, and purple Nitrile gloves. All equipment was sanitized with acetone and gloves changed between each repetition and treatment to prevent cross contamination. Once the cherries were pitted they were placed back into the original sample bag representing the sample. There were 20 ± 5 cherries randomly chosen and placed into a separate bag for the skin penetration profiling study. The cherries in the Ziploc bag were weighed to

ensure a minimum of 0.91 kg (2 lbs) per sample for the 28 day decline study. All cherries are put into a -20° C chest freezer (Kenmore®, Hoffman Estates, Ill.) and monitored to assure temperature ranges did not rise above -5 ° C for storage until homogenization procedures.

Sample Processing-Apple

Once all the apples were picked for that specific DAT, they were brought back to the lab to be processed. Apples were taken out of their labeled bags, one repetition and treatment at a time to prevent cross contamination. Apples were each cut into quarters with a clean kitchen knife and cutting board while wearing Nitrile gloves. All equipment was sanitized with acetone and gloves changed between each repetition and treatment to prevent cross contamination. Once each apple was cut, opposite quarters were placed back into one of the three original sample bags representing the 28 day decline study sample. The remaining quarters were placed into a separate bag for the skin penetration profiling experiment. All of the quarters representing the 28 day decline study in the Ziploc® bag were weighed and equaled a minimum of 1.81 kg (4 lbs) and a minimum of 28 fruit. The quarters that represented the skin penetration profiling experiment weighed approximately 1.81 kg (4 lbs). The apple samples were then put into a -20° C chest freezer (Kenmore®, Hoffman Estates, Ill.) and monitored to assure temperature ranges did not rise above -5 ° C for storage until homogenization procedures.

Homogenization Procedures-Apple and Cherry

Once all samples were collected for the harvest season, the 28 day decline study samples were ground using a commercial Hobart® food processor (Hobart Corporation, Troy, OH) beginning with the latest sample date (28 DAT) and working towards earliest DAT. Six hundred g of dry ice were added to each sample to prevent softening of the fruit while processing. Each sample was ground for 5 min. Samples were taken with a clean sanitized spoon from all four quadrants of the homogenous ground sample to fill clean labeled sample 120 ml jars (Qorpak Bottle Beakers®, Berlin Packaging, Chicago IL). Sample jars were then placed back into the freezer. The food processor was dissembled and all parts and tools were sanitized with acetone to prevent cross contamination between each treatment. Twenty four h later, the samples were taken out of the freezer and ten gram samples were removed and placed into clean labeled jars. Next, four grams of magnesium sulfate, one gram of sodium chloride, and 15 ml of HPLC grade dichloromethane were added to the new jars. The samples were placed into the refrigerator for two days to separate fruit tissue from the compound. The samples were then decanted through 12 g of reagent-grade anhydrous sodium sulfate (EMD Chemicals, Inc.) to remove water for one hour. The samples were then dried by evaporation under a chemical hood at ambient temperature and the remaining particles were brought back up with two ml of acetonitrile. The final two ml were transferred to a two ml vial (Agilent Technologies, Santa Clara, CA) for HPLC analysis.

Fruit Penetration Profiling Dissection-Apple

A sub-set of fruit samples from the 1 DAT samples were frozen and held for fruit penetration profiling dissections. Twelve apple quarters were randomly selected and one core sample per quarter taken. Following methods from Wise et al 2009, a 9 mm coring tool was used to core each frozen quarter from the inner flesh outward towards the skin. Next, each core taken was sliced into four separate slices until the correct weight for each sample was obtained. Skin slices were ± 5 g per sample, the 2 mm outer flesh were ± 7 g, the middle 10 mm flesh was ± 10 g, and the inner 5 mm flesh was ± 10 g. These cuts were made using a razor blade, and each section was placed in its own labeled jar with 10 ml of dichloromethane. The razor blade, coring tool, and cutting board, which all the cuts were made on, were sterilized using acetone between each core section cutting. Nitrile gloves were worn and changed between each core section.

Fruit Penetration Profiling Dissection-Cherry

A sub-set of fruit samples from the 1 DAT samples were frozen and held for fruit penetration profiling dissections. Twenty cherries were randomly selected, halved with a razor blade, and cored once per half. Following methods by Hoffmann et al 2009, a 5 mm coring tool was used to core each half from the inner flesh outward towards the skin. Next, each core taken was sliced into three separate slices with a razor blade until the correct weight for each sample was obtained. Skin slices were ± 0.5 g per sample, the 2 mm outer flesh were ± 0.5 g, and the inner 3 mm flesh was ± 0.5 g. The cuts and coring were conducted inside of a freezer on a cutting board which was placed on top of

extra ice packs. The freezer door was open and the cutting was conducted with hands inside to prevent thawing of cherries. Each section was placed in its own labeled jar with 10 ml of dichloromethane. The razor blade, coring tool, and cutting board were sterilized using acetone between each core section cutting. Nitrile gloves were worn and changed between each core section.

Sample Extraction-Apple and Cherry

Levels of parent compound were quantified using a waters 2695 separator module High Profile Liquid Chromatography (HPLC) equipped with a Waters MicroMass ZQ mass spectrometer detector (Waters, Milford, MA), and a C_{18} reversed phase column (50 by 3.0 mm bore, 3.5 µm particle size, (Waters, Milford, MA). The mobile phase, solvent A was with water and 0.1% formic acid. Solvent B was with acetonitrile with 0.1% formic acid (Table 17). Solvent A began at 80% and solvent B at 20% with a gradient and the column temperature of 20 degrees Celsius (Table 18). A standard was developed for each insecticide to compare the experimental concentrations. The standards of the insecticides were massed and diluted into solution with acetonitrile. The serial dilutions were made from the stock solution. The concentrations used were 7.57 g/ml, 0.155 g/ml, 0.0757 g/ml, 0.00155 g/ml, 0.000757 g/ml, and 0.0000155 g/ml.

Table 17. The mobile phase for each insecticide used for HPLC residue analysis.

Chemical	Solvent A	Solvent B	Flow Rate (ml/min)
Fenpropathrin	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.3
Cyantraniliprole	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.3
Phosmet	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.3
Spinetoram	0.1 % Formic Acid	0.1 % Formic acid in acetonitrile	0.3

Table 18. The gradient mobile phase flow used for each insecticide for HPLC residue analysis.

Active Ingredient	Time (min)	Solvent A (%)	Solvent B (%)
Fenpropathrin	0	80	20
	4	10	90
	4.5	10	90
	4.6	80	20
	10	80	20
Cyantraniliprole	0	80	20
	1	80	20
	4	20	80
	6	20	80
	6.1	80	20
	11	80	20
Phosmet	0	80	20
	1	80	20
	3	50	50
	3.1	20	80
	4	20	80
	4.1	80	20
	8	80	20
Spinetoram	0	80	20
	1	80	20
	3	50	50
	3.1	20	80
	4	20	80
	4.1	80	20
	8	80	20

Table 19. The ions (m/z) monitored, detector dwell time, and cone voltages for detection of the insecticides in HPLC residue analysis.

Chemical	Channel 1	Channel 2	Dwell (s)	Cone1 (V)	Cone2 (V)
Fenpropathrin	265	181	0.5	30	45
Cyantraniliprole	284	484	0.5	50	25
Phosmet	209	175	0.5	55	55
Spinetoram	872.2	886	0.5	55	55

The first step was to determine the range of concentrations, and highest concentration in range. The next step was to make desired concentrations with distilled acetonitrile based off the compound's molecular weight. The stock solution was used to make the next dilution, and this solution was used to make the next dilution, etc. Every other dilution was 100 fold and the dilutions in between were 50 fold. The HPLC level of quantification was 0.08 µg/g parts per million (ppm) of active ingredient, and level of detection was 0.038 ppm.

The residue data for each compound were analyzed with mixed models using the MIXED procedure in SAS 9.4 (SAS Institute, Cary, NC, 2013). The decline fruit residue studies were analyzed with repeated measures best adjusted using an unstructured and a first-order heterogeneous autoregressive covariance structure. Repetition and treatment were used as subjects of repeated measurements. When the main effects or their interactions were statistically significant ($P < 0.05$), examination i.e. slicing of interactions within main effects was performed, *F*-tests (Acimovic et al. 2014) were conducted and pairwise or specific time or treatment comparisons were conducted using *t*-tests ($\alpha = 0.05$). The residue data for the skin penetration profiling studies were analyzed with the mean proportions of residue in a particular condition were compared

across fruit tissue type by an ANOVA. Mean separation was done using least significance difference (LSD).

Results

Residue Decline Profiles-Apple

Fenpropathrin was detected throughout the 28 day decline residue profile with a general decrease in concentrations for the no adjuvant, NuFilm, and SuperSpread tank mixes (Figure 5). The no adjuvant, NuFilm, and SuperSpread tank mixes did not result in a significant difference for the overall sample set (F= 21.42, df= 12, P=0.8236). Partitioning the repeated measure analysis, there was significant effect of day main effects observed at 1 DAT with no adjuvant greater than NuFilm (F= 7.65, df= 28.03, P=0.0083) as well as no adjuvant greater than SuperSpread (F= 7.65, df= 28.03, P=0.0074). There was no significant effect of day main effects observed at 3 DAT (F= 2.37, df= 28.03, P=0.1119), 7 DAT (F= 0.75, df= 28.03, P=0.4794), 14 DAT (F= 3.21, df= 28.03, P=0.0554), 21 DAT (F= 0.77, df= 28.03, P=0.4732), and 28 DAT (F= 1.52, df= 28.03, P=0.2371) between the no adjuvant, NuFilm, and SuperSpread treatments. Mean residue values for no adjuvant, NuFilm, and SuperSpread tank mixes were above the US MRL of 5 ppm for all sample dates, but the addition of adjuvants did not exasperate fruit residue levels at the 14 day PHI. Internationally, fenpropathrin with all tank mixes and 14 day PHI would be high risk for export to most prospective countries. Fenpropathrin concentrations exceeded MRLs of Mexico at 5 ppm, Canada at 5 ppm, China at 5 ppm, Taiwan at 0.5 ppm, and Vietnam's MRL of 5 ppm and would not be

accepted for export to those countries for no adjuvant, NuFilm, or SuperSpread tank mixes with a 14 day PHI.

Cyantraniliprole was detected throughout the 28 day decline residue profile with a general decrease in concentrations for the no adjuvant, NuFilm, and SuperSpread tank mixes (Figure 5). The no adjuvant, NuFilm, and SuperSpread tank mixes did not result in a significant difference for the overall sample set (F= 0.39, df= 27.3, P=0.9394). Partitioning the repeated measure analysis, there was no significant effect of day main effects observed at 1 DAT (F= 1.21, df= 36, P=0.3112), 3 DAT (F= 1.59, df= 36, P=0.2177), 7 DAT (F= 1.25, df= 36, P=0.2993), 14 DAT (F= 0.81, df= 36, P=0.4517), 21 DAT (F= 2.96, df= 36, P=0.0648), and 28 DAT (F= 1.2, df= 36, P=0.312) between no adjuvant, NuFilm, and SuperSpread treatments. Mean residue values for no adjuvant, NuFilm, and SuperSpread tank mixes were above the US MRL of 1.5 ppm for all sample dates, but the addition of adjuvants did not exasperate fruit residue levels at the 3 day PHI. Internationally, cyantraniliprole with all tank mixes and 3 day PHI would be high risk for export to most prospective countries. Cyantraniliprole concentrations exceeded MRLs of Mexico at 1.5 ppm, Canada at 1.5 ppm, China with no established MRL, Taiwan with no established MRL, and Vietnam's MRL of 0.8 ppm and would not be accepted for export to those countries for no adjuvant, NuFilm, or SuperSpread tank mixes with a 3 day PHI.

Phosmet was detected throughout the 28 day decline residue profile with a general decrease in concentrations for the no adjuvant, NuFilm, and SuperSpread tank mixes (Figure 5). The no adjuvant, NuFilm, and SuperSpread tank mixes did not result in a significant difference for the overall sample set (F= 1.4, df= 23.1, P=0.2404).

Partitioning the repeated measure analysis, there was significant effect of day main effects observed at 1 DAT with NuFilm greater than no adjuvant (F= 3.28, df= 35.81, P=0.0482) as well as 7 DAT with SuperSpread greater than no adjuvant (F= 7.58, df= 35.81, P=0.0021). There was no significant effect of day main effects observed at 3 DAT (F= 1.86, df= 35.81, P=0.1711), 14 DAT (F= 0.39, df= 35.81, P=0.6781), 21 DAT (F= 1.11, df= 35.81, P=0.3418), and 28 DAT (F= 1.12, df= 35.81, P=0.3388) between the no adjuvant, NuFilm, and SuperSpread treatments. While residue values for phosmet with no adjuvant was below the US MRL for the 7 day PHI, the addition of SuperSpread tank resulted in residues above the US MRL of 10 ppm. Internationally, phosmet with no adjuvant and 7 day PHI would be moderate risk for export to many prospective markets. Phosmet concentrations fell below the MRL for Mexico, Canada, Saudi Arabia, Thailand, and Vietnam's MRL. Phosmet concentrations exceeded China, Taiwan, Brazil, and Israel's MRL, making international trade unacceptable. Phosmet with addition of NuFilm and 7 day PHI would be high risk for export to most prospective countries. Phosmet concentrations exceeded MRLs of Mexico at 10 ppm, Canada at 10 ppm, China at 3 ppm, Taiwan at 2 ppm, Brazil at 1 ppm, Israel at 0.5 ppm, Thailand at 10 ppm, Saudi Arabia at 10 ppm, and Vietnam's MRL of 10 ppm and would not be accepted for export to those countries with NuFilm in the tank mix at a 7 day PHI. Mean residue values for phosmet with SuperSpread and 7 day PHI would be high risk for export to most prospective countries. Phosmet concentrations exceeded MRLs of Mexico, Canada, China, Taiwan, Brazil, Israel, Thailand, Saudi Arabia, and Vietnam's MRL and would not be accepted for export to those countries with SuperSpread in the tank mix at a 7 day PHI.

Spinetoram was detected throughout the 28 day decline residue profile with a rapid decrease in concentrations for the no adjuvant, NuFilm, and SuperSpread tank mixes (Figure 5). The no adjuvant, NuFilm, and SuperSpread tank mixes resulted in a significant difference for the overall sample set (F= 39.61, df= 24, P=0.0001). Partitioning the repeated measure analysis, there was significant effect of day main effects observed at 1 DAT with no adjuvant greater than NuFilm (F= 211.75, df= 12.9, P=0.0001), SuperSpread greater than no adjuvant (F= 211.75, df= 12.9, P=0.0179), as well as SuperSpread greater than NuFilm (F= 211.75, df= 12.9, P=0.0001). There was significant effect of day main effect observed at 3 DAT with no adjuvant greater NuFilm (F= 137.52, df= 12.9, P=0.0001) as well as SuperSpread greater than NuFilm (F= 137.52, df= 12.9, P=0.0001). There was no significant effect of day main effect observed at 3 DAT between no adjuvant and SuperSpread (F= 137.52, df= 12.9, P=0.6111). There was significant effect of day main effects observed at 7 DAT with no adjuvant greater than NuFilm (F= 79.68, df= 12.9, P=0.0001), no adjuvant greater than SuperSpread (F= 79.68, df= 12.9, P=0.0002), as well as SuperSpread greater than NuFilm (F= 79.68, df= 12.9, P=0.0001). There was significant effect of day main effects observed at 14 DAT with no adjuvant greater than NuFilm (F= 66.25, df= 12.9, P=0.0001), no adjuvant greater than SuperSpread (F= 66.25, df= 12.9, P=0.0001), as well as SuperSpread greater than NuFilm (F= 66.25, df= 12.9, P=0.0223). There was significant effect of day main effect observed at 21 DAT with no adjuvant greater than NuFilm (F= 27.35, df= 12.9, P=0.0001) as well as no adjuvant greater than SuperSpread (F= 27.35, df= 12.9, P=0.0001). There was no significant effect of day main effect observed at 21 DAT between NuFilm and SuperSpread (F= 27.35, df= 12.9,

P=1). There were no significant effects of day main effect observed at 28 DAT between the no adjuvant, NuFilm, and SuperSpread treatments (*F*= 2.95, df= 12.9, *P*=0882).

Spinetoram with the addition of NuFilm or SuperSpread resulted in a significantly lower concentration of spinetoram than the spinetoram with no adjuvant at 7 DAT. Mean residue values for the no adjuvant, NuFilm, and SuperSpread tank mixes were below the US MRL of 0.2 ppm for the 7 day PHI. Internationally, no adjuvant, NuFilm and SuperSpread would be low risk for export to most prospective markets. Spinetoram concentrations fell below the MRLs of Mexico at 0.2 ppm, Canada at 0.2 ppm, Saudi Arabia at 0.05 ppm, Thailand at 0.05 ppm, Vietnam at 0.05 ppm, China with no established MRL, Taiwan at 0.2 ppm, and Israel's MRL of 0.1 ppm at 7 day PHI, making export to those countries acceptable. Mean residue values for no adjuvant and SuperSpread exceeded Brazil's MRL of 0.02 ppm and would not be accepted for export at 7 day PHI.

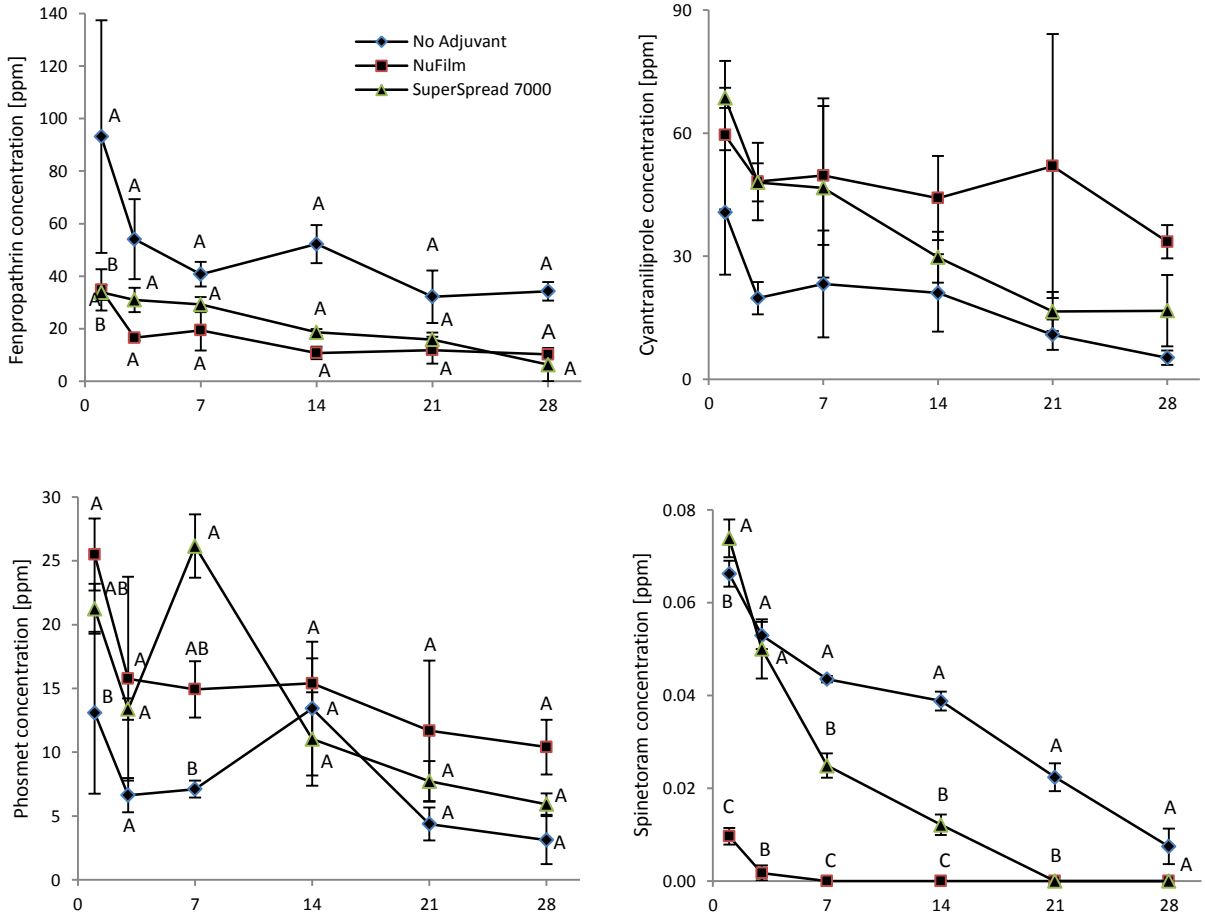

Figure 5. Fenpropathrin, cyantraniliprole, phosmet, spinetoram, alone and in combination with NuFilm and SuperSpread for 1, 3, 7, 14, 21, and 28 day decline residue profiles in 'Red Delicious' apple fruit. Concentration means within one date followed by different letters are significantly different (*t-tests p<0.05*). Error bars represent standard error of the mean (SEM). Fenpropathrin has a United States MRL of 5 ppm, while the lowest international MRL at a perspective market is 0.01 ppm in Saudi Arabia. Cyantraniliprole has a United States MRL of 1.5 ppm, while the lowest international MRL at a perspective market is 0.8 ppm in Saudi Arabia. Phosmet has a United States MRL of 10 ppm, while the lowest international MRL at a perspective market is 2 ppm in Taiwan.

Residue Decline Profiles-Cherry

Cyantraniliprole was detected throughout the 28 day decline residue profile with a general decrease in concentrations for the no adjuvant, NuFilm, and SuperSpread tank mixes (Figure 6). The no adjuvant, NuFilm, and SuperSpread tank mixes did not result in a significant difference for the overall sample set (F= 1, df= 17.7, P=0.4822). Partitioning the repeated measure analysis, there was significant effect of day main effects observed at 3 DAT with SuperSpread greater than no adjuvant (F= 3.41, df= 35.9, P=0.0442), as well as SuperSpread greater than NuFilm (F= 3.41, df= 35.9, P=0.0438). There was no significant effect of day main effects observed at 3 DAT between NuFIlm and no adjuvant (F= 3.41, df= 35.9, P=0.1078). There were no significant effects of day main effect observed at 1 DAT (F= 0.63, df= 35.9, P=0.5402), 7 DAT (F= 0.25, df= 35.9, P=0.7816), 14 DAT (F= 0.77, df= 35.9, P=0.4709), 21 DAT (F= 1.34, df= 35.9, P=0.2758), and 28 DAT (F= 0, df= 35.9, P=0.9955) between the no adjuvant, NuFilm, and SuperSpread treatments. Mean residue values for no adjuvant, NuFilm, and SuperSpread tank mixes were below the US MRL of 6 ppm at the 3 day PHI and for all sample dates. Internationally, cyantraniliprole with all tank mixes and would be low risk for export to most prospective countries. Cyantraniliprole concentrations fell below Mexico, Canada, EU, Japan, Australia, and Korea's MRLs all at 6 ppm and would be accepted for export to those countries with a 3 day PHI.

Spinetoram was detected throughout the 28 day decline residue profile with a general decrease in concentrations for the no adjuvant, NuFilm, and SuperSpread tank mixes (Figure 6). The no adjuvant, NuFilm, and SuperSpread tank mixes resulted in a significant difference for the overall sample set (F= 2.8, df= 22.8, P=0.0203). Partitioning

the repeated measure analysis, there was significant effect of day main effects observed at 1 DAT with no adjuvant greater than NuFilm (F= 7.45, df= 33.59, P=0.0113) as well as no adjuvant greater than SuperSpread (F= 7.45, df= 33.59, P=0.0055). There was no significant effect of day main effects observed at 1 DAT between NuFilm and SuperSpread (F= 7.45, df= 33.59, P=0.9486). There was a significant effect of day main effects observed at 3 DAT with no adjuvant greater than NuFilm (F= 8.84, df= 33.59, P=0.007) as well as no adjuvant greater than SuperSpread (F= 8.84, df= 33.59, P=0.0023). There was no significant effect of day main effects observed at 3 DAT between NuFilm and SuperSpread (F= 8.84, df= 33.59, P=0.8847). There was no significant effect of day main effects observed at 7 DAT (F= 1.26, df= 33.59, P=0.2961), 14 DAT (F= 0.57, df= 33.59, P=0.5704), 21 DAT (F= 2.09, df= 33.59, P=0.1395), and 28 DAT (F= 1.38, df= 33.59, P=0.2664) between no adjuvant, NuFilm, and SuperSpread treatments. Thus, while spinetoram with the addition of NuFilm and SuperSpread resulted in significantly lower concentrations of spinetoram than the no adjuvant treatment for 1 and 3 DAT, this effect diminished to non-significant levels by the 7 DAT and later sample dates. Mean residue values for the no adjuvant, NuFilm, and SuperSpread tank mixes were below the US MRL of 0.3 ppm for the 7 day PHI. Internationally, no adjuvant, NuFilm and SuperSpread would be low risk for export to most prospective markets. Spinetoram concentrations fell below Mexico at 0.3 ppm, Canada at 1 ppm, Japan at 0.5 ppm, Taiwan at 0.2 ppm, Australia at 0.2 ppm, and Korea’s MRL at 0.2 ppm, at 7 day PHI, making export to those countries acceptable. Mean residue values for no adjuvant, NuFilm, and SuperSpread exceeded the EU’s MRL of 0.05 ppm and would not be accepted for export at 7 day PHI.

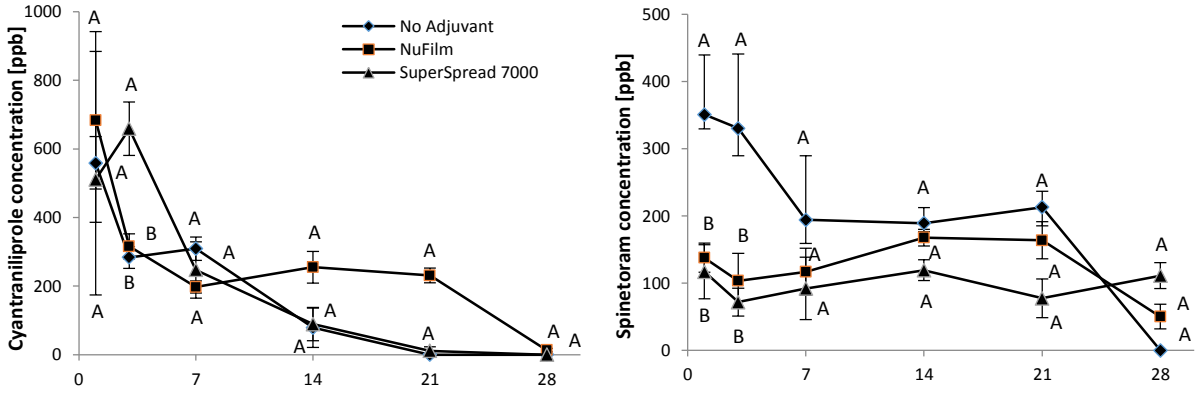

Figure 6. Cyantraniliprole and spinetoram alone and in combination with NuFilm and SuperSpread for 1, 3, 7, 14, 21, and 28 day decline residue profiles in 'Balaton' cherry fruit. Concentration means within one date followed by different letters are significantly different (*t-tests p<0.05*). Error bars represent standard error of the mean (SEM). Cyantraniliprole has a United States MRL of 6 ppm, while the lowest international MRL at a perspective market is also 6 ppm in Korea. Spinetoram has a United States MRL of 0.3 ppm, while the lowest international MRL at a perspective market is 0.05 ppm in the EU.

Residue Surface Penetration Profiles-Cherry

Cyantraniliprole was detected throughout the skin and three sub-surface layers of the cherry fruit for the no adjuvant, NuFilm, and SuperSpread tank mixes (Figure 7). The cherry skin results showed significant differences of cyantraniliprole concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 6.05, df= 3, P=0.0405). The effect of the different adjuvants resulted in significantly higher concentration of cyantraniliprole in the cherry skin for the SuperSpread-treated fruit than the no adjuvant treatment (F= 11.1, df= 3, P=0.0207).

The outer 2 mm of cherry flesh results showed no significant differences of cyantraniliprole concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.8, df= 3, P=0.5452) (Figure 7). The effect of the different adjuvants resulted in no significant difference in concentration of cyantraniliprole in the cherry skin between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.57, df= 3, P=0.5962).

The inner 3 mm of cherry flesh results showed no significant difference of cyantraniliprole concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.45, df= 3, P=0.7288) (Figure 7). The effect of the different adjuvants resulted in no significant difference in concentration of cyantraniliprole in the inner 3 mm of cherry flesh between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.54, df= 3, P=0.6108).

Mean cyantraniliprole residue values for no adjuvant and NuFilm treatments were distributed very evenly throughout the cherry skin and flesh (Figure 7). Cyantraniliprole residue values for the SuperSpread tank mix resulted in significantly more residue in the cherry skin than the outer 2 mm and inner 3 mm of flesh. The mean proportion of cyantraniliprole residue for the no adjuvant treatment was 36 % for the cherry skin, 39 % for the 2 mm of outside flesh, and 25 % for the inner 3 mm of cherry flesh. The mean proportion of cyantraniliprole residue for the NuFilm tank mix was 37 % for the cherry skin, 34 % for the 2 mm outside flesh, and 29 % for the inner 3 mm of cherry flesh. The mean proportion of cyantraniliprole residue for the SuperSpread tank mix was 80 % for the cherry skin, 12 % for the 2 mm outside flesh, and 8 % for the inner 3 mm of cherry flesh.

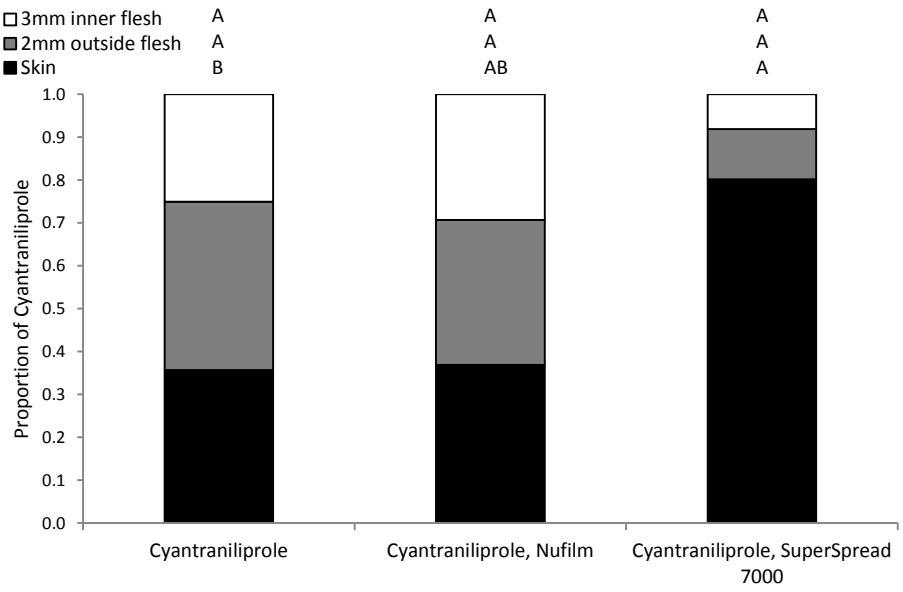

Figure 7. Mean proportion of cyantraniliprole residue for each cherry tissue type within three types of cyantraniliprole treatments of no adjuvant, NuFilm, and SuperSpread 7000. Letters above the bars within a row show significant differences among the treatment types within each cherry tissue type, and the bars with the same letter are not significantly different ($P < 0.05$). Data were analyzed by ANOVA with mean separation calculated using least significant difference (LSD) test. Data shown are non-transformed means.

Spinetoram was detected throughout the skin and three sub-surface layers of the cherry fruit for the no adjuvant, NuFilm, and SuperSpread tank mixes (Figure 8). The cherry skin results showed no significant difference of spinetoram concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 1.04, df= 3, P=0.4516). The effect of different adjuvants resulted in no significant differences in concentration of spinetoram in the cherry skin between the no adjuvant, NuFilm, and SuperSpread treatments (F= 1.12, df= 3, P=0.3953).

The outer 2 mm of cherry flesh results showed significant differences of spinetoram concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 31.96, df= 3, P=0.0011) (Figure 8). The effect of different adjuvants resulted in a significantly higher concentration of spinetoram in the outer 2 mm of cherry flesh for the no adjuvant than the NuFilm treatment (F= 41.96, df= 3, P=0.0007) and SuperSpread treatment (F= 11.98, df= 3, P=0.018). The effect of different adjuvants resulted in a significantly higher concentration of spinetoram in the SuperSpread than the NuFilm treatment (F= 11.98, df= 3, P=0.0002).

The inner 3 mm of cherry flesh results showed significant differences of spinetoram concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 11.99, df= 3, P=0.0101). (Figure 8). The effect of different adjuvants resulted in a significantly higher concentration of spinetoram in the 3 mm inner cherry flesh for the no adjuvant than the NuFilm (F= 17, df= 3, P=0.0059) and SuperSpread (F= 17, df= 3, P=0.0001).

Mean spinetoram residue values for no adjuvant, NuFilm, and SuperSpread treatments were distributed unevenly with proportions of residue indicating the majority of residue located in the cherry skin (Figure 8). The mean proportion of spinetoram residue for the no adjuvant treatment was 71 % for the cherry skin, 21 % for the 2 mm of outside flesh, and 8 % for the inner 3 mm of cherry flesh. The mean proportion of spinetoram residue for the NuFilm tank mix was 77 % for the cherry skin, 15 % for the 2 mm outside flesh, and 8 % for the inner 3 mm of cherry flesh. The mean proportion of spinetoram residue for the SuperSpread tank mix was 69 % for the cherry skin, 18 % for the 2 mm outside flesh, and 13 % for the inner 3 mm of cherry flesh.

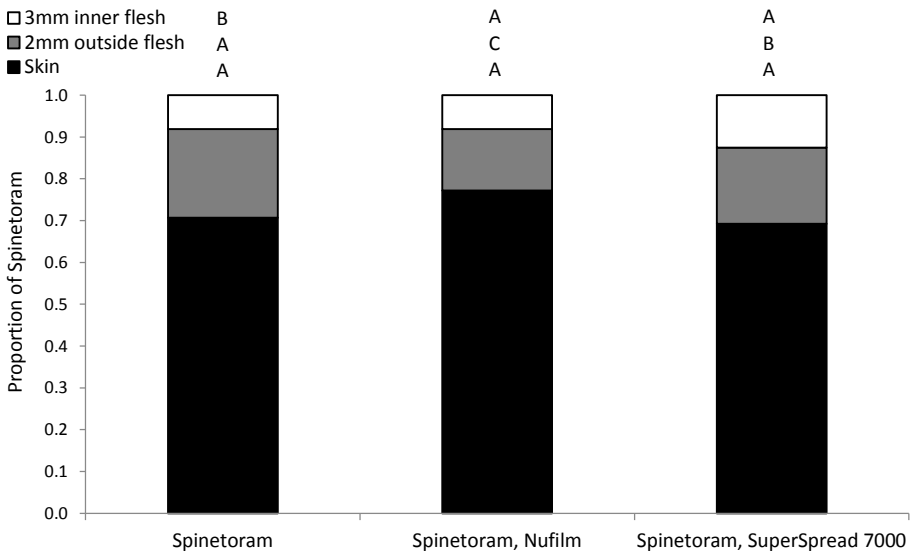

Figure 8. Mean proportion of spinetoram residue for each cherry tissue type within three types of spinetoram treatments of no adjuvant, NuFilm, and SuperSpread 7000. Letters above the bars within a row show significant differences among the treatment types within each cherry tissue type, and the bars with the same letter are not significantly different ($P < 0.05$). Data were analyzed by ANOVA with mean separation calculated using least significant difference (LSD) test. Data shown are non-transformed means.

Residue Surface Penetration Profiles-Apple

Spinetoram was detected throughout the skin and four sub-surface layers of the apple fruit for the no adjuvant, NuFilm, and SuperSpread tank mixes (Figure 9). The apple skin results showed no significant differences of spinetoram concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 2.83, df= 3, P=0.146). The effect of the different adjuvants resulted in significantly higher concentration of spinetoram in the apple skin for the no adjuvant than the SuperSpread treatments (F= 4.22, df= 3, P=0.0052).

The outer 2 mm apple flesh results showed no significant differences of spinetoram concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 1.11, df= 3, P=0.4266) (Figure 9). The effect of the different adjuvants resulted in no significant difference in concentration of spinetoram in the outer 2 mm apple flesh between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.92, df= 3, P=0.4585).

The middle 10 mm apple flesh results showed no significant differences of spinetoram concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.93, df= 3, P=0.4912) (Figure 9). The effect of the different adjuvants resulted in no significant difference in concentration of spinetoram in the middle 10 mm apple flesh between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.97, df= 3, P=0.4416).

The inner 5 mm apple flesh results showed no significant differences of spinetoram concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.23, df= 3, P=0.8727) (Figure 9). The effect of the different adjuvants resulted in no significant difference in concentration of spinetoram in the inner 5 mm apple flesh between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.29, df= 3, P=0.7568).

Mean spinetoram residue values for the no adjuvant, NuFilm and SuperSpread treatments were not distributed evenly throughout the apple skin and flesh (Figure 9). Spinetoram residue values for the no adjuvant, NuFilm, and SuperSpread treatments resulted in significantly more residue in the apple skin than the outer 2 mm, middle 10 mm, and inner 5 mm of flesh. The mean proportion of spinetoram residue for the no

adjuvant treatment was 89 % for the apple skin, 8 % for the 2 mm of outside flesh, 2 % for the middle 10 mm of apple flesh, and 1 % for the inner 5 mm of apple flesh. The mean proportion of spinetoram residue for the NuFilm tank mix was 74 % for the apple skin, 17 % for the 2 mm outside flesh, 6 % for the middle 10 mm, and 3 % for the inner 5 % of apple flesh. The mean proportion of spinetoram residue for the SuperSpread tank mix was 75 % for the apple skin, 13 % for the 2 mm outside flesh, 7 % for the middle 10 mm, and 4 % for the inner 5 mm of apple flesh.

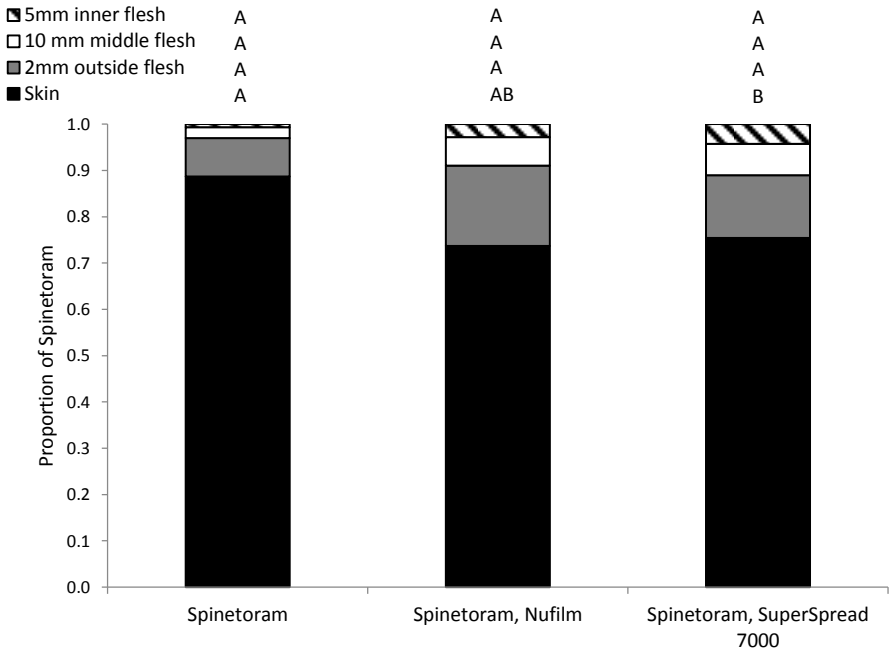

Figure 9. Mean proportion of spinetoram residue for each apple tissue type within three types of spinetoram treatments of no adjuvant, NuFilm, and SuperSpread 7000. Letters above the bars within a row show significant differences among the treatment types within each apple tissue type, and the bars with the same letter are not significantly different ($P < 0.05$). Data were analyzed by ANOVA with mean separation calculated using least significant difference (LSD) test. Data shown are non-transformed means.

Fenpropathrin was detected throughout the skin and three sub-surface layers of the apple fruit for the no adjuvant, NuFilm, and SuperSpread tank mixes (Figure 10). The apple skin resulted in no significant difference of fenpropathrin concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.23, df= 3, P=0.8692). The effect of the different adjuvants resulted in no significant difference in concentration of fenpropathrin in the apple skin between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.35, df= 4, P=0.7228).

The outer 2 mm apple flesh results showed no significant differences of fenpropathrin concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 1.71, df= 3, P=0.28) (Figure 10). The effect of the different adjuvants resulted in no significant difference in concentration of fenpropathrin in the outer 2 mm apple flesh between the no adjuvant, NuFilm, and SuperSpread treatments (F= 1.63, df= 3, P=0.2842).

The middle 10 mm apple flesh results showed no significant differences of fenpropathrin concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 5.14, df= 3, P=0.0548) (Figure 10). The effect of the different adjuvants resulted in no significant difference in concentration of fenpropathrin in the middle 10 mm apple flesh between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.98, df= 3, P=0.4381).

The inner 5 mm apple flesh results showed no significant differences of fenpropathrin concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments with all values resulting in zero (Figure 10). The effect of the different adjuvants resulted in no significant difference in concentration of

fenpropathrin in the inner 5 mm apple flesh between the no adjuvant, NuFilm, and SuperSpread treatments with all values resulting in zero.

Mean fenpropathrin residue values for the no adjuvant, NuFilm and SuperSpread treatments were not distributed evenly throughout the apple skin and flesh (Figure 10). Fenpropathrin residue values for the no adjuvant, NuFilm, and SuperSpread treatments resulted in significantly higher residue concentrations in the apple skin than the inner 5 mm of flesh. The mean proportion of fenpropathrin residue for the no adjuvant treatment was 40 % for the apple skin, 32 % for the 2 mm of outside flesh, 27 % for the middle 10 mm of apple flesh, and 0 % for the inner 5 mm of apple flesh. The mean proportion of fenpropathrin residue for the NuFilm tank mix was 32 % for the apple skin, 55 % for the 2 mm outside flesh, 14 % for the middle 10 mm, and 0 % for the inner 5 mm of apple flesh. The mean proportion of fenpropathrin residue for the SuperSpread tank mix was 44 % for the apple skin, 42 % for the 2 mm outside flesh, 14 % for the middle 10 mm, and 0 % for the inner 5 mm of apple flesh.

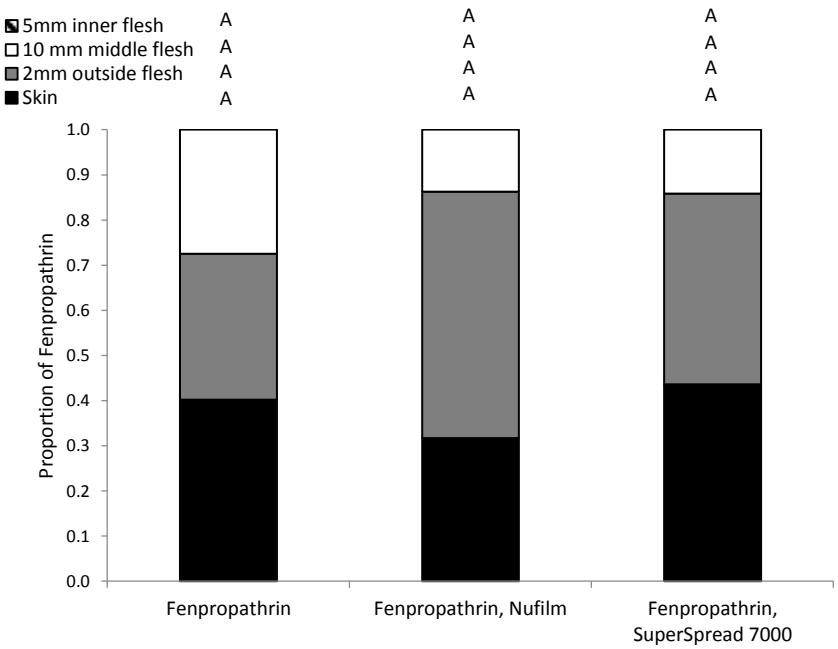

Figure 10. Mean proportion of fenpropathrin residue for each apple tissue type within three types of spinetoram treatments of no adjuvant, NuFilm, and SuperSpread 7000. Letters above the bars within a row show significant differences among the treatment types within each apple tissue type, and the bars with the same letter are not significantly different ($P < 0.05$). Data were analyzed by ANOVA with mean separation calculated using least significant difference (LSD) test. Data shown are non-transformed means.

Phosmet was detected throughout the skin and three sub-surface layers of the apple fruit for the no adjuvant, NuFilm, and SuperSpread tank mixes (Figure 11). The apple skin results showed no significant differences of phosmet concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 3.96, df= 3, P=0.0863). The effect of the different adjuvants resulted in no significant

difference in concentration of phosmet in the apple skin between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.26, df= 3, P=0.6326).

The outer 2 mm apple flesh results showed no significant differences of phosmet concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 1.42, df= 3, P=0.3417) (Figure 11). The effect of the different adjuvants resulted in no significant difference in concentration of phosmet in the outer 2 mm apple flesh between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.16, df= 3, P=0.7046).

The middle 10 mm apple flesh results showed no significant differences of phosmet concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.58, df= 3, P=0.6529) (Figure 11). The effect of the different adjuvants resulted in no significant difference in concentration of phosmet in the middle 10 mm apple flesh between the no adjuvant, NuFilm, and SuperSpread treatments (F= 0.09, df= 3, P=0.7783).

The inner 5 mm apple flesh results showed no significant differences of phosmet concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments with all values resulting in zero (Figure 11). The effect of the different adjuvants resulted in no significant difference in concentration of phosmet in the inner 5 mm apple flesh between the no adjuvant, NuFilm, and SuperSpread treatments with all values resulting in zero.

Mean phosmet residue values for the no adjuvant, NuFilm and SuperSpread treatments were not distributed evenly throughout the apple skin and flesh (Figure 11). Phosmet residue values for the no adjuvant, NuFilm, and SuperSpread treatments

resulted in significantly higher residue in the apple skin than the outer 2 mm, middle 10 mm, and inner 5 mm of apple flesh. The mean proportion of phosmet residue for the no adjuvant treatment was 83 % for the apple skin, 8 % for the 2 mm of outside flesh, 9 % for the middle 10 mm of apple flesh, and 0 % for the inner 5 mm of apple flesh. The mean proportion of phosmet residue for the NuFilm tank mix was 76 % for the apple skin, 3 % for the 2 mm outside flesh, 21 % for the middle 10 mm, and 0 % for the inner 5 % of apple flesh. The mean proportion of phosmet residue for the SuperSpread tank mix was 86 % for the apple skin, 1 % for the 2 mm outside flesh, 13 % for the middle 10 mm, and 0 % for the inner 5 mm of apple flesh.

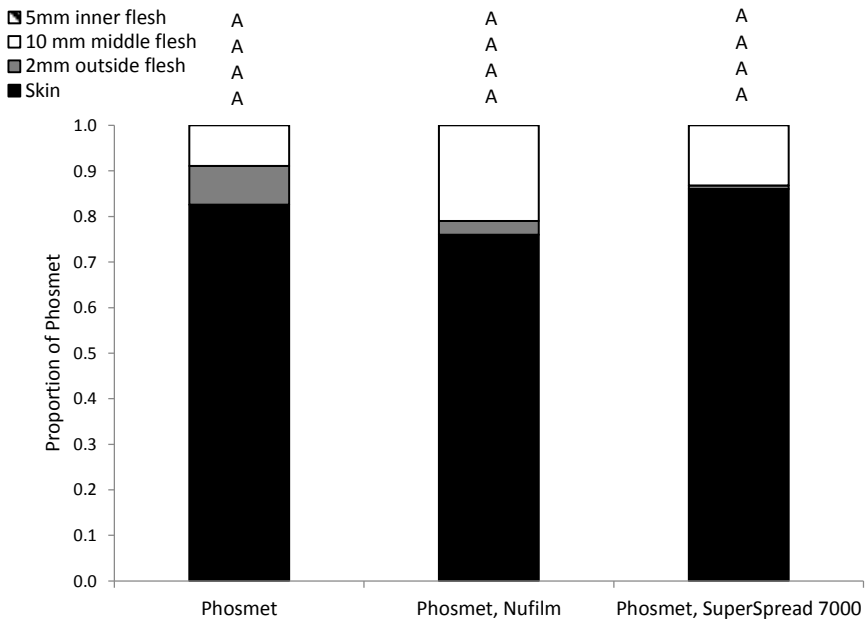

Figure 11. Mean proportion of phosmet residue for each apple tissue type within three types of spinetoram treatments of no adjuvant, NuFilm, and SuperSpread 7000. Letters above the bars within a row show significant differences among the treatment types within each apple tissue type, and the bars with the same letter are not significantly

Figure 11 (cont'd)

different ($P < 0.05$). Data were analyzed by ANOVA with mean separation calculated using least significant difference (LSD) test. Data shown are non-transformed means.

Cyantraniliprole was detected throughout the skin and sub-surface layers of the apple fruit for the no adjuvant, NuFilm, and SuperSpread tank mixes (Figure 12). The apple skin resulted in no significant difference of cyantraniliprole concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 2.68, df= 3, P=0.158). The effect of the different adjuvants resulted in no significant difference in concentration of cyantraniliprole in the apple skin between the no adjuvant, NuFilm, and SuperSpread treatments (F= 1.95, df= 3, P=0.2362).

The outer 2 mm apple flesh results showed no significant differences of cyantraniliprole concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 3.38, df= 3, P=0.1113) (Figure 12). The effect of the different adjuvants resulted in no significant difference in concentration of cyantraniliprole in the outer 2 mm apple flesh between the no adjuvant, NuFilm, and SuperSpread treatments (F= 3.16, df= 3, P=0.1299).

The middle 10 mm apple flesh results showed significant differences of cyantraniliprole concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 334.69, df= 3, P=0.0001) (Figure 12). The effect of the different adjuvants resulted in significantly higher concentration of cyantraniliprole in the middle 10 mm apple flesh for the NuFilm (F= 501.22, df= 3, P=0.0001) than the no adjuvant. The effect of the different adjuvants resulted in

significantly higher concentration of cyantraniliprole in the middle 10 mm apple flesh for the SuperSpread than the no adjuvant treatment (F= 501.22, df= 3, P=0.0001).

The inner 5 mm apple flesh results showed no significant differences of cyantraniliprole concentration for the overall sample set between the no adjuvant, NuFilm, and SuperSpread treatments (F= 3.53, df= 3, P=0.1041) (Figure 12). The effect of the different adjuvants resulted in significantly higher concentration of cyantraniliprole in the inner 5 mm apple flesh for the SuperSpread than the no adjuvant, treatment (F= 5.13, df= 3, P=0.0031).

Mean cyantraniliprole residue values for the no adjuvant, NuFilm and SuperSpread treatments were not distributed evenly throughout the apple skin and flesh (Figure 12). Cyantraniliprole residue values for the SuperSpread treatment resulted in significantly higher residue in the apple skin than the outer 2 mm, middle 10 mm, and inner 5 mm of apple flesh. The mean proportion of cyantraniliprole residue for the no adjuvant treatment was 33 % for the apple skin, 67 % for the 2 mm of outside flesh, 0 % for the middle 10 mm of apple flesh, and 0 % for the inner 5 mm of apple flesh. The mean proportion of cyantraniliprole residue for the NuFilm tank mix was 42 % for the apple skin, 24 % for the 2 mm outside flesh, 34 % for the middle 10 mm, and 0 % for the inner 5 % of apple flesh. The mean proportion of cyantraniliprole residue for the SuperSpread tank mix was 53 % for the apple skin, 18 % for the 2 mm outside flesh, 22 % for the middle 10 mm, and 7 % for the inner 5 mm of apple flesh.

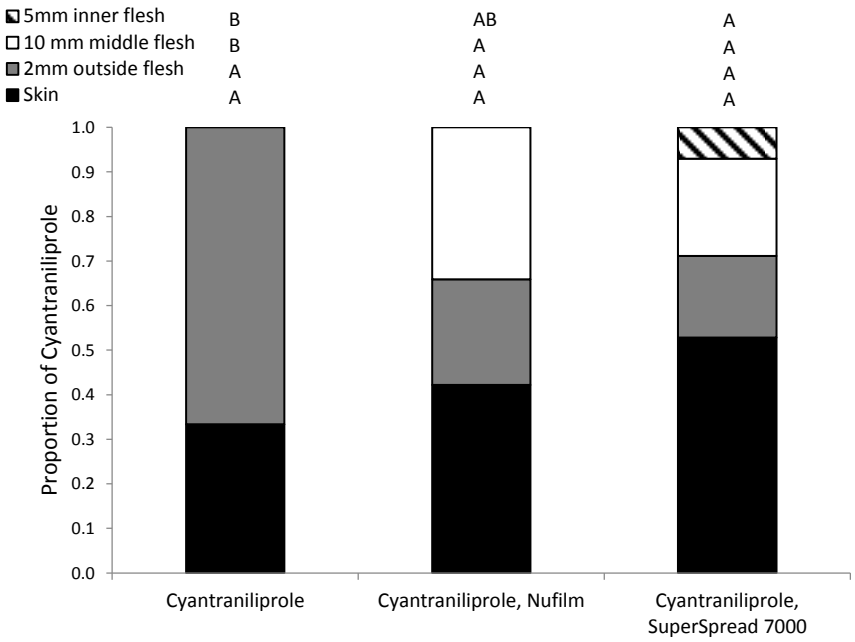

Figure 12. Mean proportion of cyantraniliprole residue for each apple tissue type within three types of cyantraniliprole treatments of no adjuvant, NuFilm, and SuperSpread 7000. Letters above the bars within a row show significant differences among the treatment types within each apple tissue type, and the bars with the same letter are not significantly different ($P < 0.05$). Data were analyzed by ANOVA with mean separation calculated using least significant difference (LSD) test. Data shown are non-transformed means.

Discussion

This research contributes important information to the insecticide residue profiling database for domestic and international apple and cherry markets. It also provides more insight to the penetrative attributes of specific insecticides with and without NuFilm and

SuperSpread adjuvants. The results also show how temporal residue profiles can be affected by the addition of NuFilm or SuperSpread, depending on the compound being applied and the type of fruit crop. In our study, compounds of the organophosphate and diamide classes showed the greatest effects from the addition of NuFilm and SuperSpread adjuvants on apple and cherry. There are several factors that influence this phenomenon. One factor is the differing plant penetration attributes of the compounds. Neonicotinoids, diamides and spinosyns have plant penetrative attributes allowing mobility into and beneath the plant cuticle (Bostanian et al. 2012, Wise et al 2017), while organophosphates and pyrethroids remain largely on the plant surface, with limited cuticle penetration. This influences the persistence of the compounds on the plant surface using different adjuvants, limiting rain wash off and other environmental degradation events such as evaporation and phytolysis,

On apple and cherry, cyantraniliprole, like most diamides, has translaminar penetration in plant tissues, thus forming a sort of reservoir from direct environmental exposure. Wise et al. (2017) demonstrated a similar diamide, chlorantraniliprole, to be moderately rainfast, which may explain why the residue profiles for cyantraniliprole in this study showed lower concentrations when applied alone than with NuFilm and SuperSpread. Cyantraniliprole is moderately persistent in normal environmental conditions (Dong et al. 2011). This is demonstrated in the cyantraniliprole residue decline study where the cyantraniliprole with the addition of NuFilm and SuperSpread resulted in numerically higher residue overall throughout all samples days than the cyantraniliprole with no adjuvant for the apple study. The penetrative study also demonstrated with the addition of SuperSpread, cyantraniliprole resulted in significantly

higher concentrations in the skin for cherry and inner flesh for apple than the other treatments. This suggests that SuperSpread increases the concentration of cyantraniliprole at harvest by enhancing the penetrative attributes of cyantraniliprole in fruit. The increased residues may also be due to the adjuvants (surfactants) causing the droplet to spread on the leaf, which will lower the mass of the active ingredient per unit area without any change in concentration until the spray solution evaporates (Castro 2014). The SuperSpread treatment was significantly higher for the cherry study at the 3 day phi, increasing MRL risks. On cherry, the residue concentrations were acceptable for export to most prospective markets at a 6 ppm MRL, but growers must still be aware that with the addition of adjuvants, cyantraniliprole residues have the potential to be higher at harvest. On apple, cyantraniliprole concentrations for all adjuvant treatments were not acceptable for export to most prospective markets at 0.8 ppm MRL and a US MRL of 1.5 ppm. Given the high residues at harvest, cyantraniliprole is high risk for international export and growers must also be aware that with the addition of adjuvants, cyantraniliprole residues have the potential to be even higher at harvest.

On apple and cherry, spinetoram, like most spinosyns, has translaminar penetration in plant tissues, thus forming a sort of reservoir from direct environmental exposure (Bostanian et al. 2012). This likely contributes to the moderate rainfastness documented for this compound (Wise et al. 2017). This may also contribute to the significant differences and variability between the different adjuvant tank mixes. The rapid degradation rate of spinetoram in this study is similar to patterns documented in other studies (DOW 2014). The spinetoram apple and cherry decline study resulted in significantly higher spinetoram concentrations for the no adjuvant treatment than the

NuFilm or SuperSpread treatments for the majority of the sample dates. For the penetrative study, spinetoram resulted in greater residues in the skin for the no adjuvant treatment than the adjuvant treatments on apple and there was greater residue for the no adjuvant treatment than the SuperSpread treatment on cherry. This suggests that Delegate WDG may be formulated with sufficient adjuvants, such that adding adjuvants to the spray tank causes run-off. The penetration profiles support the assertion that such a wash-off resulted in less overall active ingredient on the fruit.

On apple, fenpropathrin, like most pyrethroids, have limited cuticular penetration, but as lipophilic compounds have natural affinity to cuticular waxes. This is likely one of the factors responsible for the moderate rainfastness seen for pyrethroids in other studies (Hulbert et al. 2011). Fenpropathrin is relatively unstable and degrades rapidly in normal environmental conditions (Akhtar 2004). This may be reason why the fenpropathrin apple decline study resulted in significantly higher fenpropathrin concentrations for the no adjuvant treatment than the NuFilm or SuperSpread treatments for the 1 DAT sample. It was shown in a study on sweet cherries that fenpropathrin has persistent residues and growers who use it should avoid export (Haviland and Beers 2012). With similar results in this study, this work indicates that fenpropathrin is a high risk material for export. For the penetrative study, fenpropathrin resulted in fairly even distribution of residues throughout the apple tissues and between the adjuvant treatments with the highest concentrations in the outside 2 mm of apple flesh. This may indicate that fenpropathrin was not broadly affected with the addition of adjuvants. Just as suggested for spinetoram, the fenpropathrin formulation may be sufficient for optimal plant deposition. Fenpropathrin with or without the addition of

NuFilm or SuperSpread resulted in concentrations that exceeded the US MRL of 5 ppm, most prospective markets at 5 ppm, and CODEX as there is no CODEX MRL, which makes fenpropathrin high risk for those who follow it.

On apple, phosmet, like most organophosphates, has limited cuticular penetration and is considered a surface material, which makes it very susceptible to rain wash-off (Wise et al. 2017). This may explain why the residue profiles for phosmet in this study showed lower concentrations beneath the apple tissue. The phosmet apple decline study resulted in higher concentrations of phosmet with the addition of NuFilm and SuperSpread than phosmet alone at the 7 day PHI. For the penetrative study, phosmet resulted in uneven distribution of residue throughout the apple tissues with the majority located in the apple skin. There was very little phosmet residue located below the apple skin layer indicating that NuFilm and SuperSpread did not enhance phosmet penetration. This study indicated that the deposition of phosmet for the residue decline study may have increased with the addition of adjuvants, but not the penetration. Even though phosmet did not result in residues below the apple skin, the highest concentration of phosmet in the apple skin was the SuperSpread tank mix indicating that SuperSpread significantly enhanced deposition on the plant canopy's. Phosmet with no adjuvants resulted in residues below the US MRL of 10 ppm and the majority of prospective markets such as Mexico, Canada, Saudi Arabia, Thailand, and Vietnam all with MRLs of 10 ppm. With the phosmet CODEX MRL of 10 ppm, phosmet alone concentrations also fall below Colombia, Dominican Republic, Jordan, Philippines, and Singapore. Phosmet concentrations with the addition of SuperSpread and NuFilm exceeded the US MRL and all international prospective markets listed above at the 7

day PHI. This indicates that phosmet with the addition of NuFilm or SuperSpread may be high risk for international export, but phosmet with no adjuvants was moderate risk.

This MRL study presents valuable data pertaining to crop protection from invasive species (BMSB) (SWD), a crucial issue to the Michigan apple and cherry industries. Growers typically stop spraying insecticides on apples 3+ weeks before harvest and spray right to harvest for cherries (depending on product phi), but with the late season BMSB and SWD, growers are making insecticide sprays nearer to harvest, which will increase the risk of MRL violations. This research will help inform decisions of apple and cherry growers during late season pest management and what possible tactics can be used to lower the risks of violations according to specific export targets.

With the limited data available on penetrative effects that adjuvants may have in combination with insecticides, the surface and subsurface penetrative data collected will provide insight to how different adjuvants can affect the grower's ability to protect their crops and export with low risk of being rejected. Therefore, this research provides important data to the insecticide residue profiling database with the addition of adjuvants to create application and harvest regimens, as well as, degradation curves to best suit the growers' needs. This is just one of many data sets needed to reach the overall goal, but the contribution of this project was to add substantially to the MRL databases for apple and cherry. The results show that insecticide residue levels and PHI's could be predicted. These predictions could be made using specific spray rates and timings by the time of harvest in apples and cherries.

CHAPTER 5: IMPACTS OF MAXIMUM RESIDUE LIMITS AND INSECTICIDE RESIDUE REGULATIONS TO SOCIETY AND THE FRUIT INDUSTRY IN THE UPPER MIDWEST

A Historical Perspective of Pesticide Use

Pesticide Maximum Residue Limits (MRLs) of pesticides and enforcement of MRLs have had a global impact on our society and even global communities. The ripple effects of such pesticide standards can be traced back to the farmer whose livelihood relies on protecting, harvesting, and selling their crop. The societal impacts, including cost, of MRLs involve the evolution of crop protection, integrated pest management (IPM), the pesticide industry, and pesticide regulations. IPM is a strategy to control pests that was developed in response to overuse of pesticides and the associated environmental impacts. The practice entails using chemical, biological, and cultural data at a level which causes the least amount of economic and biological injury to a crop, the environment, and society (Kogan 1998).

The use of broad spectrum insecticides began in the 1940s during World War II. The use of pesticides such as DDT, an organochlorine, was a cheap and effective method to kill most insect pests in rapidly expanding crops (Stapleton 2005). (www.agrochemicals.iupac.org). It is also important to note that they controlled the insects which cause deadly diseases such as typhus and malaria Soon after their introduction, organochlorines seemed to have low toxicity to mammals and was hailed as a panacea for insect control. However, organochlorine persistence in the environment due to low degradation rate and increasing insect resistance to its toxic

effects dealt a major blow to that class of insecticides (Carson 1962). Ultimately, DDT was banned in the U.S in 1972, and the entire organochlorine class was banned in 1987 by the EPA. These actions triggered the transition to synthetic products such as organophosphates (OPs). Following the troubles with DDT and other similar products, the use of OPs appeared to be a safe alternative, broad spectrum insecticides that controlled a wide range of insects. They were easy to use, effective, and cheap compared to the other insecticides on the market.

Along with the strong pest control performance provided by these new classes of insecticides, there was also evidence of negative impacts on the environment, including reduction of natural enemies of agricultural pests and dangers as potential mutagens. Such problems fueled interest in the concept of integrated pest management (IPM), where techniques such as field scouting for insect pest, identification and enhancement of natural enemies and judicious use of pesticides at optimum times came into play.

The growing concerns with pesticide use led to the establishment of the Environmental Protection Agency in 1970. A host of regulations and registration of pesticides intensified to improve the safety of workers, the environment, and consumers (USEPA 1996).

With the implementation of stricter laws on pesticide residues, worker safety, dietary safety, and environmental safety, the number of broad spectrum compounds decreased (Wise and Whalon 2009) and newer compounds were developed. For example, a broad spectrum compound such as Guthion were phased out, while insecticide classes such as neonicotinoids, diamides, and spinosyns took their place. These types of pesticides, which are safer for workers, the environment, and humans

that consume products that have been treated with these products, are often referred to as reduced-risk pesticides. However, even reduced-risk pesticides can to carry risks or have other drawbacks. They require a stronger learning curve with the detailed application timing. The plant-insect interactions of these compounds can be difficult to grasp with many complex systems to consider. They also have high rates of degradation (slow rates if in plant material), low persistence, and resistance management can be difficult.

The Major Factors Influencing Agricultural Pest Control

The 1990s was a critical time for pesticide use with the implementation of the Food Quality Protection Act (FQPA) of 1996. This act created stricter laws regarding pesticide residues on food and residue exposure to children (Viray and Holling worth 2009). It also created risk assessments for all routes of exposure from pesticides and created a re-evaluation system of pesticide registrations periodically.

The impetus for zero tolerance and blemish free came from the food industry and a federal .marketing order implemented by the tart cherry industry (Wise and Whalon 2009). The idea of blemish-free fruits and vegetables, along with new concerns over pesticide residues resulted in additional pressure on specialty crop growers and impacted IPM implementation. Zero tolerance means no exception for any insects or blemishes on the fruit. In other words, one bad apple means the entire bushel gets tossed. This type of requirement also accelerated the rise of organic farming, adding hardships to many med-sized growers and reduced profit margins. It is quite difficult for

a grower to both implement IPM with an effort to minimize pesticide sprays and meet the pervasive zero-tolerance market standards of the 21st century food industry.

With the introduction of invasive species in fruit such as the spotted wing drosophila (*Drosophila suzukii*) (SWD) and brown marmorated stink bug (*Halyomorpha halys*) (Stal) (BMSB), IPM ibecame more difficult to implement in order to meet market standards. The advent of these persistent late-season pests left little room for integrating pest management strategies. In order to accomplish a quick, efficient, zero-insect infested fruit with low insecticide residues to stay below the tolerance level, pesticide degradation and MRL research has become imperative.

Some major developments influencing pest management and its effects on society were the Food Quality Protection Act of 1996, the concept of zero tolerance of insects and blemishes, a growing communication disconnect between the grower and the law makers (EPA), consumers, the anti-pesticide Green Movement, differing MRL calculation methods, and international MRL/ tolerance harmonization. Among these trouble spots, the FQPA seems to rise up as the key issue.

The Food Quality Protection Act of 1996 changed the dynamics of pesticide registration and pesticide residue regulations. The FQPA replaced a very different regulatory system (Batie et al. 1999). Before FQPA, pesticides on processed foods were regulated by the EPA under the Delaney Clause, which stated that no food additive would be allowed that was found to cause cancer in humans and animals but did allow low levels of pesticide residue on fresh market foods based on human toxicity. Also, pre-FQPA regulations had a refined IPM system with a systems approach to pest

management. Integrating different cultural, biological, and chemicals was an easier task since there were more options for pesticide classes and easier to follow use regulations.

The current FQPA implementation resets pesticide residue tolerances on all fresh and processed foods. It set a single standard for pesticide tolerance which increased the complications of pesticide regulation and risk assessment studies. Increased risk assessment studies include reproductive and endocrine effects on infants, children, and adults. Infants and children became the highest priority since they are most vulnerable due to body size and dietary needs (Batie et al. 1999).

Regarding the fruit industry, insecticide spray applications became more complicated due to schedule and use changes when applying reduced risk insecticides. Many growers view the reduced-risk insecticides as too selective for target organisms, too expensive with low persistence, and hard to use. Pest management strategies in an IPM program became more difficult to follow with less broad spectrum compounds on the market, longer REIs and PHIs (difficult to follow uses), and more expensive chemicals. Many growers could not risk their livelihood on the reduced-risk compounds because of their lower efficacy on pest populations and the non-target impacts were not as well understood.

Another complication was the reduction of the number of insecticide classes available for registration, a system known as risk cup. The term risk cup was developed to provide an analogy of total or aggregate pesticide exposure (Levine 2007). The fewer or smaller the risk cups are, the more difficult pesticide registration becomes with less available space for exposure. With the FQPA implementation, there is only one risk cup for all insecticides of each group that act on the human body in a particular way. There

is also a risk cup for exposure and an additional 10 fold reduction in tolerance to account for infants and children. In the original EPA system, there was a risk cup for specific insecticides under each insecticide class, uses, and crops (Batie et al. 1999). There was a risk cup for total dietary exposure, drinking water, and all of the other non-occupational sources, such as pesticides in and around the home, pets, and lawn care. The no observable effect level (NOEL) is determined by the toxicity studies required for product registration. Pesticides were tested with lab animals to find a toxological end point and then the NOEL is set to 1/100th of the NOEL to account for differences in humans and animals. There were two 10 fold reductions in exposure for humans.

FQPA Impacts on Society

Risk assessment is an essential part of the FQPA. If the risks are considered too great, pesticides can be selectively taken off the market, specific pesticide uses can be cancelled, and labeling can change to increase the PHI or decrease the use rate to reduce exposure. The decision from the registrant to remove a compound from the market is based on projected profitability, which includes how many years are left on the patent, how much of the product is sold for profit, exports, cost of studies needed to maintain the registration and manufacturing. Residue levels and insecticide uses on particular crops are often stated incorrectly at the EPA and USDA level, creating the need for better communication between the government and the farmer. The agrochemical industry takes a negative financial hit from the loss of specialty crop grower profits. There is the possibility of crop yield loss and the need for switching to a different pest management strategy, which is likely to increase costs.

The impacts of FQPA are being felt in states with large acreage of specialty crops. Fruit growers of Michigan experienced a major loss when azinphos-methyl and flubendiamide were removed from the market. Both are valuable insecticides on apple maggot (*Rhagoletis pomonella*) and San Jose scale (*Quadraspidiotus perniciosus*). There are no other products that can duplicate their efficacy on the market. There are many growers in other parts of the US who do not require the same products since they have different pest problems. There are also different pest problems internationally, which means they do not require insecticides that are restricted in the US. This situation creates competition for the US markets, since they can produce more and cheaper produce.

Globalization of agriculture has created complications that FQPA has attempted to address. The issue of different international standards has caused problems at home and abroad. Only a small portion of produce (less than one percent) imported from foreign markets to the US is tested for residues, making it easy to import to the US with restricted insecticides (GAO 2008). The basic problem is the residue limit standard (MRL) and the techniques used to measure the residue limit standards for each country.

Many of the export countries use their own pesticide residue calculation system to obtain their MRLs (Handford et al. 2015). This is also a problem for fruit growers because there are no minor use definitions with the Organization of Economic Cooperation and Development (OECD) calculation methods. Without any specific minor use definitions there are no appropriate minor use regulations to help with registration processes.

There are two different approaches that OECD member countries follow when dealing with insect pests. There is the risk assessment approach and the economic return approach. This causes delays in the MRL setting process and delays in getting the growers the right tools they need. The different methodological approaches create large issues for MRL harmonization. Another issue which causes a negative impact on the fruit industry is the low and sometimes even zero tolerance for blemishes and insects on or in the fruit (Wise and Whalon 2009). If the market does not accept insects and blemishes, then pesticides need to be sprayed at higher levels to boost that market. What are consumers willing to pay for fruit that is imperfect? Or how much are people willing to pay for fruit that is grown with less pesticide use? Some markets implement a zero tolerance policy that do not accept fruit with insects.

On a positive note, due to negative health and environmental impacts and insecticide resistance occurring before the advent of FQPA, something had to change. Unfortunately the FQPA has been considered overkill. In order to create a system which would work for everyone is a difficult task, but more research on IPM strategies, better communication with the general public (including environmentalists), industry, government, and growers are necessary in order to formulate a pest management system that would benefit agriculture worldwide.

The pesticide regulation problem is an economic, political, environmental, social, and scientific problem. The general public needs to understand the difficulties that growers face. This is where county and state extension services play an important role. Contacting and educating legislators is vital for any success in improving pest

management approaches. Finding the appropriate balance between environmentalism and industry requires education and compromise.

Changing Societies Perception

It is true. Some pesticides can be mutagenic and carcinogenic. They are toxic to a variety of human and animal tissues. But many pesticides are made from chemicals very similar to a major group of chemicals used by millions every day - pharmaceuticals. It's usually not the chemical that makes for toxicity, it's the dose in which it is applied or prescribed. This is a prevalent misconception among the general public. The FQPA, pesticide regulations, and risk assessments in the environmental, worker exposure, and dietary sections need to be reevaluated. There needs to be better and easier avenues for farmers to communicate with the EPA and voice their needs and concerns. IPM needs more support from government agencies

Better education about the value of tolerating minimal insect damage to agricultural products is a priority. Some produce sellers such as Meijer® and Whole Foods Market® now have a produce section that contains products with minimal damage for a cheaper price. Change can happen if the will is there to get it done. The innovation of organophosphates and carbamates gave us the agriculture that we have today. Research programs must continue to find new innovations. Research programs at extension research centers such as those at Michigan State University play a vital role in communication of MRLs and pesticide regulation to local growers. Facilities such as the Trevor Nichols Research Center is located in a high population of fruit growers, where the growers can communicate with researchers and gain the valuable information

needed to succeed. Local communication with growers is crucial and shown to be extremely effective over many years. The MSU Entomology department and the IR-4 program also play a major role in communication at the upper level of the regulatory sector. Communication with the EPA and politicians through IR-4 is a potential route for striking the cords with the people who make the decisions that affect society.

Future Government Pesticide Regulation

There have been major changes in pesticide legislation, which may only be the beginning. There will continue to be research towards non-chemical pest management strategies, reduced risk pesticides that are safer for humans, environment, and worker exposure. Things are very different now that the major chemical companies are merging. With these industry changes there are many complexities that the companies need to work out, which makes programs such as IR-4 and MRL research for the specialty crop grower so valuable.

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