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TRISILOXANE SURFACTANT ADJUVANTS IN POLLEN

by

Autumn Slade

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Environmental Engineering

Approved:

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William J. Doucette, Ph.D. Major Professor	Diana Cox-Foster, Ph.D. Committee Member
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Laurie McNeill, Ph.D. Committee Member	Janis L. Boettinger, Ph.D. Acting Vice Provost for Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2020

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ABSTRACT

Trisiloxane Surfactant Adjuvants in Pollen

by

Autumn Slade, Master of Science

Utah State University, 2020

Major Professor: Dr. William J. Doucette
Department: Civil and Environmental Engineering

Trisiloxanes surfactants (TSSs), used as adjuvants in agricultural applications worldwide, are presumed to be biologically inert. However, recent studies indicate that TSSs may have direct negative impacts on bees, in addition to synergistic negative effects when associated with pesticides and viruses. TSSs have been found in surface waters, pollen, and beeswax, but their environmental prevalence and fate are not well understood, in part, because of the analytical difficulty associated with the quantification of the complex commercial adjuvant mixtures.

To assess the prevalence and concentrations of TSSs in pollen collected by bees, samples collected from pollen traps were analyzed for three different TSSs oligomer groups (TSS-H, TSS-$COCH_3$, and TSS-CH_3) using liquid chromatography tandem mass spectrometry (LCMS/MS), as well as 60+ pesticides. Of the 99 pollen samples analyzed, TSS-H and a TSS-$COCH_3$ like substance were found in 43% and 15% of pollen respectively, along with nine pesticides. No TSS-CH_3 was found.

Experiments were performed to examine the volatilization and hydrolysis of

TSSs, in addition to long term recoveries of TSSs from spiked or fortified pollen. Plant uptake studies were also performed to assess the transfer of TSSs from roots to flowers.

Headspace GCMS experiments did not observe significant volatilization of TSSs but did tentatively identify several low molecular, linear volatile silicone compounds. Hydrolysis half-lives of TSSs were less than 2 days in acidic and basic aqueous solutions and less than 5.9 days at a neutral pH. The long-term recovery of TSSs in pollen was low and with sorption to pollen and polypropylene occurring. The potential transfer of TSSs from roots to flowers was relatively low based on the results of the pressure chamber studies, but TSSs were identified in the flowers of plants exposed to repeated high exposures in whole plant hydroponic studies. TSSs also did not significantly impact the root to shoot transfer of cyprodinil, one of the pesticides found in the pollen survey samples.

(120 pages)

PUBLIC ABSTRACT

Trisiloxane Surfactant Adjuvants in Pollen

Autumn Slade

Chemicals are often added to agricultural pesticide mixes to help increase the efficiency of the field spray solution, and one of the main groups of chemicals used for this purpose are trisiloxane surfactants (TSSs). Normally TSSs are considered non-toxic, but recent studies have shown TSSs to have negative impacts on organisms like honey bees. Though TSSs have recently been found in environmental sources like water, pollen, and beeswax, little is known about how TSSs behave in the environment or affects active ingredients, like pesticides.

To help determine the environmental prevalence and fate of TSSs, several studies were performed. Pollen was collected from across the United States and analyzed for TSS-H, TSS-$COCH_3$, and TSS-CH_3, where TSS-H and a TSS-$COCH_3$ like compound were found, along with nine pesticides. The likelihood for TSSs to be found in the air, water, roots, and plants was looked at as well. TSSs were determined to not be of a great concern in the air and were rapidly degradable in water in basic or acidic conditions with half of the compound disappearing in less than two days. TSSs were also found to sorb to many different surfaces, including pollen and polypropylene, a type of commonly used plastic. Lastly, TSSs were not found to readily travel from plant root solution to the leaves and flowers. This means that overall, TSSs are not stable in the environment and are not likely to travel into pollen at significant concentrations except potentially through direct contact onto the pollen or repeat exposures to the roots or leaves.

ACKNOWLEDGMENTS

First and foremost, I would like to thank Dr. William Doucette, my advisor, for his patience with all of the times I've interrupted him in his office with questions. Without his good advice, encouragement, and guidance, this thesis would never have been finished. I would also like to thank my committee members, Dr. Diana Cox-Foster and Dr. Laurie McNeill for their time they gave and the knowledge they've shared.

I am grateful for the American Honey Producers Association who started and funded a large portion of this project, as well as the Utah Water Research Laboratory, the USDA-ARS Pollinating Insect-Biology Research Unit, and the Crop Physiology Laboratory who helped provide research facilities and materials, as well as knowledge in their various areas of expertise.

I'd specifically like to thank Ellen Klinger and Mary-Kate Williams for their help with pollen samples and analysis, Joe Stewart for his guidance and technical assistance, and Terri Manwaring, Alec Hay, and Bruce Bugbee for their assistance in plant growth.

Thanks to all the friends and family who've supported me through my academic career and had the patience to listen to me rant about things I'm interested in, even if they aren't interested or don't entirely understand what I'm talking about. Lastly, a shout out of gratitude to my parents for their support, love and encouragement they give me to make my own path and follow my interests.

Autumn Slade

CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Organosilicon surfactants (OSSs) are produced for a variety of uses including foam stabilizers, wetting agents, emulsifiers, lubricants, textile and fiber chemicals, and pharmaceuticals and personal care products (PPCPs) (Powell & Carpenter 1997). In 2008, it was estimated that worldwide OSS production was 1.3 billion pounds (approximately 590,000 metric tons) and increasing yearly (Fine et al. 2017). One subset of these OSS compounds, called trisiloxane surfactants (TSSs), are frequently used as nonionic surfactant adjuvants in agricultural applications. Sometimes called "super-spreaders", TSSs have an exceptionally high degree of surface activity (i.e. strong sorption to surfaces) due to the hydrophobic siloxane backbone and hydrophilic tail(s) comprised of ethoxy (EO) or propoxy (PO) groups. The high surface activity allows the TSS adjuvant and the co-applied active ingredient to more effectively spread over a greater leaf area, reducing the amount active ingredient needed to achieve the desired effect (Figure 1).

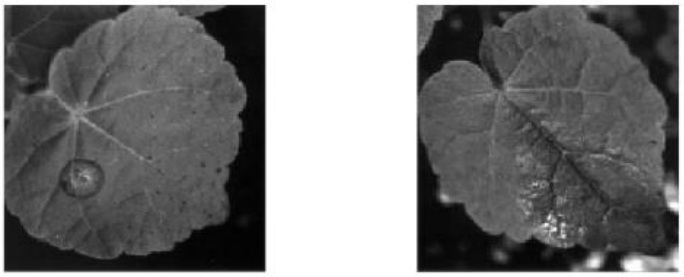

Photo 1: Herbicide with a leading nonionic surfactant (left) beads up, contacts only a small area of plant. With SYLGARD OFX-0309 Fluid (right), the herbicide rapidly coats the leaf.

Figure 1. Photo from Sylgard OFX-0309 Tech Data Sheet) illustrating decreased surface tension (from Dow 2018)

Adjuvants are assumed to be biologically inert and not required to be EPA registered. However, recent laboratory studies have shown TSSs can be toxic to soft bodied insects, impact honeybee learning, and have synergistic effects with bee viruses (Cowles et al. 2000; Ciarlo et al. 2012; Fine et al. 2017; Chen et al. 2018). Most formulations have also been shown to be more toxic than the active ingredients themselves (Mullin 2015; Mullin et al. 2015), meaning that not only can TSSs have impacts alone, but can increase the toxicities of active ingredients. Since pollinators, such as honey bees, add billions of dollars to US crops alone (Fine et al. 2017), an increase in toxicities either by TSSs alone or as a synergistic effect becomes more environmentally relevant to explore, especially with the general decline of pollinators since 2006 (Chen & Mullin 2015; Mullin et al. 2015).

While TSSs have been previously identified in pollen (Chen & Mullin 2013), little is known about the prevalence or concentrations of TSSs in pollen collected by bees. Although studies indicate that hydrolysis may be an important degradation mechanism for TSSs (Michel et al. 2014; Chen & Mullin 2015) and they are likely to have limited mobility in soils (Michel et al. 2016), information on the stability of TSSs within the pollen or the route of pollen contamination (direct contract with spray or via plant uptake) is lacking. In addition, potential impacts of TSS adjuvants on the environmental fate and transport of the active ingredients in agricultural chemical products have not been well studied. This may be in part due to the lack of analytical methods for environmental samples, where only two groups have analyzed environmental samples. Michel et al. (2012, 2014) used liquid chromatography tandem mass spectrometry (LCMS/MS) for two types of TSSs (TSS-CH_3 and TSS-H) in liquid

samples, while Chen et al. (2013, 2015) used liquid chromatography mass spectrometry (LCMS) for three different types of TSSs (TSS-CH_3, TSS-H and TSS-$COCH_3$) in solid samples, such as pollen and almond flowers.

Objectives

The purpose of this study is to determine the occurrence and concentration of TSSs in pollen, as well as provide some preliminary indication of how TSSs are accumulated in the pollen and the potential influence TSSs have on root to shoot transfer of active ingredients. In order to accomplish this, the objectives were completed.

- *Objective 1* – Develop and improve analytical methods for determining TSSs in pollen.
- *Objective 2* - Determine the frequency and concentrations of TSSs in pollen samples collected by beekeepers during California almond pollination in the spring of 2018, and collect preliminary data on select pesticides to investigate potential relationships between TSSs and pesticide detections.
- *Objective 3*: Collect data on long-term spike recoveries.
- *Objective 4*: Investigate factors impacting long-term TSS recoveries from pollen: volatility and hydrolytic stability.
- *Objective 5* - Determine the root to shoot transfer of TSSs in order to investigate potential routes of entry into pollen.

CHAPTER II

LITERATURE REVIEW

Use of Trisiloxane Surfactants Adjuvants

Adjuvants are biologically inactive ingredients added to pesticide mixes or sprays to improve the pesticide's performance or alter the physical properties of the pesticide mixture through buffering, anti-foaming, spreading, and wetting (Penn 2015). Surfactant adjuvants are specifically used to lower the surface tension of the water droplets, thus allowing them to wet or spread more easily on a plant's surface. This in turn allows the active ingredient more efficiently cover a larger surface area with less volume.

Organosilicone surfactants (OSS) are nonionic surfactants produced for a variety of uses including foam stabilizers, wetting agents, emulsifiers, lubricants, textile and fiber chemicals, and pharmaceuticals and personal care products (PPCPs) (Powell & Carpenter 1997). In agricultural applications, trisiloxane surfactants (TSSs) are an important subset of OSSs increasingly used as spray adjuvants (Chen & Mullin 2015). These "super-spreaders" are popular due to their ability to greatly reduce surface tension of tank mixes. For example, certain TSS adjuvants, such as Silwet L-77, Silwet 408, Silwet 806 and Sylgard OFX-0309, have been shown to reduce the surface tension of an aqueous mixture to less than 23 mN m^{-1} at a concentration of 0.1 wt% or less (Knoche et al. 1991; Dow 2018; Momentive 2018c; Momentive 2019), which is more than 3x less that of the normal surface tension of water at 72 mN m^{-1}.

Field loadings for TSS adjuvants are highly dependent on the intended active ingredient application (i.e. herbicides, pesticides, fungicides), as well as the other

additives in the tank mix (Momentive 2018c; Momentive 2019; Wilbur-Ellis n.d. -c; WinField Agrisolutions n.d.). The concentrations of the TSSs in the adjuvants varies widely ranging from 30 – 100% (Dow Corning 2009; Momentive 2017; Wilbur-Ellis 2017; Momentive 2018a, 2018b; Dow 2019; Dow Corning n.d.; Loveland n.d.; Wilbur-Ellis n.d. -b). Assuming 100 gallon per acre for labels not listed in a volume to volume concentration, the concentration of TSSs in a field tank mix solution ranged between 90 - 50,000 ppmv (90 - 52,000 mg/L based on adjuvant densities) for all applications, which includes the range of 1-2% (10,000-20,000 ppmv) of TSSs for pesticide spray tank mixes mentioned in the literature (Mullin et al. 2016; Chen et al. 2018). These calculations show that fields may be exposed to TSS concentrations that are widely variable and highly dependent on the discretion of the spray applicator.

Characterization of Trisiloxane Surfactants

Formulations of many TSSs adjuvents are listed as unspecified proprietary mixtures of siloxylated polyethers or copolymers (Helena 2015a, 2015b; Winfield 2018). However, Chen & Mullin (2013) found that three main TSS oligomer classes were frequently detected in commercial adjuvant mixtures including Dyne-Amic, Kinetic HV, Silkin, Silwet L-77, Sylgard 309 and Syl-Tac. These oligomer classes are comprised of a hydrophobic methylated trisiloxane backbone bonded to a variable length, hydrophilic tail comprised of ethoxy (EO) or propoxy (PO) groups. The end functional groups are either hydroxy (OH), methoxy (OCH_3), or acetoxy ($OC(O)CH_3$). The three oligomer classes are shown in Figure 2 and are referred to as TSS-H (hydroxy), TSS-CH_3 (methoxy), and TSS-$COCH_3$ (acetoxy) hereafter.

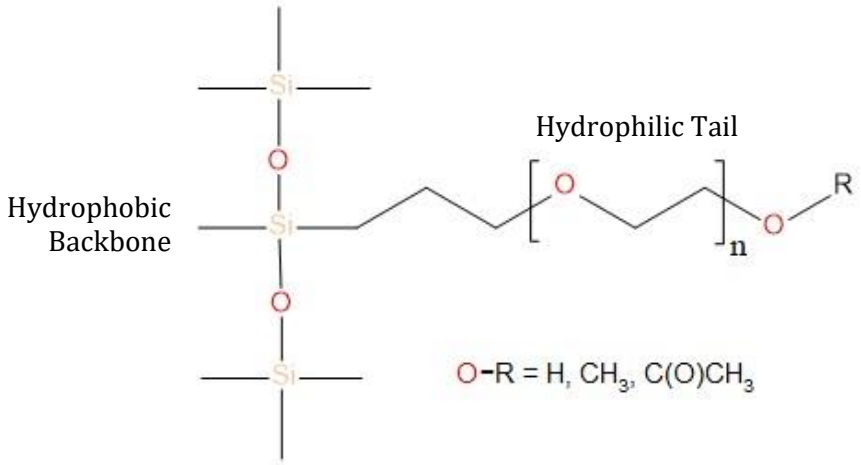

Figure 2. Common trisiloxane surfactant structure in adjuvants

The hydrophobic and hydrophilic parts allow TSSs like Silwet 806 and Silwet 408 to form aggregates, which can decrease the surface tension of the solution and allow it to spread. The concentration at which aggregates form is called the critical aggregation concentration (CAC), while micelles, a specific subset of aggregates, are formed at the critical micelle concentration (CMC). The CMC is normally the same or slightly higher than the CAC for non-ionic surfactants (Diamant & Andelman 1999). At TSS concentrations greater than the CAC, a critical wetting concentration (CWC) occurs, where the solution completely wets a hydrophobic surface. TSSs can have CMC and CAC ranging from 10 - 250 mg/L, with many on the lower end around 50 mg/L (Ivanova et al. 2010; Momentive 2018c; Momentive 2019), while the CWC is usually an order of magnitude higher around 140 - 1000 mg/L (Ivanova et al. 2010; Venzmer 2011). It should also be noted that the CAC, CMC, and CWC are dependent on the number of ethoxy groups in the oligomer (Ivanova et al. 2010; Venzmer 2011), demonstrated by Table 1. It is important to note that these compounds may interact with their environment differently depending on their concentrations and whether it is above or below the CAC, CMC, or CWC.

The quantitative analysis of TSS adjuvants is not well defined since they are

comprised of many compounds and analytical standards for individual oligomers comprising the three main classes (TSS-H, TSS-CH_3, and TSS-$COCH_3$) are not commercially available. The only available commercial standards of these TSS compounds are mixtures that are ≥90-95% pure and comprised of over 12 oligomers for each functional group with hundreds of impurities. Appendix A1 provides more information about the oligomeric distribution for the standards and impurities. The impurities in the standards are generally more polar, can be found in some of the adjuvants, and some suggested identities include other trisiloxanes, methyl polyethoxylates, and polyethylene glycols (Bonnington et al. 2004; Chen & Mullin 2013).

Table 1. Critical Aggregate and Wetting Concentrations

TSS-H Oligomer	4	6	8	9	12
CAC (mg/L)	10-50	33-52	59-253	101	-
CWC (mg/L)	119-500	271-303	390-499	333-495	1000

Sources: Ivanova et al. 2010; Venzmer 2011

Toxicities of Trisiloxane Surfactants

Currently, adjuvants, including TSS formulations, are not required to be registered with the EPA (Penn 2015), as they are considered biologically inert or non-toxic. Aquatic and oral toxicities for the trisiloxanes and adjuvants (Table B1-1), expressed as EC_{50} and ED_{50} values, found in the associated safety data sheets (SDS) and literature articles, are normally in the mg/L or mg/kg range. However, it should be noted that concentrations sprayed on crops can be orders of magnitude greater than toxicity values listed in the SDSs. In addition, recent publications (Cowles et al. 2000; Ciarlo et

al. 2012; Fine et al. 2017; Chen et al. 2018; Li et al. 2019) have found that the direct toxicities for TSSs could be higher than toxicities listed in the product information and that there are also indirect toxicities associated with many of these adjuvants. With the general decline of pollinators since 2006 (Mullin et al. 2015; Chen & Mullin 2015) and the billions of dollars honey bees add to US crops (Fine et al. 2017), an increase in toxicities by TSSs alone or synergistically with pesticides or viruses becomes more environmentally relevant to explore.

Cowles et al (2000) reported the direct toxicity of OSS and TSS adjuvants, such as Silwet L-77, Silwet 408, and Silwet 806, to soft bodied insects. An LC_{50} of 5.5-8.9 mg/L was reported for two-spotted spider mites after being dipped into the aqueous surfactant solution. Chen et al. (2018) found a honey bee mortality rate of over 75% within 10 days when orally fed 100 mg/L (0.01%) TSS-H, TSS-CH_3, or TSS-$COCH_3$ in sugar water, with the acetoxy being the most toxic, followed by the methoxy. However, Li et al. (2019) found that the toxicities of the organosilicones alone were highly dependent on the species, as four different organosilicones were found to be highly toxic (EC_{50} 3.6 - 94.9 μg/L) to *Daphnia magna,* moderately to slightly toxic (3.89 - 96.5 mg/L) to *Brachydanio rerio* (Zebra fish), and had no lethal bioactivities against *Spodoptera exigua* and *Agrotis ipsilon* (two types of moths). Comparing the toxicities reported in the SDSs to the experimental toxicities, the toxicity for Silwet-408 (a trisiloxane determined to be mainly TSS-H) was found to be over 750 times more toxic to *Daphnia magna* than reported in the product information, but the toxicity for Zebra fish was very similar. Li et al. (2019) hypothesized that the organosilicones were more toxic to *Daphnia magna* because its protective outer layer was thinner than the epidermis of the Zebra fish.

Recent studies have also shown that these TSS adjuvants may have more of an impact to pollinators and other insects than previously documented through indirect toxicities. Learning impairments for honeybees have been found at an oral dose of 20 µg for organosilicon adjuvants Dyne-Amic, Syl-Tac, Sylgard 309 and Silwet L-77 (Ciarlo et al. 2012), as well as a synergistic effect Sylgard 309 had with bee virus BQCV at a 10 ppm dose, resulting in a 44.5% increase in mortality from the 21.6% mortality rate of the virus alone or 4.1% mortality rate of the OSS alone (Fine et al. 2017). Lastly, numerous studies have found that co-formulants or tank adjuvants can cause pesticide active ingredients to have different responses (Mullin et al. 2016), but data reporting the combined toxicities of organosilicones, including TSSs, and pesticides is scarce (Li et al. 2019). Li et al. (2019) studied four organosilicones, two of which are known TSSs, and found that the joint toxicity with indoxacarb varied between organism and organosilicone. For instance, the joint toxicities ranged from being all synergistic against *Daphnia magna* to antagonistic for some organosilicones and species (Li et al. 2019). These differences in direct toxicities between reported documentation and experimentation, and the indirect effects to organisms, show the importance of determining the mode of toxic action, the potential environmental exposure concentrations and fate of TSS compounds in the environment.

Environmental Concentrations of Trisiloxane Surfactants

Within the past decade, as shown in Table 2, TSS-H, TSS-$COCH_3$, and TSS-CH_3, have been found in German surface waters (Michel et al. 2014), pollen and beeswax (Chen & Mullin 2013), and almond flowers (Chen & Mullin 2015). Of these three TSSs,

Chen & Mullin (2013) found 60% of the pollen samples (n=10) contained TSS-CH_3 and 10% contained TSS-H. However, there are many other TSSs commercially available that may also be found in the environment. For example, Chen & Mullin (2015) identified, but did not quantify, three different TSSs in almond flowers that included a PO group in addition to EO. The reported concentrations of TSSs measured in environmental samples, are the ng/g or ng/L range, which are lower than the reported toxic levels, suggesting that TSS impacts may be more important in the short term after direct spraying. Nevertheless, it is important to understand the fate and transport of these chemicals into potential sources, like pollen and water, to determine the impact they may have on foragers such as honey bees, as well as perform a larger case study to examine how widespread these TSSs may be found.

Table 2. Concentrations of TSS Surfactants Reported in Literature[a,b]

Sample	TSS-H (Hydroxy) (n = EO)	TSS-$COCH_3$ (Acetoxy) (n = EO)	TSS-CH_3 (Methoxy) (n = EO)
Pollen (ng/g)[a]	<MDL(0.78)-20 (4-13)	<MDL(0.81) (3-13)	<MDL(0.63)-22 (2-13)
Beeswax (ng/g)[a]	<MDL(0.63) (4-13)	<MDL(0.69)-390 (3-13)	<MDL(0.51)-153 (2-13)
Honey (ng/g)[a]	<MDL(0.56) (4-13)	<MDL(0.60) (3-13)	<MDL(0.53) (2-13)
Water (ng/L)[b]	NA	NA	<LOQ(1.9)-470 (4-9)

- Max TSS concentrations are sum of oligomers
- Average method detection limits (MDLs) shown for methoxy, acetoxy, and hydroxy oligomers
- Analysis was performed using high performance liquid chromatography (HPLC) mass spectrometry (MS) with either single ion monitoring (SIM)[a] or MS/MS[b] monitoring

Sources: (a) Chen & Mullin 2013, (b) Michel et al. 2014

Environmental Fate of Trisiloxane Surfactants

Many papers have been published investigating the surface properties of trisiloxanes (Hill 2002; Venzmer 2011), but very few about the fate of these compounds in the environment. Persistence and transport are two important aspects to focus on when determining environmental fate. Volatilization, sorption, photolysis, biodegradation, and hydrolysis are key processes that determine the environmental distribution and persistence of a chemical.

Based on their chemical structure, direct photolysis of these compounds is not expected. In addition, based on vapor pressure (P_v) estimates from GCMS data (Appendix B2) and air-water partition coefficient (K_{AW}) estimates obtained from the property estimaton program EPI Suite (US EPA 2019, Appendix B4), volatilization of these compounds from water is expected to be minimal. Michel et al. (2016) performed a soil leaching experiment using TSS-CH_3 and found that 85-95% of the TSS was in the first 15 cm of soil and the log K_{oc} values for oligomers 5-12 ranged from 3.3-4.1, indicating relatively high sorption to soil organic matter and low mobility in groundwater. Studies have shown that TSS compounds hydrolyze, but only a few hydrolysis products have been identified or suggested (Powell & Carpenter 1997; Radulovic et al. 2010; Michel et al. 2012; Laubie et al. 2013; Michel et al. 2014; Michel et al. 2016). TSS hydrolysis rates increase with increasing temperatures and as the pH diverges from neutral (Table 3). Though compounds that undergo hydrolysis are often biodegradable, US EPA (2019) EPI Suite estimates TSSs not to be readily biodegradable, with the half life estimated to be weeks to years (Appendix B4), indicating a need for current fate models to incorporate siloxane chemistry.

Table 3. Literature Hydrolysis Times

Structure	Half Life	pH	Temp (°C)	Source
TSS-CH_3	2 days	9	12	Michel et al. 2014
TSS-CH_3	<1 day	9	25	Michel et al. 2014
TSS-CH_3	150-300 days	7	12	Michel et al. 2014
TSS-CH_3	29-55 days	7	25	Michel et al. 2014
TSS-CH_3	1-2 days	7	50	Michel et al. 2014
TSS-CH_3	<0.35 days	5	-	Powell & Carpenter 1997
TSS-CH_3	8.35 days	7	-	Powell & Carpenter 1997
TSS-CH_3	0.39 days	9	-	Powell & Carpenter 1997
Sylwet L77 (TSS-CH_3)	43 min	3	24	Knoche et al. 1991

Though the identity of several hydrolysis products for trisiloxanes have been proposed, the physical-chemical characteristics of these compounds have not been determined and the compounds themselves are not commercially available (Powell & Carpenter 1997; Bonnington et al. 2004; Michel et al. 2014; Chen & Mullin 2015). Figure 3 shows one of the proposed hydrolysis pathways and products.

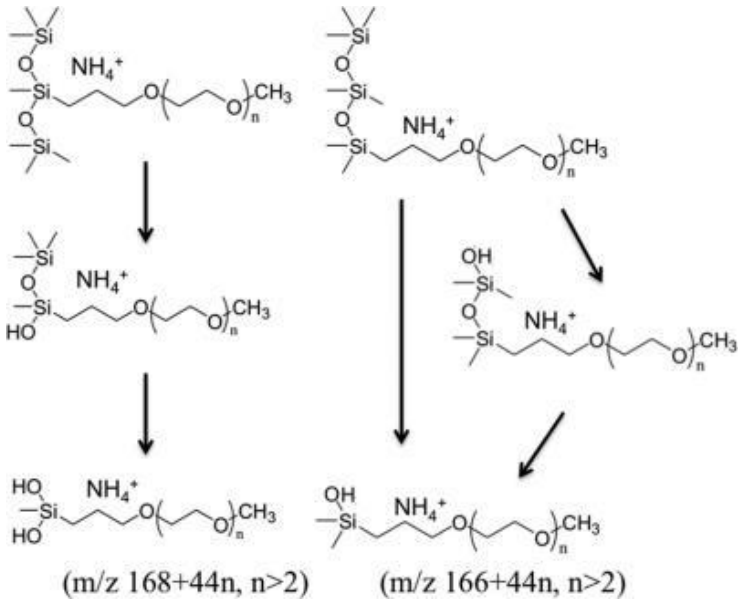

Figure 3. Proposed hydrolysis pathway (from Chen & Mullin 2015)

Using the Fugacity Level 3 model within EPI Suite (US EPA 2019) with parameters and partition coefficients listed in Table B4-1, the percentage of mass and concentration located in six different environmental compartments was estimated (Table B4-2). Using the default compartment volumes, the soil compartment was the most likely location for most of the mass to accumulate at 66-78%, with water second (22-32%) and suspended sediment the least (<0.0001%). The highest concentration is estimated to be in biota (fish), followed by soil, with air last having the lowest concentration of TSSs. With increasing ethoxy groups, the overall mass in water and concentration in biota is estimated to decrease, while mass and concentration in soil increase. The persistence for all compounds, except TSS-H 03, is expected to be more than 2 weeks.

Plant Uptake and Surfactant Effects on Pesticidal Uptake

With agricultural application of TSS adjuvants ranging in concentrations of 100 mg/L to up to 50,000 mg/L and the majority of TSSs estimated to be retained in the topsoil, plant root uptake and subsequent transfer to pollen, if significant, could be a potential pathway of concern to foragers like honeybees. In addition, the TSS adjuvants could help pesticides enter the plant, potentially affecting both foragers and human consumption. TSSs have been found in water, pollen, beeswax, and almond flowers (Chen & Mullin 2013; Michel et al. 2014; Chen & Mullin 2015), indicating there are potential routes of exposure to plants and water systems due to field spraying. Three potential pathways for TSSs to reach pollen and other parts of the plant are direct contact, uptake via the foliage and translocation through the phloem, or uptake through the root hairs and translocation through the xylem (Figure 4).

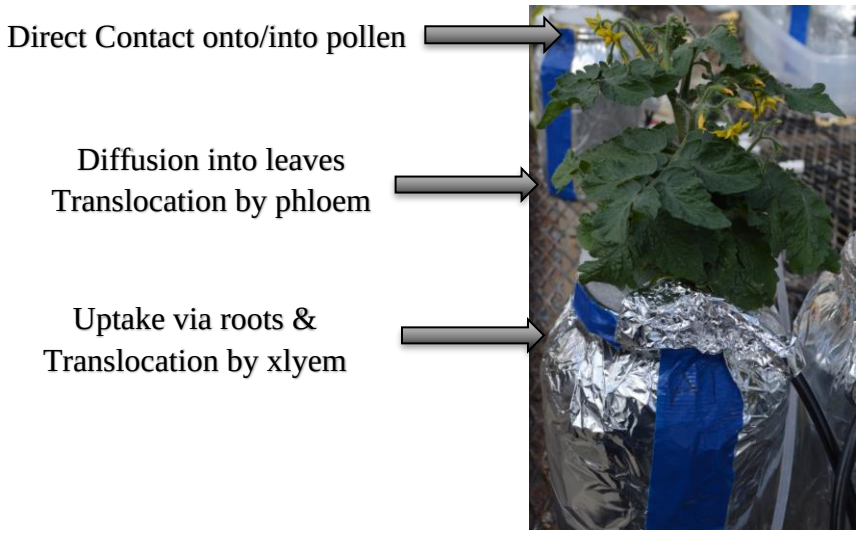

Figure 4. TSS sorption routes in plants

Since adjuvants are added to many field sprays at high concentrations, direct contact is likely the major pathway into the pollen if the spray is applied while flowering crops or weeds are in bloom. Though it is not best practice to use pesticides on blooming crop or weeds while honey bees are present, some pesticides are applied anytime (Biddinger 2017) meaning that adjuvants could be potentially applied to blooming flowers as well. This could allow the adjuvant to directly contact the pollen.

Diffusion into the foliage, then transport via the phloem is another potential route of exposure for TSSs. Studies have shown that Silwet L-77 helps in the foliar uptake of herbicides and pesticides (Zabkiewicz et al. 1993; Forster et al. 2004; Liu 2004; Mora-Garcia & Spanoghe 2016), but few studies addressed the uptake of the surfactants themselves. One study by Zabkiewicz et al (1993) showed that 51-72% of the initial concentration of Silwet L-77 applied was taken up into plant leaves within 6 hours. This indicates that the uptake of TSS adjuvants into foliage could be significant. Normally permeation through plant membranes is determined by the compound's lipophilicity

(Bromilow et al. 1987), but TSSs are thought to first rapidly enter the plant via stomates due to the low surface tension (Knoche et al. 1991; Liu 2004), then slowly diffuse through the cuticle (Zabkiewicz et al. 1993). Once inside the leaf, the compounds may reach the phloem. However, the intermediate permeability theorem by Tyree et al. (1979) states that while many compounds can enter phloem, only those that have low membrane permeability will be translocated over significant distances (Bromilow et al. 1987). This means that lipophilic compounds with log octanol-water partition coefficient (K_{OW}) greater than three or four do not generally translocate (Forster and Kimberley 2004), while more polar compounds could be translocated. Thus, trisiloxanes themselves are not likely to be transported via the phloem based on their log K_{OW} values that are estimated to be 3.5 or greater. Though trisiloxane surfactants themselves may not travel in the phloem, it has been found that surfactants significantly affect the translocation ratio, specifically for 2-deoxy-D-glucose, a commonly used metabolic tracer, and 2,4-dichlorophenoxy-acetic acid, an herbicide (Forster and Kimberley 2004).

Another method of transport of xenobiotics in a plant is uptake via the roots and transport into the shoot via the xylem. Xylem sap flows can be up to 100 times greater than that of the phloem (Bromilow et al. 1987). The ability for a compound to travel from root to shoot is often described by a transpiration stream concentration factor (TSCF), which is the ratio between the compound in root solution to the concentration in the plant, where a higher TSCF value means that more compound can be found in the above ground plant tissues. TSCF values are generally measured using intact plants or a in detopped plants using pressure chamber (Dettenmaier et al. 2009). Studies have shown TSCF to be related to the K_{OW} (Figure 5), with higher K_{OW} values often having lower TSCFs. Log

K_{OW}s for TSS oligomers based on retention times from liquid chromatography tandem mass spectrometry (LCMS/MS) were estimated to be 3.5-3.9 for TSS-H, 3.8-4.6 for TSS-CH_3, and 3.9-4.7 for TSS-$COCH_3$ (Appendix B3), meaning the TSCFs are estimated to be relatively low (Figure 5). However, even compounds having relatively low TSCF values can still reach above ground plant tissues if the exposure concentration and amount of water transpired are high (Dettenmaier et al. 2009; Orita 2012).

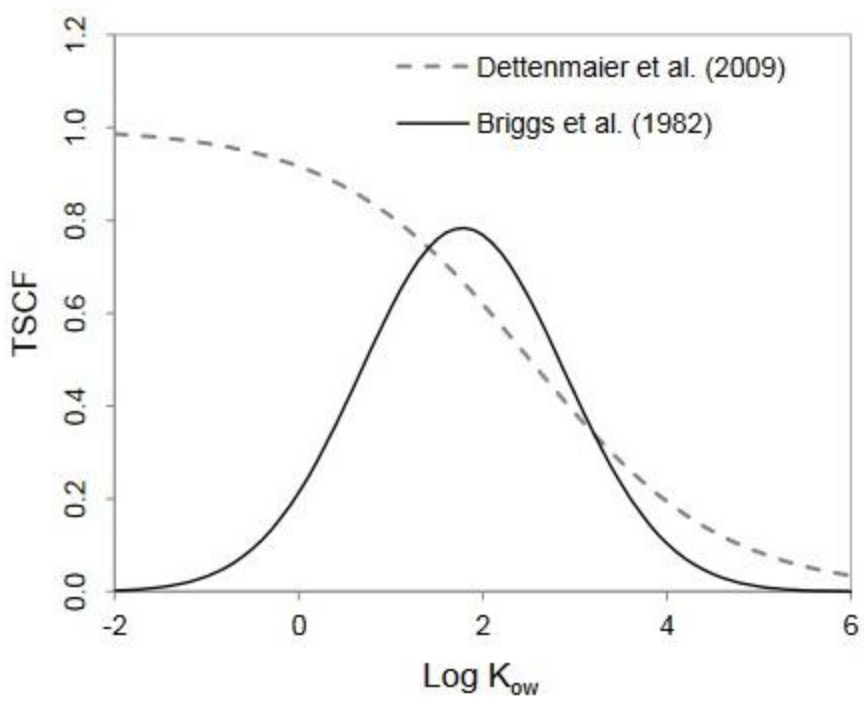

Fig. 2. Comparison of two relationships established by Briggs et al. (1982) and Dettenmaier et al. (2009) [5]

Figure 5. Log K_{OW} vs TSCF relations (from Orita 2012)

CHAPTER III

DEVELOPMENT OF ANALYTICAL METHODS FOR TSS EXTRACTION AND ANALYSIS

Methods for extraction of TSS from environmental samples are lacking in the literature. This may be due to the analytical complexity of TSS adjuvants and the standards available. In order to analyze for TSSs in pollen and other environmental media, a liquid chromatography tandem mass spectrometry (LCMS/MS) method was developed and used to evaluate several extraction methods.

Methods and Materials

Standards of TSS-H, TSS-CH_3 and TSS-$COCH_3$ were purchased from Gelest Inc. (Morrisville, PA USA) with purities ranging from ≥90-95%. The commercial adjuvants, Silwet L-77 and Xiameter OFX-309, were donated from the manufacturers. Acetonitrile (ACN), methanol (MeOH), and formic acid were obtained as LCMS grade from Fischer Chemicals. Solid ammonium formate (100% pure) from Fischer Scientific was also used. Y.S. Eco Bee Farms pollen used for method development was purchased from General Nutrition Centers (GNC) store in Logan, UT.

Liquid chromatography tandem mass spectrometry (LCMS/MS) was more sensitive and had fewer trisiloxane contamination (septa) problems than gas chromatography mass spectrometry (GCMS). The LCMS/MS system consisted of an Agilent 1290 LC connected to an Agilent QQQ 6490. The mobile phase was composed of (A) water and (B) ACN/MeOH at a 90/10 v/v ratio, with both solvents containing 2 mM ammonium formate and 0.01% formic acid (Chen & Mullin 2013). Ammonium

formate (2mM) was added to the solvent phase to create ammonium adducts and suppress other adducts that can form due to the ability of the polyethoxylated chain to trap metal ions and form complexes (Chen & Mullin 2013; Bonnington et al. 2004; Okada 1990; Okada 1993). A Phenomenex PolymerX RP-1 100Å 5 µm, 150 mm x 2 mm column was used initially but later switched to a 50 mm column that shortened analysis times while maintaining adequate chromatography separation. It was also determined that a polystyrene divinylbenzene phase was better than silica C18 at reducing column degradation caused by interactions between siloxanes and the silica backbone (Michel et al. 2012). Due to the pressure requirements of the LC pump (100 bar minimum) and the polymer column (172 bar maximum), the flow rate was not isokinetic, but increased flow rate as the solvent became more organic to keep the pressure high enough. Several different acquisition methods were used through the course of this research due to changes to the solvent programming that were made to improve chromatography separation and monitor additional ions.

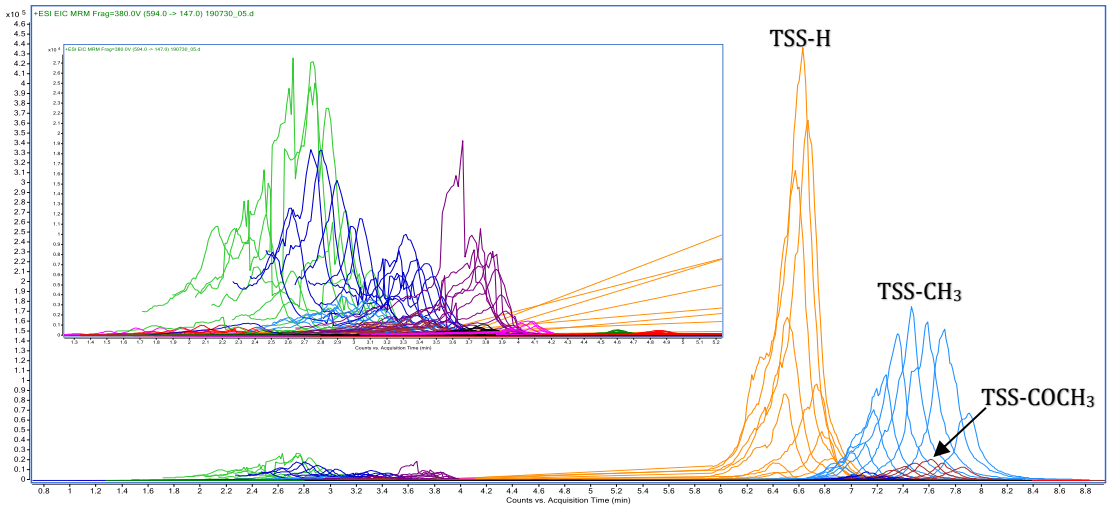

Figure 6. MRM transitions on 50 mm column for oligomeric groups
(Insert figure zoomed in on MRM transitions of oligomeric groups of impurities)

Multiple reaction monitoring (MRM) transitions were determined experimentally for each the three TSS standards, with the number of EO groups ranging from n=3 to n=15 for TSS-CH_3 and TSS-$COCH_3$ and n=3 to n=16 for TSS-H (Figure 6). The MRMs transitions are listed in Appendix C3 and TSS-CH_3 and TSS-H are similar to transitions listed previously in the literature (Michel et al. 2012). The oligomeric percentages for the three TSS standards, based on a Poisson equation from Michel et al. (2012), are given in Table 4 and details explaining how the distribution was obtained is explained in Appendix A1. Limits of quantitation (LOQ) for TSS compounds were determined based on a 10 times signal to noise (S/N) ratio using standards in ACN and more information can be found in Appendix A2.

Several methods were evaluated to determine the best approach for extracting TSSs from pollen including liquid solid extraction Chen & Mullin (2013), sonication, Accelerated Solvent Extraction (ASE) (Dionex ASE Model 150), and an Energized Dispersive Extraction (EDGE by CEM) (Figure 7). Procedures for these extraction techniques are summarized in Table 5.

Table 4. Percent of Each Oligomer in TSS Standards

Oligomer (#EO)	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
TSS-H	2.2	5.1	9.2	13.0	15.2	15.2	13.2	10.1	7.0	4.4	2.5	1.3	0.6
TSS-CH_3	2.2	5.2	9.2	13.0	15.2	15.1	13.1	10.1	7.0	4.3	2.5	1.3	0.6
TSS-$COCH_3$	2.2	5.3	9.3	13.1	15.2	15.1	13.1	10.0	6.9	4.3	2.4	1.3	0.6
TSS-H n=16 is 0.29%													

Figure 7. Instrumentation used for TSS extraction in environmental samples Dionex ASE 150 (left), CEM EDGE (right)

The initial EDGE extraction method was further evaluated using different temperatures (80, 100, and 110^{o}C), different sample holding times (0, 1, and 2 minutes), different volumes used for extraction (5, 6, 10, and 20 mL), and different sorbents added into the extraction, including sodium acetate, magnesium sulfate ($MgSO_4$), C18, PSA, Florisil, diatomaceous earth, and graphitized carbon black (GCB). In addition, several cleanup methods for after extraction were evaluated, including the use of Quechers and individual sorbents (Florisil, $MgSO_4$, PSA, and C18). The use of a Turbovap™ Automated evaporation/concentration system was evaluated to reduce solvent extract volume and increase overall method sensitivity. The necessity of homogenizing pollen samples was briefly examined by comparing pollen samples that were homogenized with liquid nitrogen in a mortar and pestle with pollen samples that were not. The pollen

sample used to evaluate the difference between homogenized and non-homogenized prior to extraction was from a local beekeeper on 3/3/2017 (labelled DC) that had previously been found to contain TSS-H.

Table 5. TSS Extraction Methods

Method	Procedure
Sonication	- 2 mL ACN to 1 g pollen in 50 mL centrifuge tube - Vortex 10 s and sonicated at room temp for 10 min - Centrifuged 5 min at 5000 rpm - Liquid extract placed in 5 mL volumetric flask - Method repeated 2 times more until 5 mL volumetric flask full
Liquid-Solid Extraction (Chen & Mullin 2013)	- 2 mL ACN and 2 mL water to 1 g pollen in 50 mL centrifuge tube - Vortexed 30 s - 2.0 g $MgSO_4$, 0.5 g sodium acetate added - Immediately vortexed 30 s - Placed in ice bath for 10 min - Centrifuged 10 min at 65000 rpm and top layer transferred to vial
ASE	- 1 g pollen in 5 mL ASE cell with approx. 0.6 g diatomaceous earth - ACN at 40°C, 5 min static cycle, 60% rinse, 60 s purge - ACN ~12 mL total volume at end of extraction
EDGE - Initial	- 1 g pollen in EDGE Q-cup with PTFE filter - 20 mL ACN, 100°C, 1 min hold - Extract filtered through Nylon 0.2 µm filter
EDGE - Reduce1 (Sorbent Cleanup and Solvent Reduction #1)	- 1 g pollen in EDGE Q-cup with PTFE filter - 20 mL ACN, 100°C, 1 min hold - Added 100 mg C18, 250 mg PSA to extract - Vortex 15 s, centrifuge 3 min at 5000 rpm - Transferred 16 mL to Turbovap™, water bath 40°C, no rinsing - Filtered through Nylon 0.2 µm filter (clogged fast w/pollen samples)
EDGE - Reduce2 (Sorbent Cleanup and Solvent Reduction #2)	- 1 g pollen in EDGE Q-cup with PTFE filter - 20 mL ACN, 100°C, 1 min hold - Transferred to Turbovap™, water bath 40°C for 30 minutes - Added 1mL concentrate to 2mL centrifuge containing 50 mg C18, 150 mg PSA - Vortex 15 s, centrifuge 3 min at 5000 rpm
EDGE - Final	- 1 g pollen in EDGE Q-cup with PTFE filter - 15 mL ACN, 100°C, 1 min hold

Results

The four extraction methods were used to compare extraction of TSSs from a field pollen samples and spikes, mailed controls and spikes (more information in Chapter IV), and control blanks and spikes without pollen were compared (Table 6). The LSE extraction method had the highest extraction of 12.8 ng TSS-H/g pollen in the sample, but the lowest spike recoveries for E09-02 and mailed samples for all TSS except TSS-$COCH_3$ for mailed samples. The final method for the EDGE was the second highest in extracting ng TSS-H/g pollen and also had relatively high extraction recoveries.

Table 6. Comparison of Extraction Techniques

Sample	Method	ng TSS/ g pollen*		TSS Spike % Recovery **			
		n	Hydroxy	n	Hydroxy	Acetoxy	Methoxy
No pollen Control	ASE	1	<LOQ	1	69	$119^{b,c}$	116^{d}
	Liquid-Solid	1	<LOQ	1	89^{a}	87^{b}	88^{e}
	Sonication	3	<LOQ	5	97 ± 5^{a}	98 ± 6^{c}	102 ± 6^{e}
	EDGE (Final Method)	9	<LOQ	3	120 ± 12	111 ± 3^{c}	112 ± 18^{d}
Mailed Controls* **	ASE	1	<LOQ	1	49	6	89^{i}
	Liquid-Solid	1	0.1	1	14	4^{g}	$28^{i,j}$
	Sonication	4	<LOQ	4	29 ± 3^{f}	3 ± 1^{h}	46 ± 3^{j}
	EDGE (Final Method)	2	<LOQ	3	28 ± 11^{f}	$8 \pm 1^{g,h}$	37 ± 12
E09-02 (E10-01 spiked for EDGE)	ASE	2	6.0 ± 6.1^{k}	2	90 ± 269^{m}	118 ± 311^{n}	113 ± 267^{o}
	Liquid-Solid	2	12.8 ± 3.4^{l}	1	41^{m}	42^{n}	40^{o}
	Sonication	2	7.6 ± 4.2^{k}	1	79^{m}	82^{n}	84^{o}
	EDGE (Final Method)	1	12.6^{l}	3	88 ± 11^{m}	70 ± 14^{n}	81 ± 17^{o}

- Average ± 95% CI with n being the number of samples analyzed
- Recoveries given are averages for oligomers above LOQ
- Superscript letters are methods that are not significantly different based on Tukey Tests between groups with the same sample and compound analyzed

*Acetoxy and methoxy TSS were all <LOQ for non-spiked samples
**Method control spikes, mailed spikes, and E10-01 spikes were spiked with 400 ng, while E09-02 spikes were spiked with 100 ng
***Mailed controls were Y.S. Eco Bee Farm pollen sent out to field and back (more information in Chapter IV)

A comparison between homogenizing and not homogenizing the pollen before extracting in the EDGE was performed (Table 7). The total average ng TSS-H/g pollen concentration found was similar between homogenized and non-homogenized pollen. However, the standard deviation was much lower with the pollen that was homogenized, demonstrating that the pollen is heterogeneous, and it is important to homogenize the sample to have lower variability and higher accuracy.

Table 7. Extraction Comparison of TSS-H in Homogenized or Non-Homogenized Pollen

Oligomer (#EO)		3	4	5	6	7	8	9	10	11	12	13	Total
ng TSS-H /g pollen	Non-Homogenized	9	16	25	45	51	55	51	43	32	21	12	358 ± 130*
	Homogenized	9	16	27	45	56	62	55	44	37	23	14	386 ± 8*

- Method: EDGE (1st method), 300317 DC Pollen used
- Oligomers 14, 15, 16 were below limit of quantitation

*n=2, standard deviations shown

In order to optimize the extraction of TSSs in the EDGE, multiple changes in sample hold time, extraction temperature, solvent volume, and sorbents were evaluated. Figure 8 shows the average extraction recovery for all of the oligomers within a compound class for the various extraction parameters. For EDGE extraction, one-minute extraction at 100^{o}C was chosen as it seemed to have the highest percent recovery overall and increasing the time by another minute did not have much impact, while increasing the temperature had a negative impact on recovery (Figure 8a). Some sorbents also had a negative impact on recovery, especially Florisil and GCB (Figure 8c-d). On the other hand, increasing solvent volume had a positive impact when extracting TSS from pollen (Figure 8b), and thus a total solvent volume of 15 mL was chosen as a compromise between extraction efficiency and sensitivity.

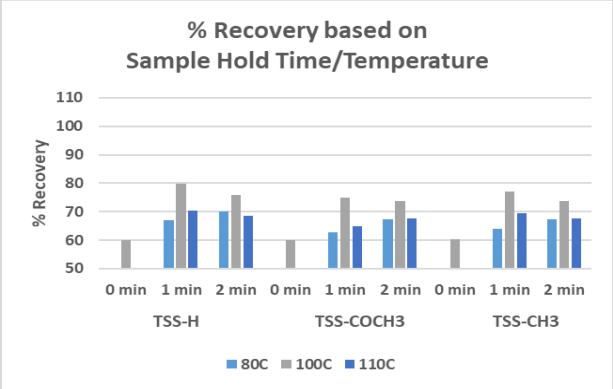

Figure 8a.
% Recovery based on Sample Hold Time and Temperature

- *500 ng/mL spike, 1g pollen, EDGE ACN extracted, 20 mL unless otherwise stated, filtered through Nylon syringe*

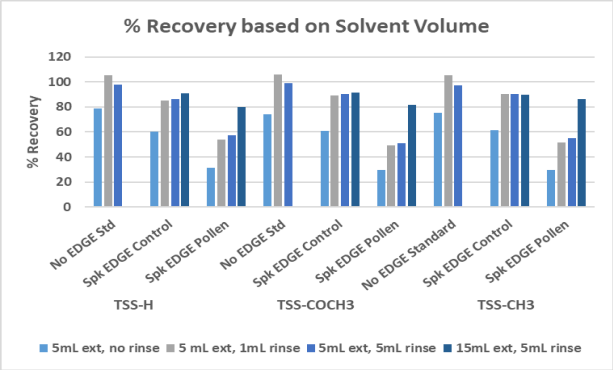

Figure 8b.
% Recovery based on Solvent Volume

- *500 ng/mL spike, 1g pollen, EDGE ACN extracted, 20 mL unless otherwise stated, filtered through Nylon syringe*

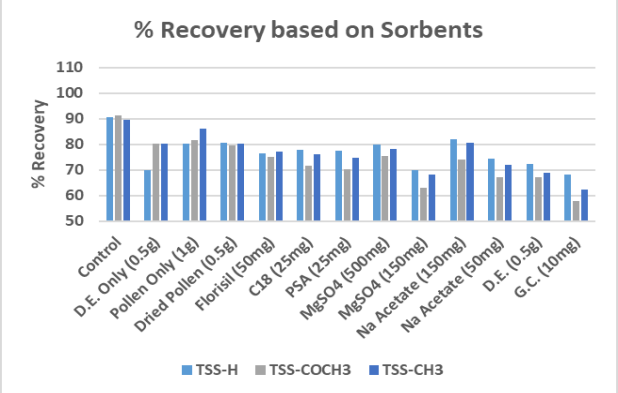

Figure 8c.
% Recovery based on Sorbents During Extraction

- *D.E. means Diatomaceous Earth, G.C. means graphitized carbon*
- *500 ng/mL spike, 1g pollen, EDGE ACN extracted, 20 mL unless otherwise stated, filtered through Nylon syringe*

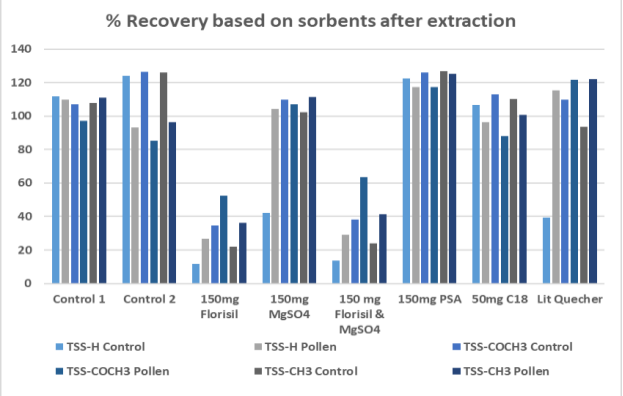

Figure 8d.
% Recovery based on Sorbents After Extraction

- *400 ng/mL spike, no pollen*

Figure 8. EDGE % Recovery of TSSs with varied parameters (n=1 for all methods, all oligomers included in calculations)

It was anticipated that reducing the solvent volume by evaporation using a nitrogen stream (Turbovap™) would lower the limit of quantitation. However, TSS appeared to be lost in the concentrations step when pollen was present as the ng TSS-H/g pollen extracted was lower for samples that had undergone solvent reduction (Reduce1 and Reduce2 methods in Table 8). However, due to low replication and high variability, many of the methods were not significantly different at 95% CI. Possible loss mechanisms during solvent reduction may include sorption to the Turbovap™ tubes, precipitates that were filtered out, or sorbents used for clean-up. Degradation due to prolonged heating is also a potential loss mechanism. Two attempts to concentrate the solvent extract to increase sensitivity are shown in Table 8.

Table 8. Comparison of EDGE Techniques with and without Solvent Reduction

Sample	EDGE Method	ng TSS/g pollen* (ng TSS/sample for blanks)			Spike Recovery***			
		n	Hydroxy	Methoxy	n	Hydroxy	Acetoxy	Methoxy
Method Control (No Pollen)	Initial	4**	5 ± 15[a]	3 ± 9[b]	2	87 ± 14[c]	68 ± 27[d]	67 ± 18[e]
	Reduce1	3**	1 ± 5[a]	1 ± 4[b]	3	76 ± 11[c]	81 ± 5[d]	81 ± 4[e]
	Reduce 2	3**	1 ± 3[a]	0.7 ± 0.4[b]	3	69 ± 33[c]	74 ± 38[d]	76 ± 38[e]
	Final	9	<LOQ	<LOQ	3	120 ± 12	111 ± 3	112 ± 18
DC Pollen	Initial	2	386 ± 74	<LOQ	1	93[g]	70[h]	72[i]
	Reduce1	3	112 ± 38[f]	<LOQ	3	36 ± 31[g]	36 ± 22[h]	40 ± 23[i]
	Reduce2	3	178 ± 130[f]	<LOQ	2	69 ± 154[g]	80 ± 125[h]	85 ± 157[i]
	Final	1	210[f]	<LOQ	0	-	-	-

- Average ± 95% CI with n being the number of samples analyzed
- Recoveries given are averages for oligomers above LOQ
- Superscript letters are methods that are not significantly different based on Tukey Tests between groups with the same sample and compound analyzed

*Acetoxy oligomers were <LOQ
**Some readings <LOQ; ng/sample calculated using 0 for samples below LOQ
***Spike recoveries were 400 ng, except for 1st method at 2000 ng

Analysis of Impurities, Hydrolysis Products, and Pesticides

MRM transitions were also determined for 15 groups of impurities in the three TSS standards and one group of unknown compounds found only in Xiameter OFX-309. More information on the MRM transitions and oligomeric distribution can be found in Appendix C3 and Appendix A1 respectively. These impurities were found to be more polar and much lower in concentration than the standards (Figure 6). After hydrolyzing the three TSS standards in water, MRM transitions were found for three additional oligomeric groups, also more polar than the standards. The groups of impurities and hydrolysis products were identified by using a full MS scan and finding compounds with m/z differences of 44, which relates to the ethoxy group. Analysis for impurities and hydrolysis products was performed during the same run as the TSS standards on the same extracts. Thus, the extraction procedure was not optimized for these compounds, but was the same as the one determined for the TSS standards.

A screening method for quantifying more than 60 pesticides was also developed to examine potential correlations with TSS adjuvants concentrations. Pesticide standards were obtained from Ultra Scientific and Phenova, chosen based on a list of common pesticides applied to California almonds. Literature pesticide MRM transitions (Mastovska et al. 2017) were then added to the LCMS/MS acquisition method (Appendix C3). A calibration curve for pesticides from 1 ng/mL to 10 ng/mL was made, and the LOQ for all pesticides was estimated to be at least 0.5 ng/mL. Pesticide analysis was performed during the same LCMS/MS run as the TSS compounds on the same extracts. Thus, the extraction procedure was not optimized for pesticides, resulting in potentially lower extraction and spike recoveries.

As the analysis was optimized for TSS standard compounds and not impurities, hydrolysis products, or pesticides. Pesticide recoveries that are above LOQ can be found under Chapter IV. No recoveries for impurities or hydrolysis products are listed, as no standards have been obtained for them

CHAPTER IV

2018 POLLEN STUDY

To determine the frequency of detections and concentrations of TSSs in pollen, samples were collected by beekeepers and sent back to the UWRL. Spiked pollen samples were sent to the field sites along with the sample collection tubes. These samples were analyzed along with the field pollen samples and were used to assess the TSSs stability during transport. The field pollen samples were also screened for a list 60+ pesticides to examine potential relationships between TSS and pesticide concentrations.

Methods and Materials

The extent of TSS-H, TSS-CH_3, and TSS-$COCH_3$ contamination in pollen was examined by analyzing samples collected by beekeepers from hives located mainly in California during the 2018 spring almond pollination. Five pre-weighed 50 mL polypropylene centrifuge tubes were sent to various beekeepers: three were empty, one contained 1 g of Y.S. Eco Bee Farms pollen (analyzed and found to be TSS free), and the last one contained 1 g of Y.S. Eco Bee Farms pollen spiked with 400 ng of each of the three TSS standards. Ninety-nine pollen samples were collected by beekeepers from pollen traps fitted over hive entrances. These samples were collected in California (88), Wisconsin (9), and Kentucky (2) were then mailed back to the Utah Water Research Laboratory (UWRL) mainly during February and March 2018, with a few sent back in July and August. Examples of mailed tubes and pollen samples can be seen in Figure 9.

Pollen samples were homogenized with a mortar and pestle using liquid nitrogen, and one-gram sub-samples were extracted with 15mL ACN at 100^{o}C using the EDGE®

system by CEM. Aliquots (1mL) were transferred to amber autosampler vials and analyzed by LC-MS/MS in EI mode and MRM transition mode using method TSS_50mm_MRM20uL-Hp (Appendix C2). Linear calibration curves for each oligomeric TSS standard was used with concentrations ranging from 0.5 - 50 ng/mL Concentrations of TSS oligomers were then summed together to simplify data presentation and provide a general overview of concentrations and recoveries.

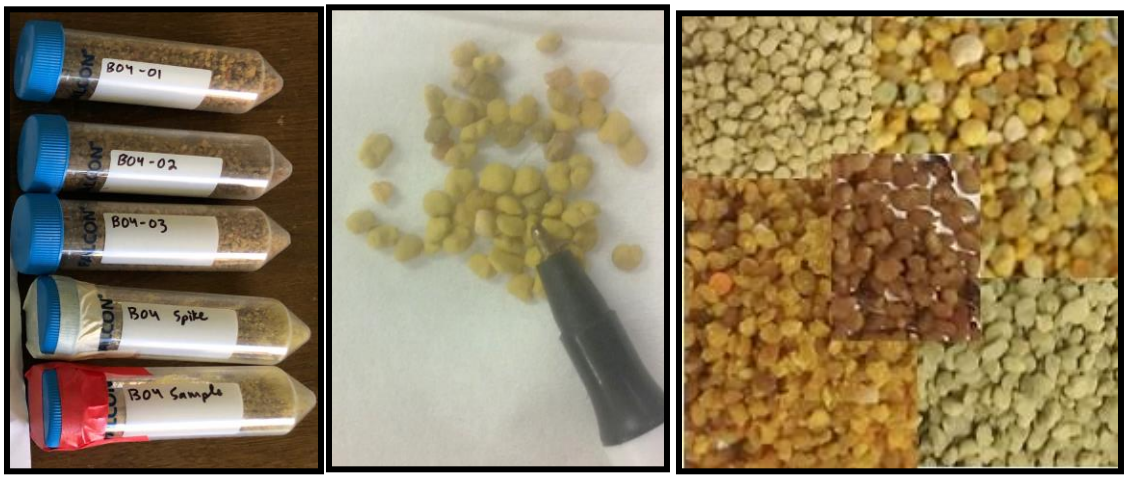

Figure 9. Pollen tubes with mailed samples (left)
Y.S. Eco Bee Farms pollen size compared to pen tip (middle)
Pictures of pollens received from field before homogenization (right)

Concentrations of more than 60 pesticides, commonly used in California almond orchards, were also determined in addition to the three TSS standards (Table 9). The pesticides were purchased from Ultra Scientific and Phenova as components of several standard mixtures. Nine commercial pesticide standard mixtures were combined to generate four standard mixtures that were used to develop calibration curves and determine a single LOQ of 7.5 ng/g for all pesticides analyzed. One of the four standard mixtures was then used as a continuing calibration verification standard (CCV)

throughout sample analysis. Spike recoveries were determined by spiking 400 ng/g onto Y.S. Eco Bee Farms Pollen.

Table 9. List of Analyzed Pesticides

3-Hydroxycarbofuran	Demeton-S	Imazalil	Prochloraz
Acephate	Diazinon	Imidacloprid	Propamocarb
Acetamiprid	Diflubenzuron	Malathion	Propiconazole
Aldicarb sulfoxide	Dimethoate	Mandipropamid	Pyraclostrobin
Atrazine	Dioxacarb	Metaflumizone	Pyrimethanil
Azinphos methyl	Diuron	Metalaxyl	Rotenone
Azoxystrobin	Ethoprofos	Methomyl	Spinesad - A/D
Bendiocarb	Fenazaquin	Methoxyfenozide	Spinetoram - Spinosyn J/L
Boscalid	Fenpyroximate	Monocrotophos	Spirotetramat
Carbaryl	Fensulfothion	Myclobutanil	Tebufenozide
Carbofuran	Fenthion	Novaluron	Teflubenzuron
Chlorfluazuron	Flonicamid	Omethoate	Tetrachlorvinphos
Chlorpyrifos	Flubendiamide	Oxamyl	Thiabendazole
Chlothianidin	Fludioxonil	Paclobutrazol	Thiacloprid
Coumaphos S	Flufenoxuron	Phosmet	Tribufos
Cyazofamid	Flutolanil	Phoxim	
Cyprodinil	Formetanate HCl	Piperonyl butoxide	

- Pesticides in blue commonly used on almonds

Results

Ninety-nine pollen samples – 88 samples from 3 different beekeepers in CA, 9 samples from a beekeeper in WI, and 2 samples from a beekeeper from KY – were analyzed for TSSs and more than 60 pesticides/fungicides. The concentration range for each of the TSS groups are given in Table 10 as a sum of all of the oligomers found in a given TSS class. Not surprisingly, the concentrations of oligomers in the pollen samples followed a similar distribution as the standards with the middle oligomers being most frequently identified and in the highest concentrations. The hydroxy (RO-H) trisiloxane, found in 43% of the samples, was the most commonly identified TSS (Table 10) and was found in pollen from four of the five beekeepers (Figure 10).

Table 10. TSS Concentrations in Pollen Samples

Functional Group	%Pollen with TSS	TSS Concentration* (ng/g)	Oligomers Found (n = EO)	%Recovery (400 ng/g) (immediate)**	%Recovery (400 ng/g) (mailed)***
Hydroxy	43%	<LOQ - 62.9	5-13	62.7 ± 19.5	27.8 ± 4.5
Acetoxy**	15%	<LOQ - 722.7	4-15	56.3 ± 15.5	8.1 ± 0.4
Methoxy	0%	<LOQ	-	64.8 ± 15.3	36.7 ± 5.0

Functional Group	Oligomers Analyzed (n = EO)	Oligomeric LOQ Range (ng/g)	Oligomeric LOG Avg ± Stdev (ng/g)
Hydroxy	3-16	0.40 - 3.86	1.63 ± 1.15
Acetoxy	4-15	1.87 - 15.04	7.28 ± 3.80
Methoxy	3-15	0.65 - 3.91	2.13 ± 1.00

*Sum of oligomers, pollen not on a dry weight basis
**Average and std dev of recovered sum of oligomers of 12 different pollens
***Average and std dev of recovered sum of oligomers of spiked store-bought pollen mailed to beekeepers
****Quant/Qual ions do not match for samples – acetoxy TSS-like compound

Fifteen percent of pollen contained unidentified compounds that had the same retention times (RTs) as the acetoxy TSS oligomers, but had different quant and qual ion ratios than the standards, indicating either a slightly different compound or an interference. However, it should be noted that the ratio for the quant/qual ions in Xiameter OFX-309 do not exactly match the standard ratio either, though the CAS # is the same for both the standard and Xiameter (Appendix B1), implying that there may be an acetoxy TSS-like compound in the samples. All of the pollen with the acetoxy TSS like compound were from the same beekeeper. No methoxy TSS was found in any pollen.

Overall, TSS-H was more prevalent in the pollen, but at relatively low concentrations, while the TSS-$COCH_3$ like compound was less prevalent, but was present at higher concentrations (Figure 11). Pollen containing TSS-H were found in samples from four of the five beekeepers, while the TSS-$COCH_3$ like compound was only found in samples obtained from one beekeeper, indicating different TSS adjuvants were used

(Figure 10). The pollen sample results in this study are different than those found in Chen et al. (2013), where the methoxy standard was the most commonly found standard with hydroxy being next, and no acetoxy found.

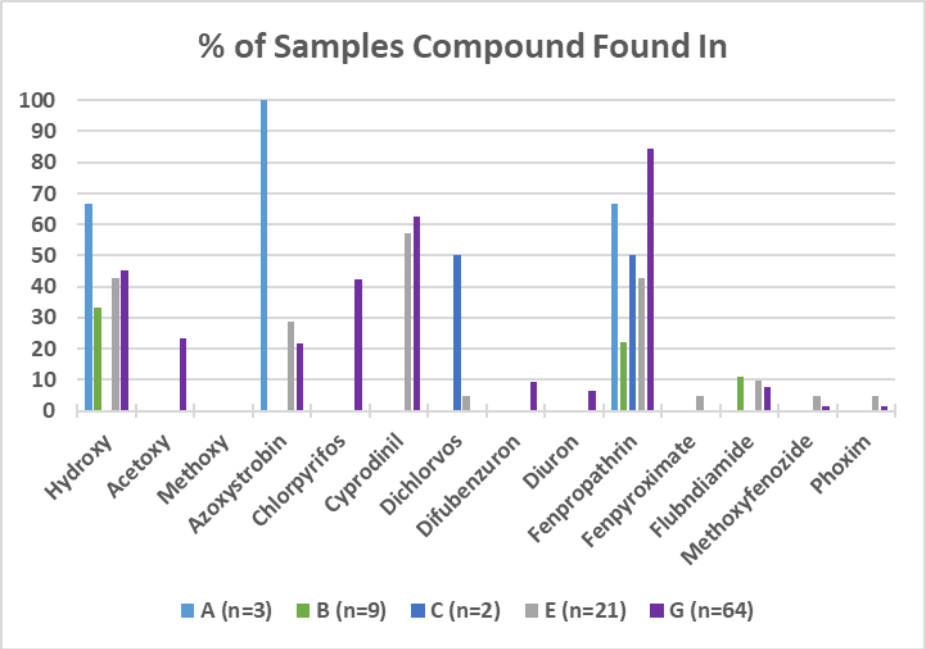

Figure 10. Distribution of analytes across beekeepers
(A, E, G - Samples from California, B - Samples from Wisconsin, C - Samples from Kentucky, D - Samples never received back/analyzed, F - Tubes never sent out)

In addition to the three TSS standards, sample extracts were also qualitatively analyzed for more than 60 pesticides. Nine pesticides were found in the pollen with varying occurrences and concentrations (Table 11). Since the focus of this project was quantifying the TSS compounds in pollen, no attempt was made to optimize extraction efficiencies for the individual pesticides and single LOQ for all pesticides was approximated to be 7.5 ng/g using a low concentration standard. Overall, the pesticides identified and their concentrations found in pollen varied between beekeepers, which is to be expected, as different growers have different practices. However, a correlation

between cyprodinil and the TSS-$COCH_3$ like compound (Figure 12) was observed, indicating these compounds were likely sprayed together.

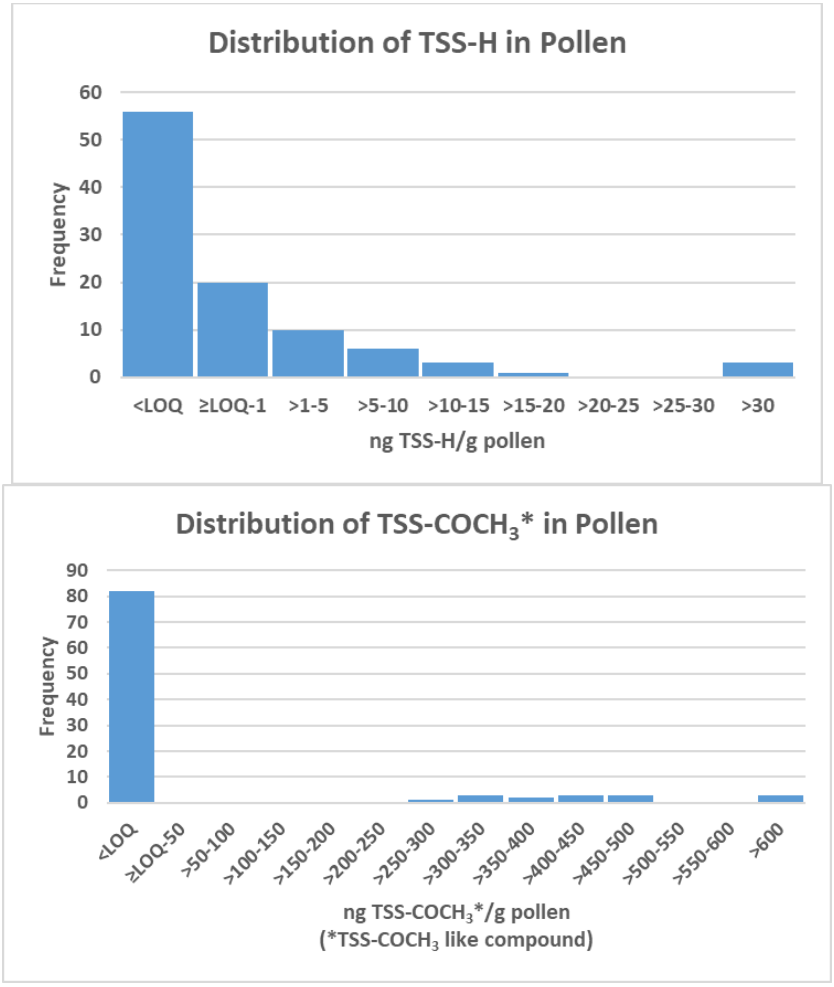

Figure 11. Distribution of TSSs in pollen samples (TSS-H: <LOQ is ≤0.4, TSS-$COCH_3$: <LOQ is ≤1.9)

It should be noted that the long-term (6 month) recoveries for the TSS spiked pollen were much less than the freshly spiked pollen samples, especially for the acetoxy oligomers. A follow up laboratory study, described in Chapter V, was performed to look at potential losses associated with storage conditions.

Table 11. Qualitative Pesticide Concentrations in Pollen Samples

Compound	Type	%Pollen with Pesticide	Pesticide Concentration (ng/g)*	%Recovery** (75 ng/g)
Azoxystrobin	Fungicide	23%	<LOQ - 489.1	47 ± 1
Chlorpyrifos	Pesticide	27%	<LOQ – 70.5	22 ± 4
Cyprodinil	Fungicide	53%	<LOQ – 5888.8	37 ± 3
Diflubenzuron	Pesticide	6%	<LOQ – 53.4	3 ± 2
Diuron	Herbicide	4%	<LOQ – 14.1	18 ± 3
Fenpyroximate	Acaricide	1%	<LOQ – 23.7	15 ± 2
Flubendiamide	Insecticide	8%	<LOQ – 9.5	28 ± 5
Methoxyfenozide	Insecticide	2%	<LOQ – 11.1	26 ± 3
Phoxim	Insecticide	2%	<LOQ – 10.0	9 ± 1

*LOQ for all pesticides set at 7.5 ng/g
**Y.S. Eco Bee Farms pollen used

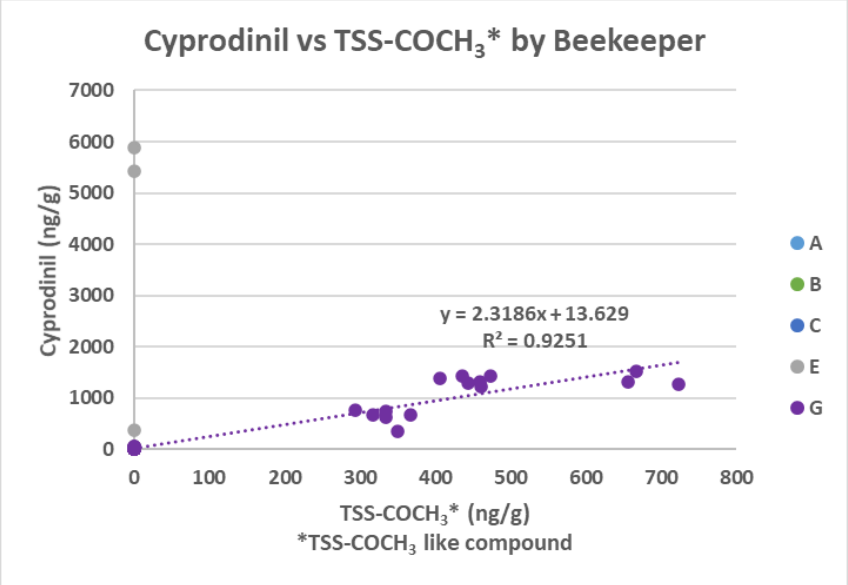

Figure 12. Correlation between Cyprodinil and TSS-COCH$_3$ like compound in pollen (A, E, G - Samples from California, B - Samples from Wisconsin, C - Samples from Kentucky, D - Samples never received back/analyzed, F - Tubes never sent out)

CHAPTER V

ADDITIONAL LONG-TERM RECOVERIES

In the 2018 pollen study, the TSS pollen spikes sent to the field and returned with the samples were found have much lower recoveries than the spikes that were extracted shortly after preparation. To better understand the low recoveries, an additional TSS spike recovery study was performed. Pollen samples were spiked with TSSs and stored for 4 ½ weeks under three different conditions (in ACN at room temperature, at room temperature without ACN, and in liquid nitrogen). Recoveries of TSS from the three storage conditions were compared to a spiked pollen controls that were extracted within 24 hours. A mass balance exercise was also conducted in an attempt to identify where the TSS was lost.

Methods and Materials

To further investigate the stability of TSSs in pollen, 15 mL polyprolyene centrifuge tubes containing either 0 or 1 g of Y.S. Eco Bee Farms pollen were spiked with 400 ng of TSS-H, TSS-$COCH_3$, and TSS-CH_3 in triplicate and stored for approximately 4 ½ weeks under three different conditions: 1) room temperature, 2) under liquid nitrogen, and 3) in 2 mL ACN at room temperature. In addition, triplicate spikes were analyzed at time zero. In order to determine where the TSSs ends up and how much is lost, the centrifuge tube rinsate, pollen, and ACN were all analyzed where applicable. For the spiked samples stored in 2mL of ACN, subsamples were removed with a syringe and transferred directly into autosampler vials. To quantify any TSS sorbed to the tube after the sample had been removed, 5mL of ACN was added to the tube, vortexed for 30

seconds, centrifuged for 5 min at 5000 rpm, then transfered to a vial and repeated twice more for a total of 15mL ACN. An aliquot of this solution was then transferred to an autosampler vial and analyzed. The pollen was analyzed by weighing what could be scraped out of the tube into a Qcup and then extracted on the EDGE using 15mL ACN at 100^{o}C with a 1 min hold time. An aliquot was then transferred to an autosampler vial. All samples were then analyzed by LCMS/MS and a mass balance determined.

Results

Fifteen EDGE method controls (Qcups run through EDGE without any pollen during run) were analyzed, resulting in contamination of 3 ± 3 ng TSS-H/sample, 85 ± 57 ng TSS-$COCH_3$/sample, and ng TSS-CH_3 below detection limit. This showed that the EDGE throughout the run had occasional TSS contamination between samples. The results for triplicate controls for the four treatments without TSSs being spiked are shown in Figure 13. Though TSS-CH_3 values were below detection limit, there was slight TSS-H contamination (Figure 13a) and even more TSS-$COCH_3$ contamination (Figure 13b), especially samples containing the control pollen. Thus, the EDGE and pollen appeared to attribute contamination of both forms of TSS, while the polypropylene tube or glass syringe used may have attributed to TSS-$COCH_3$ contamination. The EDGE method control was subtracted from all EDGE samples, while the controls for various storage treatments without pollen were subtracted from the those with pollen. Therefore, all recoveries take into account contamination from the different methods.

After 4 ½ weeks of storage, the lowest average recoveries for TSSs from spiked tubes without pollen (methoxy - 25% (Figure 14c), acetoxy - 69% (Figure 14b), and

hydroxy - 28% (Figure 14a)) or samples stored with pollen (methoxy -33% (Figure 14c), acetoxy - 17% (Figure 14b), and hydroxy 31% (Figure 14a) was stored at ambient room temperature, sometimes with ACN and sometimes without.

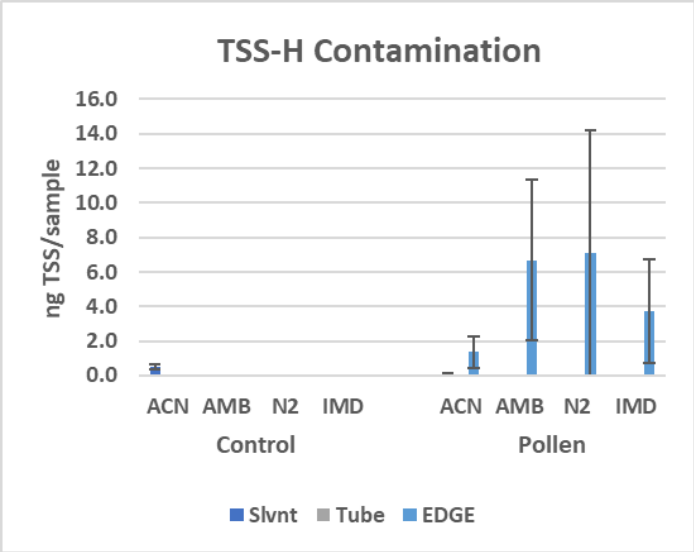

Figure 13a.
TSS-H Contamination

Legend:

- *ACN: 2mL of ACN added to spike at room temperature*
- *AMB: stored at room temperature*
- *N2: stored in liquid nitrogen*
- *IMD: spiked and extracted within 24 hours*

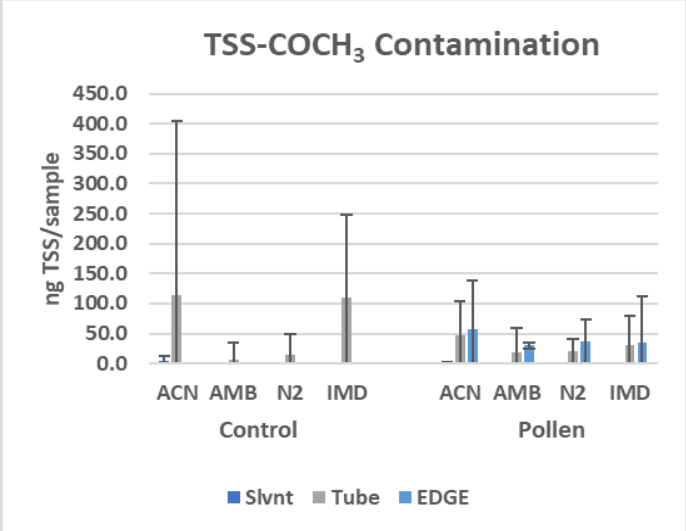

Figure 13b.
TSS-H Contamination

Legend:

- *ACN: 2mL of ACN added to spike at room temperature*
- *AMB: stored at room temperature*
- *N2: stored in liquid nitrogen*
- *IMD: spiked and extracted within 24 hours*

Figure 13. TSS contamination in long-term spike study
(n=3, 95% CI shown by error bars, TSS-CH_3 below LOQ)

EDGE method controls and no pollen controls subtracted from associated values
EDGE Method Control (Qcup with no pollen) (ng/sample, n=15, 95% CI):
TSS-H: 3 ± 3, TSS-$COCH_3$: 85 ± 57, TSS-CH_3: <LOQ

The recoveries for TSS-H and TSS-CH_3 at ambient temperatures without ACN were significantly lower than other storage treatments. The highest average recoveries for TSS from samples without pollen (methoxy - 95% (Figure 14c), acetoxy - 99% (Figure 14b), and hydroxy - 93% (Figure 14a) were from samples with different treatments, though none of them were stored at ambient temperature without ACN. The highest recoveries from samples stored with pollen (methoxy - 74% (Figure 14c), acetoxy - 96% (Figure 14b), and hydroxy 83% (Figure 14a) were all stored with liquid nitrogen.

While recoveries from pollen that were extracted within 24 hours after spiking had the second highest recoveries (methoxy - 59% (Figure 14c), acetoxy - 85% (Figure 14b), and hydroxy 67% (Figure 14a), the recoveries were not significantly different from using liquid nitrogen. Pollen that was stored at room temperature had 11-54% less mass recovered than samples stored in liquid N_2 or extracted almost immediately after spiking, though only TSS-H had significantly different recoveries for the two room temperature extractions versus the N_2 and extraction within 24 hours. The recoveries from samples containing pollen versus those not containing pollen were significantly different for all TSS if methods were also included as a factor within the Tukey Test. Though recovery for TSS-H and TSS-CH_3 was also low for samples stored at room temperature without ACN or pollen, the fact that recovery for TSS in samples stored in ACN without pollen was 44 - 60% higher in mass recovery indicates that something about pollen itself causes long term recoveries to be low.

The location of where the TSSs were recovered from was also analyzed for. The potential locations were labeled EDGE for TSS extracted off the pollen by the EDGE, Tube for TSS desorbed from the centrifuge tube (after pollen was removed), or solvent if

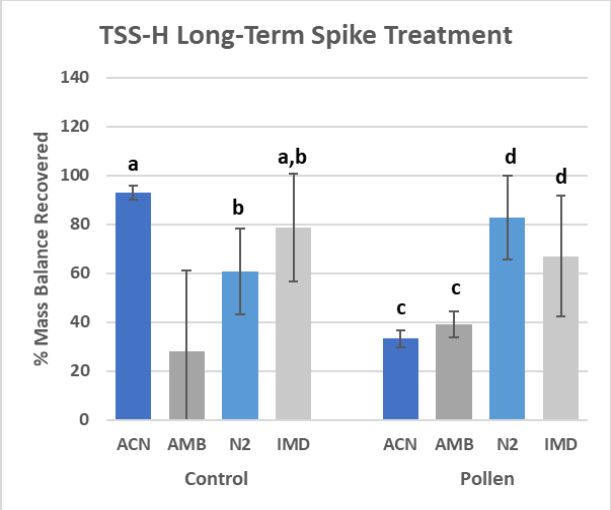

Figure 14a.
TSS-H Treatment Recoveries

Legend:

- *ACN: 2mL of ACN added to spike at room temperature*
- *AMB: stored at room temperature*
- *N2: stored in liquid nitrogen*
- *IMD: spiked and extracted within 24 hours*

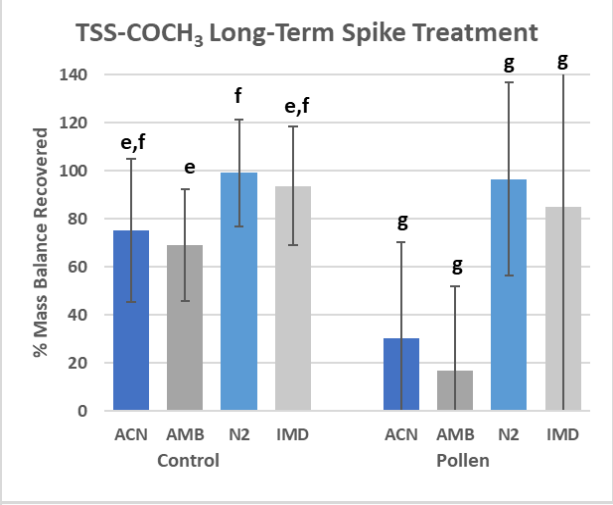

Figure 14b.
TSS-$COCH_3$ Treatment Recoveries

Legend:

- *ACN: 2mL of ACN added to spike at room temperature*
- *AMB: stored at room temperature*
- *N2: stored in liquid nitrogen*
- *IMD: spiked and extracted within 24 hours*

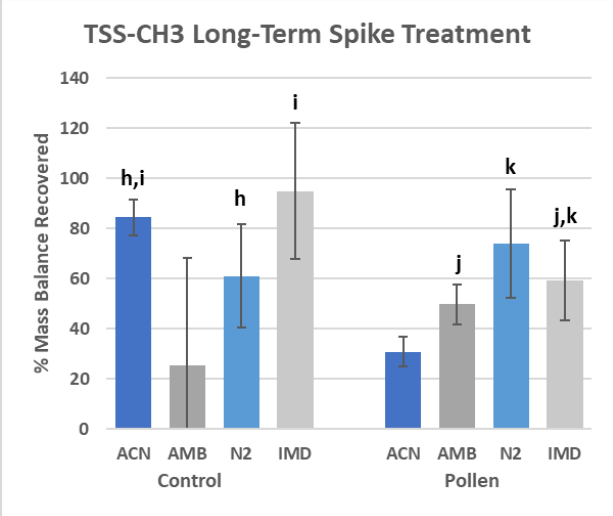

Figure 14c.
TSS-CH_3 Treatment Recoveries

Legend:

- *ACN: 2mL of ACN added to spike at room temperature*
- *AMB: stored at room temperature*
- *N2: stored in liquid nitrogen*
- *IMD: spiked and extracted within 24 hours*

Figure 14. % mass TSS spike recovered after 4 ½ week for four treatments (error bars are 95% CI with n=3, methods with the same letters are not significantly different based on ANOVA and Tukey Test within same control or pollen group)

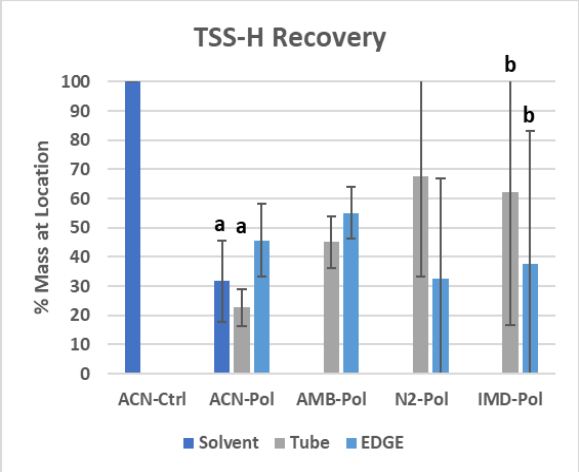

Figure 15a.
TSS-H Recovery by Location

Legend (Treatments):
- *ACN: 2mL of ACN added to spike at room temperature*
- *AMB: stored at room temperature*
- *N2: stored in liquid nitrogen*
- *IMD: spiked and extracted within 24 hrs*

Legend (Locations):
- *Solvent: 2 mL ACN in ACN treatment*
- *Tube: rinsate from 15 mL tube*
- *EDGE: pollen extracted on EDGE*

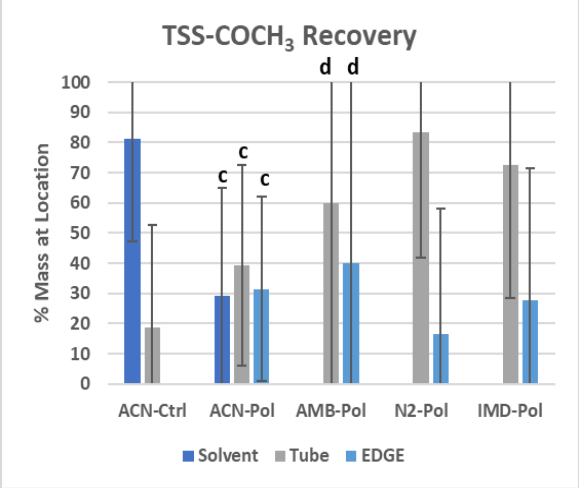

Figure 15b.
TSS-$COCH_3$ by Location

Legend (Treatments):
- *ACN: 2mL of ACN added to spike at room temperature*
- *AMB: stored at room temperature*
- *N2: stored in liquid nitrogen*
- *IMD: spiked and extracted within 24 hrs*

Legend (Locations):
- *Solvent: 2 mL ACN in ACN treatment*
- *Tube: rinsate from 15 mL tube*
- *EDGE: pollen extracted on EDGE*

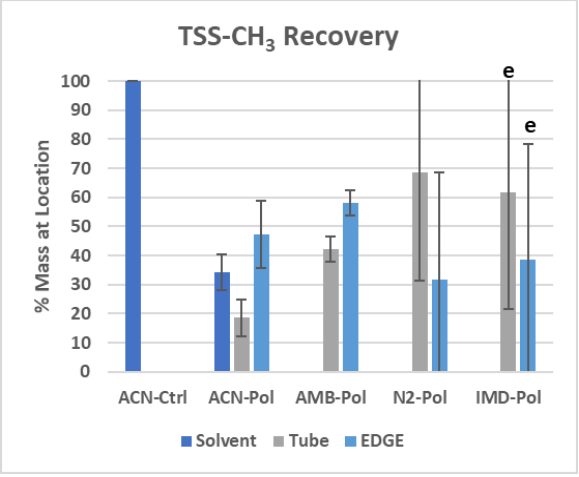

Figure 15c.
TSS-CH_3 by Location

Legend (Treatments):
- *ACN: 2mL of ACN added to spike at room temperature*
- *AMB: stored at room temperature*
- *N2: stored in liquid nitrogen*
- *IMD: spiked and extracted within 24 hrs*

Legend (Locations):
- *Solvent: 2 mL ACN in ACN treatment*
- *Tube: rinsate from 15 mL tube*
- *EDGE: pollen extracted on EDGE*

Figure 15. Location of mass of TSSs recovered (oligomer sums) after 4 ½ week for four different treatments
(error bars are 95% CI with n=3, methods with the same letters are not significantly different based on ANOVA and Tukey Test within same method)

solvent was added to the treatment. The only control without pollen that is shown is the treatment in which ACN was used, as all TSSs recovered from other control methods would have come solely from off the centrifuge tube. Sorption to the containers can be seen by Figure 15, where a significant portion of the mass recovered is from the container sometimes even significantly more than from the pollen, as is the case for all TSSs stored in liquid nitrogen. Though the TSSs were spiked directly onto the pollen, the TSSs may have transferred from the pollen to the centrifuge tube if the TSSs did not absorb into the pollen, but rather just adsorbed to the surface, which could then transfer to the centrifuge tube. In addition, as the TSSs were not uniformly dispersed through the pollen as it was spiked on top, the pollen that contained the majority of the TSSs may have been the fraction of the pollen most likely to stick to the side of the tube and thus not be extracted in the EDGE, but rather with the tube analysis. Overall, the instability of TSSs in pollen at room temperature and the transition of TSSs from spiked pollen to the tube may help explain the low long-term pollen recoveries indicated previously in the 2018 pollen study.

CHAPTER VI

POTENTIAL LOSSES OF TSS FROM LONG-TERM POLLEN SPIKES

To investigate the two most likely potential loss mechanisms associated with the low recoveries of TSSs in the long-term pollen spike samples at ambient temperatures, two studies were conducted. A qualitative headspace analysis was performed to investigate the relative volatility of TSS compounds, impurities or degradation products and a hydrolysis study was performed to look at the stability of TSSs as a function of pH.

Materials and Methods: Volatility Study

One drop of each of the three TSS standards were added to a 20 mL headspace vial resulting in the addition of 3 mg TSS-$COCH_3$, 2 mg TSS-H, and 7 mg TSS-CH_3. The vials were capped and left for approximately two days at room temperature. At the same time 100 µL of 1000 µg TSS/mL ACN (total of 0.1 mg) of the three standards were added to separate 20 mL vials that were either blank or contained 200 mg diatomaceous earth, 200 mg pollen or 10 mg carbon sorbent. This was done to compare how the volatility of the standards was affected by the different matrixes.

For the headspace analysis, 1 mL with a 10:1 split was injected on an Agilent GCMS (Figure 16) with a Rtx-5ms column set for electron impact (EI) mode and scanned from m/z 43-500. Full details of the method 2108_TSSHEADSPACE can be found in Table C1-1. The headspace GCMS analysis results were compared to direct injection of the same standards dissolved in ACN. Fifty mg/L standards of TSS-H, TSS-$COCH_3$, and TSS-CH_3 in ACN were run on the GCMS using liquid injection. 180124_BeePesticideMethod (Table C1-2) was used, where the temperatures and MS

programming were set at the same parameters as the headspace analysis, but the inlet liner was changed to a double gooseneck with glass wool and the injection volume was decreased to a splitless 1 µL injection.

Figure 16. Agilent GCMS

Results: Volatility Study

The headspace in vials containing two concentrations (2-7 mg and 0.1 mg) of the three TSS standards were analyzed by GCMS. None of the congeners associated with the three TSS standards were found in concentrations above an estimated detection limit of 10 µg/mL. Based on the liquid phase TSS concentrations of about 1 g/mL and the estimated detection limit in the headspace, vapor pressure for the TSS compounds would be 10^{-5} or lower which matches with the P_v estimates previously determined (Appendix B3).

An unidentified siloxane was found in the headspace of both the acetoxy and methoxy standards at a retention time of 5.7 min. In addition, a compound at 8.9 min, tentatively identified as tetradecamethyl-hexasiloxane (L6) (NIST 2008), was found in the headspace of all three standards. A L6 standard was not available at the time of this

study and would be needed to verify identity of the compound. L6 is a water insoluble linear siloxane that has been found in sewage sludge and indoor dust. It has an estimated acute toxicity to fish at 2.7 parts per quadrillion (Gelest 2014b; ChemSpider 2015; Tran et al. 2015; Thomaidi et al. 2016; ECHA 2019). At the higher concentration of TSS-$COCH_3$, the headspace also contained other unidentified siloxanes that did not appear in the other two standards (Figure 17). Since the TSS compounds were not found in the headspace the impact of the sorbents on volatilization could not be quantified.

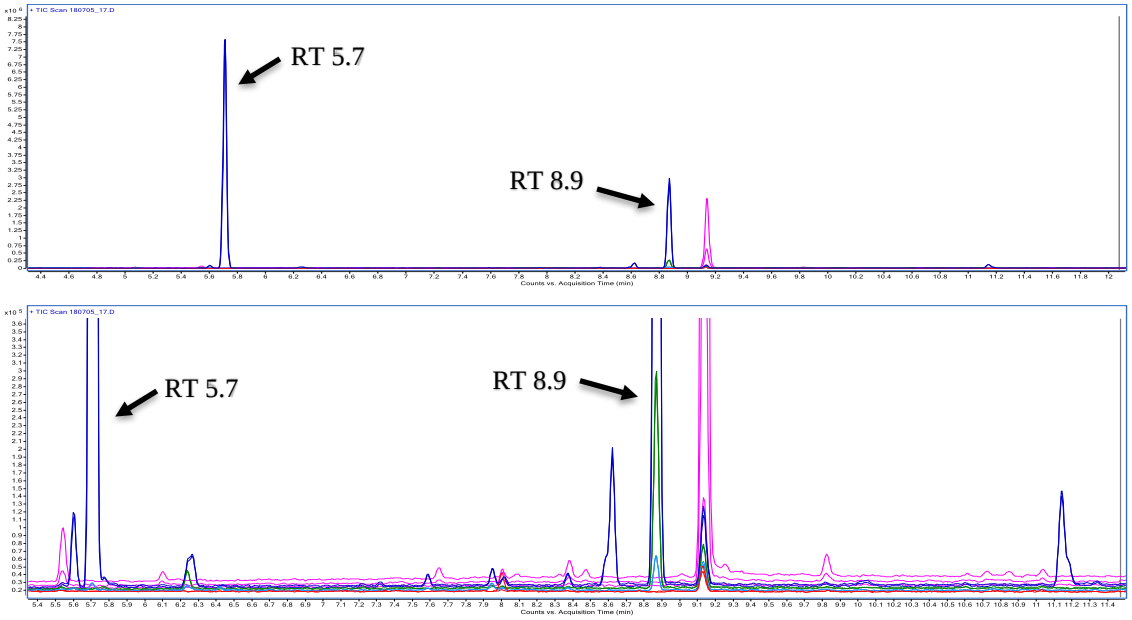

Figure 17. Total ion chromatogram (TIC) headspace analysis
(Top graph shows overview, Bottom graph zoomed in to show smaller peaks)
3 mg TSS-$COCH_3$, 2 mg TSS-H, and 7 mg TSS-CH_3 in 20mL headspace vials
Dark Blue – TSS-$COCH_3$, Dark Green – TSS-H, Light Blue – TSS-CH_3,
Pink – First blanks, Red – Last blanks

Overall, the results indicate that significant volatilization of the TSS compounds is unlikely and the volatile siloxanes present in the headspace are most likely impurities or breakdown products, with TSS-$COCH_3$ having the most volatile compounds. The lack of volatility indicates that low recoveries of long-term spikes was not due to volatilization

of TSSs, though volatilization of impurities or degradation products is still possible.

Methods and Materials: Hydrolysis Study

A hydrolysis study was conducted to monitor the change in TSS solution concentrations in pH 5, 7, and 9 buffers and in unbuffered deionized water. Buffer solutions at pH 5 and pH 9 were made with 10 mM ammonium acetate and adjusted to the correct pH using 0.1 N acetic acid. The pH 7 buffer solution was made using 10 mM K_2HPO_4 and adjusted to correct pH with 0.1 N acetic acid. Solutions were loosely covered with parafilm and placed in an incubator at 20^{o}C overnight to allow them to equilibrate before the experiment started and pH was measured. LCMS grade TSS standards made in ACN were used as controls. A 100 mg/L (0.01%) concentration of each of the three standards was made up in the three buffers, deionized water and ACN, then separated into two separate vials for duplicates and incubated at 20^{o}C. Eight samples were taken over the course of four days. The samples (10 uL) were placed into 1 mL ACN and stored in the freezer until analysis by LCMS/MS.

Results: Hydrolysis Study

Concentrations were calculated using a weighted non-linear calibration curve corrected for any CCV drift by assuming linear drift between samples. Oligomeric concentrations were added together and an exponential curve (Equation 1) was fit to the data (Figure 18) after removing the last three values of TSS-$COCH_3$ in the pH 9 buffer (Figure 18b). Half-lives were then calculated equation 2 and shown in Table 12.

Equation 1: $y = a*e^{k*x}$

Equation 2: $t_{1/2} = \frac{-0.693}{k}$

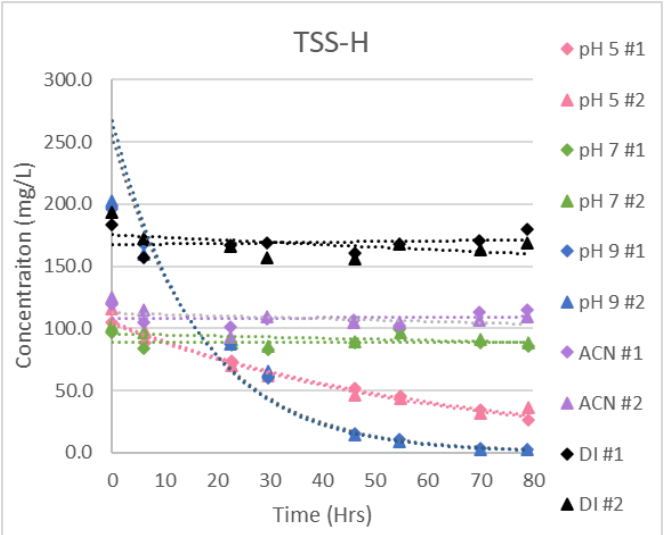

Figure 18a. Hydrolysis of TSS-H at various pHs

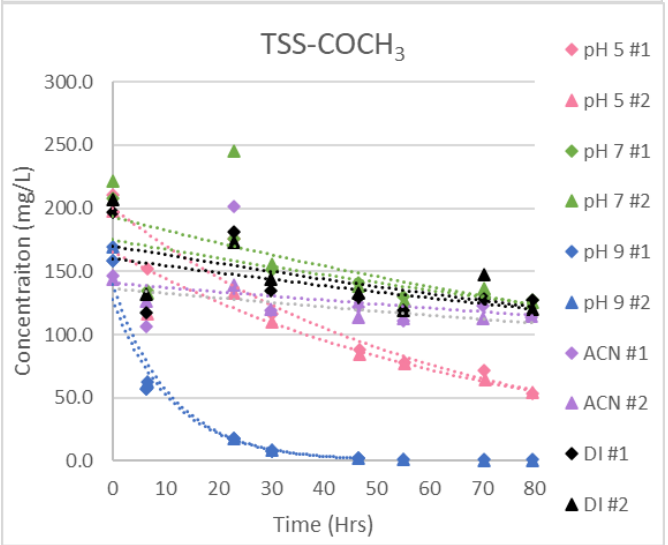

Figure 18b. Hydrolysis of TSS-$COCH_3$ at various pHs

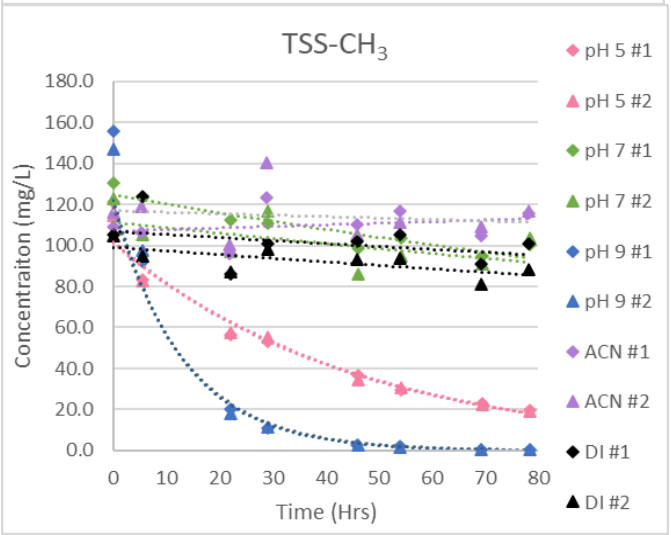

Figure 18c. Hydrolysis of TSS-CH_3 at various pHs

Figure 18. Hydrolysis of TSSs shown by summed oligomers and exponential fit curves

Some of the initial solution measured concentrations were higher than the nominal concentration of 100 mg/L this may have been due to the solutions being near or above the critical micelle concentration (CMC), yielding solutions that were slightly heterogenous with a higher concentration near the top where bubbles may form. Solution pHs were measured at the beginning and end of the experiment with the initial pHs starting at 5.19, 7.20, 7.02, and 9.28. At the end of the experiment, the average shift in pH (with standard deviation) was 0.20 ± 0.12 units, where all of the pHs shifted to be more acidic except at pH 5 where they shifted to be more basic (Figure 19).

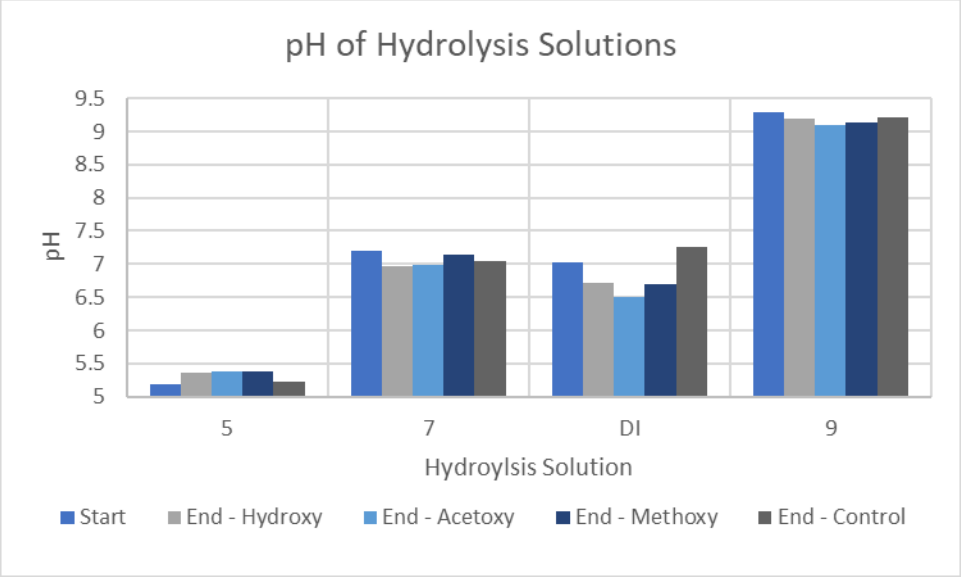

Figure 19. pH of hydrolysis solutions at start and end of experiment

The TSS standards were more rapidly hydrolyzed in basic solutions than acidic solutions and were fairly stable in neutral conditions, though TSS-$COCH_3$ was less stable at neutral conditions than the other two standards. The TSS-$COCH_3$ also was less stable in the acetonitrile control than the other two standards, which had lives greater than four weeks. However, the half-life of TSS-$COCH_3$ in acetonitrile (10.9 days) was still greater

than the half-lives in any of the other aqueous solutions, indicating water speeds up the degradation process, whether through hydrolysis or another process. TSS-$COCH_3$ degraded most rapidly in basic conditions with a half-life of 0.3 days, followed by methoxy and hydroxy TSS at 0.4 and 0.5 days respectively (Table 12). In acidic conditions, the half-lives were about 3-6x slower than the basic half-lives with half-halves at 1.3, 1.8, and 1.9 days for methoxy, hydroxy, and acetoxy TSS respectively. This is similar to the literature, except that Powell et al. (1997) has the acidic reaction slightly faster than the basic.

Table 12. Experimental Hydrolysis Half Lives (Days)

Solvent\TSS	TSS-H	TSS-$COCH_3$	TSS-CH_3	TSS-CH_3 (Literature)*
DI	>25**	7.5 ± 7.1	18 ± 32	-
ACN	>28**	10.9 ± 7.0	>47**	-
pH 5	1.8 ± 0.3	1.9 ± 1.8	1.3 ± 0.1	<0.4 days
pH 7	>30.2***	5.9 ± 10.2	9.9 ± 24.0	8.4 – 55 days
pH 9	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	<1 day

- 95% CI, n=2

*Sources: Powell & Carpenter 1997, Michel et al. 2014
**Values contained both positive and negative half-lives making averages and 95% CI unreasonable to report
***Values were 1650 and 30 days making 95% CI unreasonable to report

Overall, half-lives indicate that hydrolysis is more rapid for the acetoxy TSS compounds, followed by methoxy and hydroxy TSS. Acetoxy also had the lowest recoveries for the long-term spikes, followed by hydroxy and methoxy TSS, indicating that hydrolysis could play an important part in the low long-term spike recoveries and stability.

CHAPTER VII

PLANT UPTAKE

After finding TSSs in pollen, two plant uptake studies were performed to evaluate the extent of TSS root to shoot transfer as described by the TSCF. Prior to the TSCF experiments, an additional study was performed to determine a root concentration factor (RCF) and how long it takes for equilibrium with the roots to be established (Appendix D). TSCF was first measured using a pressure chamber method where the roots of a plant are placed in a chamber and a nutrient solution containing TSS compounds is forced through them until steady state is reached. Caffeine was used as a reference compound since values measured in previous study using the same method were available for comparison.

After being unable to attain a steady state TSCF value using the pressure chamber approach, a whole plant TSCF experiment was performed. To simplify the analysis, only acetoxy TSS was added to the plants in two different concentrations. The fungicide cyprodinil was also added to determine if the acetoxy TSS could impact the uptake of this compound. Cyprodinil is a foliar fungicide that was found in the 2018 pollen study at levels that correlated with TSS-$COCH_3$ concentrations. Cyprodinil has a log K_{OW} value of 3.9 (Millipore 2019) and a field application of around 0.2 g/L assuming 100 gal/acre (EPA 1998).

Materials and Methods: Chamber Study

TSCF values for the TSS compounds and caffeine were determined using the pressure chamber approach similar to that described by Orita (2012). Hoyt Soybeans

(*Glycine max*) were grown hydroponically in 1 gallon glass jars containing pH 5.6 Nutrient Solution Starter (Appendix C4) for approximately 1 week, then changed to pH 5.6 Nutrient Solution Vegetative (Appendix C4) and grown until the roots and stems are large enough to perform the study, a total of approximately 5-9 weeks. The plant was then cut just below the first cotyledonary node and placed into the pressure chamber containing nutrient solution either spiked only with approximately 50 µg/L caffeine as a reference compound, or 50 µg/L caffeine and 1 mg/L mix of TSS-H, TSS-CH_3, and TSS-$COCH_3$. A piece of rubber tubing was used to connect the plant stem to a fitting that leads to a fraction collector (Figure 20). Air was forced into the chamber, pressurizing the chamber until the nutrient solution flowed through the roots into the xylem and collected via an autosampler. A sample of the nutrient solution the roots were immersed in was taken at the same time via a septa at the bottom of the pressure chamber. By measuring the concentration of the analyte in the root nutrient solution and the xylem sap at steady state, the TSCF can then be determined using Equation 3.

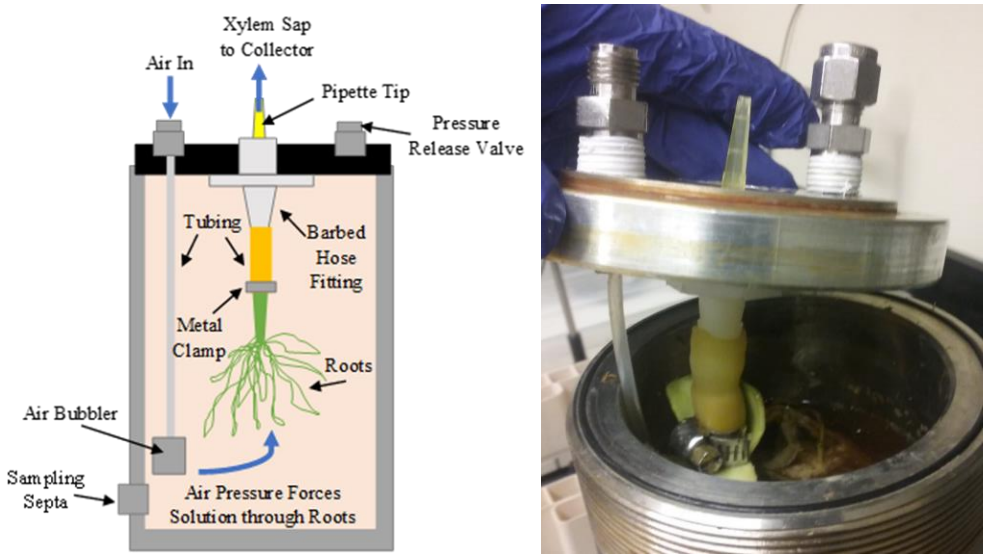

Figure 20. (Left) Diagram of pressure chamber, (Right) Top of pressure chamber

Equation 3: $TSCF = \frac{xylem\ sap\ concentration}{pressure\ chamber\ concentration}$

Results: Chamber Study

TSCF is the ratio of the concentration of an analyte in the xylem sap to that in solution taken up by the plant at steady state. In order to measure TSCF during a pressure chamber experiment, the concentration of xylem sap samples are compared to the nutrient solution concentration until steady state is reached. A TSCF of 1 indicates that a compound is uptaken into the plant at the same rate as water, while TSCF values less than one indicate that a compound does not move from root to shoot as readily as water. Figure 21 shows the TSCF versus time curves over the course of the run. Caffeine was added as a reference compound to both the control and TSS spiked plants to help determine how well plants were sealed into the chamber. Literature values for radio-labelled C^{14}-caffeine are between 0.78 and 0.83 (Dettenmaier et al. 2009; Orita 2012), while the pressure chamber TSCF values in this study were much lower at an average and 95% CI (n=3) of 0.21 ± 0.53 for control plants (caffeine only) and 0.42 ± 0.54 (TSS plus caffeine). Though the TSCF for caffeine for the controls and TSS spikes appeared to be different, they were not significantly different at a 95% CI level based on an ANOVA and Tukey Test. The lower TSCF value obtained here may indicate steady state was not attained. It is also possible that values reported by Dettenmaier et al. (2009) and Orita (2012), using C^{14}-caffeine, were higher due to the presence of more mobile 14C-metabolites in the xylem sap. Analysis by HPLC-MS/MS reduces the potential for false positives.

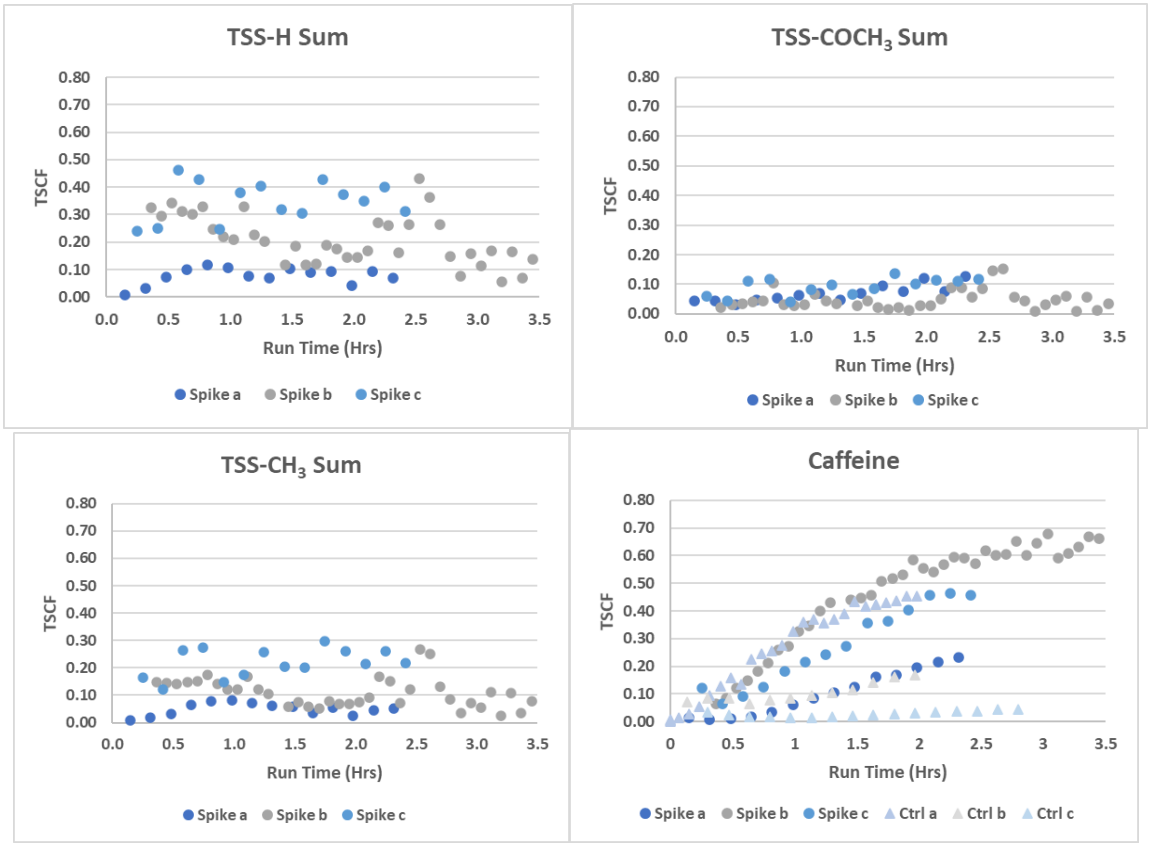

Figure 21. TSCF of caffeine and oligomer sums of TSS classes in Hoyt Soybean over the course of pressure chamber run

The concentrations of the TSS compounds varied over time resulting in a significant scatter in the TSCF (Figure 21) making it difficult to determine if steady state was attained. This is not surprising since the hydrolytic stability of TSSs in aqueous media is much less than caffeine. For each plant, the TSCF for an TSS oligomer was considered to be the average of the last five TSCF values in the run and the overall TSCF for a TSS standard was calculated to be the average of all of the oligomers for that standard. The averages and 95% CI confidence intervals for the TSCFs oligomer sums for the two groups, controls (only caffeine) and spikes (caffiene and TSS), are given in Table 13. The hydroxy TSS is estimated to have the lowest log K_{OW} (3.6) and the highest

TSCF at 0.15 ± 0.31. This agrees with previous log Kow vs TSCF correlations presented Dettenmaier et al. (2009) and Briggs et al. (1982) (Figure 5). Methoxy and acetoxy TSSs also have TSCFs in the right range with estimated log K_{OW}s at 4.1 and 4.2 respectively, and lower TSCFS at 0.09 and 0.06 respectively. The log K_{OW} for acetoxy TSS was estimated to be slightly more non-polar than the other TSS standards, which correlates with it having a slightly lower TSCF than the other standards.

Table 13. Pressure Chamber Transpiration Stream Concentration Factor (TSCF)

	TSS-H	**TSS-$COCH_3$**	**TSS-CH_3**	**Caffeine**
Pressure Chamber Control (Soybean)	-	-	-	0.21 ± 0.53
Pressure Chamber TSS (Soybean)	0.15 ± 0.31	0.06 ± 0.09	0.09 ± 0.21	0.42 ± 0.54

- 95% CI, n=3
- TSS concentration presented as oligomer sums

Materials and Methods: Whole Plant Experiment

Dwarf tomatoes (*Solanum lycopersicum*) were chosen because they grow well hydroponically, mature and flower quickly, and are good at obtaining iron in a neutral pH nutrient solution. The seeds were sprouted in a paper towel, then transferred about 1 ½ weeks later to one-gallon glass jars containing pH 7.1 Research Greenhouse Nutrient Solution Starter (Appendix C4). Four gallon jars and one quart jar were also filled with nutrient solution, but without the additon of a plant. Nutrient solution was added to the jars as needed and measured to determine evaporation. Evapotranspiration (evaporation and transpiration) was measured in the jars containing plants using the same approach. Two sets of experiments were performed at different concentrations of TSS.

For the first set of experiments, plants were spiked one time with the TSS-$COCH_3$ and cyprodinil at weeks 5-6. TSS-$COCH_3$ was used because it was found at the highest concentrations in the 2018 Pollen study and cyprodinil since its pollen concentrations were found to be correlated with TSS-$COCH_3$. Cyprodinil, dissolved in ACN, was added to five 1-gallon jars, three with plants and two without, to yield concentations of 1 mg cyprodinil/L in the aqueous nutrient solutions. Another set of five jars were prepared in a similar manner with 1 mg/L TSS-$COCH_3$ in addition to the cyprodinil. Two additional 1-gallon jars containing plants were spiked with ACN to determine if the ACN (~0.72%), the solvent used to add the cyprodinil and TSS-$COCH_3$, impacted the plants. A quart jar was also filled with nutrient solution and spiked with 1.9 mL ACN as a control blank. An overview of the three treatment groups for round 1 can be seen in Table 14.

Table 14. Treatments for Plants Grown for TSCF

	Treatment			
	Control	**Cyprodinil Only**	**TSS-$COCH_3$ 1 mg/L**	**TSS-$COCH_3$ 10 mg/L**
Number of Plantless Controls (Round 1 | 2)	1 | 1	2 | 2	2 | 0	0 | 2
Number of Plants (Round 1 | 2)	2 | 1	3 | 1	3 | 0	0 | 5
Theoretical Initial Concentration of ACN (mL ACN/L water)	7.6	7.6	7.6	7.6
Theoretical Initial Concentration of Cyprodinil (mg Cyprodinil/L water)	0	1	1	1
Theoretical Initial Concentration of TSS-$COCH_3$ (mg TSS-$COCH_3$/L water)	0	0	1	10

Table 14 (cont). Treatments for Plants Grown for TSCF

	Treatment			
	Control	**Cyprodinil Only**	**TSS-$COCH_3$ 1 mg/L**	**TSS-$COCH_3$ 10 mg/L**
Total mL ACN added (Round 1 | 2)	1.9 | 5.5	7.6 | 22.8	7.6 | NA	NA | 22.8
Total mg Cyprodinil added	0	3.8	3.8	3.8
Total mg TSS-$COCH_3$ added	0	0	1	50

- Round 2 had four subsequent spikes of 3.8 mL of 10 mg TSS-$COCH_3$/mL ACN in order to try to keep a steady concentration of TSS-$COCH_3$. Controls and Cyprodinil only was spiked with 3.8 mL ACN to keep ACN concentrations between treatment groups the same.
- There was no TSS only treatment as cyprodinil was used as a reference compound.

Nutrient (root exposure) solution samples were taken before and after the analytes were added and before and after nutrient solution was added to replace solution lost to evapotranspiration. These samples were taken by pipetting 0.5 mL of nutrient solution and diluting into 0.5 mL of ACN. pH of the nutrient solution was also measured on the days samples were taken and ~1 M HNO_3 was added to keep the nutrient solution close to pH 7 where TSS hydrolysis reaction rates are slower nearer.

In the second round, the initial nominal exposure concentration was increased to 10 mg/L and TSS was added to the root exposure solutions at multiple times in an attempt to keep the concentrations more constant. Besides a different number of each replicate, the remainder of the experimental design was the same as previously described. Subsequent spikes were made every other day and consisted of 3.8 mL of ACN for the blank and cyprodinil only plants and blanks or 3.8 mL of 10 mg/mL TSS-$COCH_3$ for the

remaining plants and blanks. A quart jar was again used as a control blank, filled with nutrient solution and spiked with 1.9 mL ACN initially, then 0.9 mL ACN each time a spike was added. The nominal exposure concentrations, masses of analyte and ACN added and are summarized in Table 14 for both experiments.

Once again, nutrient solution samples were taken before the jars were spiked, after the spike was added and every time before and after nutrient solution and spikes were added. These samples were collected by pipetting 0.05 mL of nutrient solution and diluting into 0.95 mL of ACN. The pH of the nutrient solution was also measured before and after nutrient solution and spikes were added and ~1 M HNO_3 was added more often than round 1 to help keep the pH closer to pH 7 to help minimize loss by hydrolysis.

After spiking, the plants were grown for approximately two more weeks until about 1-2 L had been transpired. The plants were then cut and divided into root, stem, leaves, flowers, and fruit categories and weighed. Before being weighed, the wet roots were allowed to drip into an empty jar, then rinsed in 500 mL of DI water for a few minutes, and allowed to drip again. The water used to rinse the roots was sampled, diluted with ACN (0.5:0.5 mL) and analyzed by LCMS/MS. The mass desorbed off of the roots was then calculated and divided by dry root mass to determine a µg TSS-$COCH_3$/g root desorbed. Triplicate subsamples of roots, stems, and leaves were also placed in the oven at approximately 80^{o}C to get a dry weight only (were not analyzed for TSS or cyprodinil). After air drying for a few days and being crushed with mortar and pestle without liquid nitrogen, approximately 0.1 g dry weight of flower, leaf, and root sample was extracted via the EDGE using the same method as the 2018 pollen samples and analyzed on the LCMS/MS. To determine the TSCF of the whole plant, Equation 4

below was used. The µg analyte in the plant was determined by Equation 5, where concentration of analyte in the stem is assumed to be similar to the leaf and concentration in fruit is assumed to be similar to the flower. RCFs were also determined by Equation 6.

Equation 4: $$TSCF = \frac{\mu g\ analyte\ in\ plant \div mL\ water\ transpired}{concentration\ analyte\ in\ water\ (\frac{\mu g}{mL})}$$

Equation 5: $$\mu g\ analyte\ in\ plant = \left(leaf\ weight\ (g) + stem\ weight\ (g)\right) * (analyte\ in\ leaves\ \left(\frac{\mu g}{g}\right)) + \left(flower\ weight\ (g) + fruit\ weight\ (g)\right) * (analyte\ in\ flower\ \left(\frac{\mu g}{g}\right))$$

Equation 6: $$RCF\ \left(\frac{mL}{g}\ or\ \frac{L}{kg}\right) = \frac{\left(\frac{\mu g}{g} root\ rinsate + \frac{\mu g}{g} root\ EDGE\ extract\right) * 1000\ \frac{ng}{\mu g}}{\frac{ng}{mL} Final\ NS\ Concentration}$$

Results: Whole Plant Uptake

Since the TSCF values in the pressure chamber experiment did not appear to reach a steady equilibrium possibly due to the stability of TSS-$COCH_3$ in aqueous media, a second TSCF experiment was performed using the whole plant and simplified to only contain the acetoxy TSS, which was found at the highest concentrations in the 2018 pollen study. Cyprodinil was also added to act as a reference compound, as well as potentially show if TSSs have an affect on the uptake of cyprodinil. For the tomatoes that were grown to calculate a whole plant TSCF, the pH of the nutrient solution fluctuated between a pH of about 6.6 to 8.7 and became slightly basic over the course of the run, even though acid was added to try and keep the pH nearer neutral. This drift from neutral pH may explain part of the reason why acetoxy TSS concentration degraded quickly, reaching below detection limit for most of the oligomers between sampling events (every other day). As more than 90% of the concentration of most acetoxy oligomers disappeared within 48-55 hours, this is slightly faster than the half-life of 3.2 days

calculated previously at pH 7 but is reasonable at pH 9 with a calculated half-life of 0.2 days.

Transpiration of the plants was calculated by subtracting evaporation, assumed to be the average solution lost from the five plantless control jars, from the solution lost from each jar containing plants. As larger plants transpire more, a relative growth rate (RGR) was calculated to help normalize growth rates over time in order to make sure the TSS and cyprodinil spikes did not have a toxic effect on the plants (Figure 22). The RGRs were calculated based on transpiration using Equation 7 below, where "mL" is the volume of water transpired (mL) at times t_2 and t_1.

Equation 7: $$RGR = \frac{\ln\left(\frac{mL_2}{mL_1}\right)}{t2-t1}$$

RGR helps normalize growth rates between sizes of plants over time, and the change in RGR over time, or the slope, indicates if the plant is growing faster (positive slope) or slower (negative slope) over time. Though plants in round 2 seemed to have slightly more trouble growing consistently, there was no significant difference at 95% CI between the linear slope of RGR over the course of the whole experiment and any of the treatments. There is also no significant difference between RGR linear slope of different treatments during spiking treatment (day 29-end for round 1 and day 34-end for round 2).

All of the plants, except for TSS 2a, developed fruit by the time they were harvested, which may indicate why RGR appears to be slowing down or had a negative trend. As can be seen in Figure 23, TSS 2a never grew well and TSS 2b was dying, if not dead, right before it was cut. Though both of these were spiked with 10 mg/L TSS concentrations, it cannot be said that the 10 mg/L TSS concentration caused the decline

in plant growth, because TSS 2a was not growing well before it was spiked, while TSS 2e seemed to be doing okay even after it was spiked. Also, most of the RGRs between plant groups appear to be similar, with a couple of the plants with 10 mg/L TSS concentrations having transpired the most water.

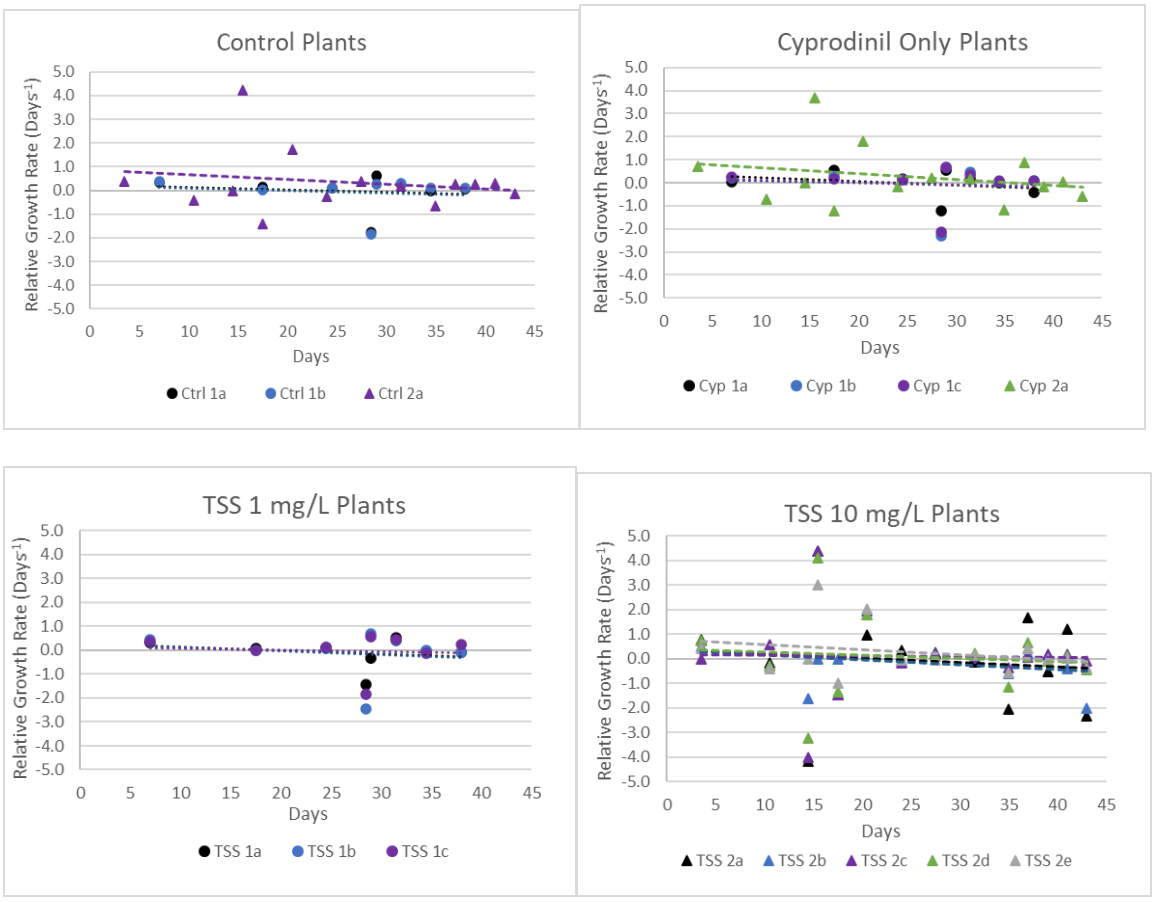

Figure 22. Relative growth rate (RGR) based on transpiration during plant growth Round 1(Circles) Spiked on Day 29, Round 2 (Triangles) Spiked on Day 34

The concentration of TSS-$COCH_3$ and cyprodinil did not stay constant over the course of plant growth. In the first round, the plants were spiked once at 1 mg TSS-$COCH_3$ and cyprodinil/L water and the acetoxy disappeared within a few days, which is faster than the pH 7 half-life of 5.9 days would indicate, but not entirely unexpected as

the nutrient solution became slightly basic over time and is a more complex matrix of nutrient solution as compared to DI water or DI water with a simple buffer. In the second round of plants, TSS-$COCH_3$ was spiked every other day at a higher concentration of 10 mg TSS-$COCH_3$/L water to try and help keep some analyte in solution. Though the plants were spiked at a theoretical concentration 10 mg/L and the samples showed concentrations of about 15-35 mg/L directly after spiking, the concentration for many oligomers were below detection when measured again two days later (Figure 24a). It should be noted that the sample concentrations are higher than the theoretical concentrations most likely due to heterogeneous mixing as TSS-$COCH_3$ is a surfactant and can form micelles at high concentrations and 10 mg/L is around the CAC for some oligomers, which was shown by the formation of bubbles immediately after spiking.

Though TSS-H was not added to the nutrient solution, the concentrations of TSS-H measured one and two days after being spiked were on or almost at the same order of magnitude of how much TSS-$COCH_3$ was initially spiked (Figure 24b). This strongly

Figure 23. Dwarf tomato plants TSS 2e (left), TSS 2a (middle), and TSS 2b (right) before being cut

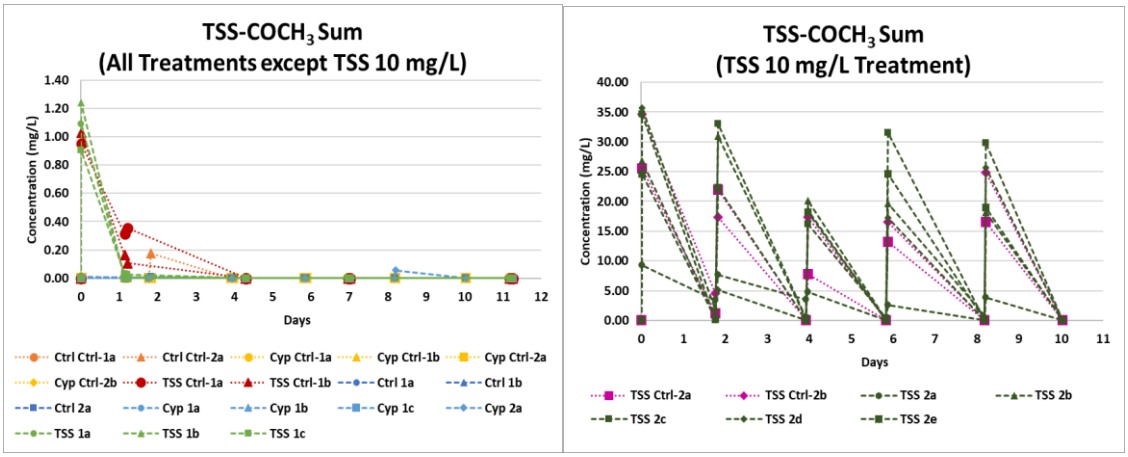

Figure 24a. Acetoxy TSS concentration over experiment

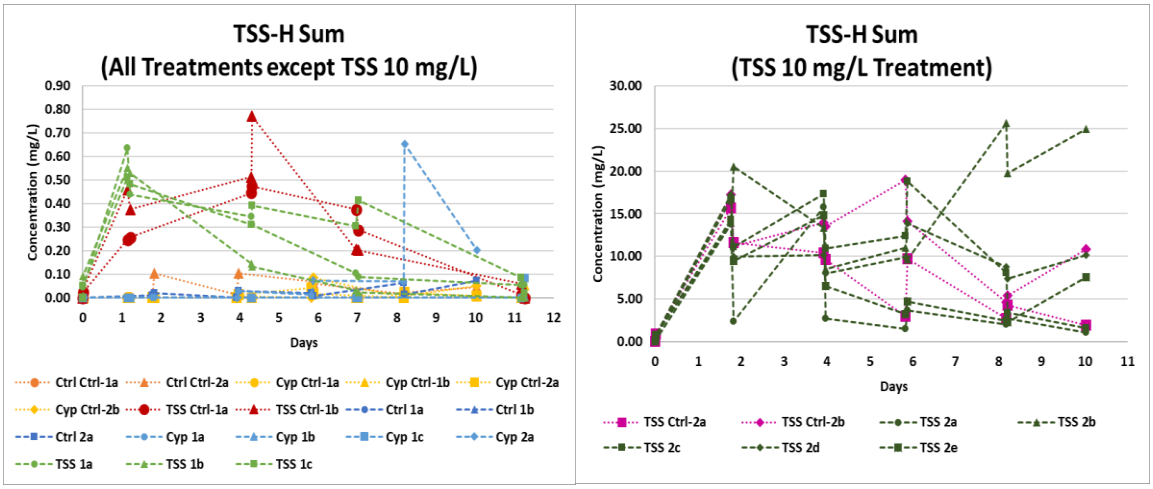

Figure 24b. Hydroxy TSS concentration over experiment

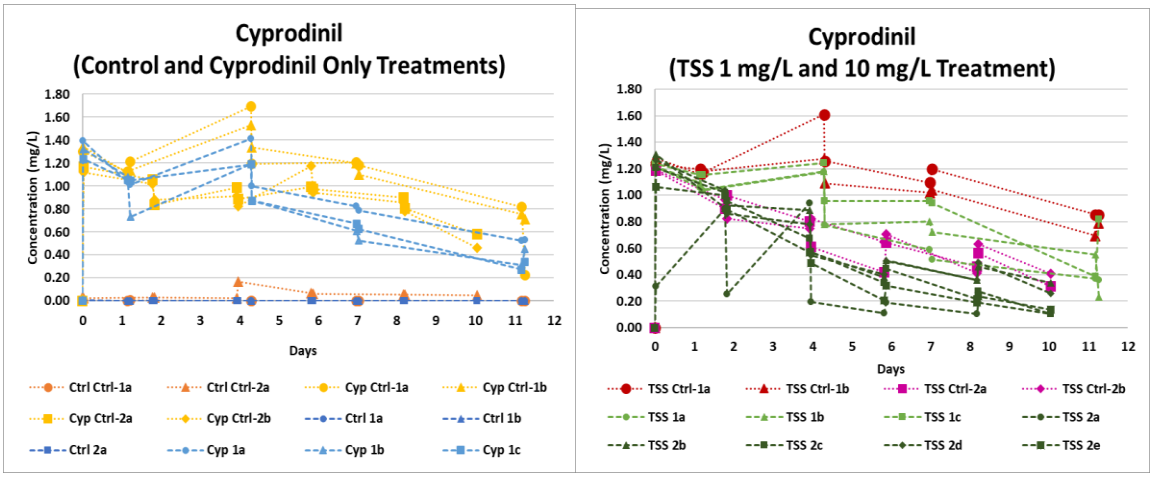

Figure 24c. Cyprodinil concentration over experiment

Figure 24. Analyte concentrations during experiment (Day 0 is initial spike)

indicates that TSS-$COCH_3$ tends to transform into TSS-H before degrading further. It is interesting to note that TSS-H in the samples that were spiked with a lower concentration of TSS-$COCH_3$ did not disappear as rapidly as the acetoxy TSS did, but instead persisted at a concentration that can be seen over most of the plant growth. This indicates a slower hydrolysis time, which is supported by the half-life of 8 and 31 days calculated for TSS-H in DI water and pH 7 buffer, respectively. However, much the TSS-H does seem to reach an equilibrium in the nutrient solution, as even though TSS-$COCH_3$ was spiked into the solutions every other day which is quicker than TSS-H's experimental half-life, TSS-H does not seem to grow over the course of the run, but instead decreases, indicating a concentration dependent mechanism with either degradation pathways or sorption. The cyprodinil concentrations also declined by 48-91% over the course of plant growth, though it did not occur as rapidly as the acetoxy TSS did as cyprodinil was only spiked once and can still be seen in the solution by the end of the run. This may be due to either a slower sorption rate or slower degradation rate.

Analyte calculations for uptake and TSCFs exclude plants TSS 2a and TSS 2b in the TSS-10 mg/L group, as TSS 2a did not grow very well, and TSS 2b was close to dead when harvested. However, plant TSS 2b measurements are listed in Table 15 as a worst-case scenario comparison for uptake, as plants that are stressed or close to dying sometimes allow more contaminants past membranes and thus have a higher uptake and TSCF. Plant Cyp 2a in the cyprodinil only treatment group is also excluded from calculations due to contamination of TSS in the nutrient solution. TSS-CH_3 is below detection limit or signal to noise for almost every measurement in every group, and is thus not presented in any calculations or table.

Table 15. Analyte Plant Concentrations (Dry Weight Basis)

Sample	Analyte Sums	Final nutrient solution conc (mg/L)	DI water desorption (µg/g root)	Root EDGE Extract (µg/g root)	Leaf EDGE Extract (µg/g leaf)	Flower EDGE Extract (µg/g flower)
Cyprodinil Only	Hydroxy	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Acetoxy	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Cyprodinil	0.37 ± 0.33	16 ± 28	71 ± 124	6.5 ± 2.8	0.32 ± 0.33
TSS-$COCH_3$ 1 mg/L	Hydroxy	0.05 ± 0.11	3.1 ± 6.8	1.1 ± 3.5	<LOQ	<LOQ
	Acetoxy	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Cyprodinil	0.44 ± 0.25	15 ± 16	61 ± 96	4.8 ± 3.9	0.45 ± 0.28
TSS-$COCH_3$ 10 mg/L	Hydroxy	6.4 ± 10.9	40 ± 121	14 ± 15	20 ± 35	1.9 ± 4.3
	Acetoxy	<LOQ	<LOQ	<LOQ	0.09 ± 0.26	<LOQ
	Cyprodinil	0.17 ± 0.08	6.7 ± 3.2	63 ± 31	8.4 ± 7.0	0.19 ± 0.15
TSS 2b (Plant Stressed)	Hydroxy	25	314	44	50	87
	Acetoxy	0.17	0.10	<LOQ	3.7	3.7
	Cyprodinil	0.34	13	62	12	0.33

- 95% CI with, n sample = 3 per group (<LOQ were counted as zeroes)
- TSS concentrations are presented as oligomer sums

Cyprodinil was found in all parts of the plant analyzed for all three groups that were spiked with the fungicide, while TSS-$COCH_3$ was only found in the plants spiked continuously with 10 mg/L concentrations (Table 15). This group also contained TSS-H in all parts of the plants, while TSS-H was found only in the root zone of the plants that were spiked once with a 1 mg/L concentration of TSS-$COCH_3$. Across the three groups, the concentrations of cyprodinil extracted from the plants via the EDGE were similar with averages for the three treatment groups ranging between 61-71 µg/g, 4.8-8.4 µg/g, and 0.19-0.45 µg/g for roots, leaves, and flowers respectively (Table 15). The concentration of cyprodinil that desorbed from the root in water was less than the concentration extracted via the EDGE at 6.7-16 µg/g root (Table 15), which is most

likely due to cyprodinil being more lipophilic than hydrophilic, and thus most likely preferring ACN to water. Though cyprodinil has a high log K_{OW} of 3.9 and is not expected to cross through the phloem easily, cyprodinil was found in the tomato flowers at a concentration of 2.5 - 9.6% relative to the leaf concentration (Table 16).

When plants were spiked with a 1 mg/L concentration of acetoxy TSS, a small amount of hydroxy TSS can be seen in the root zone of the plant, but not in the upper part of the plant (Table 15), and is thus most likely due to sorption. When a larger amount of acetoxy TSS is spiked, however, TSS-H can be seen through all parts of the plants, unlike TSS-$COCH_3$ which can only be seen slightly above detection limit (~0.019 µg/g) in the leaves. However, when the plant is stressed like TSS 2b was, the concentrations of TSS-H and TSS-$COCH_3$ are much larger, indicating that these compounds can travel from root to shoot, but only when plant membranes are compromised, indicating increased potential in uptake in areas that are saline or drought stressed.

An RCF was determined by comparing the concentration of the final analyte nutrient solution concentration to the concentration found in the roots by adding both what was rinsed off of the roots with DI water and extracted with ACN in the EDGE. The TSS-H RCF calculated from this experiment with average and 95% CI at 15 ± 16 was almost 20 times less than the RCF calculated in Appendix D at 253 ± 315 and is relatively low for a compound with a log K_{OW} of around 4. The two different values for RCF, as well as the large 95% CI in the previous experiment, show that the environment in which TSSs is in plays a large role in how it will behave, especially when it comes to live organisms in the system.

Table 16. Analyte Recoveries, Comparisons, and TSCF

Treatment	Analyte Sums	Mass Recovered (mg)	% Mass Recovered	Flower to Leaf % Ratio	RCF (L/kg)	TSCF
Cyprodinil Only	TSS-H	-	-	-	-	-
	TSS-$COCH_3$	-	-	-	-	-
	Cyprodinil	1.6 ± 1.4	41 ± 36	5.4 ± 8.0	223 ± 274	0.031 ± 0.014
TSS-$COCH_3$ 1 mg/L	TSS-H	0.18 ± 0.41	(4.8 ± 10.8 of $COCH_3$)	-	66 ± 178	< LOQ
	TSS-$COCH_3$	<LOQ	<LOQ	-	-	-
	Cyprodinil	1.8 ± 0.9	46 ± 25	9.6 ± 6.2	174 ± 272	0.025 ± 0.024
TSS-$COCH_3$ 10 mg/L	TSS-H	25 ± 42	(13 ± 22 of $COCH_3$)	9.0 ± 10.8	15 ± 16	0.012 ± 0.029
	TSS-$COCH_3$	0.0004 ± 0.0013	0.0002 ± 0.0007	-	-	< 0.0006 ± 0.0032
	Cyprodinil	0.89 ± 0.69	23 ± 18	2.5 ± 3.4	468 ± 444	0.031 ± 0.006
C3 (TSS-$COCH_3$ 10 mg/L & Plant Stressed)	TSS-H	95	50	174	14	0.084
	TSS-$COCH_3$	0.67	0.35	99	0.56	<0.006
	Cyprodinil	1.5	39	2.8	223	0.059

95% CI with, n sample = 3 per group (<LOQ were counted as zeroes)
TSS concentrations are presented as oligomer sums

Though most of the analytes were concentrated in the leaves, the concentration of analytes in the flowers was non-negligible, especially in the stressed plant TSS 2b. Thus, the concentration of analyte the plant took up for a TSCF was calculated using Equations 4 & 5 and assumed that the concentration of analyte in the stem was the same as the concentration in the leaves, and the concentration in the fruit was the same as the flower, as the fruit was not analyzed and both parts of the plant are fed by the phloem. Concentrations in the roots were not included as most of the concentration in the roots is assumed to be due to sorption. The TSCF for oligomer classes for a plant were determined by taking the average of each oligomer TSCF and the averages and 95% CIs

of these averages for each plant group are listed in Table 16.

The determined TSCFs for TSS-H, TSS-$COCH_3$, and cyprodinil for all groups, including plant stressed TSS 2b, are lower than 0.1, with cyprodinil TSCFs between the three groups similar to each other at between 0.025 and 0.031. The TSCF for TSS-H and TSS-$COCH_3$ could only be calculated for the plants spiked with a high amount of TSS-$COCH_3$, and those were determined to be 0.025 and less than 0.0006 for hydroxy and acetoxy TSS respectively. All TSCF values were slightly lower than predicted based on models like those in Figure 5, but were not unreasonable for compounds with log K_{OWs} around 3.5 to 5. The TSCF values are also lower than the pressure chamber values, which is also normal, as TSCF values found using pressure chambers do not allow for plant metabolism within the upper part of the plant. The TSCF values for both the pressure chamber and whole plant experiments were relatively low, indicating root to shoot uptake not to be extremely high. However, it should be noted that at the 90 - 52,000 mg/L concentrations potentially used as field sprays, as previously mentioned in Chapter II, there is a potential for root to shoot transfer of TSS compounds to be significant, even though low percentages of the root solution concentration are expected to be taken up.

CHAPTER VIII

CONCLUSIONS

To investigate the prevalence of TSS adjuvants in pollens collected by honey bees, a field sampling survey was conducted, with the focus on California almond orchards. Prior to field sampling, several extraction methods were evaluated for TSS recoveries. An acetonitrile extraction method, implemented using the EDGE system by CEM, was found to provide the best combination of simplicity, speed and TSS recovery.

The field survey analyzed 99 pollen samples from five different sites across three different U.S. states found that 43% of the samples contained TSS-H with a maximum concentration of 63 ng/g dry wt. Samples containing TSS-H were located at four of the five sites. Fifteen percent of the pollen samples contained an TSS-$COCH_3$ like compound with a maximum concentration of 723 ng/g. All of the pollen samples containing TSS-$COCH_3$ were collected by the same beekeeper at multiple sites. Nine pesticides were also found in the pollen samples and a positive correlation between cyprodinil (a fungicide) and the TSS-$COCH_3$-like compounds was discovered. Overall, the results indicate that TSSs can be found in pollen at a variety of concentrations and orchards, with concentrations potentially correlated with pesticides.

To better understand the low TSS recoveries in field spikes in the pollen field study, three studies were subsequently performed to investigate the volatility, hydrolytic stability and longer-term sample storage stability. It was determined that TSS is not likely to volatize, but is unstable in water with half lives in the hours to days range, depending on the pH of the solution. TSS recoveries from polypropylene tubes and pollen stored at room temperature were low relative to spiked samples extracted and analyzed

immediately but similar to those stored in liquid nitrogen, indicating a time and temperature dependent loss mechanism. This loss over time at room temperatures indicates that concentrations in pollen samples collected from the field may underestimate the concentrations present shortly after spraying. Further studies are also needed to determine the fate of breakdown products in pollen and their toxicity to bees and other pollinators.

To determine the potential for TSSs to be taken up by plant roots and contaminate pollen, two different plant uptake studies were performed. TSCF values measured using a pressure chamber method yields average values below 0.2 for the three TSS classes, while whole plant experiments measured TSCF values that were 10-100 times lower. However, hydroponic plant uptake experiments conducted with repeated exposures dose of approximately 10 mg/L TSS-$COCH_3$ resulted in the presence of TSS-H in the flower of Dwarf tomatoes at a low ug/g levels, indicating that there is a potential for TSS-$COCH_3$ to be transformed into TSS-H in the hydroponic solution and move from roots to flower or pollen. Preliminary results also found that TSSs did not impact the root to shoot transfer of caffeine or cyprodinil but additional replication is needed to verify that observation. Experiments also indicated that stressed plants may also see an increased uptake of TSSs into plants and therefore pollen. Further studies would be needed to determine the impact of saline and drought conditions on the uptake of TSSs into plants and ultimately pollen.

The toxicities and prevalence of hydrolysis products and the more polar unknown compounds in the adjuvants should also be investigated to determine their potential impact on honey bees.

ENGINEERING SIGNIFICANCE

Trisiloxane surfactants (TSSs) are widely used as adjuvants, or additives, in agricultural pesticide applications to help decrease the surface tension of solutions, which allows the pesticide solution to more efficiently spread on leaves. Though adjuvants are assumed to be inert by regulatory definition, recent studies have shown these TSS adjuvants to impact honey bees, which are an important general pollinator and provide billions of dollars to US crops alone. This study attempted to help determine the concentration and fate of these adjuvants in pollen collected by honey bees.

This study found TSSs in pollen samples collected from various orchards despite finding that these compounds are relatively unstable to hydrolysis at room temperatures. Because of their low stabilities, the field concentrations of TSS compounds in the pollen could be higher than reported. Experimentally determined TSCFs values for these compounds indicated a relatively low tendency for root to shoot transfer but TSSs were observed in tomato flowers subjected to repeated root zone doses. In addition, the concentration of field tank mixes can be several orders of magnitude above the those used in this thesis. Finally, TSS concentrations applied directly to the field are several orders of magnitude above the levels shown to negatively impact honey bees, meaning that direct contact may be an issue if spraying occurs while pollinators are present.

Thus, it is recommended to not spray TSSs when pollinators are out or when flowers are in bloom. It is also recommended to limit the use of TSS adjuvants to only what is necessary and not consider them biologically “inert.” If TSSs are used during flower bloom, it is also recommended to wait for a short period of time to allow some of the TSSs to hydrolyze before pollinators are allowed to pollinate the crops.

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APPENDICES

APPENDIX A

ANALYSIS CHARACTERISTICS

A1 - TSS Oligomeric Distribution

Commercial TSS standards consist of a distribution of oligomeric compounds as the result of trisiloxanes synthesis process. The percent of each individual oligomer in the was determined theoretically using the Poisson distribution equation explained in Michel et al. (2012). The theoretical values given by Michel et al. (2012) compare relatively well to the experimental values calculated using the MRM ion areas, though they are slightly different from the values given by Chen et al. (2013) (Figure A1-1).

Experimental values using single ion monitoring (SIM) were also compared for both hydroxy and methoxy standards (the acetoxy oligomer SIM is not shown as EO = 12 SIM transition was entered wrong). The number of replicates for each concentration distribution ranged from one to eighteen and included runs on different days. The SIM values were closer to the theoretical values, which may be due to the differences in optimization for each individual transition when acquiring MRM data.

Because the TSS standards are only available at a 90-95% purity, several oligomeric groups with m/z differences of 44 (relating to EO group) that were not TSS standards (hereafter called impurities) were found, and the oligomeric distribution of these compound classes are shown in Figure A1-2. As the original molecular weight is unknown, the m/z used for MRMs were assumed to be the molecular weights plus an ammonium ion, thus allowing for the Poisson distribution equation to be used as it was with the TSS standards.

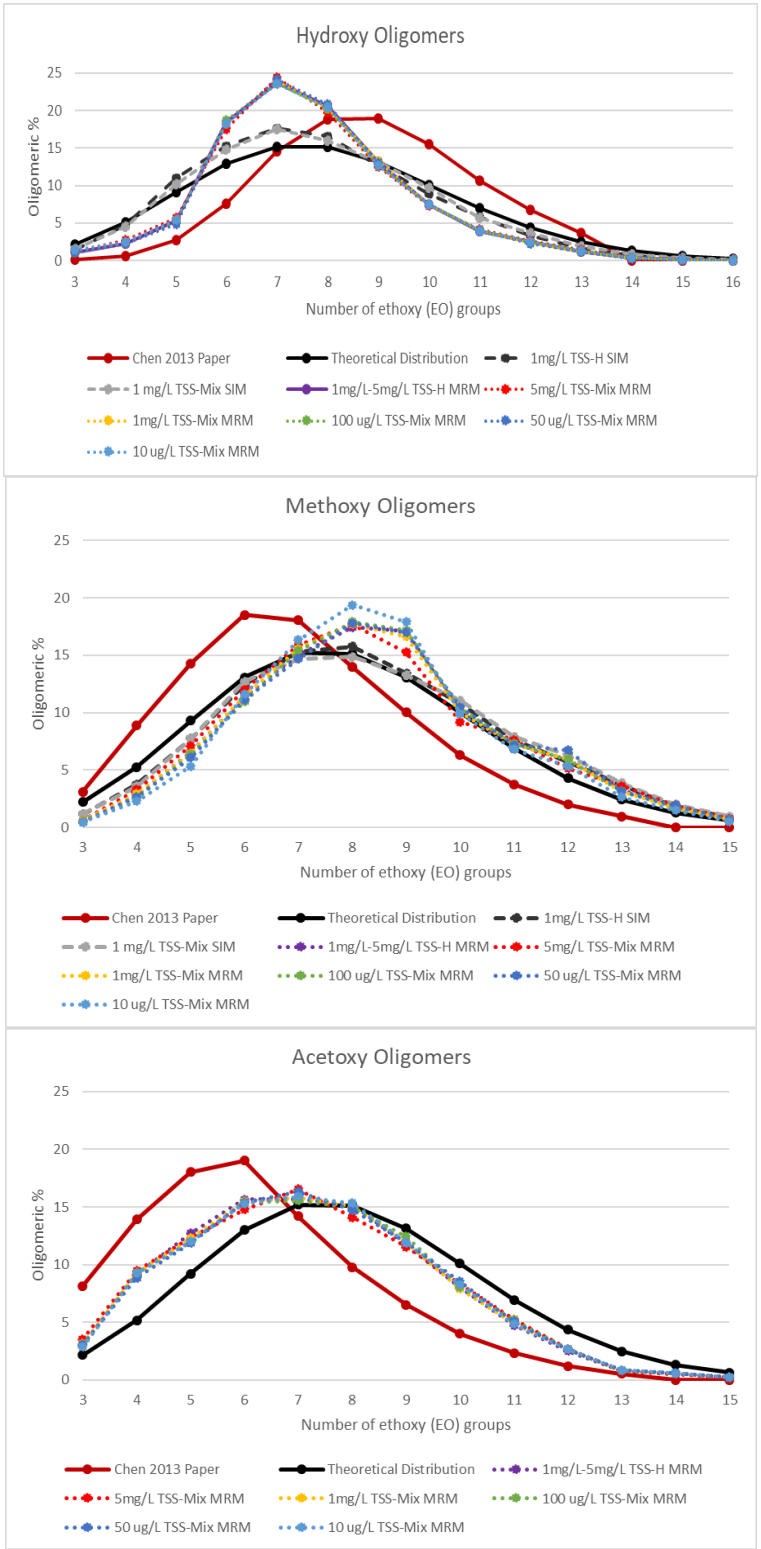

Figure A1-1. Oligomeric % distribution of TSS-H, TSS-CH_3, and TSS-$COCH_3$ (Error bars not shown as highest error for 95% CI is <2.7% and 95% of CI errors are less than 1%.)

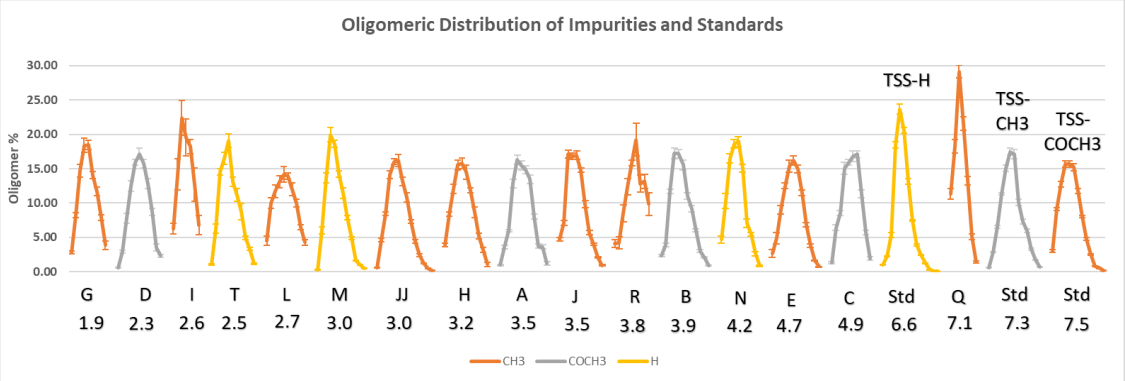

Figure A1-2. Oligomeric % distribution of impurities and standards in TSS-H, TSS-CH_3, and TSS-$COCH_3$ in approximate order of retention time.
Values from 1 ug/mL standard with 95% CI. Retention times listed are averages for the retention times in an oligomeric group from method TSS_50mm_MRM20uL-hp (Appendix C2).

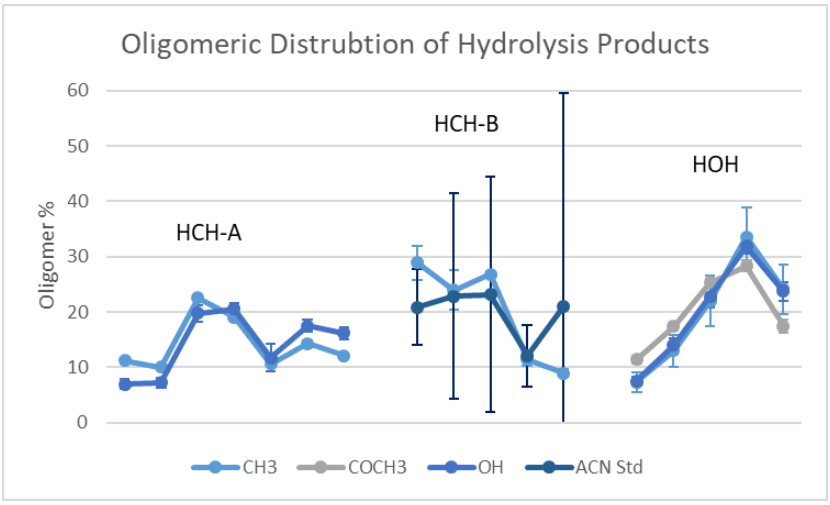

Figure A1-3. Oligomeric % distribution of hydrolysis products in TSS-H, TSS-CH_3, and TSS-$COCH_3$.
Values only included if all oligomers had area count above 1000 counts. 95% CI shown.

Three major hydrolysis products were also found after the standards were left in acidic or basic water over time. Like the impurities, the molecular weight is unknown, so the m/z used for MRMs were assumed to be the molecular weights plus an ammonium ion, thus allowing for the Poisson distribution equation to be used as it was with the TSS

standards and impurities. Unlike the standards and impurities, the hydrolysis products did not seem to follow a Gaussian like curve in their oligomer % composition (Figure A1-3). This may be due to the kinetics of the hydrolysis of each oligomer.

A2 - Signal to Noise (S/N) Ratios

S/N ratios were calculated using Agilent MassHunter Qualitative Analysis program for a series of decreasing concentrations. For example, Figure A2-1 shows the quant ion for TSS-H Oligomer n = 6 where the detection limit was determined to be the 0.5 ng/mL standard. Using the Poisson distribution in Table 4, TSS-H oligomer is 12.96% of the total concentration. Thus, the detection limit is 0.065 ng/mL for TSS-H.

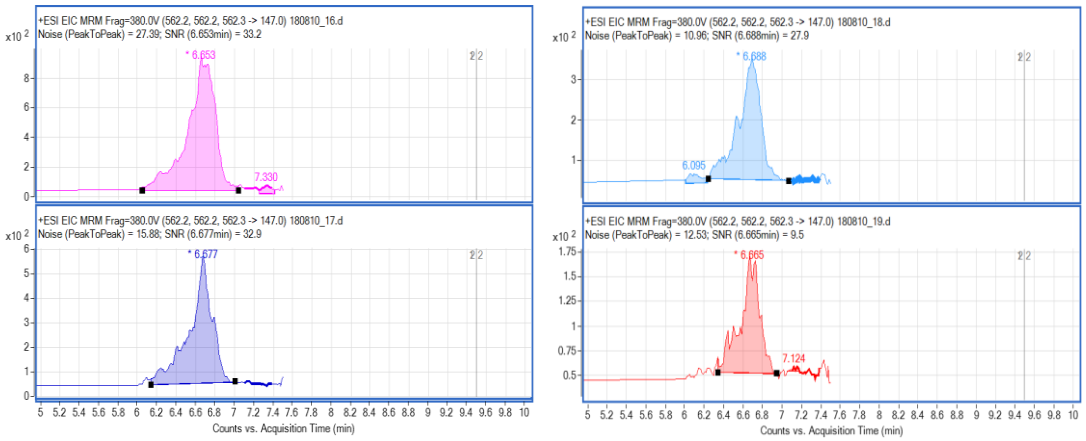

Figure A2-1. LOQ based on MRMs and SNR

APPENDIX B

LITERATURE AND ESTIMATED VALUES FOR ENVIRONMENTAL PROPERTIES

B1 - Toxicity

Table B1-1 lists the toxicities found in adjuvant and chemical safety data sheets (SDSs) and mentioned in literature papers. A recent paper published by Li et al. (2019) performed their own toxicity study and found toxicities for fish similar to those mentioned in SDSs in the mg/L range, but the EC_{50} for *Daphnia magna* was in the µg/L range, lower than SDSs reports, indicating potential toxic impact on specific organisms.

Table B1-1. Trisiloxanes in Adjuvants and their Listed Toxicities

Adjuvant or Standard	Trisiloxane (CAS) % Trisiloxane	OSS Conc applied (mg/L)	Aquatic Toxicity (mg/L)	Oral toxicity (mg/kg)
TSS-$COCH_3$	TSS-$COCH_3$ (125997-17-3) >95%	-	-	LD_{50} rat: >2000
TSS-CH_3	TSS-CH_3 (27306-78-1) >95%	-	NOEC Zebra fish, 96hr: 0.56 NOEC rainbow trout, 96hr: 3.2 NOEC Daphnia magna, 48hr: 25 NOEC Daphnia similis, 48hr: 10 NOEC algae**, 96hr: 1	LD50 dust, vapor: 2 mg/l/4h
TSS-H	TSS-H (67674-67-3) >90%	-	-	-
Silwet L-77®	TSS-CH_3 (27306-78-1) 50-<100% TSS-H (67674-67-3) 5-<10%	100 – <49,652 10 – <4,965	LC_{50} Zebra fish, 96hr: 2.75, 6.8 NOEC Zebrafish: 0.56 EC_{50} Daphnia magna, 48hr: 6.2-23.4, 25 NOEC Daphnia, 48hr: 10 EC_{50} algae*, 96hr: 32	LD_{50} rat: >2,000 LC_{50} rat inhalation: >11.78 mg/L [5% aqueous solution] LC_{50} rat inhalation: 2 mg/L [aerosols]
Silwet* 408	TSS-H (67674-67-3) 50-<100%	123 – <49,020	LC_{50} Zebra fish: 6.8, 5.61-6.93 EC_{50} Daphnia magna: 25, 0.032 EC_{50} algae*: 32	LD_{50} rat: > 2,000 Inhalation: ATEmix 13.1 mg/L
Sylgard® OFX-0309	TSS-$COCH_3$ (125997-17-3) 75-85%	181 – 3,286	LC_{50} Fathead minnow****, 96hr: 4.3 LC_{50} Daphnia magna, 48hr: 41	LD_{50} rat: estimated >2,000
Syl-Tac®	TSS-$COCH_3$ (125997-17-3) 30-<40%	101 – <430***	LC_{50} Rainbow trout, 96hr: >5 EC_{50} Daphnia, 48hr: >5	-

Table B1-1 (cont). Trisiloxanes in Adjuvants and their Listed Toxicities

Adjuvant or Standard	Adjuvant or Standard	Adjuvant or Standard	Adjuvant or Standard	Adjuvant or Standard
Xiameter® OFX-0309	TSS-$COCH_3$ (125997-17-3) 80%	388 – 2,910	LC_{50} Fathead minnow****, 96hr: 4.3 LC_{50} Daphnia magna, 48hr: 41	LD_{50}: estimated 3,125
Sylgard 309®	Trisiloxane (134180-76-0) 75-100%	227 – 48,544	LC_{50} Fathead minnow: >4.6 EC_{50} Daphnia, 48hr: 22.9->41	-
Silwet 806®	Trisiloxane (134180-76-0) 50-<100%	125 – <49,900	LC_{50} Rainbow trout, 96hr: 2.1 NOEC Rainbow trout, 96hr: 1 EC_{50} Daphnia magna: 0.015 EC_{50} Zebra fish: 3.89-4.45	LD_{50} rat: > 2,000 Inhalation: >11.78 mg/L
Dyne-Amic®	Proprietary ≤99%	≤3,992 - 42,969	LC_{50} Bluegill, 96hr: 26.9	LD_{50} rat: > 5,050
Kinetic® (HV)	Proprietary ≤99%	≤1,190 – 4,901	LC_{50} Bluegill, 96hr: 19.8 EC_{50} Daphnia, 48hr: 111	LD_{50} rat: > 2,890
Silkin®	Proprietary ≤99%	≤295 – 2,357	-	LD_{50}: 2,360

- Values in blue are from Li et al. (2019) paper to show toxicity differences

Sources: Bakke et al. (2008), Chen & Mullin (2013), Dow (2018), Dow (2019), , Dow Corning (2009), Dow Corning (2016), Dow Corning (n.d.), Gelest (2014a), Gelest (2019a, 2019b), Helena (2015a, 2015b), Helena Holding (2014), Helena Holding (2015), Li et al. (2019), Loveland Industries Inc (n.d.), Michel et al. (2016), Momentive (2017), Momentive (2018a, 2018b, 2018c), Momentive (2019), Mullin et al. (2016), Wilbur-Ellis (2017), Wilbur-Ellis (n.d. -a,-b,-c), Winfield (2018), WinField Agrisolutions (n.d.)

*** Pseudokirchneriella subcapitata** ****Selenastrum capriconutum**
*****assumed 100 gal/acre** ******Pimephales promelas**

B2 - P_v Estimate

TSS standards consist of mixtures of oligomers and the oligomers fragment into very similar mass spectra. Thus, the gas chromatography mass spectrometry (GCMS) peak relating to which oligomer was estimated is not definitive. The synthetic average for the TSS oligomers is 7.5; thus it was assumed that the largest peak was either oligomer number 7 or 8. In addition, the order of molecular weights and polarity for the standards go H < CH_3 < $COCH_3$; thus the oligomers the relationship between the standards, oligomers and retention time (RT) was determined to be as shown by Figure B2-1.

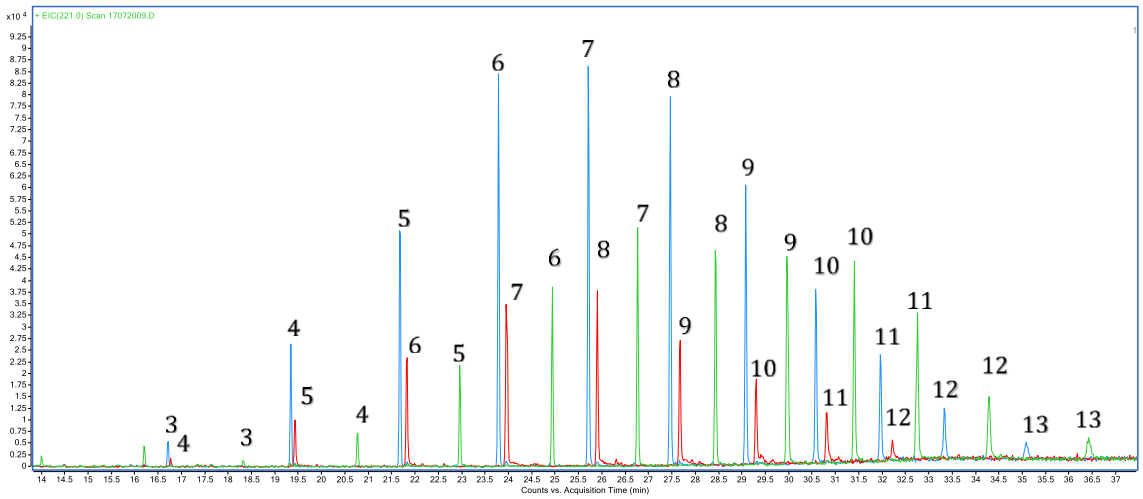

Figure B2-1. Estimation of TSS RT on GCMS
Method: TSS_MDEV (Appendix C1)
TSS-CH_3 163 µg/mL (blue), TSS-H 115 µg/mL (red), TSS-$COCH_3$ 144 µg/mL

Using a Hewlett-Packard GCMS and the method 180124_BeePesticide Method (Appendix C1), vapor pressure (P_v) values were estimated for the TSS standards (Table B2-2) by comparing retention times (RT) of known pesticides (Table B2-1) and extrapolating from an exponential graph (Figure B2-2).

Table B2-1. Pesticide P_v vs GCMS RT

Compound	RT	P_v (mm Hg)	Temp (ºC)	Compound	RT	P_v (mm Hg)	Temp (ºC)
Dichlorvos	6.10	1.58E-02	25	**Pentachlorophenol**	14.68	1.10E-04	25
Propamocarb	8.31	5.48E-02	25	**Lindane**	14.81	4.20E-05	20
Mevinphos	8.96	1.23E-04	20	**Pyrimethanil**	15.31	1.65E-05	25
4-Nitrophenol	10.57	5.00E-04	25	**Diazinon**	15.50	9.00E-05	25
Picloram	10.75	4.50E-07	20	**Disulfoton**	15.60	9.75E-05	25
Dichlorprop	12.06	7.50E-08	20	**Methyl Parathion**	17.14	3.50E-06	25
Ethoprofos	12.42	3.80E-04	25	**Heptachlor**	17.34	4.00E-04	25
Naled	12.85	2.00E-04	20	**Metalaxyl**	17.62	5.62E-06	25
Monocrotophos	13.29	7.35E-06	20	**Ronnel**	17.65	7.50E-05	20
Phorate	13.51	6.38E-04	25	**Malathion**	18.53	3.38E-06	25
Dimethoate	14.13	1.88E-05	25	**Aldrin**	18.54	1.20E-04	25

Table B2-1 (cont). Pesticide P_v vs GCMS RT

Compound	RT	P_v (mm Hg)	Temp (ºC)	Compound	RT	P_v (mm Hg)	Temp (ºC)
Fenthion	18.80	1.05E-05	25	**Dieldrin**	21.49	5.98E-06	25
Chlorpyrifos	18.86	2.03E-05	20	**Myclobutanil**	21.66	1.60E-06	25
Bentazone	19.19	3.45E-06	20	**Endrin**	22.02	5.89E-06	25
Trichloronate	19.23	1.50E-05	20	**Fensulfothion**	22.33	5.00E-05	25
Cyprodinil	19.63	3.68E-06	25	**Endrin aldehyde (1)**	22.68	2.00E-07	25
Heptachlor epoxide	19.82	1.95E-05	30	**Propiconazole (1)**	23.19	4.20E-07	20
Hexythiazox	20.47	2.55E-08	20	**Endosulfan sulfate**	23.21	2.80E-07	25
trans-Chlordane	20.49	9.75E-06	25	**Propiconazole (2)**	23.33	4.20E-07	20
Tetrachlorvinphos	20.83	4.20E-08	20	**Phosmet**	24.29	4.90E-07	25
cis-Chlordane	20.90	9.75E-06	25	**Coumaphos**	26.46	9.70E-08	20
Flutolanil	21.24	4.88E-08	25	**Prochloraz**	26.51	1.13E-06	25
Fludioxonil	21.45	2.93E-09	25	**Azoxystrobin**	29.03	8.25E-13	25

- P_v Source: US EPA (2019) Database

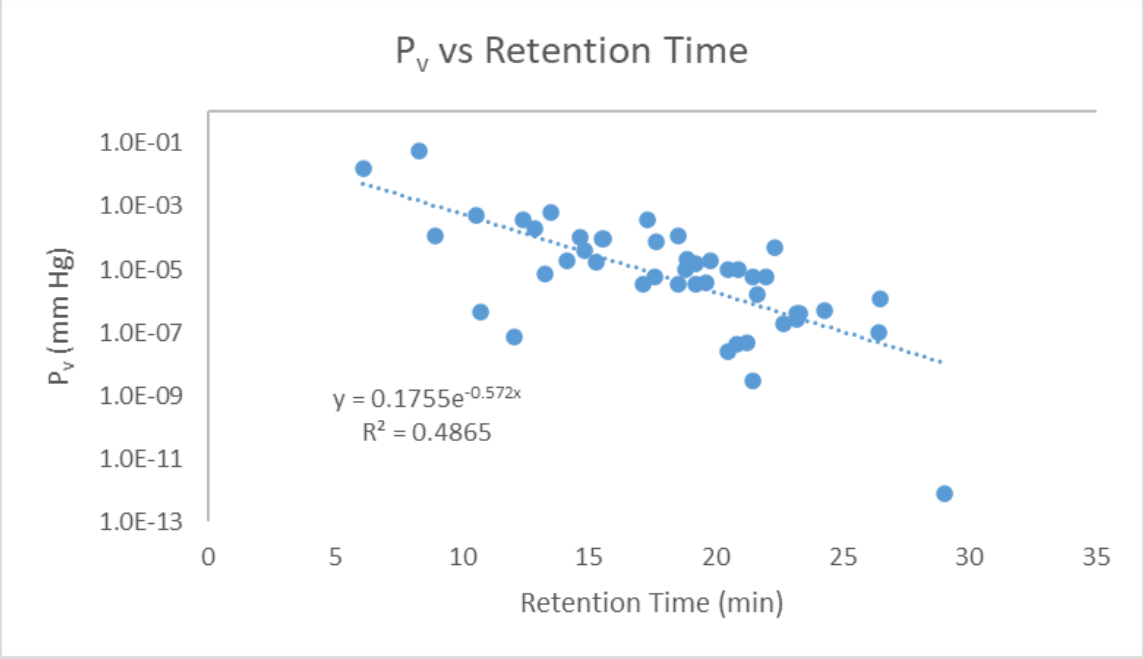

Figure B2-2. Pesticide P_v vs GCMS RT

Table B2-2. Estimated TSS Pv based on RT

TSS	RT (min)	Estimated P_v (mm Hg)	TSS	RT (min)	Estimated P_v (mm Hg)	TSS	RT (min)	Estimated P_v (mm Hg)
TSS-H_04	14.06	5.64E-05	**TSS-$COCH_3$_03**	16.82	1.16E-05	**TSS-CH_3_03**	14.00	5.84E-05
TSS-H_05	18.76	3.84E-06	**TSS-$COCH_3$_04**	20.80	1.19E-06	**TSS-CH_3_04**	18.65	4.09E-06
TSS-H_06	22.12	5.61E-07	**TSS-$COCH_3$_05**	23.48	2.58E-07	**TSS-CH_3_05**	21.98	6.08E-07
TSS-H_07	24.58	1.37E-07	**TSS-$COCH_3$_06**	25.63	7.54E-08	**TSS-CH_3_06**	24.41	1.52E-07
TSS-H_08	26.62	4.28E-08	**TSS-$COCH_3$_07**	27.49	2.60E-08	**TSS-CH_3_07**	26.42	4.80E-08
TSS-H_09	28.41	1.54E-08	**TSS-$COCH_3$_08**	29.16	1.00E-08	**TSS-CH_3_08**	28.20	1.73E-08
TSS-H_10	30.18	5.59E-09	**TSS-$COCH_3$_09**	31.10	3.30E-09	**TSS-CH_3_09**	29.89	6.59E-09
TSS-H_11	32.58	1.42E-09	**TSS-$COCH_3$_10**	33.94	6.50E-10	**TSS-CH_3_10**	32.14	1.82E-09

B3 - K_{OW} Estimates

Octanol-water partition coefficients (K_{OW}) were estimated for the TSS standards (Table B3-2) by comparing retention times (RT) on a LCMS/MS using method TSS_50mm_MRM20uL-hp (Appendix C2) and literature K_{OW} of known pesticides (Table B3-1) and extrapolating from a linear graph (Figure B3-1).

Table B3-1. Pesticide LCMS RT vs Literature K_{OW}

Compound Name	RT (min)	Kow (Lit)	Compound Name	RT (min)	Kow (Lit)
Omethoate	1.20	-0.9	**Methomyl**	1.79	0.09
Acephate	1.20	-0.85	**Flonicamid**	1.99	-0.24
Dinotefuran	1.20	-0.549	**3-Hydroxycarbofuran**	2.39	2.32
Formetanate HCl	1.20	-0.0014	**Chlothianidin**	2.56	0.7
Cyromazine	1.20	0.0069	**Thiabendazole**	2.72	2.39
Propamocarb	1.20	0.84	**Dimethoate**	2.79	0.75
Aldicarb sulfoxide	1.20	1.15[a]	**Dioxacarb**	2.81	0.67
Mevinphos	1.40	0.127	**Imidacloprid**	2.91	0.57
Monocrotophos	1.51	-0.22	**Acetamiprid**	2.96	0.8
Oxamyl	1.63	-0.44	**Sulfamethoxazole**	3.10	0.89

Table B3-1 (cont). Pesticide LCMS RT vs Literature K_{OW}

Compound Name	RT (min)	Kow (Lit)	Compound Name	RT (min)	Kow (Lit)
Bentazone	3.18	-0.46	**Tebufenozide**	6.40	4.25
Thiacloprid	3.69	1.26	**Boscalid**	6.44	2.96
Dichlorvos	3.69	1.9	**Propiconazole**	6.46	2
Carbamazepine	3.80	2.45	**Prochloraz**	6.48	3.5
Imazalil	4.03	2.56	**Phosmet II**	6.50	2.8
Metalaxyl	4.23	1.75	**Flubendiamide**	6.52	4.14
Carbofuran	4.23	1.8	**Malathion II**	6.53	2.75
Fluoxetine	4.26	4.05	**Azinphos methyl**	6.55	2.96
Bendiocarb	4.28	1.7	**Tetrachlorvinphos**	6.55	3.53
Phosmet I	4.43	2.8	**Diflubenzuron 1**	6.55	3.89
Atrazine	4.48	2.7	**Cyazofamid**	6.89	3.2
Malathion I	4.60	2.75	**Fenchlorphos oxon**	7.02	4.88[f]
Methamidophos	4.78	-0.79	**Diazinon**	7.09	3.69
Diuron	4.82	2.87	**Rotenone**	7.18	4.1
Diflubenzuron 2	4.84	3.89	**Novaluron**	7.23	4.3
Demeton-S	4.89	1.32[b]	**Cyprodinil**	7.25	4
Fensulfothion	4.89	2.23	**Teflubenzuron**	7.33	4.3
Carbaryl	4.95	2.36	**Tolyfluanid**	7.48	3.9
Paclobutrazol	5.13	3.11	**Coumaphos S**	7.68	4.13[d]
Naled	5.15	2.18	**Metaflumizone**	7.70	4.6
Spirotetramat	5.22	2.51	**Trichlosan**	7.70	4.76
Coumaphos O	5.28	4.13[d]	**Phoxim**	7.73	3.38
Myclobutanil	5.35	2.89	**Fenthion**	7.73	4.84
Spinesad - A	5.54	4.1[c]	**Piperonyl butoxide**	7.75	4.75
Fludioxinil	5.74	4.12	**Pyraclostrobin**	7.85	3.99
Ethoprofos	5.75	2.99	**Disulfoton**	7.88	3.95
Spinesad - D	5.78	4.1[c]	**Flufenoxuron**	8.11	5.11
Pyrimethanil	6.07	2.84	**Chlorfluazuron**	8.28	2.8
Spinetoram - Spinosyn J	6.09	4.2[e]	**Chlorpyrifos**	8.59	4.7
Methoxyfenozide	6.11	3.72	**Fenpropathrin**	8.61	6.04
Azoxystrobin	6.15	2.5	**Fenazaquin**	8.66	5.51
Iprodione	6.18	3	**Fenproximate**	8.73	5.01
Spinetoram - Spinosyn L	6.22	4.2[e]	**Tribufos**	8.95	5.52
Mandipropamid	6.25	3.2	**Hexythiazox**	8.98	2.67
Flutolanil	6.31	3.17	**Bifenthrin**	9.00	6.6
Gemfibrozil	6.40	3.4	**Etofenprox**	9.00	6.9

- K_{ow} for (a) aldicarb (b) demeton-S-methyl (c) Spinosad (d) Coumaphos (e) Spinetoram (f) Fenchlorphos

Sources: NIH (2020), University of Hertfordshire (2020a, 2020b)

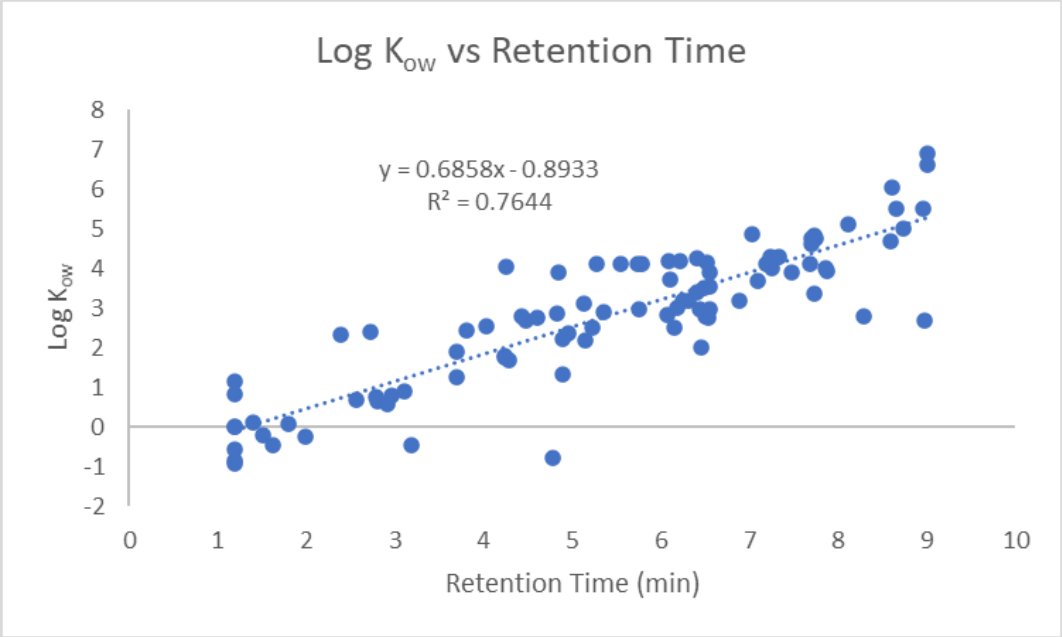

Figure B3-1. Pesticide LCMS RT vs K_{OW} known compounds

Table B3-2. Estimated TSS K_{OW} Based on RT

Compound Name	RT (min)	Est Kow	Compound Name	RT (min)	Est Kow	Compound Name	RT (min)	Est Kow
TSS-H 03	6.93	3.9	**TSS-$COCH_3$ 03**	8.20	4.7	**TSS-CH_3 03**	8.08	4.6
TSS-H 04	6.90	3.8	**TSS-$COCH_3$ 04**	8.02	4.6	**TSS-CH_3 04**	7.88	4.5
TSS-H 05	6.80	3.8	**TSS-$COCH_3$ 05**	7.88	4.5	**TSS-CH_3 05**	7.73	4.4
TSS-H 06	6.75	3.7	**TSS-$COCH_3$ 06**	7.77	4.4	**TSS-CH_3 06**	7.58	4.3
TSS-H 07	6.68	3.7	**TSS-$COCH_3$ 07**	7.64	4.3	**TSS-CH_3 07**	7.46	4.2
TSS-H 08	6.64	3.7	**TSS-$COCH_3$ 08**	7.54	4.3	**TSS-CH_3 08**	7.35	4.1
TSS-H 09	6.58	3.6	**TSS-$COCH_3$ 09**	7.44	4.2	**TSS-CH_3 09**	7.26	4.1
TSS-H 10	6.56	3.6	**TSS-$COCH_3$ 10**	7.36	4.2	**TSS-CH_3 10**	7.18	4.0
TSS-H 11	6.51	3.6	**TSS-$COCH_3$ 11**	7.30	4.1	**TSS-CH_3 11**	7.09	4.0
TSS-H 12	6.49	3.6	**TSS-$COCH_3$ 12**	7.20	4.0	**TSS-CH_3 12**	7.01	3.9

Table B3-2 (cont). Estimated TSS K_{OW} Based on RT

Compound Name	RT (min)	Est Kow	Compound Name	RT (min)	Est Kow	Compound Name	RT (min)	Est Kow
TSS-H 13	6.46	3.5	**TSS-$COCH_3$ 13**	7.14	4.0	**TSS-CH_3 13**	6.98	3.9
TSS-H 14	6.45	3.5	**TSS-$COCH_3$ 14**	7.09	4.0	**TSS-CH_3 14**	6.93	3.9
TSS-H 15	6.41	3.5	**TSS-$COCH_3$ 15**	7.05	3.9	**TSS-CH_3 15**	6.88	3.8
TSS-H 16	6.40	3.5	-	-	-	-	-	-

B4 - US EPA EPI Suite Estimates

Using US EPA (2019) EPI Suite's fugacity level III modeling tool with inputs defined in Table B4-1, the % of TSS in air, water, fish, sediment, and soil were calculated (Table B4-2).

Table B4-1 – Estimated Values Used for EPI Suite Fugacity Model

n*	MW	P_v (mmHg)	Log Kow	Log K_{OC}	Log K_{AW}	Log BCF	Air Half Life (Hr)	Water Half Life (Day)	Soil** Half Life (Week)	Sediment Half Life (Years)
					TSS-H					
3	412.8	7.59E-04	3.86	2.7	-6.74	2.21	4.9	8.3	10.7	0.9
4	456.8	5.64E-05	3.84	2.7	-8.55	2.20	3.8	8.3	10.7	0.9
5	500.9	3.84E-06	3.77	2.7	-10.36	2.15	3.2	8.3	17.1	1.5
6	544.9	5.61E-07	3.74	2.6	-12.17	2.14	2.7	8.3	17.1	1.5
7	589.0	1.37E-07	3.69	2.6	-13.98	2.10	2.4	8.3	17.1	1.5
8	633.0	4.28E-08	3.66	2.7	-15.79	2.08	2.1	8.3	17.1	1.5
9	677.1	1.54E-08	3.62	2.8	-17.60	2.06	1.9	8.3	17.1	1.5
10	721.1	5.59E-09	3.61	2.9	-19.40	2.05	1.7	8.3	51.4	4.4
11	765.2	1.42E-09	3.57	3.1	-21.21	2.02	1.6	8.3	51.4	4.4
12	809.2	7.10E-10	3.56	3.3	-23.02	2.02	1.4	8.3	51.4	4.4
13	853.3	3.19E-10	3.54	3.4	-24.83	2.00	1.3	8.3	51.4	4.4
14	897.3	1.52E-10	3.53	3.6	-26.64	2.00	1.2	8.3	51.4	4.4
15	941.4	7.60E-11	3.50	3.8	-28.45	1.98	1.2	8.3	51.4	4.4
16	985.5	3.98E-11	3.50	4.0	-30.26	1.98	1.1	8.3	51.4	4.4

Table B4-1 (cont). Estimated Values Used for EPI Suite Fugacity Model

n*	MW	P_v (mmHg)	Log Kow	Log K_{OC}	Log K_{AW}	Log BCF	Air Half Life (Hr)	Water Half Life (Day)	Soil** Half Life (Week)	Sediment Half Life (Years)
					TSS-$COCH_3$					
3	454.8	1.16E-05	4.73	3.4	-4.93	1.41	5.1	8.3	10.7	0.9
4	498.8	1.19E-06	4.61	3.4	-6.74	1.33	4.0	8.3	17.1	1.5
5	542.9	2.58E-07	4.51	3.4	-8.55	1.27	3.3	8.3	17.1	1.5
6	587.0	7.54E-08	4.44	3.3	-10.36	1.22	2.8	8.3	17.1	1.5
7	631.0	2.60E-08	4.35	3.3	-12.17	1.16	2.4	8.3	17.1	1.5
8	675.0	1.00E-08	4.28	3.4	-13.97	1.12	2.1	8.3	17.1	1.5
9	719.1	3.30E-09	4.21	3.5	-15.78	1.07	1.9	8.3	51.4	4.4
10	763.2	6.50E-10	4.15	3.6	-17.59	1.03	1.7	8.3	51.4	4.4
11	807.2	6.15E-10	4.11	3.8	-19.40	1.00	1.6	8.3	51.4	4.4
12	851.3	3.16E-10	4.04	4.0	-21.21	0.96	1.5	8.3	51.4	4.4
13	895.3	1.71E-10	4.00	4.1	-23.02	2.31	1.3	8.3	51.4	4.4
14	939.4	9.70E-11	3.97	4.3	-24.83	2.29	1.2	8.3	51.4	4.4
15	983.4	5.72E-11	3.94	4.5	-26.63	2.27	1.2	8.3	51.4	4.4
					TSS-CH_3					
3	426.8	5.84E-05	4.65	3.4	-4.24	1.36	4.6	8.3	17.1	1.5
4	470.8	4.09E-06	4.51	3.4	-6.05	1.27	3.6	8.3	17.1	1.5
5	514.9	6.08E-07	4.41	3.4	-7.86	1.20	3.0	8.3	17.1	1.5
6	558.9	1.52E-07	4.31	3.3	-9.66	1.14	2.6	8.3	17.1	1.5
7	603.0	4.80E-08	4.22	3.3	-11.47	1.08	2.3	8.3	17.1	1.5
8	647.1	1.73E-08	4.15	3.4	-13.28	1.03	2.0	8.3	51.4	4.4
9	691.1	6.59E-09	4.09	3.5	-15.09	0.99	1.8	8.3	51.4	4.4
10	735.2	1.82E-09	4.03	3.6	-16.90	0.95	1.7	8.3	51.4	4.4
11	779.2	1.04E-09	3.97	3.8	-18.71	2.29	1.5	8.3	51.4	4.4
12	823.3	5.03E-10	3.91	4.0	-20.52	2.25	1.4	8.3	51.4	4.4
13	867.3	2.59E-10	3.89	4.1	-22.32	2.23	1.3	8.3	51.4	4.4
14	911.4	1.40E-10	3.86	4.3	-24.13	2.21	1.2	8.3	51.4	4.4
15	955.4	7.88E-11	3.83	4.5	-25.94	2.19	1.2	8.3	51.4	4.4

Parameter Sources:

- P_v (GCMS RT Data), Log K_{OW} (LCMS RT Data), Log K_{OC} (Michel et al. 2016 and estimated values), Water Half Life (Powell & Carpenter 1997: TSS-CH_3 pH 7)
- Boiling Point/ Melting Point: TSS-H = -18/205, TSS-CH_3/$COCH_3$ = 0/205 (Sources: Gelest (2014a), Gelest (2019a, 2019b))
- Solubility set at 10 mg/L (lowest literature CAC value), Emissions set at 1kg/hr for water, air, and soil

*n = number of EO in oligomer

**Soil half-life changes from 17.1 weeks to 51.4 weeks when ultimate biodegradation in model changes from months to recalcitrant

Table B4-2. EPI Suite Fugacity Level III Estimations

		Air	Water	Fish	Suspended Sediment	Soil	Sediment
Compartment Volume (m^3)		1.0E+14	2.0E+11	2.0E+05	1.0E+06	1.8E+10	5.0E+08
n*	**Persistence (Days)**	**Air % mass**	**Water % mass**	**Fish % mass**	**Suspended Sediment % mass**	**Soil % mass**	**Sediment % mass**
				TSS-H			
3	12.6	1.06	32.40	0.01	0.00	66.40	0.07
4	16.2	0.52	29.80	0.01	0.00	69.60	0.06
5	19.5	0.33	27.20	0.01	0.00	72.40	0.06
6	21.5	0.22	26.00	0.01	0.00	73.70	0.05
7	22.5	0.18	25.50	0.01	0.00	74.20	0.05
8	22.7	0.15	25.40	0.01	0.00	74.40	0.05
9	22.6	0.14	25.30	0.01	0.00	74.50	0.05
10	26.6	0.11	23.40	0.00	0.00	76.40	0.05
11	26.7	0.10	23.20	0.00	0.00	76.60	0.05
12	26.7	0.10	23.10	0.00	0.00	76.80	0.05
13	26.6	0.09	23.10	0.00	0.00	76.80	0.05
14	26.7	0.08	22.90	0.00	0.00	77.00	0.05
15	26.8	0.08	22.70	0.00	0.00	77.10	0.05
16	26.9	0.07	22.60	0.00	0.00	77.30	0.05
n*	**Persistence (Days)**	**Air % mass**	**Water % mass**	**Fish % mass**	**Suspended Sediment % mass**	**Soil % mass**	**Sediment % mass**
				TSS-CH_3			
3	17.4	0.59	28.30	0.08	0.00	71.00	0.06
4	21.8	0.30	25.40	0.05	0.00	74.20	0.05
5	23.3	0.21	24.60	0.04	0.00	75.10	0.05
6	23.7	0.19	24.50	0.03	0.00	75.30	0.05
7	23.5	0.17	24.50	0.03	0.00	75.20	0.05
8	23.4	0.15	24.50	0.02	0.00	75.20	0.05
9	27.6	0.12	22.70	0.02	0.00	77.20	0.05
10	27.5	0.11	22.60	0.02	0.00	77.20	0.05
11	27.5	0.10	22.50	0.01	0.00	77.30	0.05
12	27.5	0.09	22.40	0.01	0.00	77.50	0.05
13	27.4	0.09	22.00	0.01	0.00	77.50	0.05
14	27.5	0.08	22.20	0.01	0.00	77.70	0.05
15	27.6	0.08	22.00	0.01	0.00	77.80	0.05

Table B4-2 (cont). EPI Suite Fugacity Level III Estimations

n*	Persistence (Days)	Air % mass	Water % mass	Fish % mass	Suspended Sediment % mass	Soil % mass	Sediment % mass
				TSS-$COCH_3$			
3	18.4	0.54	27.30	0.06	0.00	72.00	0.06
4	20.1	0.36	26.30	0.04	0.00	73.20	0.06
5	22.3	0.24	25.10	0.03	0.00	74.60	0.05
6	23.3	0.19	24.70	0.03	0.00	75.10	0.05
7	23.3	0.16	24.60	0.02	0.00	75.10	0.05
8	27.6	0.12	22.70	0.02	0.00	77.10	0.05
9	27.5	0.11	22.70	0.01	0.00	77.10	0.05
10	27.4	0.11	22.70	0.01	0.00	77.20	0.05
11	27.4	0.10	22.50	0.01	0.00	77.30	0.05
12	27.5	0.09	22.40	0.01	0.00	77.50	0.05
13	27.4	0.09	22.40	0.01	0.00	77.50	0.05
14	27.5	0.08	22.20	0.01	0.00	77.70	0.05
15	27.5	0.08	22.00	0.01	0.00	77.80	0.05

- EPI Suite Values Entered: See Table B4-1, Emissions were set at 1 kg/hr in each section

*n = number of EO in oligomer

APPENDIX C

ADDITIONAL METHODS INFORMATION

C1 - GCMS Programs

Table C1-1. 2018_TSSHEADSPACE

GCMS	Agilent 7890A GC/5975C MS
Column	RTx-5ms 30m x 0.25 mm 0.25 µm
Oven Temp	80 °C for 0 min then 30 °C/min to 110 °C for 3 min then 10 °C/min to 150 °C for 0 min then 5 °C/min to 200 °C for 0 min then 10 °C/min to 310 °C for 5 min
Inlet Temp	290ºC
Air	UHP Helium
Injection Volume	1 mL, 10:1 split
Inlet Liner	Straight
Solvent	ACN
Auxiliary Temp	290ºC
MS Source	230ºC
MS Quad	150ºC
Solvent Delay	4 min
M/Z Scan Parameters	43 - 500

Table C1-2. 180124_BeePesticideMethod

GCMS	Agilent 7890A GC/5975C MS
Column	RTx-5ms 30m x 0.25 mm 0.25 µm
Oven Temp	80 °C for 0 min then 30 °C/min to 110 °C for 3 min then 10 °C/min to 150 °C for 0 min then 5 °C/min to 200 °C for 0 min then 10 °C/min to 310 °C for 5 min
Inlet Temp	290ºC
Air	UHP Helium
Injection Volume	1 µL, splitless
Inlet Liner	Double gooseneck with glass wool
Solvent	ACN
Auxiliary Temp	290ºC
MS Source	230ºC
MS Quad	150ºC
Solvent Delay	4 min
M/Z Scan Parameters	43 - 500

Table C1-3. TSS_MDEV	
GCMS	Hewlett Packard 6890N GC/5973 MS
Column	Rtx-5ms 30m x 0.32 mm 0.5 µm
Oven Temp	35°C for 1 min then 10 °C/min to 350 °C for 5 min
Inlet Temp	310ºC
Air	UHP Helium
Injection Volume	1 µL
Solvent	$MeCl_2$
Auxiliary Temp	310ºC
MS Source	230ºC
MS Quad	150ºC
Solvent Delay	4 min
M/Z Scan Parameters	34 - 800

C2 - LCMS/MS Program

Table C2-1. TSS_50mm_MRM20uL-hp	
LCMS/MS	Agilent 1290 LC with QQQ 6400
Column	Phenomenex PolymerX RP-1 100Å, 5µm, 50mm x 2mm
Injection Volume	20 µL
Solvent A	DI water with 0.01% formic acid and 2mM ammonium formate
Solvent B	90:10 ACN:MeOH with 0.01% formic acid and 2mM ammonium formate
Column temperature	25ºC
Solvent Programming	Time [min] / A [%] / B [%] / Flow [mL/min] / Max. Pressure Limit [bar] 0.00 / 95.00 / 5.00 / 0.600 / 172.00 6.00 / 40.00 / 60.00 / 0.600 / 172.00 8.00 / 25.00 / 75.00 / 0.800 / 172.00 9.00 / 25.00 / 75.00 / 0.800 / 172.00 9.50 / 0.00 / 100.00 / 1.200 / 172.00 10.50 / 0.00 / 100.00 / 1.400 / 172.00 11.40 / 95.00 / 5.00 / 0.450 / 172.00 11.50 / 95.00 / 5.00 / 0.600 / 172.00
Post time	2.5 minutes
Gas temperature	200ºC
Gas flow	18 L/min
Nebulizer	40 psi
Sheath Gas Temperature	350ºC
Sheath Gas Flow	12 L/min
Fragmentor	380
Cell Acceleration Voltage	5

C3 - MRM transitions

Table C3-1. LCMS/MS MRM Transitions

Compound Name	Ret Time* (min)	Delta Ret Time	Transition 1	Collision Energy (CE) 1	Transition 2	CE 2
TSS-H 03	6.93	1.5	430.2 -> 191	20	430.2 -> 147	25
TSS-H 04	6.90	1.5	474.3 -> 191	22	474.3 -> 147	26
TSS-H 05	6.80	1.5	518.3 -> 191	25	518.3 -> 147	28
TSS-H 06	6.75	1.5	562.3 -> 191	30	562.3 -> 147	25
TSS-H 07	6.68	1.5	606.4 -> 191	30	606.4 -> 147	25
TSS-H 08	6.64	1.5	650.4 -> 191	30	650.4 -> 147	25
TSS-H 09	6.58	1.5	694.4 -> 191	30	694.4 -> 147	25
TSS-H 10	6.56	1.5	738.4 -> 191	30	738.4 -> 147	25
TSS-H 11	6.51	1.5	782.5 -> 191	30	782.5 -> 147	25
TSS-H 12	6.49	1.5	826.5 -> 191	30	826.5 -> 147	30
TSS-H 13	6.46	1.5	870.5 -> 191	30	870.5 -> 147	35
TSS-H 14	6.45	1.5	914.5 -> 191	30	914.5 -> 147	30
TSS-H 15	6.41	1.5	958.6 -> 191	30	958.6 -> 147	30
TSS-H 16	6.40	1.5	1002.6 -> 191	30	1002.6 -> 147	30
TSS-COCH$_3$ 03	8.20	1.5	472.3 -> 221	20	472.3 -> 131	20
TSS-COCH$_3$ 04	8.02	1.5	516.3 -> 221	30	516.3 -> 131	21
TSS-COCH$_3$ 05	7.88	1.5	560.3 -> 221	32	560.3 -> 131	22
TSS-COCH$_3$ 06	7.77	1.5	604.3 -> 221	35	604.3 -> 131	25
TSS-COCH$_3$ 07	7.64	1.5	648.4 -> 221	35	648.4 -> 131	27
TSS-COCH$_3$ 08	7.54	1.5	692.4 -> 221	35	692.4 -> 131	27
TSS-COCH$_3$ 09	7.44	1.5	736.4 -> 221	35	736.4 -> 131	30
TSS-COCH$_3$ 10	7.36	1.5	780.4 -> 221	30	780.4 -> 131	30
TSS-COCH$_3$ 11	7.30	1.5	824.5 -> 221	30	824.5 -> 131	30
TSS-COCH$_3$ 12	7.20	1.5	868.5 -> 221	33	868.5 -> 131	33
TSS-COCH$_3$ 13	7.14	1.5	912.5 -> 221	33	912.5 -> 131	33
TSS-COCH$_3$ 14	7.09	1.5	956.5 -> 221	35	956.5 -> 131	35
TSS-COCH$_3$ 15	7.05	1.5	1000.6 -> 221	35	1000.6 -> 131	35
TSS-CH$_3$ 03	8.08	1.5	444.3 -> 147	15	444.3 -> 103	25
TSS-CH$_3$ 04	7.88	1.5	488.3 -> 147	20	488.3 -> 103	23
TSS-CH$_3$ 05	7.73	1.5	532.3 -> 147	21	532.3 -> 103	25
TSS-CH$_3$ 06	7.58	1.5	576.3 -> 147	22	576.3 -> 103	27
TSS-CH$_3$ 07	7.46	1.5	620.4 -> 147	24	620.4 -> 103	28
TSS-CH$_3$ 08	7.35	1.5	664.4 -> 147	26	664.4 -> 103	29
TSS-CH$_3$ 09	7.26	1.5	708.4 -> 147	29	708.4 -> 103	30
TSS-CH$_3$ 10	7.18	1.5	752.4 -> 147	30	752.4 -> 103	30
TSS-CH$_3$ 11	7.09	1.5	796.5 -> 147	30	796.5 -> 103	30
TSS-CH$_3$ 12	7.01	1.5	840.5 -> 147	30	840.5 -> 103	30
TSS-CH$_3$ 13	6.98	1.5	884.5 -> 147	35	884.5 -> 103	25
TSS-CH$_3$ 14	6.93	1.5	928.6 -> 147	35	928.6 -> 103	30
TSS-CH$_3$ 15	6.88	1.5	972.6 -> 147	35	972.6 -> 103	35
Carbamazepine	3.80	0.7	237 -> 193.9	15	237 -> 179	35
Fluoxetine	4.26	0.7	310.1 -> 148.1	5	-	-
Sulfamethoxazole	3.10	0.7	254 -> 155.9	10	254 -> 92	30

Table C3-1 (cont). LCMS/MS MRM Transitions

Compound Name	Ret Time* (min)	Delta Ret Time	Transition 1	Collision Energy (CE) 1	Transition 2	CE 2
3-Hydroxycarbofuran	2.39	1.0	255.2 -> 181	15	255.2 -> 163	18
Acephate	1.20	1.0	184.1 -> 143	12	184.1 -> 125	16
Acetamiprid	2.96	1.0	223 -> 126	16	223 -> 56	16
Aldicarb sulfoxide	1.20	1.0	207 -> 132	10	207 -> 89	14
Atrazine	4.48	1.0	216 -> 174	16	216 -> 68.1	36
Azinphos methyl	6.55	1.0	317.7 -> 131.9	30	317.7 -> 124.7	35
Azoxystrobin	6.15	1.0	404.1 -> 372.1	14	404.1 -> 344.2	26
Bendiocarb	4.28	1.0	224.1 -> 167	4	224.1 -> 109	12
Bentazone	3.18	1.0	241.1 -> 198.9	8	241.1 -> 80	56
Bifenthrin	9.00	1.0	440.2 -> 181.2	20	440.2 -> 166.2	52
Boscalid	6.44	1.0	342.8 -> 306.7	19	342.8 -> 271.3	33
Carbaryl	4.95	1.0	202.2 -> 145	15	202.2 -> 127	28
Carbofuran	4.23	1.0	222.2 -> 165	13	222.2 -> 123	23
Chlorfluazuron	8.28	1.0	540 -> 382.9	28	540 -> 158	24
Chlorpyrifos	8.59	1.0	349.9 -> 197.8	28	349.9 -> 97	40
Chlothianidin	2.56	1.0	250.1 -> 168.6	15	250.1 -> 132.1	29
Coumaphos O	5.28	1.0	347 -> 291	22	347 -> 211	34
Coumaphos S	7.68	1.0	363 -> 307	16	363 -> 227	24
Cyazofamid	6.89	1.0	325.1 -> 108.1	20	325.1 -> 44.1	36
Cyprodinil	7.25	1.0	226.1 -> 93	36	226.1 -> 77.1	56
Cyromazine	1.20	1.0	167.1 -> 85	16	167.1 -> 68	40
Demeton-S	4.89	1.0	259.1 -> 89	4	259.1 -> 61	44
Diazinon	7.09	1.0	305.1 -> 169.1	18	305.1 -> 153.1	22
Dichlorvos	3.69	1.0	220.7 -> 144.8	11	220.7 -> 108.9	19
Diflubenzuron 1	6.55	1.0	311 -> 158.2	15	311 -> 141.1	32
Diflubenzuron 2	4.84	1.0	311 -> 158.2	15	311 -> 141.1	32
Dimethoate	2.79	1.0	230 -> 199	10	230 -> 125	20
Dioxacarb	2.81	1.0	224.1 -> 167.1	12	224.1 -> 123.1	20
Diuron	4.82	1.0	233 -> 160	28	233 -> 72.1	15
Disulfoton	7.88	1.0	275.1 -> 89	8	275.1 -> 61	40
Ethoprofos	5.75	1.0	243.1 -> 131.1	20	243.1 -> 97.1	32
Etofenprox	9.00	1.0	394.2 -> 177.1	16	394.2 -> 107	44
Fenazaquin	8.66	1.0	307.2 -> 161.1	14	307.2 -> 57.1	28
Fenchlorphos oxon	7.02	1.0	306.9 -> 109.1	20	306.9 -> 109	20
Fenpropathrin	8.61	1.0	350.2 -> 125.1	12	350.2 -> 55.1	44
Fenpyroximate	8.73	1.0	422.2 -> 366.1	16	422.2 -> 135.1	36
Fensulfothion	4.89	1.0	309 -> 173	24	309 -> 157	28
Fenthion	7.73	1.0	279 -> 169	16	279 -> 105.2	24
Flonicamid	1.99	1.0	230.1 -> 203.1	16	230.1 -> 174.1	16
Flubendiamide	6.52	1.0	683 -> 408	8	683 -> 273.9	40
Fludioxinil	5.74	1.0	266.1 -> 229.1	8	266.1 -> 158.1	36
Flufenoxuron	8.11	1.0	489.1 -> 158.1	16	489.1 -> 141.1	56
Flutolanil	6.31	1.0	324.1 -> 262	20	324.1 -> 242	28
Formetanate HCl	1.20	1.0	222.1 -> 165.1	12	222.1 -> 46.2	28
Hexythiazox	8.98	1.0	353 -> 228.1	14	353 -> 168.1	26

Table C3-1 (cont). LCMS/MS MRM Transitions

Compound Name	Ret Time* (min)	Delta Ret Time	Transition 1	Collision Energy (CE) 1	Transition 2	CE 2
Imazalil	4.03	1.0	297 -> 255	20	297 -> 159	36
Imidacloprid	2.91	1.0	256.1 -> 209	14	256.1 -> 175	18
Iprodione	6.18	1.0	330 -> 246.9	16	330 -> 244.9	16
Malathion I	4.60	1.0	330.7 -> 327.1	7	330.7 -> 126.8	11
Malathion II	6.53	1.0	330.7 -> 284.7	7	330.7 -> 126.8	11
Mandipropamid	6.25	1.0	412.1 -> 328.1	16	412.1 -> 125.1	44
Metaflumizone	7.70	1.0	507.1 -> 178	36	507.1 -> 116	52
Metalaxyl	4.23	1.0	280.1 -> 220.1	13	280.1 -> 192.2	18
Methamidophos	4.78	1.0	142 -> 125	13	142 -> 94	14
Methomyl	1.79	1.0	163.1 -> 106	9	163.1 -> 88	9
Methoxyfenozide	6.11	1.0	369.1 -> 149.2	18	369.1 -> 91.1	47
Mevinphos	1.40	1.0	225 -> 193.1	8	225 -> 127.1	20
Monocrotophos	1.51	1.0	224.1 -> 127.1	16	224.1 -> 98.1	12
Myclobutanil	5.35	1.0	289.1 -> 125.1	30	289.1 -> 70.1	18
Naled	5.15	1.0	382.8 -> 127	12	380.8 -> 126.9	12
Novaluron	7.23	1.0	493 -> 158	20	493 -> 141	52
Omethoate	1.20	1.0	214 -> 183	12	214 -> 155	14
Oxamyl	1.63	1.0	237.1 -> 90.1	12	237.1 -> 72.1	12
Paclobutrazol	5.13	1.0	294.1 -> 125.1	48	294.1 -> 70.1	16
Phosmet I	4.43	1.0	318 -> 159.9	16	318 -> 133	40
Phosmet II	6.50	1.0	318 -> 159.9	16	318 -> 133	40
Phoxim	7.73	1.0	299.1 -> 129.1	16	299.1 -> 77.1	32
Piperonyl butoxide	7.75	1.0	356 -> 176.8	13	356 -> 118.9	37
Prochloraz	6.48	1.0	376 -> 307.9	12	376 -> 70.1	20
Propamocarb	1.20	1.0	189.2 -> 102	16	189.2 -> 74	24
Propiconazole	6.46	1.0	341.8 -> 158.8	27	341.8 -> 68.9	21
Pyraclostrobin	7.85	1.0	387.8 -> 194.1	10	387.8 -> 163.7	12
Pyrimethanil	6.07	1.0	200.1 -> 107.2	24	200.1 -> 82	28
Rotenone	7.18	1.0	395.2 -> 213	24	395.2 -> 192	20
Spinesad - A	5.54	1.0	732.5 -> 142	32	732.5 -> 98.3	52
Spinesad - D	5.78	1.0	746.5 -> 142.1	32	746.5 -> 98.4	60
Spinetoram - Spinosyn J	6.09	1.0	748.5 -> 142.1	36	748.5 -> 98.1	72
Spinetoram - Spinosyn L	6.22	1.0	760.5 -> 142.1	36	760.5 -> 98.1	72
Spirotetramat	5.22	1.0	374.2 -> 302.2	20	374.2 -> 216.1	40
Tebufenozide	6.40	1.0	353.1 -> 133.1	22	353.1 -> 105	50
Teflubenzuron	7.33	1.0	381 -> 158	16	381 -> 141	48
Tetrachlorvinphos	6.55	1.0	366.5 -> 240.6	17	366.5 -> 126.7	17
Thiabendazole	2.72	1.0	202.1 -> 175	24	202.1 -> 131	33
Thiacloprid	3.69	1.0	253 -> 126.1	24	253 -> 90.1	48
Tolyfluanid	7.48	1.0	347 -> 237.9	8	347 -> 137	36
Tribufos	8.95	1.0	315.1 -> 169	14	315.1 -> 57.1	32
HOH1	2.00	0.5	594 -> 540	15	594 -> 147	27
HOH2	2.00	0.5	550 -> 497	14	550 -> 147	26
HOH3	2.00	0.5	506 -> 453	12	506 -> 147	23

Table C3-1 (cont). LCMS/MS MRM Transitions

Compound Name	Ret Time* (min)	Delta Ret Time	Transition 1	Collision Energy (CE) 1	Transition 2	CE 2
HOH4	2.00	0.5	462 -> 409	10	462 -> 147	20
HOH5	2.00	0.5	418 -> 365	8	418 -> 147	20
HCH-A1	2.30	1.0	388 -> 147	16	388 -> 103	18
HCH-A2	2.30	1.0	432 -> 147	18	432 -> 103	22
HCH-A3	2.30	1.0	476 -> 147	20	476 -> 103	25
HCH-A4	2.30	1.0	520 -> 147	23	520 -> 103	26
HCH-A5	2.30	1.0	564 -> 147	25	564 -> 103	27
HCH-A6	2.30	1.0	608 -> 147	25	608 -> 103	28
HCH-A7	2.30	1.0	652 -> 147	25	652 -> 103	30
HCH-B1	3.25	0.6	606 -> 147	23	606 -> 103	30
HCH-B2	3.25	0.6	650 -> 147	24	650 -> 103	30
HCH-B3	3.25	0.6	694 -> 147	25	694 -> 103	30
HCH-B4	3.25	0.6	738 -> 147	27	738 -> 103	30
HCH-B5	3.25	0.6	782 -> 147	30	782 -> 103	30
Q01	7.47	1.0	480.3 -> 147	20	480.3 -> 103	22
Q02	7.33	1.0	524.1 -> 147	22	524.1 -> 103	24
Q03	7.22	1.0	568.4 -> 147	24	568.4 -> 103	26
Q04	7.11	1.0	612.4 -> 147	26	612.4 -> 103	28
Q05	7.04	1.0	656.4 -> 147	27	656.4 -> 103	28
Q06	6.95	1.0	700.4 -> 147	29	700.4 -> 103	29
Q07	6.89	1.0	744.3 -> 147	31	744.3 -> 103	30
C01	4.96	0.6	488.2 -> 219	20	488.2 -> 131	22
C02	4.92	0.6	532.2 -> 219	16	532.2 -> 131	24
C03	4.91	0.6	576.3 -> 219	20	576.3 -> 131	24
C04	4.90	0.6	620.3 -> 219	20	620.3 -> 131	25
C05	4.87	0.6	664.2 -> 219	23	664.2 -> 131	26
C06	4.86	0.6	708.2 -> 219	25	708.2 -> 131	28
C07	4.86	0.6	752.3 -> 219	25	752.3 -> 131	28
C08	4.86	0.6	796.4 -> 219	26	796.4 -> 131	29
C09	4.83	0.6	840.2 -> 219	27	840.2 -> 131	30
C10	4.85	0.6	884.1 -> 219	26	884.1 -> 131	30
E01	4.71	0.6	416.3 -> 147	20	416.3 -> 103	20
E02	4.68	0.6	460.2 -> 147	20	460.2 -> 103	21
E03	4.67	0.6	504.2 -> 147	20	504.2 -> 103	22
E04	4.65	0.6	548.2 -> 147	22	548.2 -> 103	24
E05	4.63	0.6	592.2 -> 147	25	592.2 -> 103	27
E06	4.62	0.6	636.2 -> 147	25	636.2 -> 103	28
E07	4.64	0.6	680.3 -> 147	25	680.3 -> 103	30
E08	4.63	0.6	724.3 -> 147	25	724.3 -> 103	30
E09	4.64	0.6	768.3 -> 147	26	768.3 -> 103	30
E10	4.63	0.6	812.4 -> 147	29	812.4 -> 103	32
E11	4.65	0.6	856.3 -> 147	32	856.3 -> 103	35
E12	4.65	0.6	900.5 -> 147	35	900.5 -> 103	35
N0	4.05	0.6	490 -> 365	15	490 -> 147	15
N01	4.09	0.6	534.1 -> 409.1	15	534.1 -> 147	15
N0-1	4.03	0.6	446 -> 321	15	446 -> 147	15

Table C3-1 (cont). LCMS/MS MRM Transitions

Compound Name	Ret Time* (min)	Delta Ret Time	Transition 1	Collision Energy (CE) 1	Transition 2	CE 2
N02	4.12	0.6	578.2 -> 453.2	16	578.2 -> 147	15
N03	4.16	0.6	622.3 -> 497.3	17	622.3 -> 147	20
N04	4.18	0.6	666.2 -> 541.2	18	666.2 -> 147	20
N05	4.20	0.6	710 -> 585	19	710 -> 147	25
N06	4.23	0.6	754.2 -> 629.2	20	754.2 -> 147	25
N07	4.25	0.6	798.4 -> 673.2	20	798.4 -> 147	25
N08	4.27	0.6	842.4 -> 717.2	20	842.4 -> 147	25
B01	3.80	0.6	294.1 -> 219	15	294.1 -> 131	15
B02	3.82	0.6	338.2 -> 219	15	338.2 -> 131	15
B03	3.82	0.6	382.2 -> 219	15	382.2 -> 131	15
B04	3.83	0.6	426.3 -> 219	17	426.3 -> 131	15
B05	3.86	0.6	470.2 -> 219	20	470.2 -> 131	15
B06	3.86	0.6	514.1 -> 219	20	514.1 -> 131	18
B07	3.89	0.6	558.2 -> 219	20	558.2 -> 131	21
B08	3.92	0.6	602.3 -> 219	21	602.3 -> 131	23
B09	3.92	0.6	646.2 -> 219	22	646.2 -> 131	25
B10	3.94	0.6	690.2 -> 219	24	690.2 -> 131	30
B11	3.97	0.6	734.4 -> 219	24	734.4 -> 131	27
B12	3.99	0.6	778.4 -> 219	25	778.4 -> 131	27
R01	3.72	0.6	500.1 -> 147	25	500.1 -> 103	25
R02	3.74	0.6	544.5 -> 147	25	544.5 -> 103	27
R03	3.78	0.6	588.4 -> 147	26	588.4 -> 103	28
R04	3.80	0.6	632.4 -> 147	27	632.4 -> 103	29
R05	3.79	0.6	676.5 -> 147	27	676.5 -> 103	30
R06	3.82	0.6	720.3 -> 147	28	720.3 -> 103	31
R07	3.86	0.6	764.3 -> 147	29	764.3 -> 103	32
R08	3.87	0.6	808.5 -> 147	30	808.5 -> 103	33
R09	3.89	0.6	852.5 -> 147	31	852.5 -> 103	35
J01	3.30	0.6	266.1 -> 147	10	266.1 -> 103	15
J02	3.36	0.6	310.2 -> 147	10	310.2 -> 103	15
J03	3.40	0.6	354.2 -> 147	10	354.2 -> 103	16
J04	3.44	0.6	398.2 -> 147	10	398.2 -> 103	17
J05	3.48	0.6	442.3 -> 147	12	442.3 -> 103	18
J06	3.52	0.6	486.3 -> 147	15	486.3 -> 103	20
J07	3.52	0.6	530.2 -> 147	17	530.2 -> 103	22
J08	3.60	0.6	574.1 -> 147	20	574.1 -> 103	25
J09	3.64	0.6	618.3 -> 147	22	618.3 -> 103	26
J10	3.67	0.6	662.3 -> 147	25	662.3 -> 103	28
J11	3.74	0.6	706.3 -> 147	30	706.3 -> 103	30
A01	3.21	0.6	296.2 -> 175	10	296.2 -> 131	14
A02	3.26	0.6	340.2 -> 175	10	340.2 -> 131	15
A03	3.32	0.6	384 -> 175	12	384 -> 131	17
A04	3.34	0.6	428.2 -> 175	15	428.2 -> 131	19
A05	3.40	0.6	472.2 -> 175	17	472.2 -> 131	20
A06	3.45	0.6	516.1 -> 175	20	516.1 -> 131	22
A07	3.49	0.6	560.1 -> 175	21	560.1 -> 131	23

Table C3-1 (cont). LCMS/MS MRM Transitions

Compound Name	Ret Time* (min)	Delta Ret Time	Transition 1	Collision Energy (CE) 1	Transition 2	CE 2
A08	3.55	0.6	604.2 -> 175	23	604.2 -> 131	25
A09	3.59	0.6	648.1 -> 175	24	648.1 -> 131	25
A10	3.60	0.6	692 -> 175	25	692 -> 131	25
A11	3.70	1.0	736.2 -> 175	27	736.2 -> 131	25
A12	3.64	0.6	780.1 -> 175	30	780.1 -> 131	25
O01	3.05	1.0	294.1 -> 219	10	294.1 -> 129	15
O02	3.10	1.0	338.1 -> 219	10	338.1 -> 129	15
O03	3.14	1.0	382.2 -> 219	12	382.2 -> 129	16
O04	3.22	1.0	426.1 -> 219	15	426.1 -> 129	18
O05	3.27	1.0	470.1 -> 219	16	470.1 -> 129	19
O06	3.33	1.0	514.1 -> 219	18	514.1 -> 129	20
O07	3.38	1.0	558.1 -> 219	19	558.1 -> 129	22
O08	3.44	1.0	602.3 -> 219	20	602.3 -> 129	25
O09	3.48	1.0	646.3 -> 219	21	646.3 -> 129	25
O10	3.52	1.0	690.4 -> 219	23	690.4 -> 129	27
O11	3.54	1.0	734.2 -> 219	25	734.2 -> 129	30
O12	3.57	1.0	778.1 -> 219	25	778.1 -> 129	30
H01	2.85	1.0	268.1 -> 147	10	268.1 -> 103	15
H02	2.90	1.0	312.2 -> 147	12	312.2 -> 103	15
H03	3.00	1.0	356.2 -> 147	14	356.2 -> 103	17
H04	3.10	1.0	400.2 -> 147	15	400.2 -> 103	20
H05	3.25	1.0	444.3 -> 147	18	444.3 -> 103	22
H06	3.25	1.0	488.3 -> 147	19	488.3 -> 103	23
H07	3.30	1.0	532.3 -> 147	20	532.3 -> 103	25
H08	3.40	1.0	576.3 -> 147	23	576.3 -> 103	25
H09	3.45	1.0	620.4 -> 147	25	620.4 -> 103	25
H10	3.50	1.0	664.4 -> 147	25	664.4 -> 103	30
H11	3.50	1.0	708 -> 147	25	708 -> 103	30
JJ0	2.48	0.6	222 -> 147	10	222 -> 103	10
JJ01	2.56	0.6	266.1 -> 147	10	266.1 -> 103	10
JJ02	2.67	0.6	310.2 -> 147	15	310.2 -> 103	15
JJ03	2.76	0.6	354.2 -> 147	15	354.2 -> 103	15
JJ04	2.86	0.6	398.2 -> 147	16	398.2 -> 103	18
JJ05	2.95	0.6	442.2 -> 147	17	442.2 -> 103	20
JJ06	3.01	0.6	486.2 -> 147	18	486.2 -> 103	22
JJ07	3.08	0.6	530.3 -> 147	20	530.3 -> 103	25
JJ08	3.13	0.6	574.3 -> 147	22	574.3 -> 103	26
JJ09	3.17	0.6	618.3 -> 147	25	618.3 -> 103	27
JJ10	3.24	0.6	662.4 -> 147	27	662.4 -> 103	28
JJ11	3.31	0.6	706.4 -> 147	30	706.4 -> 103	30
JJ12	3.35	0.6	750.4 -> 147	30	750.4 -> 103	30
JJ13	3.39	0.6	794.3 -> 147	32	794.3 -> 103	32
M0	2.52	0.6	296 -> 177	15	296 -> 133	15
M01	2.66	0.6	340.1 -> 177	20	340.1 -> 133	20
M0-1	2.34	0.6	252 -> 177	15	252 -> 133	15
M02	2.81	0.6	384.2 -> 177	15	384.2 -> 133	15

Table C3-1 (cont). LCMS/MS MRM Transitions

Compound Name	Ret Time* (min)	Delta Ret Time	Transition 1	Collision Energy (CE) 1	Transition 2	CE 2
M03	2.89	0.6	428.2 -> 177	15	428.2 -> 133	15
M04	2.99	0.6	472.1 -> 177	16	472.1 -> 133	16
M05	3.06	0.6	516.3 -> 177	17	516.3 -> 133	17
M06	3.13	0.6	560.2 -> 177	20	560.2 -> 133	20
M07	3.20	0.6	604.3 -> 177	20	604.3 -> 133	20
M08	3.27	0.6	648 -> 177	20	648 -> 133	20
M09	3.23	0.6	692.2 -> 177	22	692.2 -> 133	22
M10	3.36	0.6	736.4 -> 177	25	736.4 -> 133	25
L0	2.40	0.6	460 -> 147	20	460 -> 103	25
L01	2.46	0.6	504.3 -> 147	20	504.3 -> 103	25
L02	2.57	0.6	548.3 -> 147	20	548.3 -> 103	25
L03	2.66	0.6	592.3 -> 147	25	592.3 -> 103	29
L04	2.73	0.6	636.3 -> 147	26	636.3 -> 103	30
L05	2.80	0.6	680.4 -> 147	28	680.4 -> 103	32
L06	2.85	0.6	724.4 -> 147	29	724.4 -> 103	32
L07	2.94	0.6	768.5 -> 147	30	768.5 -> 103	33
L08	2.98	0.6	812.4 -> 147	30	812.4 -> 103	30
L09	3.03	0.6	856.5 -> 147	30	856.5 -> 103	30
T0	1.83	0.6	252 -> 133	10	252 -> 129	10
T01	2.03	0.6	296.1 -> 133	15	296.1 -> 129	15
T02	2.19	0.6	340.2 -> 133	15	340.2 -> 129	15
T03	2.32	0.6	384.3 -> 133	19	384.3 -> 129	17
T04	2.45	0.6	428.2 -> 133	21	428.2 -> 129	18
T05	2.56	0.6	472.2 -> 133	26	472.2 -> 129	20
T06	2.66	0.6	516.2 -> 133	27	516.2 -> 129	21
T07	2.74	0.6	560.2 -> 133	28	560.2 -> 129	22
T08	2.80	0.6	604.1 -> 133	29	604.1 -> 129	23
T09	2.87	0.6	648.2 -> 133	30	648.2 -> 129	25
T10	2.93	0.6	692 -> 133	30	692 -> 129	25
I01	2.12	1.0	298.2 -> 147	15	298.2 -> 103	15
I02	2.42	1.2	342.2 -> 147	15	342.2 -> 103	18
I03	2.58	1.2	386.2 -> 147	16	386.2 -> 103	19
I04	2.60	1.2	430.2 -> 147	18	430.2 -> 103	21
I05	2.70	1.0	474.3 -> 147	20	474.3 -> 103	24
I06	2.82	1.0	518.2 -> 147	20	518.2 -> 103	25
I07	2.90	1.0	562.2 -> 147	20	562.2 -> 103	25
D01	1.64	0.6	298.1 -> 219	10	298.1 -> 133	15
D02	1.81	0.6	342.2 -> 219	13	342.2 -> 133	16
D03	1.97	0.6	386.2 -> 219	15	386.2 -> 133	17
D04	2.10	0.6	430.2 -> 219	17	430.2 -> 133	18
D05	2.23	0.6	474.2 -> 219	18	474.2 -> 133	19
D06	2.33	0.6	518.2 -> 219	20	518.2 -> 133	21
D07	2.43	0.6	562.2 -> 219	21	562.2 -> 133	23
D08	2.52	0.6	606.2 -> 219	23	606.2 -> 133	25
D09	2.59	0.6	650.3 -> 219	24	650.3 -> 133	26
D10	2.66	0.6	694.1 -> 219	25	694.1 -> 133	25

Table C3-1 (cont). LCMS/MS MRM Transitions

Compound Name	Ret Time* (min)	Delta Ret Time	Transition 1	Collision Energy (CE) 1	Transition 2	CE 2
D11	2.75	0.6	738.3 -> 219	25	738.3 -> 133	25
G0	1.35	0.6	270 -> 133	17	270 -> 103	20
G0_5	1.52	0.6	314.1 -> 133	18	314.1 -> 103	20
G01	1.69	0.6	358.3 -> 133	18	358.3 -> 103	20
G02	1.85	0.6	402.2 -> 133	19	402.2 -> 103	21
G03	1.97	0.6	446.2 -> 133	15	446.2 -> 103	20
G04	2.09	0.6	490.2 -> 133	22	490.2 -> 103	26
G05	2.20	0.6	534.3 -> 133	20	534.3 -> 103	27
G06	2.29	0.6	578.3 -> 133	23	578.3 -> 103	27
G07	2.38	0.6	622.3 -> 133	27	622.3 -> 103	35
Caffeine**	1.56	0.7	195.1 -> 138.1	20	195.1 -> 109.9	20

- Gemfibrozil, Trichlosan, and Dinotefuran were added wrongly to method and not used
- All transitions listed were positive polarity
- B & O transitions 1 combined, J & JJ transitions 1 & 2 combined, and M transitions 2 & T transition 1 combined for long term study analysis method
- Most pesticide transitions from Mastovska et al. 2017

*Retention time for TSS_50mm_MRM20uL-hp method
**MRM added later methods

C4 - Nutrient Solution

Nutrient solutions were used in order to grow soybeans and tomatoes hydroponically. Initially, a lower pH solution based off of Orita (2012) paper was used. A newer nutrient solution based off of the recommendations from USU Research Greenhouse was used for later experiments and changed to pH 7 in order to reduce hydrolysis rates.

Table C4-1. Nutrient Solution Concentrations

	Orita 2012		Research Greenhouse	
	Nutrient Solution Starter	Nutrient Solution Vegetative	Nutrient Solution Starter	Nutrient Solution Vegetative
	mM	mM	mM	mM
1M $Ca(NO_3)_2$	1	2	1	1
2M KNO_3	1	3	1	4
0.2M KH_2PO_4	0.5	0.125	0.2	0.4
0.5M $MgSO_4$	0.5	1.5	0.05	1

Table C4-1 (cont). Nutrient Solution Concentrations

	Orita 2012		Research Greenhouse	
	Nutrient Solution Starter	**Nutrient Solution Vegetative**	**Nutrient Solution Starter**	**Nutrient Solution Vegetative**
	µM	**µM**	**µM**	**µM**
50mM $FeCl_3$	5	1.5	5	2.5
25mM EDDHA	40	10	20	5*
20mM $MnCl_2$	6	9	2	2
30mM $ZnCl_2$	6	4	3	3
400mM H_3BO_4	4	40	40	40
40mM $CuCl_2$	4	4	2	2
1mM $NaMoO_4$	0.1	0.1	0.1	0.1
pH adjustment (nitric acid)	5.6	5.6-5.7	5.6 or 7.1	5.6 or 7.1

*Iron EDDHA sometimes doubled at neutral pH to allow for more availability

APPENDIX D

ROOT SORPTION STUDY

Materials and Methods

Sorption of TSSs to plant roots is important for understanding plant availability and uptake. Two different compound mixtures - a solution of 100 ng/mL caffeine and a solution of 100 ng/mL caffeine with 100 ng/mL TSS-H - were made in pH 5.6 Research Greenhouse Nutrient Solution (Appendix C4), as well as DI water. Caffeine was added as a reference compound as it is more stable compound with some sorption data for it. Forty mL of either one of the spiked nutrient solution or blank nutrient solution was then added to 50mL polypropylene centrifuge tubes containing one of four different Soybean root weights - 0, 1, 2, and 4 grams - making a total of 12 samples in nutrient solution. An additional 3 controls were then made by adding 40 mL of the two DI solutions, as well as blank DI water to 50mL polypropylene centrifuge tubes. An initial 100 µL aliquot was taken out and placed in the freezer, then the samples were shaken using an Eberbach table top shaker and shaken on low continuously, except when aliquots were taken out. Four aliquots were taken over the course of three days and placed in the freezer until analysis. Analysis was performed using LCMS/MS and a method similar to TSS_50mm_MRM20uL-hp (Appendix C2), except with caffeine MRM added.

Concentrations were determined using a linear calibration curve between 0.5 ng/mL (or 5 ng/mL for some oligomers) and 120 ng/mL. Class sums were obtained by adding the area of each oligomer and calculating concentrations from a linear calibration curve. Sorption density (q) is calculated using Equation D-1, where C_o is the initial

concentration, C_e is the concentration at equilibrium, V is volume of solution, and m is mass of sorbent. An RCF can then be determined by relating the concentration of chemicals on the roots to the concentration in the solution (Equation D-2).

Equation D-1: $q = \frac{(C_0 - C_e)V}{m}$

Equation D-2: RCF$= \frac{q}{C_e}$

Results

Fifty ng/mL mix of the three TSS standards and caffeine in both ACN and nutrient solution were run as CCVs, and stability of CCVs throughout the run was highly dependent on compound. The TSS-H concentrations in the nutrient solution decreased by about 70% for some CCVs, but their concentrations in ACN drifted less than 15%. This shows a great instability in analysis for TSS compounds in pH 5.6 nutrient solution, even with a relative stability in ACN. Caffeine, on the other hand, stayed constant until about halfway through the run in which it suddenly decreased by about 40-50% in both nutrient solution and ACN, and stayed constant for the rest of the run. Several impurities stayed more constant throughout the run with deviations less than ± 20-25% in either nutrient solution or water, while other CCVs, like hydrolysis products, had deviations in the 40-50% range. Though there were sudden drifts in the CCVs, samples were not corrected for it, RCF is a ratio between samples collected around the same time, which were run on the LCMS/MS together, and thus should be relatively comparable to each other, though overall concentrations could be wrong (as in Figure D-1). Blanks containing no TSS or caffeine but containing 0, 1, 2 or 4 g roots were subtracted from samples containing the same weight of roots.

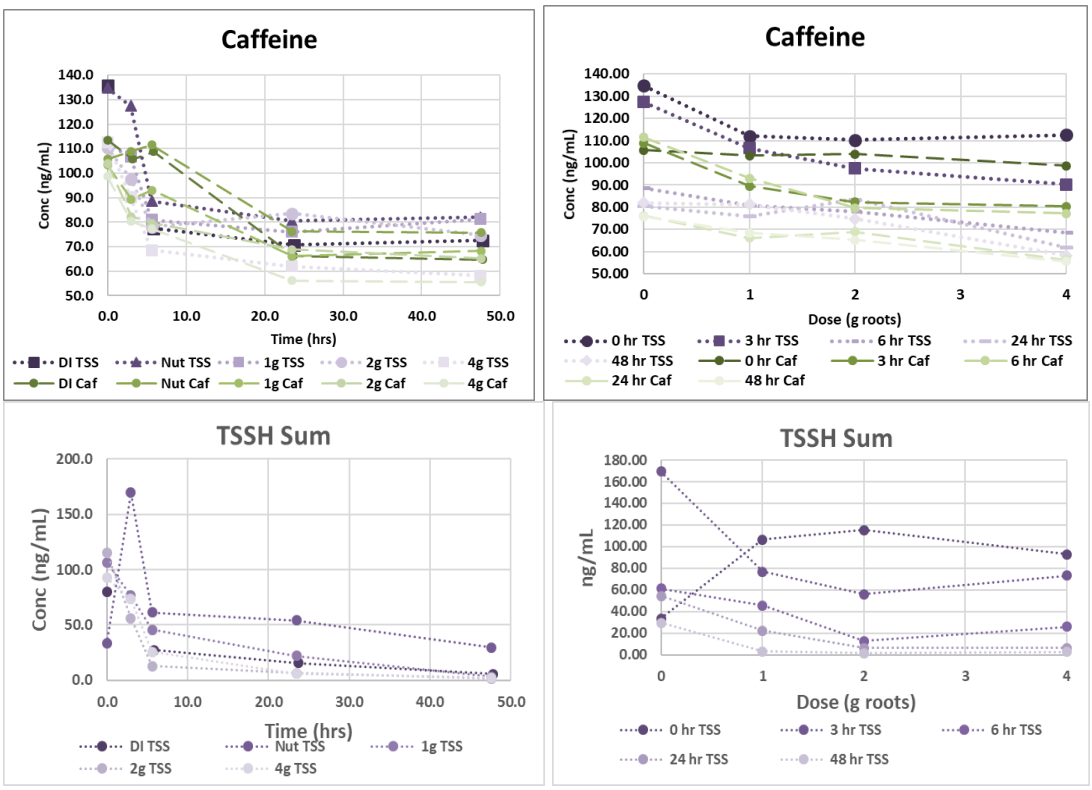

Figure D-1. Concentration of caffeine or TSS-H by time or dose

Equilibrium was assumed to be reached after 48 hrs (Figure D-1). Sorption density (q) was calculated using Equation D-1, where C_o was the concentration of TSSs in the nutrient solution sample containing no roots, C_e was the concentration of TSSs at 48 hrs in the samples containing root weights of 1, 2 and 4 g. With the use of C_o as the concentration of TSSs in nutrient solution which had been shaken for 48 hrs, the effects of hydrolysis or sorption to the centrifuge tube was accounted for. The results for q at the three different root weights are listed in Table D-1, along with the partition coefficients with those doses as well. The average and standard deviations for the RCF were also determined and listed in Table D-1.

The log RCF for caffeine alone was slightly lower at 0.6 ± 0.2 than the EPI Suite estimate of 0.98 log Koc (ratio between organic matter and solution) (Appendix B4). The

addition of TSSs lowered the average RCF, but increased the variability of RCF based on root amount. This is probably due to TSSs being a surfactant, which negates some of the hydrophobicity between non-polar compounds and water. An interesting note is the dependence of RCF and dose of roots in the solution, where for the sum of TSS-H oligomers, more roots in solution meant a lower RCF. As RCF is a ratio between the roots and the nutrient solution, a higher RCF indicates that the equilibrium concentration in the roots will be higher than the concentration in the nutrient solution. Since the log RCF for TSS-H is 2.4 ± 2.5, this indicates that the surfactant prefers to sorb to the roots, but has a large variability of how much it sorbs based on the ratio of root weight to analyte concentration due to the RCF at 4 g root being much lower than the RCF at 1 and 2 g roots. This suggests that equilibrium wasn't reached in the 4 g root samples.

Table D-1. Root Sorption Densities and Root Concentration Factors (q and RCF) (µg/g and L/kg)

Root Dose (g)		1.0	2.0	4.0	avg RCF	± 95% CI (n=3)	Log RCF	± 95% CI (n=3)
Caf only	q	0.3	0.2	0.2	-	-	-	-
	RCF	4.4	3.3	3.7	3.8	1.4	0.6	0.2
Caf w/TSS	q	0.0	0.1	0.2	-	-	-	-
	RCF	0.3	1.9	4.1	2.1	4.7	0.3	0.7
TSS-H Sum	q	1.1	0.6	0.3	-	-	-	-
	RCF	319.7	331.9	106.5	252.7	314.7	2.4	2.5

RCF values were also highly dependent on oligomer and root dose (Figure D-2). Expectations were that oligomers with longer chains should have higher RCF values, as shown for TSS-CH_3 in the literature (Michel 2016). However, the log RCF decreased as the oligomer chain grew longer for oligomers for samples with higher root doses, meaning that oligomers with longer EO chains are less likely to sorb to roots than shorter

oligomers with a shorter EO chain. It should be noted that for the lower oligomers (EO 3-5), the RCF and q were positively correlated with root dose, while the higher oligomers (EO 7-15) had the opposite trend with RCF and q negatively correlated with root dose.

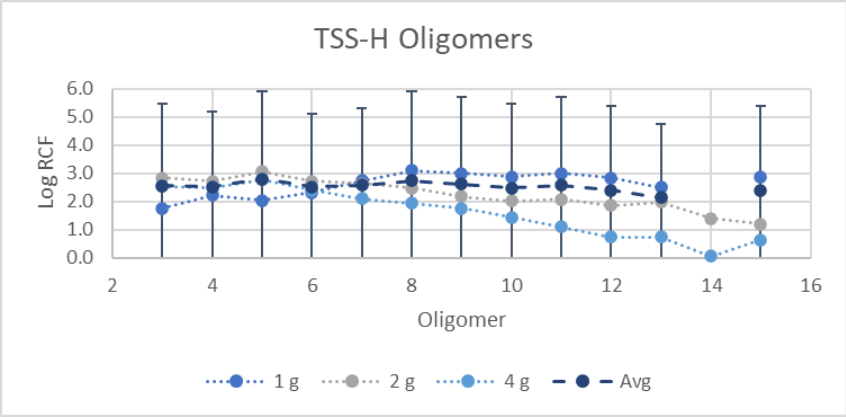

Figure D-2. TSS-H Oligomer vs Log RCF
(± 95% CI, n=3)