

INFLUENCE OF NEONICOTINOID SEED TREATMENT ON TWO CO-OCCURRING  
ARTHROPOD PESTS, TWO-SPOTTED SPIDER MITE (*TETRANYCHUS URTICAE*) AND  
WESTERN FLOWER THRIPS (*FRANKLINIELLA OCCIDENTALIS*)

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**Title**

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**MASTER OF SCIENCE**

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## ABSTRACT

Insecticidal neonicotinoid seed treatments in agriculture is a common insect pest management strategy. Seed treatments have systemic and residual toxicity, which are effective against target insect pests. However, effects on other arthropod pests is less straightforward. We evaluated the effects of a neonicotinoid seed treatment, thiamethoxam, on two soybean pests, herbivorous two-spotted spider mites (*Tetranychus urticae* Koch) and an omnivore and facultative predator of spider mite eggs, western flower thrips (*Frankliniella occidentalis* Pergande). We used greenhouse and laboratory experiments to evaluate thiamethoxam soybean seed treatments on spider mite densities and thrips omnivorous feeding behavior. Our results suggest seed treatment may encourage spider mite reproduction and influence thrips herbivory over egg predation when both resources are available. Implications of this study highlight the use of thiamethoxam soybean seed treatment as a potential contributor to increased spider mite populations, thrips reduced role as spider mite predator, and increased role as herbivorous pest.

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## CHAPTER 1. LITERATURE REVIEW

### 1.1. General properties of neonicotinoid insecticides

Use of agrochemicals against arthropod crop pests is a common practice to minimize economic losses, and scientists are continually looking for new chemistries to improve management of arthropod pests. Neonicotinoids are a newer class of insecticides that were developed, in part, because of limitations of older chemistries, such as poor application versatility, target pest specificity, and limited residual activity (Elbert et al. 2008, Simon-Delso et al. 2015). In addition, several studies have indicated older insecticides negatively affected natural insect communities including beneficial insects such as predators and pollinators within immediate and surrounding areas (Desneux et al. 2007). Furthermore, increased incidences of insecticide resistance and insensitivity presented agricultural producers with ongoing problems associated with pest management through insecticide use (Nauen and Denholm 2005). Recognition of the limitations of older insecticides encouraged agrochemical companies to develop that would be compatible and sustainable within an agricultural and environmental framework (Elbert et al. 2008). A novel chemical class of insecticide that would address potential resistance, target specific pests, and reduce risks to non-target taxa by keeping active ingredients localized were focal points of neonicotinoid class compounds (Nauen and Denholm 2005, Cloyd and Bethke 2011, Douglas and Tooker 2015).

### 1.2. Historical background of neonicotinoids

Neonicotinoid class insecticides were developed as an alternative to earlier insecticides, such as organophosphates and carbamates (Jeschke and Nauen 2008). The first commercially available neonicotinoid compound, imidacloprid, was developed in 1991 by Bayer Crop Science, Monheim, Germany (Nauen and Denholm 2005, Elbert et al. 2008). Several neonicotinoid analogues followed imidacloprid, each providing growers with different options in terms of

toxicity to a broad range of arthropod or insect pests and application methods (Nauen and Denholm 2005, Elbert et al. 2008, Goulson 2013). All neonicotinoid class compounds are classified by their chemical ring structure and functional groups (Tomizawa and Casida 2005, Shimomura et al. 2006, Jeschke and Nauen 2008). Common cyclic compounds include imidacloprid, thiacloprid, and thiamethoxam, and noncyclic compounds include nitenpyram, acetamiprid, clothianidin, and dinotefuran (Shimomura et al. 2006, Jeschke and Nauen 2008). Differences in neonicotinoid chemical compound structures determine their physical and chemical properties, including: water solubility, stability, plant uptake rate and metabolism, insecticidal activity, selective toxicity, and insect metabolism (Tomizawa and Casida 2005, Cloyd and Bethke 2010, Jeschke et al. 2010).

Compared to other classes of broad-spectrum insecticides (e.g., organophosphates, carbamates, synthetic pyrethroids), primary factors leading to neonicotinoid adoption were plant systemicity and high selectivity against target pests (Elbert et al. 2008, Simon-Delso et al. 2015). When used within the framework of an integrated pest management system, neonicotinoids may delay the probability of insects developing resistance (Tomizawa and Casida 2003; Jeschke and Nauen 2008), while simultaneously reducing risks to non-target organisms (Jeske and Nauen 2008), and decreasing the probability of drift outside of the treated area (Elbert et al. 2008, Jeschke and Nauen 2008). They also have persistent activity against crop pests, are economical, easy to use, and are available in several different formulations (Elbert et al. 2008, Jeschke and Nauen 2008, Simon-Delson et al. 2015, North et al. 2016). Overall, neonicotinoid insecticides offered several distinct advantages over previously existing insecticides.

### **1.3. Current use of neonicotinoids**

Since being released for commercial use, neonicotinoid class insecticides have quickly become a preferred method of chemical pest control (Goulson 2013, Douglas and Tooker 2015, Hurley and Mitchell 2016). By 1999, imidacloprid was the most widely used insecticide in the world, with sales in the United States estimated at nearly a half billion dollars (Maienfisch et al. 2001). Thiamethoxam is now the second-most used neonicotinoid and is commonly applied as seed coatings for 115 crops (Elbert et al. 2008, Jeschke et al. 2010, Hurley and Mitchell 2016). Neonicotinoids are currently licensed for use in over 120 countries (Bonmatin et al. 2015), most notably by the largest crop-producing areas within Latin America, Asia, and the United States (Bass et al. 2015, Simon-Delso et al. 2015). These three regions account for approximately 75 percent of global neonicotinoid use in crop protection (Bass et al. 2015). A large majority of major field crops in United States, including soybean, maize, and wheat are grown using neonicotinoid treatments (Jeschke et al. 2010). Neonicotinoid usage trends continued at an increasing rate, from 2000 to 2012, nearly all major row crops grown in United States received a neonicotinoid seed treatment (Douglas and Tooker 2015). In addition, neonicotinoids were applied to an estimated 80 percent of soybean grown within the southern states of the United States (Reisig et al. 2012). Neonicotinoids are also commonly used in non-agricultural plant protection, such as for ornamentals and turf grasses (Elbert et al. 2008).

### **1.4. Neonicotinoid formulations**

Neonicotinoids are applied to plants using several methods, including: foliar sprays, soil drenches, direct injections into the trunks of woody plants, and seed coatings (Goulson 2013). Currently, in an agricultural setting most neonicotinoids are applied as soil drenches and seed dressings (Douglas and Tooker 2015). For example, 60 percent of global agriculture production

of cereals and vegetables crops commonly receive neonicotinoid applications of soil or seed treatments (Jeschke et al. 2011). Each neonicotinoid formulation used in agriculture possesses different effective ranges against arthropod pests, from broad to limited (Elbert et al. 2015). Neonicotinoid efficacy is dependent upon several factors, some of which include plant growth stage, soil type, and type of target pest (Buchholz and Nauen 2002).

### **1.5. The selective nature of neonicotinoids**

Neonicotinoids are marketed as reduced risk insecticides, which is primarily because the compound is transported directly and systemically into plant tissues (Elbert et al. 2008). Systemic uptake and transport of neonicotinoids allow for the compound to be transported and distributed within the plant, thereby minimizing exposure to non-target organisms, including vertebrates (Tomizawa and Casida 2003, Elbert et al. 2008). Seed coated and soil drench neonicotinoids are directly absorbed by plant root systems and transported to all plant structures (Elbert et al. 2008, Goulson 2013, Hurley and Mitchell 2017), including xylem transport to new growth (Elbert et al. 2013, Moscardini et al. 2015). Neonicotinoid foliar sprays are absorbed by plant leaves through translaminar movement (Buchholz and Nauen 2002, Elbert et al. 2008) and remain active on both contacted leaf surfaces and within foliage (Buchholz and Nauen 2002). However, active toxic concentrations decline over time as a treated plant grows (Elbert et al. 2008, Magalhaes et al. 2009, Goulson 2013), although this can depend on the formulation used, plant species, leaf morphology (Buchholz and Nauen 2002), and environmental stressors such as nearby weeds or temperature (Elbert et al. 2008, Goulson 2013). Despite potential abiotic and biotic effects on neonicotinoid activity, the systemic nature of neonicotinoid insecticides provide plants with more persistent and uniform protection against herbivorous insects compared to other types of insecticides (Elbert et al. 2008, Goulson 2013).

## 1.6. Mode of action and specificity to target pests

Major agricultural crop arthropod pests targeted by neonicotinoids include species within the following insect orders: Coleoptera, Diptera, Hemiptera, Lepidoptera, and Thysanoptera (Nauen and Denholm 2005, Elbert et al. 2008, Jeschke et al. 2011, Culliney 2014). Exposure to neonicotinoids occurs when target pests ingest the neurotoxin while feeding on a treated plant, but can also occur through direct contact with neonicotinoid residues (Goulson 2013).

Neonicotinoids belong to a class of insecticides that are modeled after a naturally occurring alkaloid, nicotine, (Tomizawa and Casida 2003), and act as nicotinic acetylcholine receptor agonists (Marquini et al. 2003, Tomizawa and Casida 2005, Jeschke and Nauen 2008). Numerous nicotinic acetylcholine receptors are distributed throughout an insect's central nervous system, and are responsible for neurotransmissions (Tomizawa and Casida 2003).

Neonicotinoids antagonistically bind with nicotinic acetylcholine receptors on the post-synaptic membrane, and thus depolarize membrane potentials which are responsible for neurotransmitter release along central nervous synaptic pathways (Matsuda et al. 2001, Shimomura et al. 2006, Jeschke and Nauen 2008). For susceptible insects, the ultimate result of neonicotinoid exposure is paralysis and death.

One of the reasons neonicotinoids target specific insect groups (e.g. soybean aphids) is that the post synaptic neuron membrane binding sites are more numerous and strongly binding in insects (Matsuda et al. 2001, Goulson 2013). The toxicological profile of neonicotinoids can be directly attributed to both transport into an insect's central nervous system, which is facilitated by compound hydrophobicity, and high binding site affinity of neonicotinoids to the molecular structure of insect nicotinic acetylcholine receptors (Tomizawa and Casida 2003). Not all neonicotinoids act on insects similarly, for instance, target site selectively and toxicity is greater

in aphids and leafhoppers (Tomizawa and Casida 2003). In addition, distribution and retention locations of neonicotinoids are variable among formulations, for example, thiamethoxam is translocated through plant xylem but is not phloem mobile (Maienfisch et al. 2001).

### **1.7. Resistance of target pests to neonicotinoids**

The success of neonicotinoids as a singular solution to crop protection is currently a debated topic. Although neonicotinoids are considered to be highly effective at managing pest populations, some arthropod species have developed resistance or decreased susceptibility to neonicotinoids (Nauen and Denholm 2005, Bass et al. 2015). In 1996, five years after the introduction of imidacloprid, the first report of neonicotinoid resistance was recorded within cotton whitefly populations, *Bemisia tabaci* (Homoptera: Aleyrodidae) (Bass, et al. 2015). Currently, the Arthropod Pesticide Resistance Database (<http://www.pesticideresistance.org/>) lists over 500 arthropod species resistant to neonicotinoids, including western flower thrips (Thysanoptera: Thripidae, *Frankliniella occidentalis*) (Gao et al. 2012, Wang et al. 2016). Most species of insects displaying neonicotinoid resistance are those that have developed resistance to other insecticides (Nauen and Denholm 2005, Bass et al. 2015). Chronic pest exposure to neonicotinoids may also encourage resistance and insensitivity (Nauen and Denholm 2005, Goulson 2013).

### **1.8. Exposure of non-target arthropods to neonicotinoids**

Field cropping systems are composed of not only herbivorous pests that are targets of neonicotinoid insecticides, but a community of non-target arthropods. Several arthropod groups, such as non-target herbivores, predators, and pollinators, utilize field crop plants as foraging or oviposition habitats. The same chemical properties that enhance neonicotinoid effectiveness against crop pests also contribute to their potential to harm non-target arthropods (Goulson 2013,

Bonmatin et al. 2015, Pisa et al. 2015, Botias et al. 2016, Douglas and Tooker 2016). Exposure risks and effects on non-target arthropods are variable and may involve several factors such as exposure route and non-target traits (Desneux et al. 2007). Resource use, feeding strategies and life stage of non-targeted groups can influence the level of exposure and impact to exposed organisms within areas affected by neonicotinoids (Gontijo et al. 2015).

Exposure of non-target arthropods to neonicotinoids can occur through direct contact from foliar sprays or residues, ingestion of intentionally or unintentionally treated plant materials, or via consuming contaminated prey items (Cloyd and Bethke 2011, Douglas and Tooker 2016). Neonicotinoids remain active in soils, thereby increasing the risks of movement of contaminated dust to nearby areas (Sanchez-Bayo et al. 2007, Bonmantin et al. 2015, Pisa et al. 2015, Botias et al. 2016). For example, between 80 and 98 percent of the active compound is not taken up by crop plants developing from neonicotinoid treated seed (Goulson 2013).

Neonicotinoids can persist and remain active in the soil for up to a year (Goulson 2013, Bonmantin et al. 2015). Also, neonicotinoids are highly water soluble, which increases the likelihood that non-crop vegetation within or adjacent to fields will be contaminated through leaching (Sanchez-Bayo et al. 2007, Goulson 2013, Pisa et al. 2015, Botias et al. 2016).

Neonicotinoid uptake by field margin vegetation increases the risk that arthropod natural enemies will be exposed, because an estimated 60 percent of them utilize non-crop field edges as refuges and alternative habitats (Bianchi et al. 2006).

After plant uptake, neonicotinoids will be present in most tissues, such as leaves, stems, roots, and pollen, as well as exudates (Cloyd and Bethke 2011, Gontijo et al. 2014, Botias et al. 2016). Exposure can occur when non-target arthropods move across plant surfaces or feed on plant tissues or other resources (Seagraves and Lundgren 2012), including critical nutrient

sources such as extra floral nectaries (Moscardini et al. 2015). Exposure of predatory arthropods can also occur from consuming contaminated herbivorous prey (Szczepaniec et al. 2011).

Likewise, several generalist predator taxa are facultative omnivores, and have been observed avoiding feeding on neonicotinoid treated plant tissue. It may be possible neonicotinoids may influence diet switching from plant to prey (Seagraves and Lundgren 2012).

### **1.9. Lethal and sub-lethal effects of neonicotinoids on non-target arthropods**

Neonicotinoids have variable effects across non-target taxa, and both direct and indirect exposure can result in increased mortality rates (Moser and Obryki 2009, Cloyd and Bethke 2011). The effects neonicotinoids have on non-target arthropods are not always fatal, but can be sub-lethal (Moser and Obryki 2009, Goulson 2013, Martinou and Stavrinides 2015, Gontijo et al. 2015). Sub-lethal effects are defined as disrupted or altered physiology and behavior upon being exposed to an insecticide (Cloyd and Bethke 2011, Goulson 2013). Physiologically, sub-lethal effects of neonicotinoids can positively or negatively influence reproduction and development (Desneux et al. 2007, Seagraves and Lundgren 2011). Behaviorally, sub-lethal effects of neonicotinoids can negatively influence mobility, orientation, and foraging (Desneux et al. 2007, Moser and Obryki 2009, Szczepaniec et al. 2011, Goulson 2013, Fernandes et al. 2016). Not surprisingly, sub-lethal effects on non-target taxa have the potential to destabilize and restructure community interactions (Cloyd and Bethke 2011, Szczepaniec et al. 2011). Likewise, other neonicotinoid exposed non-targeted arthropods, including minor or occasional pests, can benefit from relaxed top-down control, thereby increasing the potential for pest outbreaks (Szczepaniec et al. 2011).

Strictly predaceous arthropods can be negatively affected by neonicotinoids via ingesting contaminated prey, facilitating a dietary transfer of toxins which has been termed secondary poisoning (Pozzebon et al. 2011, Douglas et al. 2015, Martinou and Stavrinides 2015).

Szczepaniec et al. (2011) observed reduced mobility, feeding rates, and longevity of two key spider mite predators (Coccinellidae, Chrysopidae) after they consumed spider mites *Tetranychus schoenei* (Acari: Tetranychidae) that had fed on imidacloprid treated leaves. In addition, no debilitating effects for both predators were observed when maintained on imidacloprid-treated leaves without contaminated prey, thereby suggesting impairment through predation.

Several hemipteran predators known to supplement their diet through herbivory have been used as study organisms to evaluate non-target effects of neonicotinoids from various routes of exposure (Prabhaker et al. 2011, Gontijo et al. 2015). Exposure potential to hemipterans can occur within neonicotinoid-treated cropping systems through contact, ingestion of contaminated plant tissue or prey (Gontijo et al. 2015). Torres and Ruberson (2004) found that survival rates of facultative plant-feeding and generalist predator stinkbug nymphs, *Podisus nigrispinus* (Heteroptera: Pentatomidae) were lower after direct contact with imidacloprid and thiamethoxam, and after ingestion of residues on cotton leaves. Prabhaker et al. (2011) demonstrated sub-lethal effects on two beneficial species of insects, big-eyed bugs, *Geocoris punctipes* (Hemiptera: Geocoridae) and minute pirate bugs, *Orius insidiosus* (Hemiptera: Anthocoridae). Both species are omnivorous and natural enemies of a wide range of crop pests, including spider mites, soybean aphids, and whiteflies (Fernandes et al. 2016, Prabhaker et al. 2016). Prabhaker et al. (2016) found that when exposed to low doses of imidacloprid and thiamethoxam by direct contact with guttation droplets and leaf feeding, *O. insidiosus* and *G. punctipes* had increased mortality rates, reduced prey consumption, and lower fecundity. Similarly, Seagraves and Lundgren (2012) evaluated effects of neonicotinoid seed treatments (*i.e.*, imidacloprid and thiamethoxam) on soybean aphids, *Aphis glycines* (Hemiptera: Aphididae)

and several predators, including damsel bugs (Hemiptera: Nabidae), lacewings (Neuroptera: Chrysopidae), and pirate bugs *Orius insidiosus* (Hemiptera: Anthocoridae). Their field study showed inconsistent season-long efficacy of the chemicals against soybean aphids, while predator populations were more abundant in untreated fields. Laboratory toxicity trials revealed *O. insidiosus* had reduced survival when feeding on thiamethoxam-treated leaves, and the insects avoided feeding on treated leaves when uncontaminated prey was available.

Previous and current research on neonicotinoid effects on omnivorous arthropods has focused on altered herbivore pest suppression. Neonicotinoid effects on omnivorous arthropods such as western flower thrips, which primarily consume plant tissue but opportunistically preys on a co-occurring herbivore (*i.e.*, twospotted spider mites), is limited. Herbivory and predation responses of western flower thrips within neonicotinoid-treated systems have yet to be evaluated.

### **1.9.1. Effects of neonicotinoids on herbivorous spider mites and a facultative omnivore, western flower thrips**

In recent years researchers have observed increased populations of non-target herbivores such as spider mites on plants treated with neonicotinoids (Szczepaniec et al. 2011, Szczepaniec and Raupp 2012). Hypotheses to explain this phenomenon include: chemically-stimulated spider mite reproduction, chemically-altered host plant physiology, reduced interspecific competition, and decreased natural enemy abundances.

Much of the work on how neonicotinoids affects interactions between non-target herbivores and natural enemies have focused on strictly predatory species (*i.e.*, predatory phytoseiid mites, ladybeetles, and other generalist predators). We were interested in how neonicotinoids impact interactions between spider mites and a facultative omnivore that, in

addition to feeding on spider mites, primarily feeds on plant tissue and is a target pest of neonicotinoids (*i.e.*, western flower thrips, WFT).

### **1.9.2. Life history of western flower thrips (WFT)**

*Frankliniella occidentalis*, common name western flower thrips, represents one of over 5000 species of plant-feeding thrips belonging to order Thysanoptera, family Thripidae (Reitz 2009). Western flower thrips have haplo-diplo reproductive systems in which haploid males are produced from unfertilized eggs and diploid females develop from fertilized eggs (Reitz 2009, Cook et al. 2011). Eggs are oviposited by females directly under the plant epidermal tissues of leaves (Reitz 2009). First instars emerge from eggs 2-4 days after oviposition and begin feeding (Reitz 2009). The second instar stage will migrate to the base of its host plant and the prepupa and pupal stages are found in soil are non-feeding (Reitz 2009). Adults emerge from soil between 1-3 days of completion of pupal stages (Reitz 2009, Cook et al. 2011). The size range of adults is typically between 1-2 mm, with females being 1.66-1.70 mm and males slightly smaller at 1.26-1.31 mm (EPPO 2002). The average life span of an adult is about 3 weeks (Reitz 2009). Adult females continue to produce eggs throughout adulthood and populations are composed of several generations (Reitz et al. 2011). Previous studies have suggested that temperature and host plant quality influence thrips biology, including oviposition preference, development time, and longevity (Zhang et al. 2007, Reitz et al. 2011).

### **1.9.3. Feeding behavior of WFT**

WFT are considered a significant agricultural pest with a broad host range (Reitz 2009). Including over 250 different crops and 60 plant families (Reitz 2009, Reitz et al. 2011), such as cotton, soybean, and numerous ornamental plants. Several weed species within and outside of cropping areas have also been identified as alternate feeding and reproductive hosts for thrips (Cook et al. 2011, Reitz et al. 2011). Direct damage to plants occurs from both feeding and

oviposition (Reitz and Funderburk 2012). Adults feed on plant pollen and localized leaf patches by inserting stylet mouthparts into leaves and withdrawing cellular contents (Reitz 2009). Feeding causes deformation of leaves and reduces plant growth; chlorosis and characteristic necrotic scarring are commonly observed with thrips damage (Zhang et al. 2007, Cook et al. 2011, Reitz and Funderburk 2012).

Western flower thrips have been described as opportunistic or facultative predators. They commonly occupy host plants with other agricultural pest arthropods, including pest species of aphids and spider mites (Wilson et al. 1996, Men, et al. 2003, Xu et al. 2006, Zhi et al. 2006, Reitz et al. 2011, Martini et al. 2013). Previous studies have shown both larval and adult WFT will consume eggs of some of its natural enemies, like predatory mites (Acari: Phytoseiidae), as well as eggs of herbivorous pests, like greenhouse whiteflies (Hemiptera: Aleyrodidae) and twospotted spider mites, *Tetranychus urticae* (Trichilo and Leigh 1986, Wilson et al. 1996, Agrawal and Klein 2000, Janssen et al. 2003, Reitz 2009, Reitz et al. 2011, van Maanen et al. 2012, Martini et al. 2013). Plant volatiles released from pest herbivory may additionally influence thrips foraging and residency on suitable host plants (Martini et al. 2013). Thrips predation and herbivory impact pest populations as well as thrips development and reproduction (Agrawal et al. 1999, Janssen et al. 2011, Reitz et al. 2011, van Maanen et al. 2012).

Host plant quality has been identified as a significant factor affecting WFT behavior, development, and longevity (Maris et al. 2004, Zhang et al. 2007, Shan et al. 2012). WFT have demonstrated differential preference and host finding behavior between susceptible and resistant plants (Maris et al. 2004). Thrips feeding decisions (*e.g.*, plant material versus arthropod prey) may be influenced by plant host quality, prey availability and density, and prey quality (Wilson et al. 1996, Agrawal et al. 1999, Agrawal and Klein 2000, Janssen et al. 2003, Martini et al.

2013). Induced plant resistance resulting from herbivory by other arthropods such as spider mites can affect thrips diet switching between herbivory and predation (Agrawal et al. 2000). Agrawal et al. (1999) found that thrips mortality increased in the absence of spider mite eggs on herbivore-induced cotton leaves, while plant feeding was reduced by 50 percent, and numbers of eggs consumed doubled compared to non-induced leaves. In addition, eggs produced from spider mites females reared on induced plants were less preferred by thrips (Agrawal and Klein 2000).

Identifying the interactive effects of host plant and prey quality as predictive measures influencing thrips feeding decisions may have significant implications for neonicotinoid-treated systems. Szczepaniec et al. (2013) found that the neonicotinoids imidacloprid, clothianidin, and thiamethoxam altered plant phytohormone concentrations and expression of genes associated with plant defense, thereby reducing plant responses to herbivory. The direct and indirect effects of neonicotinoid treated plants may extend to thrips omnivory. Plants with reduced resistance are potentially more susceptible to herbivorous arthropods such as spider mites. Thus, within treated systems, neonicotinoids may influence thrips foraging and feeding decisions and overall performance via multiple mechanisms.

#### **1.9.4. Effects of neonicotinoids on WFT**

WFT currently are listed as resistant to seven chemical classes mode of insecticidal action categorized by the Insecticide Resistance Action committee, including neonicotinoids (Bielza 2008, Gao et al. 2012). Several factors may contribute to thrips resistance. Thrips polyphagy, thigmotactic behavior, and life history place many limitations on insecticide exposure and/or efficacy while encouraging resistance (Reitz et al. 2011). In addition, thrips resistance may be further enhanced by metabolic detoxification, target site modification, toxicant penetration of the cuticle or synergistic mechanisms of combined resistant responses (Biezla et

al. 2008, Reitz 2009, Gao et al. 2012, Mouden et al. 2017). Although several cropping systems have transitioned to neonicotinoid seed treatments (e.g., imidacloprid, thiamethoxam), additional information about WFT responses other than mortality, density reductions and resistance management is needed (Herron and James 2007, Resig et al. 2012, Wang et al. 2016). Limited information is available about neonicotinoid sub-lethal effects influencing WFT omnivory, host finding, and interactions with other herbivorous arthropods.

### **1.9.5. Life history of twospotted spider mites (TSSM)**

*Tetranychus urticae* Koch, common name twospotted spider mite, is a species belonging to the class Arachnida, order Acari, and family Tetranychidae (Helle and Sabelis 1985, Borror et al. 1992). Spider mites share several physical characteristics with their arachnid relatives, including two distinct body regions, a prosoma, opisthosoma and four pairs of legs (Helle and Sabelis 1985, Borror et al 1992). Spider mites also produce silk, which is used to create micro-habitats, protect eggs, and for aerial dispersal (Helle and Sabelis 1985). Female spider mites reproduce via haplodiploidy, and progeny sex ratios are significantly female biased (Helle and Sabelis 1985, Macke et al. 2012). Adult female mites usually oviposit on the abaxial surface of leaves, which is where offspring often remain and develop (Helle and Sabelis 1985). A single female can produce 10 to 150 eggs, each approximately 100-150 micrometers in diameter (Helle and Sabelis 1985). The duration of embryonic development can vary between 3-10 days (Helle and Sabelis 1985). The three juvenile developmental stages: larva, protonymph, and deutonymph (Helle and Sabelis 1985). During each of these immature stages are actively moving and feeding, coupled with inactive periods for molting of new cuticles (Helle and Sabelis 1985). Immatures reach adulthood between 6-10 days after egg emergence. Adults measure less than a millimeter (Helle and Sabelis 1985). Several biotic and abiotic factors influence spider mite reproduction

and development (Helle and Sabelis 1985). Temperature range, host plant nutrient quality, and humidity are critical determinants of spider mite life history (Helle and Sabelis 1985, El Taj et al. 2016).

#### **1.9.6. Feeding behavior of TSSM**

The mouthparts of spider mites are protractible and retractable chelicerae, which penetrate leaf surfaces when feeding (Helle and Sabelis 1985). The most common feeding areas for spider mites is the underside surface of leaves (Helle and Sabelis 1985). Mites insert their stylet mouthparts into the leaf epidermis and extract cell contents (Helle and Sabelis 1985). This feeding behavior produces a characteristic stippling pattern of discoloration (Helle and Sabelis 1985). High spider mite densities injure plants through damage-induced physiological changes leading to decreased photosynthesis, leaf water loss, and reduced growth (Helle and Sabelis 1985). Spider mites migrate to more suitable plants hosts depending on plant phytochemical properties and leaf morphology (van den Boom et al. 2002). Additionally, host plant quality influences spider mite oviposition and colonization (Yano et al. 1998). Plants infested with conspecifics or damaged are less preferred compared to undamaged plants (Egas et al. 2003).

Spider mites are considered a major herbivorous pest of a field crops and greenhouse plants (Helle and Sabelis 1985, El-Taj et al. 2016). Spider mite pest status is influenced by a number of factors, including crop type, geographic region, and seasonal conditions (Helle and Sabelis 1985, Fry 1989, Klubertanz et al. 1990, Navajas 1998, van den Boom et al. 2003). This species is highly polyphagous and can colonizing over 1000 different plant species from more than 140 plant families (Navajas 1998, Grbic et al. 2011, El-Taj et al. 2016). Once established, spider mite populations can rapidly attain damaging levels resulting in reduced crop yields (Helle and Sabelis 1985). As generalist herbivores, spider mites possess the ability to accept and

successfully populate marginal or low quality plant hosts (Fry 1989, Yano et al. 1998, Agrawal 2000, Egas et al. 2003).

### **1.9.7. Effects of neonicotinoids on spider mites (SM)**

Researchers having observed increased densities of spider mites within neonicotinoid treated systems, and have postulated several possible mechanisms that may contribute to this phenomenon (Sclar et al. 1998, Ako et al. 2006, Szczepaniec and Raupp 2012, Szczepaniec et al. 2011, Smith et al. 2013, Barati and Hejazi 2015). However, other studies have found negative or non-significant effects of neonicotinoids on spider mite fecundity (Ako et al. 2004, Duso et al. 2008, Pozzebon et al. 2011). One idea is that the chemical stimulated the female mite's reproductive system (*i.e.*, hormoligosis; James and Price 2002). Secondly, neonicotinoids were thought to disrupt or suppress plant resistance and defense responses, thus weakening resistance elicitors to spider mite herbivory (Szczepaniec et al. 2013). Lastly, lethal or sub-lethal effects on spider mite natural enemies were thought to reduce predator pressure, thus leading to increased spider mite densities (Sclar et al. 1998, Pozzebon et al. 2011, Szczepaniec et al. 2011).

### **1.9.8. Neonicotinoid effects on SM fecundity**

Neonicotinoids may increase spider mite populations through hormoligosis, a positive reproductive response (James and Price 2002), which may be additionally influenced by environmental conditions (Helle and Sabelis 1985). The hormoligant influence of neonicotinoids on spider mite reproduction can involve several critical factors such as spider mite biotype, chemical formulation used, exposure route, and dosage (Ako et al. 2006, Barati and Hejazi 2015). Treatment effects can be quantified by comparing number of eggs produced and lifetime fecundity of exposed female adults to untreated controls (Smith et al. 2013). James and Price (2002) showed increased fecundity of female spider mites exposed to imidacloprid through spray

contact and ingestion of treated excised bean leaves. This study showed increases in egg production between 10-26 percent, which translated to 30-70 more eggs over a female's lifetime. Egg production responses differed based on how spider mites were exposed to imidacloprid, with individuals directly sprayed with the chemical producing more eggs immediately, continuing for 12 days while mites exposed through ingestion of systemically treated leaves showed a delayed response, with increased egg production not observed until day six. Similarly, Barati and Hejazi (2015) directly and indirectly exposed cohorts of adult female spider mites to three different neonicotinoids, thiamethoxam, acetamiprid, and thiacloprid. Individual mites were placed on common bean leaf pieces (*Phaseolus vulgaris* L.) and directly sprayed, and individuals were also placed on leaves taken from plants on which drench solutions were used. Fecundity life table results from these experiments indicated increased reproductive rates across all neonicotinoid formulations used and methods of exposure. Conversely, field experiments by Smith et al. (2013) did not indicate significant differences in spider mite fecundity for populations on seed-treated cotton plants (imidacloprid, thiamethoxam), but foliar applications of thiamethoxam did show a positive fecundity response. In laboratory trials, exposing spider mites via cotton leaf discs taken from plants treated with systemic and foliar applications also resulted in higher fecundity, especially for foliar-applied thiamethoxam (Smith et al. 2013).

### **1.9.9. Neonicotinoid effects on SM via host plants**

Another potential mechanism that may contribute to higher spider mite population on plants treated with neonicotinoids led researchers to consider the effects of these insecticides on host plant physiology (Szczepaniec et al. 2013). Szczepaniec et al. (2013) conducted a series of experiments to evaluate neonicotinoid effects on host plant (cotton, corn, tomato) defense responses to spider mite herbivory. Gene expression regulation (jasmonic acid, salicylic acid)

and concentrations of several phytohormones involved in induced defenses were quantified as a response to spider mite herbivory alone and following treatment of thiamethoxam, clothianidin, and imidacloprid. Overall, these studies demonstrated changed expression of genes associated with plant defense and altered phytohormone concentrations across all host plants after exposure to neonicotinoids imidacloprid and thiamethoxam. Furthermore, spider mite population growth was observed on all treated host plants. These experiments provided evidence supporting the hypothesis of plant-mediated effects of neonicotinoid treated host plants and spider mite interactions through altered induced defenses.

Plant mediated effects were also examined by Szczepaniec and Raupp (2013) using imidacloprid treated boxwoods and boxwood spider mites *Eurytetranychus buxi* (Acari: Tetranychidae) in the absence of natural enemies. Spider mites ingesting treated plant material produced more eggs compared to non-treated, while spider mites exposed to imidacloprid through contact sprays showed no increases in elevated fecundity. These experiments further supported the possible mechanisms of host plant mediated effects leading to spider mite increased population growth.

#### **1.9.9.1. Neonicotinoid effects on SM and predator interactions**

Several studies have highlighted the effects of neonicotinoids as a contributor of disrupted pest suppression through top down regulation (Sclar et al. 1998, Torres and Ruberson 2004, Pozzebon et al. 2011, Prabhaker et al. 2011, Szczepaniec et al. 2011, Fernandes et al. 2016). Elimination or reductions in natural enemy abundances due to neonicotinoid exposure have been supported as one mechanism potentially enhancing spider mite population growth (Szczepaniec et al. 2011, Smith et al. 2013). Many spider mite predators, including several hemipterans and predatory mites, can be exposed to neonicotinoids through several routes, most

notably prey or plant material consumption (Pozzebon et al. 2011, Gontijo et al. 2015).

Variability of environmental conditions, community structure, resources available and chemical formulation may influence exposure pathways. Neonicotinoid-exposed predators have responded differently to neonicotinoids under field and simulated laboratory conditions (Torres and Ruberson 2004, Cloyd and Bethke 2011, Krischik et al. 2015). According to Fernanades et al. (2016), variable toxic effects on predators categorized as lethal or sub-lethal may be related to chemical degradation in field conditions. Predator exposure can occur through direct contact or indirectly from ingesting contaminated prey. Direct and indirect effects of neonicotinoids on natural enemies may lead to increased mortality rates resulting from both lethal and sub-lethal exposure (Moser and Obryki 2009, Ohnesorg et al. 2009, Pozzebon et al. 2011, Szczepaniec et al. 2011, Gontijo et al. 2015, Martinou et al. 2015, Moscardini et al. 2015, Pisa et 2015, Fernandes et al. 2016). Factors such as plant species, formulation type, route of exposure, predator species and life stage not only influence neonicotinoid effects on natural enemies, but their role in predator prey interactions. Evaluating the potential impact of these factors may therefore be critical when applied to spider mite and natural enemy responses to neonicotinoid exposure.

Measuring neonicotinoid effects on predator-prey relationships may be dependent on how these groups are exposed. As previously mentioned, predators may be exposed through direct contact and dietary transfer of consumed prey. Effects of predator exposure to neonicotinoids can result in decreased survival, longevity, impaired mobility, and foraging efficiency. Pozzebon et al. (2011) assessed neonicotinoid (thiamethoxam) effects between twospotted spider mites, *T. urticae* and one of its predators, *Phytoseiulus persimilis* (Acari: Phytoseiidae), using different routes of exposure. Both *T. urticae* and *P. persimilis* were exposed topically, placed on leaves

with foliar residues, and provided leaves obtained from drench treated plants. By comparing single and combinations of exposure routes, residual and contaminated food sources had the greatest sub-lethal effects on both mite species. Overall, single routes of exposure resulted in non-constant lethal, sub-lethal effects on predator and prey but increased synergistically.

*Phytoseiulus persimilis* ingestion of contaminated *T. urticae* prey combined with exposure to foliar residues resulted in decreased survival of the predatory mite. Increased mortality was observed for *T. urticae* through contact with foliar residues and feeding on systemically treated leaves, while direct topical contact had no effect. These experiments demonstrate how routes of exposure may potentially shift prey populations and interactions, specifically for those in which neonicotinoids can alter density dependent relationships. More notably, systemic neonicotinoids such as thiamethoxam seed treatments, may provide additional exposure risks to non-target omnivorous arthropods, including WFT, because omnivores can be exposed to insecticides two ways – via treated foliage and contaminated prey.

Previous and recent research of negative effects of neonicotinoid insecticides on non-target arthropods have focused on generalist predators and pollinators. Currently, information on about how omnivorous arthropods, such as target pest WFT, may be affected is limited.

Although WFT are described as opportunists, neonicotinoids may potentially shift feeding preferences within environments where both treated plant materials and toxic prey like SM are present, which can ultimately impact the survival and degree of plant damage caused by each type of pest.

## 1.10. References

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## CHAPTER 2. INFLUENCE OF NEONICOTINOID SEED TREATED SOYBEAN ON TWO CO-OCCURRING ARTHROPOD PESTS, TWO-SPOTTED SPIDER MITE (*TETRANYCHUS URTICAE*) AND WESTERN FLOWER THRIPS (*FRANKLINIELLA OCCIDENTALIS*)

**Key Words:** thiamethoxam, omnivory, feeding preference, pest management

### 2.1. Introduction

Use of neonicotinoids has steadily increased within agricultural field crops (Goulson 2013, Douglas and Tooker 2015, Hurley and Mitchell 2016). Neonicotinoids are an attractive pest management option because they are systemic insecticides, and thus highly effective for most sucking and chewing insect pests (Elbert et al. 2008, Jeschke et al. 2010). Although neonicotinoids can be sprayed on foliage, injected into tree trunks, added to the soil, and applied to seeds (Goulson 2013), use of the latter method contributes to their prevalence within field crops due to protection against early season pests (Hurley and Mitchell 2016, North et al. 2016). A recent meta-analysis focusing on the United States revealed that seed applications accounted for 80 percent of neonicotinoid use, and that between 2006 and 2011, 34 to 44 percent of soybean acreage was grown using neonicotinoid (thiamethoxam, imidacloprid, clothianidin) treated seeds (Douglas and Tooker 2016). Despite the systemic nature of neonicotinoids, exposure of non-target arthropods still occurs.

Insect communities in annual cropping systems are composed of herbivorous pests that are targets of neonicotinoid insecticides and a community of non-target arthropods (*e.g.*, other herbivores, omnivores, predators, pollinators) that utilize field crop plants as foraging or oviposition habitats (Altieri 1999, Sanchez-Bayo et al. 2007, Goulson 2013). Exposure of non-target arthropods to neonicotinoids can occur through a variety of means, including direct contact

with foliar sprays or residues, ingestion of treated plant material, or feeding on contaminated prey (Cloyd and Bethke 2011, Szczepaniec et al. 2011, Gontijo et al. 2014, Botias et al. 2016). Several generalist predator taxa are facultative omnivores, and have been observed avoiding feeding on neonicotinoid treated plant tissue, which suggests neonicotinoids may impact diet switching (Seagraves and Lundgren 2012, Gontijo et al. 2015). Effects of neonicotinoids on non-target arthropods can be lethal or sub-lethal, the latter including altered biology and behavior (e.g., reduced mobility, disorientation, or diet switching (Cloyd and Bethke 2011, Seagraves and Lundgren 2012, Martinou and Stavrindides 2015, Gontijo et al. 2018). Szczepaniec et al. (2011) attributed decreased generalist predator diversity and abundance to secondary poisoning from consumption of herbivorous spider mites from neonicotinoid-treated foliage.

Not all effects of neonicotinoids on non-target arthropods are detrimental. Spider mites (Acari: Tetranychidae) are polyphagous herbivorous pests that are not target pests of neonicotinoids (Elbert et al. 2008). Several studies have found enhanced densities of spider mites on neonicotinoid-treated plants (Sclar et al. 1998, Szczepaniec et al. 2011, Smith et al. 2013), although other research has shown neutral (Pozzebon et al. 2011) or negative effects (Ako et al. 2006) on spider mite populations. Mechanisms that have been proposed to contribute to increased spider mite populations following neonicotinoid applications include hormoligosis (Sclar et al. 1998, James and Price 2002, Smith et al. 2013), altered plant defense (Szczepaniec et al. 2013, Wulff et al. 2019), and reductions in natural enemy populations via poisoning by ingestion of contaminated prey (Szczepaniec et al. 2011). Cropping systems utilizing neonicotinoids to suppress populations of primary insect pests may therefore experience greater problems with secondary pests like spider mites.

Western flower thrips, *Frankliniella occidentalis* Pergande, are insect pests of multiple cropping systems (Reitz and Funderburk, 2012 Mouden et al. 2016) and are target pests of neonicotinoids (Broughton and Herron 2009, Reitz et al. 2011, Knight et al. 2015). Thrips feed on pollen and by inserting stylets into leaf tissue and extracting cellular contents. Feeding damage can cause leaf deformations and delayed plant growth. (Cook et al. 2011, Reitz et al. 2011). Field, greenhouse, and laboratory bioassay studies have demonstrated neonicotinoids can be effective at increasing thrips mortality. However, neonicotinoid efficacy may be associated with plant type, thrips developmental stage, and exposure dosage (Broughton and Herron 2009, Shan et al. 2012). Thrips response to neonicotinoid application type and compound is also variable such as foliar versus seed treatment. For example, imidacloprid, thiamethoxam seed treatment can be effective at controlling thrips populations early season but inconsistent over time (Cook et al. 2011, Knight et al. 2015). Additionally, low concentration exposure can increase development time and reproductive rates generationally (Cao et al. 2019), thereby potentially encouraging resistance. Western flower thrips resistance to seven chemical class insecticides, including neonicotinoids has been recently reported by the Arthropod Pesticide Resistance Database (Gao et al. 2012). Although primarily herbivorous, western flower thrips are opportunistic, or facultative, predators that supplement their diet with arthropod prey, and also consume spider mite eggs (Trichilo and Leigh 1986, Wilson et al. 1996, Agrawal and Klein 2000, Janssen et al. 2003) and be attracted to plants colonized by spider mites (Agrawal and Klein 2000). The predatory rate of WFT on TSSM eggs has been observed in previous studies to be a functional response to TSSM egg density, therefore suggesting WFT as opportunist predators (Trichilo and Leigh 1986). Thrips feeding decisions may be influenced by plant host

quality, prey availability and density, and prey quality (Wilson et al. 1996, Agrawal et al. 1999, Agrawal and Klein 2000, Janssen et al. 2003, Magalhaes et al. 2015, Martini et al. 2013).

Studies about potential negative effects of neonicotinoids on non-target arthropods have primarily focused on strict herbivores like spider mites or beneficial arthropods (*e.g.*, pollinators, predators; Sclar et al. 1998, Sanchez–Bayo et al. 2007, van Lexmond et al. 2015, Pisa et al. 2015, Douglas and Tooker 2016, Wood and Goulson 2017). Impacts of neonicotinoids on omnivorous insects are understudied, although Prabhaker et al. (2016) found that when exposed to low doses of imidacloprid and thiamethoxam by direct contact with treated leaves, including guttation droplets and leaf feeding, omnivorous big-eyed bugs, *Geocoris punctipes* Say (Hemiptera: Geocoridae) and minute pirate bugs, *Orius insidiosus* Say (Hemiptera: Anthocoridae), had increased mortality rates, reduced prey consumption, and lower fecundity. Similarly, Seagraves and Lundgren (2012) found survival of *O. insidiosus* was reduced when feeding on thiamethoxam treated leaves, and the insects avoided feeding on treated leaves when uncontaminated prey was available.

The goal of this study was to evaluate the effects of thiamethoxam seed treated soybean on densities of twospotted spider mites (*Tetranychus urticae* Koch) and feeding behavior of western flower thrips. Our objectives included: (1) using soybean aphids to verify the insecticidal activity of the seed treatment, (2) quantifying spider mite fecundity on treated and non-treated intact soybean plants when mite movement was restricted and unrestricted, and (3) quantifying the impact of the insecticidal seed treatment on the amount of leaf tissue and/or spider mite eggs eaten by adult female thrips reared on treated versus non-treated soybeans.

## 2.2. Materials and methods

We conducted a series of greenhouse experiments to assess effects of an insecticidal neonicotinoid seed treatment on herbivorous twospotted spider mites and western flower thrips on soybean. After verifying the insecticidal activity of the seed treatment, the first set of experiments explored effects of soybean host plant (NST = no seed treatment, ST = with the seed treatment) on spider mite densities in the absence of thrips. Subsequent experiments investigated effects of soybean host plant and spider mite and thrips origin (reared on NST soybean versus ST soybean) on thrips feeding by quantifying consumption of spider mite eggs and soybean leaf tissue.

Soybean plants [*Glycine max* (L.) Merr.]. The soybean line used in experiments was susceptible to soybean aphids (*i.e.*, *rag1rag1rag2rag2*), and was developed at Iowa State University from a cross between A08-1243074 and LD08-89051a (Wiarda et al. 2012, McCarville and O’Neal 2013). Seed treated (ST) soybean plants had a neonicotinoid insecticidal seed treatment applied prior to planting (thiamethoxam, Cruiser®5FS, Syngenta Crop Protection, Inc., Greensboro NC; lowest rate for soybean of 0.0756 mg AI per seed; McCarville and O’Neal 2013). Control soybean plants (NST) did not receive a seed treatment.

Soybean plants used to rear insects and for experiments were grown in a greenhouse (20 ± 2°C, 16:8 L:D, RH = 40-65%) in plastic molded pots (7.62 deep x W8.89 x H9.53 cm) in a soilless growing medium (PRO-MIX BX, Premier Horticulture Inc., Quakertown, PA). Plants were not fertilized but were watered with tap water twice a week (100-200 mL). To prevent unintended infestation by arthropod pests from adjacent greenhouse rooms, NST and ST plants were grown separately within thrips-proof cages (150 x 150 per cm<sup>2</sup> nylon mesh count, 160 µm aperture, W47.5 x D47.5 x H47.5 cm, BugDorm-44545F, MegaView Science Co. Ltd.,

Taichung, Taiwan), and yellow sticky cards (7.62 x 12.7 cm) were placed around cages. Cages were spaced approximately one m apart, and cage location rotated twice a week to ensure plants experienced similar growing conditions. All plants used for experiments were at the V2 vegetative growth stage (*i.e.*, two fully developed trifoliates above the unifoliate nodes, Fehr and Caviness 1977).

TSSM: Twospotted spider mites (*Tetranychus urticae* Koch). TSSM were originally collected from a greenhouse in 2013, and maintained on soybean (14R09N, 0.9 maturity group, Genuity® Roundup Ready 2 Yield®, Peterson Farms Seed, Harwood, ND) in a laboratory under hydroponic conditions with fluorescent lighting (960305-T5, Sun Blaze®, Intertek, USA) (25 ± 2°C, 16:8 L:D, RH = 60-80%). TSSM lab colony plants were watered twice a week with tap water (100-200 mL), and did not receive any chemicals, including fertilizers. TSSM used for experiments were identified by mounting specimens on microscope slides and assessing morphological features using a stereomicroscope (Stemi 2000-C, Zeiss, Jena, Germany) and dichotomous keys (Helle and Sabelis 1985).

Previous studies indicated host plant identity and quality may influence TSSM behavior, development and reproduction (Yano et al. 1998, Agrawal 2000, Boom et al. 2003, Taj et al. 2016). Therefore, we used TSSM (eggs, larvae, nymphs, adults) from our standard lab colony to establish two new spider mite colonies for a minimum of 1 month, thereby producing two TSSM populations, one reared on NST soybean plants (TSSM-NST) and another reared on ST soybean plants (TSSM-ST). Colonies were maintained on NST or ST soybean plants within thrips-proof cages in a greenhouse setting, as previously described, with one m separating cages and yellow sticky cards surrounding cages to minimize infestation by unintended arthropod pests. New plants were added to cages once a week, with care taken to prevent cross-cage contamination.

TSSM used in experiments were transferred onto rectangular soybean leaf strips (14R09N, 1 x 3 cm) by hand using fine point paintbrushes, with one brush used for mites from the NST colony and another for mites from the ST colony. A tweezers was used to move the strips into plastic cups (P125, 36.9 mL, Solo®, Solo Cup Company, Lake Forest, IL) containing cotton moistened with tap water, and cups were covered with plastic lids. Cups containing TSSM were placed in a cooler ( $4 \pm 2^{\circ}\text{C}$ ) for approximately 24 h prior to use in experiments.

WFT: Western flower thrips [*Frankliniella occidentalis* (Pergande)]. WFT used in experiments were initially collected in from durum wheat plants (*Triticum* sp.) grown in a greenhouse for a maximum of six yrs. WFT collection involved shaking durum plants to dislodge WFT individuals (nymphs, adults) into plastic containers (946 mL, 3.8 cm diam., H15 cm) containing excised soybean leaves (variety 14R09N). WFT containers were placed into a cooler ( $4 \pm 2^{\circ}\text{C}$ ) for 24 h prior to transfer for WFT-NST and WFT-ST colony establishment. WFT genus and species identity was confirmed prior to experiments by slide mounting individuals and microscopically observing diagnostic characters detailed by Mound and Kibby (1998).

WFT colonies were established and maintained within caged (BugDorm-44545F) soybean plants grown from NST and ST seeds under previously described greenhouse conditions. WFT colony cage spacing, sticky card placement, and watering protocols were also as previously described for TSSM colonies. WFT colonies were allowed to establish for a minimum of one month, allowing for multiple generations (egg to adult development 9 to 13 d, adult female longevity 26 d to 5 wks; Reitz 2009) of NST and ST populations, prior to experiments involving WFT individuals. New NST and ST plants (nine per cage) were replaced

approximately once every two wks, with caution to avoid unintended transfer of ST WFT into NST colonies or vice versa.

WFT used in experiments were collected by randomly selecting leaves from each WFT colony (NST and ST), placing leaves into lid covered plastic containers (946 mL, 3.8 cm diam., H15 cm). Containers containing WFT were placed in a cooler ( $4 \pm 2^\circ\text{C}$ ) for approximately 24 h prior to use in experiments. We selected adult females for experiments because they are readily identified using morphological differences (i.e., body size, coloration and shape of abdominal segments, presence of ovipositor; Mound and Kirby 1998, EPPO Bulletin 2002), and they are an active feeding stage known to consume spider mite eggs (Trichilo and Leigh 1986, Agrawal et al. 1999).

At the end of experiments, each living or dead WFT was removed from experimental arenas using a small paintbrush and placed in individual 1.5 mL plastic microtubes filled with 70% ethanol. WFT sex was microscopically confirmed, and measurements of WFT body size were taken to evaluate potential influences of WFT origin (i.e., reared on NST or ST soybeans) on body size, and relationships between body size and feeding. WFT were mounted dorsal side up on microscope slides, and length and width measurements (mm) taken using a stereomicroscope (Wild Heerbrugg, Switzerland) equipped with an optical measuring grid eyepiece (1 mm at 10X magnification). Body length was measured from the anterior vertex of the head to the terminal margin of the last abdominal segment, and body width assessed by measuring the lateral width of the widest abdominal segment.

Soybean aphids (*Aphis glycines* Matsumura). Soybean aphids used in experiments to confirm the efficacy of insecticidal seed treatments were collected from soybean grown near Prosper, ND in 2009, and were maintained on a susceptible soybean variety under laboratory

conditions (RG607RR, 25±2°C, 16:8 L:D, RH = 60-80%). New soybean aphids were added to the colony on a yearly basis to ensure genetic diversity.

Verification of seed treatment efficacy. To assess the insecticidal activity of the thiamethoxam seed treatment, three adult soybean aphids were placed on each of three NST and three ST plants grown to the V2 stage. Aphids were individually placed on the center adaxial leaf surface of the first trifoliolate using a fine paintbrush. All plants grouped and remained caged by treatment (BugDorm-44545F) under laboratory conditions (25± 2°C, 16:8 L:D, RH = 60-80%) for approximately 72 h, after which the total number of aphids on each plant was immediately quantified for all structures of each plant. Excised leaves and stems were placed under a stereomicroscope and the number of live individuals recorded. ANOVA (JMP© 13.2.1) was used to analyze data from the seed treatment verification experiment, with *Plant Identity* (NST, ST) as the independent variable and aphids per plant as the dependent variable.

Reproduction of spider mites on thiamethoxam-treated soybean plants. *TSSM clip cage experiment*. The first experiment focused on quantifying spider mite reproduction when mites were spatially confined on living, non-damaged NST and ST soybeans. We used female spider mites from an established colony reared on non-neonicotinoid treated soybean plants (14R09N), thereby creating conditions in which all spider mites had no previous direct or indirect exposure to thiamethoxam. Individual plants (V2 growth stage) were considered replicates, and two trials were conducted; in the first trial there were 12 replicates of each seed treatment (NST and ST), and 15 replicates in the second trial.

In the first trial, one adult female spider mite (versus three adult female spider mites in the second trial) was placed on a single rectangular soybean leaf strip (as described earlier) and transferred to the adaxial surface of the middle leaflet of the first trifoliolate of each experimental

plant. Spider mites were immediately enclosed within a circular plastic clip cage (H1.0cm x 5.0 cm diam.) placed over the leaf, leaving a gap (~2 mm) so that mites could move to the abaxial leaf surface. The top and bottom of each clip cage was covered with mesh (150 mesh per cm<sup>2</sup>) and glued to a hair clip (L11.0 x W1.4 cm). The inner edges of the cage in contact with the leaf were ringed with foam (thickness 0.33 cm) to minimize potential leaf damage. Each clip cage was supported with wire (16 gauge) connected to a wooden stake (L15.0 x W1.3 cm) positioned within the potting soil so that plant petioles were not visibly twisted or stressed. Plants were not fertilized, although tap water was added twice a week (100-200 mL), and the bottom of each pot was placed within a plastic self-sealing bag (L16.5 x W14.9 cm) to prevent potential interpot movement of the chemical seed treatment during watering. Plants were caged (BugDorm-44545F), and pot and cage position within the greenhouse rotated every 24 h.

Spider mites were allowed to oviposit for 10 d. At the end of the experiment, each trifoliolate with a clip cage was detached from the plant, placed in a labeled plastic bag, and refrigerated ( $4 \pm 2^\circ\text{C}$ ) until processed (within 24 h). Processing involved removing the clip cage and counting all spider mite life stages (egg, larva, protonymph, deutonymph, adult) using a stereo microscope.

### **2.2.1. TSSM whole plant experiment.**

To further investigate the effects of thiamethoxam on TSSM, we added adult females to intact NST and ST soybean plants and did not restrict mite movement. This experiment followed the same design, protocols, and conditions as the TSSM clip cage experiment, but mites were allowed to move freely on the plant, and there were 15 replicates of each treatment. After ten d, above ground plant structures were cut, placed into labeled bags, and refrigerated ( $4 \pm 2^\circ\text{C}$ ) until processed (within 24 h). The densities and within-plant location of all TSSM life stages (egg,

larva, protonymph, deutonymph, adult) were assessed by scanning plant material under a dissecting microscope.

*Statistical analysis: spider mite only experiments.* All data were analyzed using JMP© 13.2.1 (SAS Institute, Cary, NC, USA), and histograms, Shapiro-Wilk W test, Levene's test, and residual plots used to ensure data met the assumptions needed for parametric analysis. ANOVA was used to analyze data from the TSSM-only clip cage experiment, with *Plant Identity* (NST, ST) as the independent variable, trial as a random effect, and offspring per female or total mites per clip cage as the dependent variable. Three data points (trial 1: NST  $n = 2$ , ST  $n = 1$ ) were excluded from the analysis because mites escaped from clip cages. Offspring per female could not be calculated from arenas lacking females at the end of the experiment (trial 1: NST  $n = 0$ , ST  $n = 1$ ; trial 2: NST  $n = 0$ , ST  $n = 2$ ), and thus data from those arenas were not included in that analysis. In the whole plant experiment, *Seed Treatment* was the independent variable, and offspring per female, total mites per plant (log transformed), or percentage of mites found on the original infestation site (arcsin transformed) was the dependent variable.

### **2.2.2. Influence of thiamethoxam on WFT feeding: TSSM eggs only.**

Influence of thiamethoxam on WFT consumption of TSSM eggs and/or soybean leaf tissue. We conducted a no-choice lab experiment to evaluate adult female thrips (WFT) consumption of spider mite eggs from TSSM females reared on NST or ST plants in the absence of plant cues. We also examined whether WFT rearing origin (NST or ST soybeans) influenced feeding preferences. There were two separate blocks, with each block consisting of 12 replicates of each treatment combination (*TSSM origin x WFT origin*).

Experimental arenas consisted of plastic Petri dishes (38.5 mm diameter) with holes (1.6 mm) drilled in the lid to reduce condensation. Mesh (150 x 150 mesh per  $\text{cm}^2$ ) was glued over

the holes, and the bottom lid ringed with modeling clay (W2.0 mm; Plastina Von Aken®, Von Aken International, Rancho Cucamonga, CA) to prevent arthropods from leaving arenas. Experimental arenas were partially filled with a plaster powder (Plaster of Paris, Dap Products Inc., Baltimore, MD) and water mixture (1:1 g/mL, dried for 48 hr) to serve as moisture reservoirs (Rohde Jr. 1956). Arenas were reused by blocks only for repeated experiments following the same order and treatment assignments, otherwise new arenas were constructed. Experimental arenas were saturated with water immediately prior to experimental trials. Green paper circles (89 g/m<sup>2</sup>, r = 15.8 mm, area = 784 mm<sup>2</sup>; Earthchoice Colors®, Domtar) were placed into the center of arenas to mimic leaf discs. Paper discs absorbed water from contacting saturated plaster, thereby creating conditions in which desiccation of TSSM eggs and WFT was minimized and thrips could not get under the paper disc.

In order to collect a same age cohort of TSSM eggs for use in experiments, three female TSSM adults were selected from a colony NST or ST soybean plant. TSSM females were placed on the middle leaflet of the first trifoliolate of a caged soybean (NST or ST, respectively), and allowed to oviposit for 24 h. Leaves with eggs were removed from plants, adult female removed, and leaves refrigerated within self-sealing plastic bags (4 ± 2°C, L16.5 x W14.9 cm) for approximately 24 h prior to experiments. Ten spider mite eggs were transferred to the center of each green paper circle using a fine point paintbrush and arranged such that eggs were evenly spaced (~ 1 mm apart).

Adult female thrips (time elapsed from parental generation approximately 12 weeks) were collected from colonies reared on NST and ST soybean plants as described previously. One thrips was added to the center of each experimental arena using a fine point paintbrush. Experimental arenas were placed in an incubator (MIR-253, Sanyo Electric Co. Ltd., Osaka,

Japan) under constant light ( $22 \pm 2^\circ\text{C}$ , RH = 60-80%) for 72 h, after which arenas were removed and thrips survival, size (as previously described), and number of spider mite eggs remaining were recorded.

### **2.2.3. Influence of thiamethoxam on WFT feeding: soybean leaf tissue only.**

WFT herbivory and feeding behavior may be influenced by thiamethoxam seed treatments and previous exposure to thiamethoxam. We conducted an experiment using a 2 X 2 factorial design with NST and ST soybean leaf discs and adult female WFT from NST and ST colonies. WFT individuals used for this experiment originated from colonies reared on NST and ST plants for approximately six months. We used similar methods to the TSSM egg only experiment, and there were two blocks with 12 replicates of each treatment. Circular leaf discs (radius = 15.8 mm, total area =  $784 \text{ mm}^2$ ) were cut from first trifoliolate soybean leaves using a hollow metal dowel, and placed into experimental arenas (38.5 mm diam.) with underside of each leaf facing up. Modeling clay was pressed around the perimeter of each arena, thereby sealing the leaf and preventing WFT from moving underneath the leaf disc. Individual WFT were placed within an experimental arena using a fine point paintbrush. Lids were placed on experimental arenas, which were then placed into an incubator under constant light for 72 h ( $22 \pm 2^\circ\text{C}$ , RH = 60-80%).

At the end of the experiment WFT from each arena were documented as alive, dead, or missing, and placed into labeled ethanol filled vials until body size was quantified as previously described. We used a stereo microscope to quantify the number of visible leaf scars, which we described as discolored necrotic patches on leaf surfaces (Reitz 2009) beginning at minimum  $1.60 \text{ mm}^2$  of visibly distinct damaged tissue enclosed by undamaged tissue, and total leaf area damaged. A transparent plastic sheet with a grid (10x10 grid squares per square inch, individual

square area = 6.5 mm<sup>2</sup>; ALVIN Cross Section Paper, ALVIN & Co. Inc. Bloomfield, CT) was placed over the abaxial surface of each leaf disc. We documented leaf damage by tracing each leaf scar on the transparency sheet with a permanent marker and then calculated total leaf area consumed, and leaf area per scar.

#### **2.2.4. Influence of thiamethoxam on WFT feeding: combination of TSSM eggs, soybean leaf.**

We evaluated thiamethoxam effect on WFT feeding when both TSSM eggs and soybean leaf tissue were present by providing WFT with both food items simultaneously within experimental arenas. We followed similar protocols as outlined in TSSM only and soybean leaf tissue only experiments. We used a full factorial design combining NST and ST for each factor (*WFT origin* x *TSSM origin* x *plant identity*), with 6 replicates in two separate blocks ( $n = 96$ ). WFT survival, body size, number of TSSM eggs remaining, number of leaf scars, and total leaf area damaged were assessed after 72 h.

*Statistical analyses: WFT feeding trials.* All data were analyzed using JMP© 13.2.1 (SAS Institute, Cary, NC, USA), and histograms, Shapiro-Wilk  $W$  test, Levene's test, and residual plots used to ensure data met the assumptions needed for parametric analysis. Data from arenas where thrips were dead or missing at the end of the experiment were excluded from all analyses, except those concerning the number of missing and dead thrips.

We used correlation analysis to examine relationships between thrips length and width. Body size and mass can impact the volumetric amount of food an insect consumes (Chown and Nicolson 2004) and initially we had planned to use this parameter as a covariate in our analyses of treatment effects (*Plant Identity*, *TSSM origin*, and/or *WFT origin*) on thrips feeding; however, when we used factorial ANOVA to investigate treatment effects on thrips length, we found that

in some cases treatments impacted thrips size (see results section), thus preventing the use of thrips size as a covariate (Urquhart 1982, Quinn and Keough 2002, Huitema 2011). Therefore, in analyses of treatment effects on thrips feeding, we adjusted the amount of food consumed (*i.e.*, number of TSSM eggs, area of leaf tissue eaten, number of leaf scars, or area per leaf scar) by dividing by thrips length to get amount of food consumed per unit thrips length. We felt that length was a more appropriate metric to use for the adjustment of food consumption, as width, which was measured at the widest point of the abdomen at the end of the experiment, might have been impacted by the amount of food consumed.

Contingency analyses and Pearson's chi-squared test were used to test the impact of independent variables on the incidence of WFT missing and dead at the end of experiments and the likelihood of thrips feeding (*i.e.*, creating at least one feeding scar, at least on spider mite egg consumed). For experiments examining WFT feeding, factorial ANOVA was used to test the impact of independent variables (*Plant Identity*, *TSSM origin* and/or *WFT origin*), on the dependent variables: number of TSSM eggs per unit thrips length (SQRT X+0.5 transformed), leaf area damaged per unit thrips length (LOG X+1 transformed), number of leaf scars per unit thrips length (SQRT X+0.5 transformed), and leaf area damage per leaf scar (SQRT X+0.5 transformed) with block as a random variable. Each dependent variable was analyzed separately.

In order to assess how experimental treatments impacted total thrips feeding (TSSM eggs + leaf tissue), and the degree to which thrips fed on TSSM eggs versus leaf tissue, we first converted each type of data to total volume of food consumed. The volume of soybean leaf tissue consumed was calculated by multiplying the total damaged leaf surface area (mm<sup>2</sup>) by soybean leaf thickness. Pfeifer et al. (2018) found the average thickness of a soybean leaf (tissue not directly adjacent to the midrib, as per our leaf arenas), to be approximately 0.20 mm, and since

thrips prefer foraging from the abaxial side of leaves (Fiene et al. 2013), we estimated soybean leaf thickness eaten to be 0.10 mm. TSSM eggs are spherical, and so we calculated the volume (V) of TSSM eggs consumed by using  $V = 4/3 \pi r^3$ , with  $r = 0.07$  mm, since the diameter of TSSM eggs has been reported as 0.140 mm (Helle and Sabelis 1985). Volume of TSSM eggs eaten was then added to volume of leaf tissue eaten to calculate a total cubic volume of food eaten per arena. Data were analyzed using factorial ANOVA, with total volume of food consumed per arena (LOG X+1 transformed) as the dependent variable, *Plant Identity*, *TSSM origin*, and *WFT origin* as independent variables, and block as a random variable.

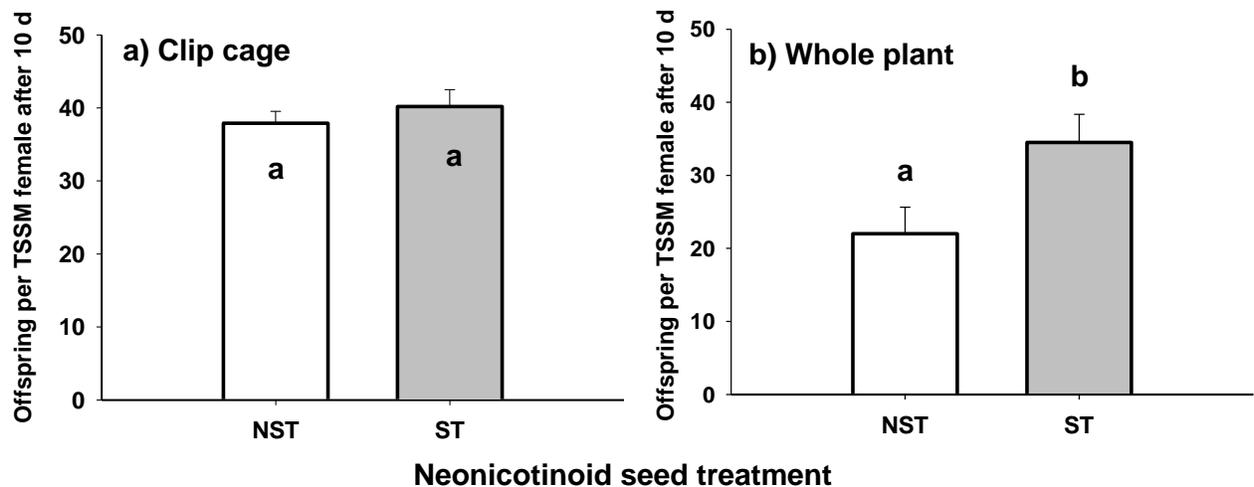
To evaluate the degree to which thrips fed on TSSM eggs versus leaf tissue, we considered each TSSM egg eaten and each leaf scar as a distinct feeding event. We then divided the number of leaf scars by the total number of feeding events (leaf scars + eggs eaten), which was adjusted by WFT length and calculated the percentage of 'herbivorous feeding events,' and arcsine transformed. Data were analyzed using factorial ANOVA, with *Plant Identity*, *TSSM origin* and *WFT origin* as independent variables, and block as a random variable.

### 2.3. Results

Verification of seed treatment efficacy. We used soybean aphid density as a proxy to verify the efficacy of thiamethoxam seed treatment. Three d after infesting plants with three adult aphids, there were no aphids on any of the ST plants, whereas  $28.67 \pm 2.46$  were present on NST plants ( $df_{1,4}$ ,  $F = 67.85$ ,  $P = 0.001$ ), which strongly suggests thiamethoxam was present in plant tissues.

Effects of ingesting thiamethoxam-treated soybean leaves on spider mite fecundity. After 10 d, densities of offspring per TSSM female within clip cages on NST plants and ST plants were equivalent (Fig. 1a; *Plant Identity*:  $df_{1,45}$ ,  $F = 0.560$ ,  $P = 0.458$ ). Results were similar when

data on the total number of mites per cage were analyzed with both trials together ( $df_{1,45}$ ,  $F = 1.40$ ,  $P = 0.243$ ), or each trial separately (Trial 1:  $P = 0.891$ ; Trial 2:  $P = 0.241$ ; data not shown). Overall, the number of offspring per spider mite female was lower in the whole plant experiment than the clip cage experiment, even though both lasted 10 d (Fig. 1a-b). In the whole plant experiment, offspring per female was higher on ST plants (*Plant Identity*:  $df_{1,29}$ ,  $F = 5.58$ ,  $P = 0.028$ ). Results were similar for total mites per plant ( $df_{1,29}$ ,  $F = 8.28$ ,  $P = 0.008$ ; data not shown). After 10 d, the majority of the mite population was on the leaf (abaxial or adaxial surface) to which the adult females had been added (NST = 71.1%, ST = 86.1%), which was similar among seed treatments ( $df_{1,29}$ ,  $F < 0.001$ ,  $P = 0.993$ ; data not shown).

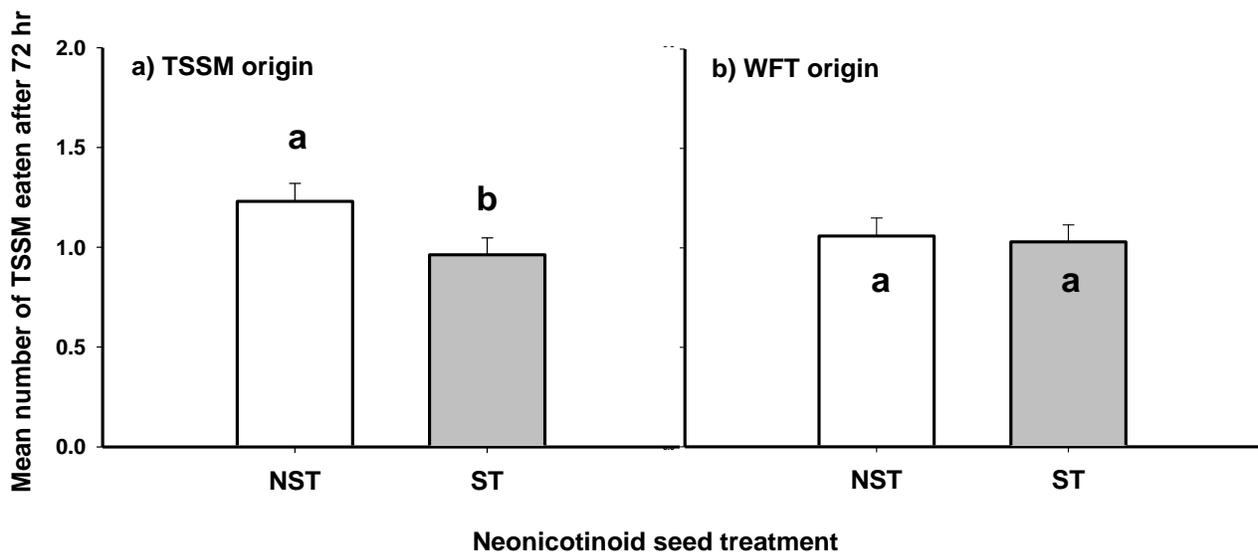


**Figure 1.** Mean number of offspring  $\pm$  SEM per recovered adult female spider mite at the end of 10 d in a) the clip cage experiment, b) the whole-plant experiment. Different letters indicate significance at  $P \leq 0.05$ .

Influence of thiamethoxam on WFT feeding: TSSM eggs only. At the end of the experiment, 6.3% of arenas ( $n = 96$ ) had a dead thrips ( $n = 6$ ), and the thrips could not be found (*i.e.*, was missing) in 10.4% of arenas ( $n = 10$ ). The number of missing or dead thrips was not impacted by TSSM origin (Pearson  $\chi^2 = 2.72$ ,  $P = 0.257$ ), WFT origin (Pearson  $\chi^2 = 2.32$ ,  $P =$

0.314), or block (Pearson  $\chi^2 = 1.27$ ,  $P = 0.531$ ). Thrips body length averaged  $1.38 \pm 0.02$  mm (mean  $\pm$  SE), and was strongly correlated with thrips body width ( $R^2 = 0.834$ ,  $P < 0.0001$ ; data not shown). Thrips were slightly longer in arenas that received TSSM eggs reared on treated soybeans (*TSSM origin*:  $df_{1,77}$ ,  $F = 3.877$ ,  $P = 0.053$ ), although thrips origin did not impact thrips length (*TSSM origin x WFT origin*:  $df_{1,77}$ ,  $F = 3.517$ ,  $P = 0.065$ ; *WFT origin*:  $df_{1,77}$ ,  $F = 0.902$ ,  $P = 0.345$ ; data not shown).

Consumption of TSSM eggs was limited during the 72 h trial period, although more TSSM eggs (per unit thrips length) were eaten in arenas where eggs originated from TSSM females reared on NST plants (Fig. 2a; *TSSM origin*:  $df_{1,74}$ ,  $F = 4.40$ ,  $P = 0.039$ ), which was not impacted by thrips origin (Fig. 2b; *TSSM origin x WFT origin*:  $df_{1,74}$ ,  $F = 0.751$ ,  $P = 0.389$ ; *WFT origin*:  $df_{1,73}$ ,  $F = 0.147$ ,  $P = 0.702$ ).

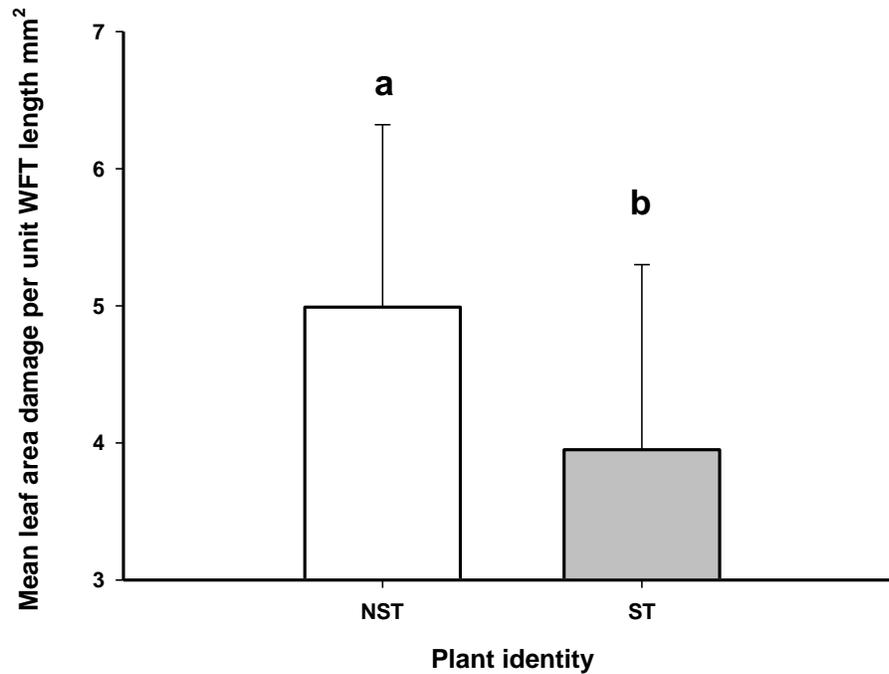


**Figure 2.** Mean number of TSSM eggs  $\pm$  SEM eaten within arenas at the end of 72 h in the absence of plant cues per unit thrips length, as impacted by a) TSSM origin, b) WFT origin. Different letters indicate significance at  $P \leq 0.05$ .

Influence of thiamethoxam on WFT feeding: soybean leaf tissue only. At the end of the experiment, we analyzed possible treatment effects on the number of dead ( $n = 8$ ) and missing ( $n = 5$ ) thrips. The number of missing or dead thrips was not impacted by WFT origin (Pearson  $\chi^2 = 0.80$ ,  $P = 0.371$ ), but was lower on ST leaf arenas (Pearson  $\chi^2 = 4.36$ ,  $P = 0.037$ ), and in trial 2 (Pearson  $\chi^2 = 4.36$ ,  $P = 0.037$ ).

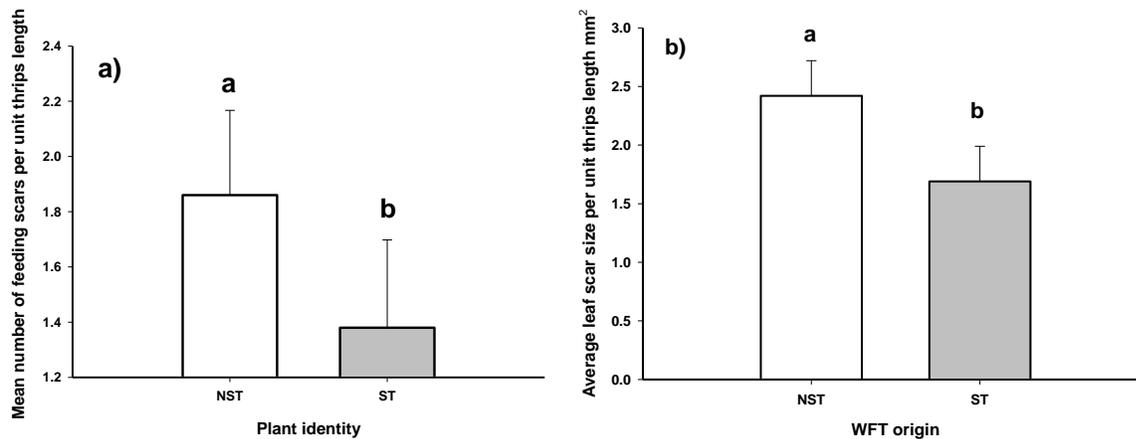
Thrips body length averaged  $1.38 \pm 0.02$  mm (mean  $\pm$  SE), and was strongly correlated with thrips body width ( $R^2 = 0.713$ ,  $P < 0.0001$ ; data not shown). Thrips originating from ST plants were longer in length (*WFT origin*: NST =  $1.33 \pm 0.06$ , ST =  $1.44 \pm 0.06$ ;  $df_{1,78}$ ,  $F = 5.674$ ,  $P = 0.020$ ), regardless of what type of leaf disc (NST or ST) thrips were placed on (*Plant Identity x WFT origin*:  $df_{1,78}$ ,  $F = 3.220$ ,  $P = 0.077$ ; *Plant Identity*:  $df_{1,78}$ ,  $F = 0.110$ ,  $P = 0.741$ ).

There was a higher likelihood that thrips placed on treated ST leaf discs would not feed (*i.e.*, did not produce any feeding scars; Pearson  $\chi^2 = 4.91$ ,  $P = 0.027$ ) regardless of thrips origin (Pearson  $\chi^2 = 1.18$ ,  $P = 0.278$ ). Overall, the average leaf area damaged was  $6.56 \pm 0.63$  (mean  $\pm$  SEM) mm<sup>2</sup> per leaf disc ( $4.59 \pm 0.40$  per unit thrips length). The leaf area damaged (per unit length thrips) was lower on ST leaf discs (*Plant Identity*:  $df_{1,78}$ ,  $F = 4.516$ ,  $P = 0.037$ ; Fig. 3), regardless of thrips origin (*Plant Identity x WFT origin*:  $df_{1,78}$ ,  $F = 0.130$ ,  $P = 0.720$ ; *WFT origin*:  $df_{1,78}$ ,  $F = 0.117$ ,  $P = 0.734$ ). The total leaf area damaged per unit thrips length was positively correlated with the number of leaf scars per unit thrips length ( $R^2 = 0.784$ ,  $P < 0.0001$ ; data not shown).



**Figure 3.** Mean leaf area damaged  $\pm$  SEM per unit WFT length on NST and ST soybean leaf discs after 72 h in the absence of spider mites. Different letters indicate significance at  $P \leq 0.05$ .

Overall, thrips produced an average of  $2.34 \pm 0.20$  (mean  $\pm$ SE) feeding scars per leaf disc ( $1.66 \pm 0.13$  per unit thrips length). The total number of leaf scars (per unit length thrips) was lower on ST leaf discs (*Plant Identity*:  $df_{1,78}$ ,  $F = 5.061$ ,  $P = 0.027$ ; Fig. 4a), although there was no impact of thrips origin (*Plant Identity*  $\times$  *WFT origin*:  $df_{1,78}$ ,  $F = 0.468$ ,  $P = 0.496$ ; *WFT origin*:  $df_{1,78}$ ,  $F = 0.697$ ,  $P = 0.406$ ). The average leaf scar size was  $2.92 \pm 2.00$  (mean  $\pm$ SEM) mm<sup>2</sup> per leaf disc ( $2.11 \pm 1.32$  per unit thrips length). Average feeding scar size (per unit length thrips) was lower for WFT originating from ST colonies (*WFT origin*:  $df_{1,71}$ ,  $F = 7.373$ ,  $P = 0.008$ ; Fig. 4b), although other main and interaction effects were not significant (*Plant Identity*:  $df_{1,71}$ ,  $F = 0.559$ ,  $P = 0.456$ ; *Plant Identity*  $\times$  *WFT origin*:  $df_{1,71}$ ,  $F = 1.719$ ,  $P = 0.194$ ).



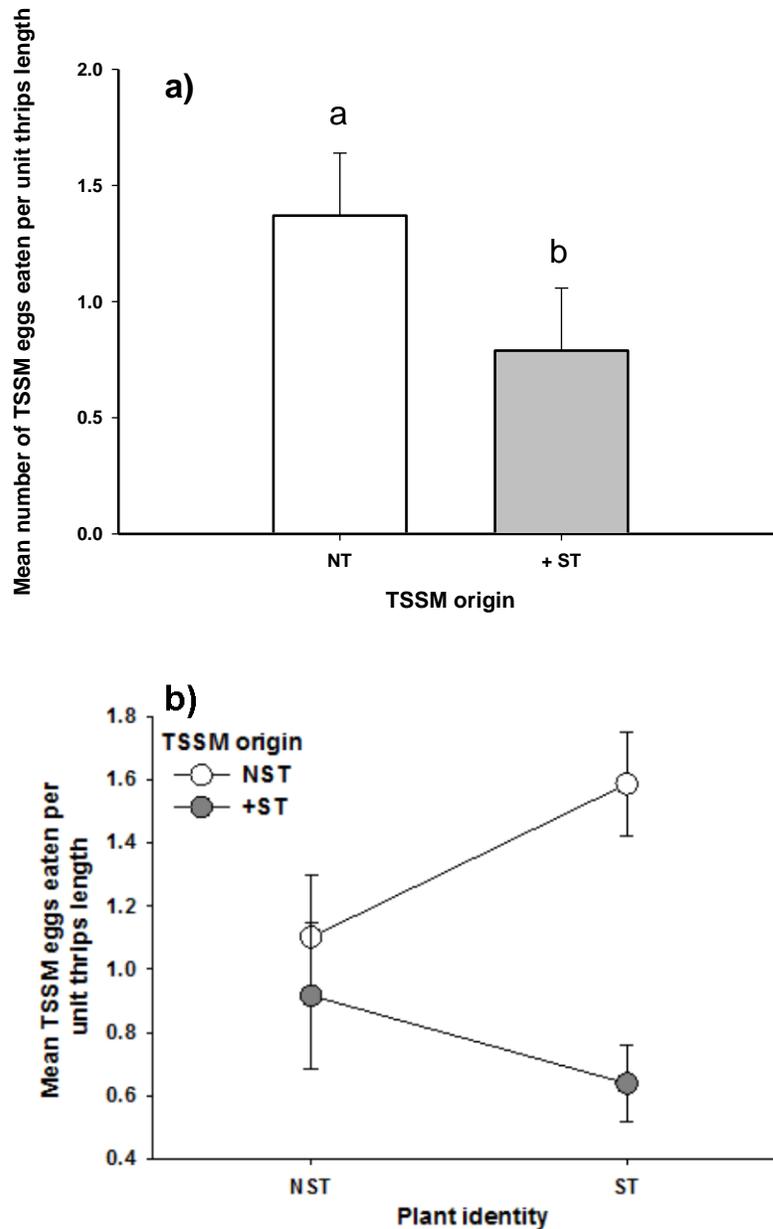
**Figure 4.** Mean number of a) leaf scars  $\pm$  SEM b) average size of leaf scar per unit WFT length on NST and ST soybean leaf discs after 72 h in the absence of spider mite eggs. Different letters indicate significance at  $P \leq 0.05$

Influence of thiamethoxam on WFT feeding: combination of TSSM eggs and soybean leaf tissue. At the end of the experiment, 9.37% of arenas had a dead thrips ( $n = 9$  of 96) and the thrips could not be found (*i.e.*, was missing) in 6.25% of arenas ( $n = 6$  of 96). However, the total number of arenas with missing or dead thrips were not affected by plant identity (Pearson  $\chi^2 = 1.970$ ,  $P = 0.159$ ), TSSM origin (Pearson  $\chi^2 = 0.079$ ,  $P = 0.779$ ), or WFT origin (Pearson  $\chi^2 = 0.790$ ,  $P = 0.790$ ). Thrips body length averaged  $1.35 \pm 0.03$  mm (mean  $\pm$  SE), and was strongly correlated with thrips body width ( $R^2 = 0.802$ ,  $P < 0.0001$ ; data not shown). Unlike previous experiments, thrips body length was not impacted by any of the treatments (all main effects and interactions,  $P > 0.220$ ).

In general, the number of TSSM eggs consumed per unit thrips length was similar to the experiment when eggs were presented to thrips on green paper instead of a leaf disc. Thrips ate more TSSM eggs when eggs originated from female spider mites reared on NST plants (TSSM origin:  $df_{1,60}$ ,  $F = 9.362$ ,  $P = 0.003$ ; Fig. 5a), however, this effect was more pronounced when

thrips were on ST leaf discs (*Plant Identity* x *TSSM origin*:  $df_{1,60}$ ,  $F = 2.199$ ,  $P = 0.043$ ; Fig. 5b).

All other main and interactive effects were not significant (Table 1).

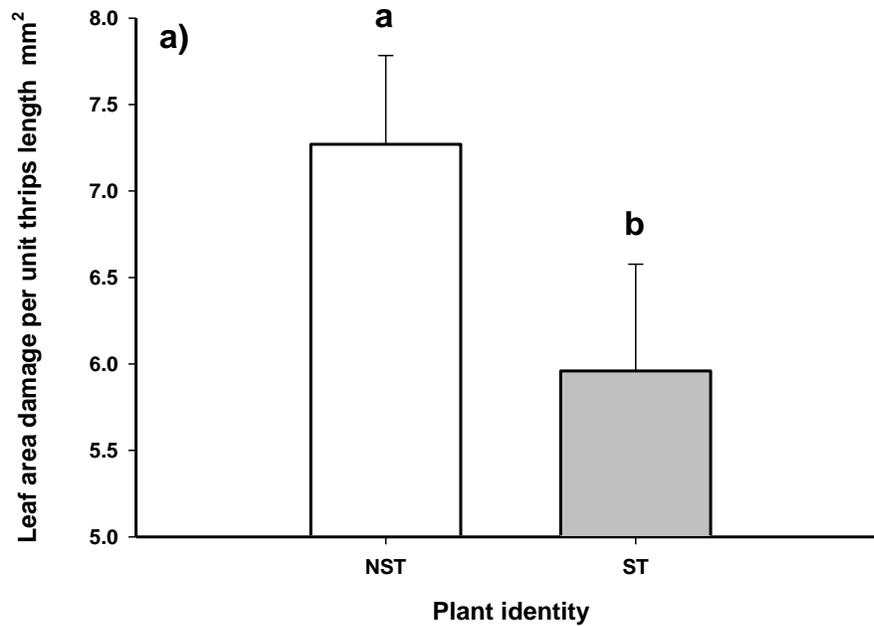


**Figure 5.** Mean number of a) TSSM eggs consumed  $\pm$  SEM by WFT after 72 h on soybean leaf discs by TSSM origin b) Interaction between plant identity and TSSM origin on mean number of eggs eaten by WFT per unit thrips length. Different letters indicate significance at  $P \leq 0.05$ .

**Table 1.** Factorial analysis of variance for effects of plant identity, TSSM origin, and WFT origin on number of TSSM eggs per unit thrips length consumed after 72 h.

Source	df	F	P
<i>Plant identity</i>	1, 60	0.894	0.348
<i>TSSM origin</i>	1, 60	9.362	<b>0.003</b>
<i>WFT origin</i>	1, 60	0.142	0.708
<i>Plant identity X TSSM origin</i>	1, 60	4.283	<b>0.043</b>
<i>Plant identity x WFT origin</i>	1, 60	0.069	0.794
<i>TSSM origin x WFT origin</i>	1, 60	0.464	0.497
<i>Plant identity x TSSM origin x WFT origin</i>	1, 60	2.199	0.143

The total leaf area damaged was positively associated with the total number of leaf scars ( $R^2 = 0.720$ ,  $P < 0.0001$ ; data not shown). Overall, the average leaf area damaged was  $8.91 \pm 0.63$  (mean  $\pm$ SE) mm<sup>2</sup> per leaf disc ( $6.56 \pm 0.49$  per unit thrips length), which was slightly higher than the previous leaf-only experiment. Leaf area damaged per thrips length was lower on ST leaf discs (*Plant Identity*:  $df_{1,60}$ ,  $F = 6.143$ ,  $P = 0.016$ ; Fig. 6), and was not impacted by the other treatments (Table 2).

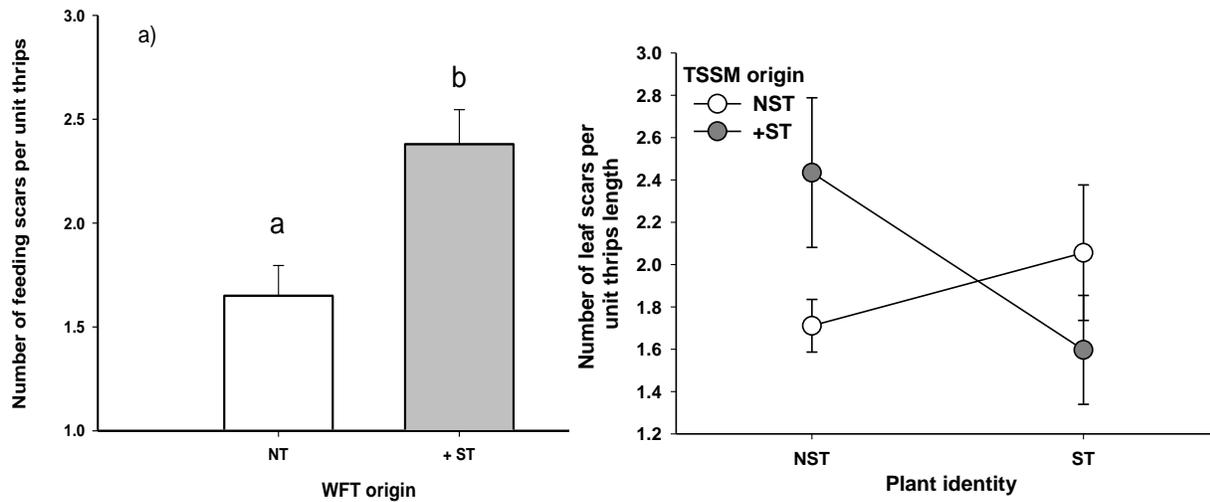


**Figure 6.** Mean leaf area damaged per unit WFT length  $\pm$  SEM on NST and ST soybean leaf discs after combination of TSSM eggs and soybean leaf tissue were available to WFT for 72 hr. Different letters indicate significance at  $P \leq 0.05$ .

**Table 2.** Factorial analysis of variance for effects of plant identity, TSSM origin, and WFT origin on total leaf area damage per unit thrips length after 72 h.

Source	df	F	P
<i>Plant identity</i>	1, 60	6.143	<b>0.016</b>
<i>TSSM origin</i>	1, 60	0.144	0.706
<i>WFT origin</i>	1, 60	0.407	0.526
<i>Plant identity X TSSM origin</i>	1, 60	1.926	0.170
<i>Plant identity x WFT origin</i>	1, 60	0.512	0.477
<i>TSSM origin x WFT origin</i>	1, 60	0.917	0.342
<i>Plant identity x TSSM origin x WFT origin</i>	1, 60	1.329	0.254

None of the treatments impacted the likelihood of thrips feeding on leaf discs (*i.e.*, no feeding scars) (for all effects: Pearson  $\chi^2 < 2.34$ ,  $P > 0.125$ ). Overall, thrips produced an average of  $2.65 \pm 0.17$  (mean  $\pm$ SE) feeding scars per leaf disc ( $1.92 \pm 0.13$  per unit thrips length), which was slightly higher than the previous leaf-only experiment. The total number of leaf scars (per unit length thrips) was higher for thrips reared on ST soybeans (*WFT origin*:  $df_{1,61}$ ,  $F = 6.207$ ,  $P = 0.016$ ; Fig. 7a). However, the number of leaf scars depended on both plant identity and TSSM origin (*Plant Identity*  $\times$  *TSSM origin*:  $df_{1,61}$ ,  $F = 4.807$ ,  $P = 0.032$ ). When TSSM eggs from females reared on NST soybeans were present, the number of leaf scars was slightly higher on ST leaf discs compared to NST leaf discs. This pattern was reversed on ST leaf discs, with the highest number of leaf scars on arenas with TSSM eggs from females reared on ST soybeans (Fig. 7b). All other main and interactive effects were not significant (Table 3).



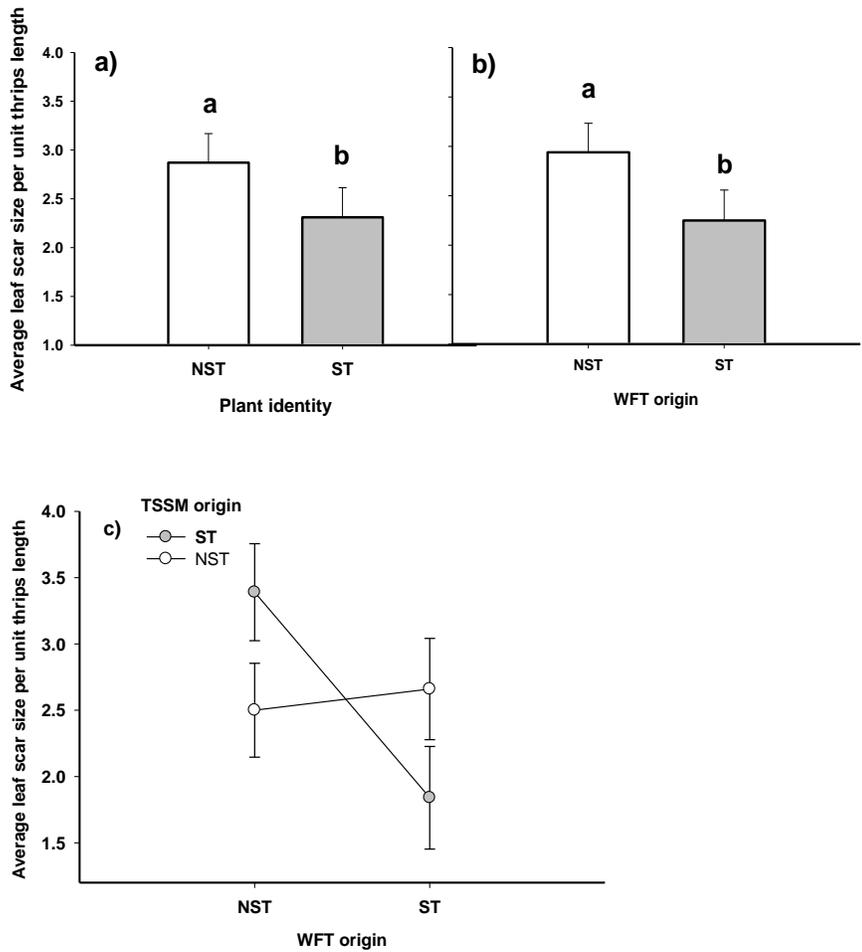
**Figure 7.** Mean number of a) feeding scars  $\pm$  SEM produced by WFT after 72 h b) Interaction between plant identity and TSSM egg origin on number of feeding scars. Different letters indicate significance at  $P \leq 0.05$ .

**Table 3.** Factorial analysis of variance for effects of plant identity, TSSM origin, and WFT origin on total number of leaf scars per unit thrips length after 72 h.

Source	df	F	P
<i>Plant identity</i>	1, 60	0.876	0.353
<i>TSSM origin</i>	1, 61	0.291	0.592
<i>WFT origin</i>	1, 60	6.207	<b>0.016</b>
<i>Plant identity X TSSM origin</i>	1, 61	4.807	<b>0.032</b>
<i>Plant identity x WFT origin</i>	1, 60	0.000	0.864
<i>TSSM origin x WFT origin</i>	1, 61	0.084	0.542
<i>Plant identity x TSSM origin x WFT origin</i>	1, 61	2.506	0.115

Overall, the size of an average thrips feeding scar was  $3.56 \pm 0.17$  (mean  $\pm$  SE) per leaf disc ( $2.69 \pm 0.16$  per unit thrips length), which was higher than the leaf-only experiment. Leaf scar size (per unit length thrips) was lower on ST leaf discs ( $2.32 \pm 0.32$ ; *Plant Identity*:  $df_{1,56}$ ,  $F = 4.070$ ,  $P = 0.048$ ; Figure 8a). However, average leaf scar size was dependent on TSSM and

WFT origin (*TSSM origin x WFT origin*:  $df_{1,56}$ ,  $F = 6.821$ ,  $P = 0.011$ ; Fig. 8b) Although NST thrips produced lower scar sizes, ST thrips were comparatively similar when NST eggs were available to both NST, ST thrips regardless of plant identity. However, when eggs from females reared on ST plants were present to NST thrips, average scar size was highest. This pattern was reversed when ST thrips were given ST eggs, resulting in lowest average scar size. All other main and interactive effects were not significant (Table 4).

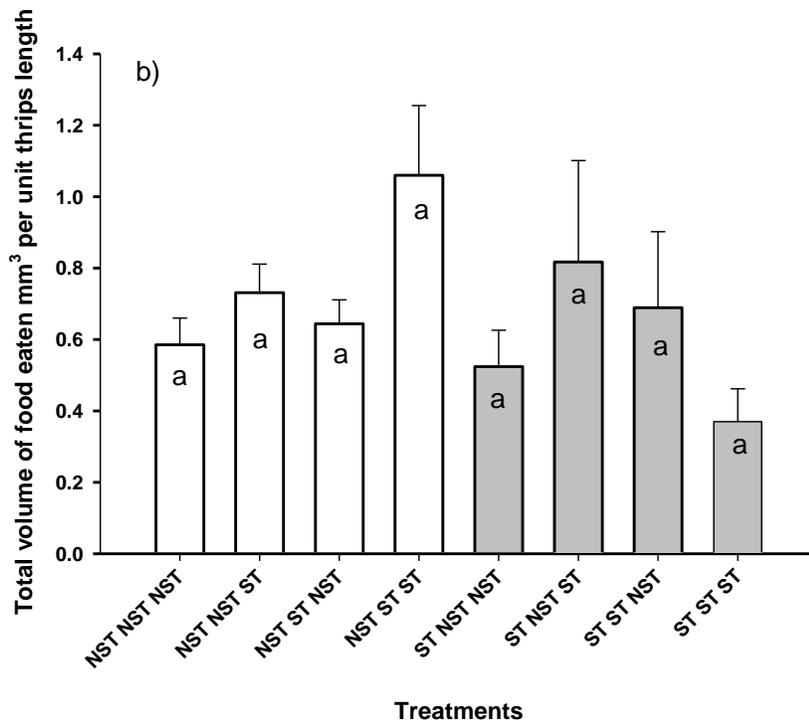
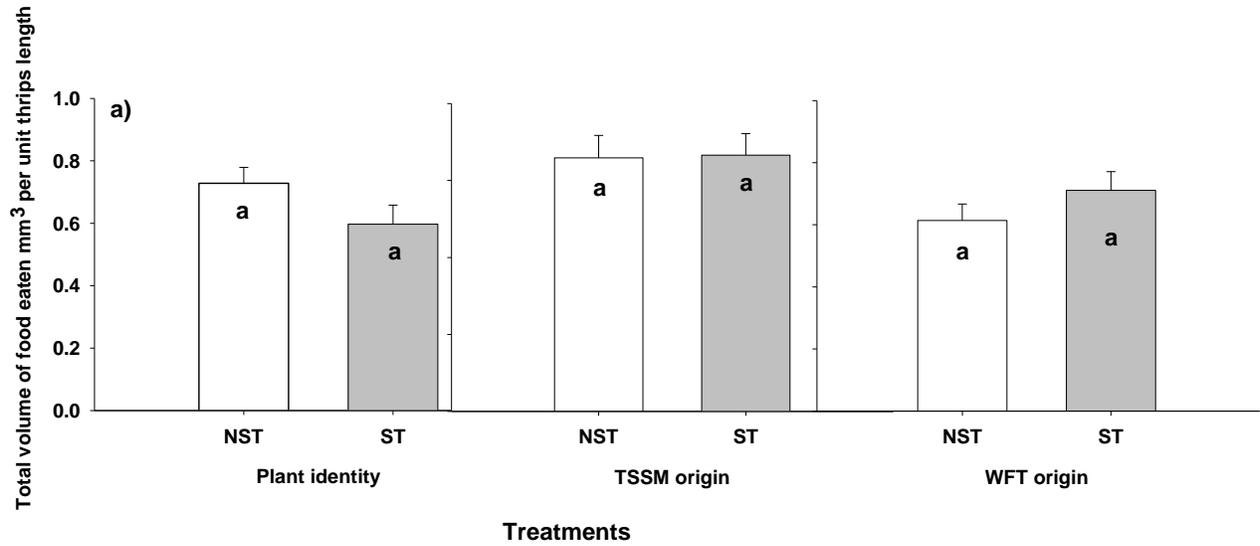


**Figure 8.** Average size of leaf scar  $\pm$  SEM per unit WFT length by a) plant identity, b) WFT origin c) Interaction between TSSM egg and WFT origin on average scar size after 72 h. Different letters indicate significance at  $P \leq 0.05$ .

**Table 4.** Factorial analysis of variance for effects of plant identity, TSSM egg origin, and WFT origin on mean leaf scar size per WFT length after 72 h.

Source	df	F	P
<i>Plant identity</i>	1, 56	4.070	<b>0.048</b>
<i>TSSM origin</i>	1, 56	0.003	0.956
<i>WFT origin</i>	1, 56	5.483	<b>0.022</b>
<i>Plant identity X TSSM origin</i>	1, 56	0.509	0.478
<i>Plant identity x WFT origin</i>	1, 56	1.735	0.193
<i>TSSM origin x WFT origin</i>	1, 56	6.821	<b>0.011</b>
<i>Plant identity x TSSM origin x WFT origin</i>	1, 56	0.016	0.897

Overall, the total volume of food consumed by WFT was  $0.89 \pm 0.56 \text{ mm}^3$  (mean  $\pm$  SE) per arena ( $0.65 \pm 0.43$ ) per unit thrips length. Leaf material accounted for almost all of the volume from combined food items, with  $0.89 \pm 0.56 \text{ mm}^3$  (mean  $\pm$  SE) of leaf tissue eaten ( $0.66 \pm 0.40$  per unit thrips length), compared to  $0.002 \pm 0.001 \text{ mm}^3$  of TSSM egg consumed. There was no main or interactive effects of *Plant identity*, *TSSM origin*, or *WFT origin* on the volume of combined food sources consumed (Fig. 9, Table 5).



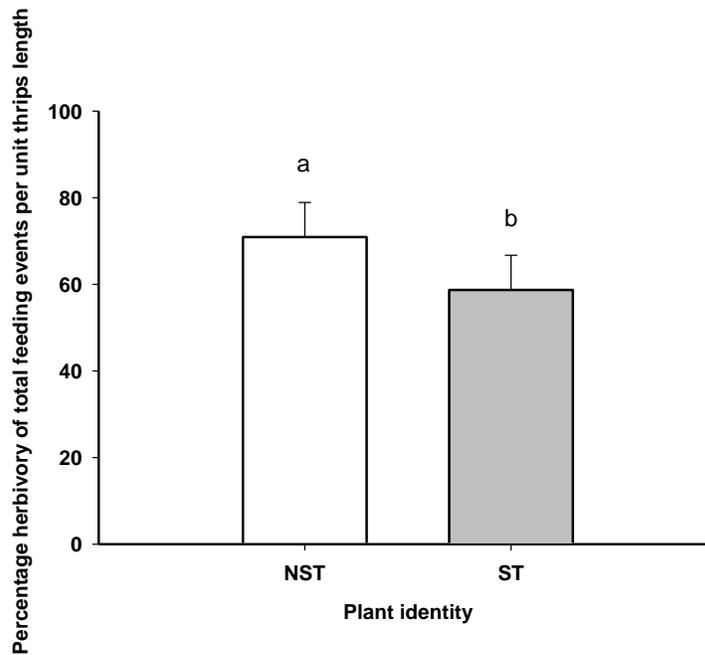
**Figure 9.** Mean total volume food  $\pm$  SEM consumed (TSSM eggs + leaf tissue) per unit WFT length after 72 h by a) independent treatment factors b) combined treatment factors.

**Table 5.** Factorial analysis of variance for effects of plant identity, TSSM origin, WFT origin on total volume of food eaten, leaf and TSSM eggs by WFT per unit thrips length after 72 h.

Source	df	F	P
<i>Plant identity</i>	1, 60	3.403	0.070
<i>TSSM origin</i>	1, 60	0.015	0.901
<i>WFT origin</i>	1, 60	0.770	0.383
<i>Plant identity X TSSM origin</i>	1, 60	2.092	0.153
<i>Plant identity x WFT origin</i>	1, 60	0.958	0.331
<i>TSSM origin x WFT origin</i>	1, 60	1.357	0.248
<i>Plant identity x TSSM origin x WFT origin</i>	1, 60	2.285	0.135

With regard to WFT feeding preference, thrips primarily ate a mixed diet where both TSSM eggs and leaf tissue was consumed (56 of 80 arenas = 70%), whereas in a quarter of arenas they only consumed leaf tissue (20 of 80 = 25%), and only ate TSSM eggs in 4 of 80 arenas (5%). There was only one arena where thrips did not eat any food (1 of 80 arenas = 0.1%). Plant identity, TSSM origin, and WFT origin did not affect the feeding preference ratios of different diet selections (Pearson  $\chi^2 = 3.89$ ,  $P = 0.143$ ,  $\chi^2 = 1.40$ ,  $P = 0.497$ ,  $\chi^2 = 1.92$ ,  $P = 0.382$ ).

Overall, total feeding events per arena (leaf scars + eggs) was  $4.11 \pm 2.07$  (mean  $\pm$  SE), or  $2.96 \pm 1.45$  per unit thrips length. Thrips fed significantly more on leaf tissue compared to consuming eggs. Here, herbivory, as defined as number of leaf scars, was strongly favored by thrips by percentage for all feeding events ( $0.647 \pm 0.26$ ,  $0.352 \pm 0.265$ ). A higher percentage of herbivorous feeding events occurred on NST ( $0.709 \pm 0.080$ ) leaf discs compared to ST leaf discs,  $0.587 \pm 0.083$ ; Fig. 10; *Plant identity*:  $df_{1,59}$ ,  $F = 4.129$ ,  $P = 0.046$ ) All other main and interactive effects were not significant (Table 6).



**Figure 10.** Percentage of herbivorous feeding events per unit WFT length  $\pm$  SEM on NST and ST soybean leaf discs after 72 h. Different letters indicate significance at  $P \leq 0.05$ .

**Table 6.** Factorial analysis of variance for effects of plant identity, TSSM origin, and WFT origin on percentage herbivory of feeding events per unit thrips length after 72 h.

Source	df	F	P
<i>Plant identity</i>	1, 59	4.129	<b>0.046</b>
<i>TSSM origin</i>	1, 59	2.552	0.115
<i>WFT origin</i>	1, 59	0.892	0.511
<i>Plant identity X TSSM origin</i>	1, 59	0.435	0.478
<i>Plant identity x WFT origin</i>	1, 59	0.792	0.376
<i>TSSM origin x WFT origin</i>	1, 59	0.740	0.393
<i>Plant identity x TSSM origin x WFT origin</i>	1, 59	0.001	0.968

## 2.4. Discussion

Use of neonicotinoid seed treatments continues to increase within agricultural production systems, including soybeans (Douglas and Tooker 2015). In North America, neonicotinoid seed treatment have increasingly become a common strategy of crop protection against early season target pests such as soybean aphids and bean leaf beetles (Magalhaes et al. 2009, Douglas and Tooker 2015, North et al. 2016). Although effective against a broad range of economically important pests, the net value of using seed treatments may be diminished if their use leads to increased problems with pest resistance (Gao and Reitz 2012, Bass et al. 2015), or increased densities of minor or non-target herbivorous pests such as thrips and spider mites. Increased spider mite populations following neonicotinoid treatment have been previously reported in multiple growing systems (e.g. agricultural, urban) (Sclar et al. 1998, Ako et al. 2006, Szczepaniec and Raupp 2012, Szczepaniec et al. 2011, Smith et al. 2013, Barati and Hejazi 2015). Twospotted spider mites can be found co-occurring with a facultative predator of spider mite eggs, western flower thrips (Wilson et al. 1996). One mechanism contributing to increased spider mite populations is secondary poisoning of generalist arthropod predators of prey feeding of neonicotinoid treated plants (Pozzebon et al. 2011, Szczepaniec et al. 2011). Predation of neonicotinoid tainted arthropod prey has been linked with both lethal and sub-lethal effects on natural enemies. Insect omnivores, including thrips can acquire nutrition from multiple sources (e.g. pollen, leaves, spider mite eggs). Thrips food selection are influenced by source quality (Agrawal and Klein 2000), however thrips feeding response to neonicotinoid treated sources of plant and prey is unclear. Given the widespread use of neonicotinoids, we investigated a neonicotinoid soybean seed treatment, thiamethoxam effects on twospotted spider mite reproductive response and influence on western flower thrips omnivorous feeding responses as

degree of herbivory, spider mite egg predation and diet shifting between both herbivory and predation.

Several mechanisms underlying increased spider mite populations following neonicotinoid treatments have been proposed, including decreased performance, diversity, and abundance of exposed natural enemies (Sanchez-Bayo et al. 2007, Seagraves and Lundgren 2011, Szczepaniec et al. 2011), altered plant defenses (Szczepaniec et al. 2013, Wulff et al. 2019), and physiological stimulation of mite reproduction (James and Price 2002, Barati and Hejazi 2015) and these mechanisms may be operating simultaneously. Predators of herbivorous arthropod pests can be directly exposed to neonicotinoids by contact on plant surfaces or indirectly by consuming prey feeding on treated plants (Torres and Ruberson 2004, Cloyd and Bethke 2011, Szczepaniec et al. 2011, Bredeson et al. 2015, Douglas et al. 2015). Neonicotinoid seed treatments do not require direct contact with insect pests to be effective, as they are systemic insecticides (Tomizawa and Casida 2005) however this property can promote exposure to omnivorous insects. Effects of omnivorous insects are understudied despite increased risks and may respond differently than predatory arthropods. Omnivores are exposed by direct feeding on treated plant resources or indirectly through predation of contaminated prey (Cloyd and Bethke et al. 2011, Prabhaker et al. 2011, Seagraves and Lundgren 2012, Gontijo et al. 2015). How exposed omnivorous insects feeding decisions will be affected may have implications for both shared host plant and herbivorous arthropod prey through increased or decreased feeding of both food sources.

The initial part of this study quantified spider mite reproduction on thiamethoxam-seed treated soybeans when mites were spatially restricted versus free to move over the entire plant. We found increased levels of spider mite reproduction on treated soybeans, compared to non-

treated which parallels results from studies using detached leaf arenas receiving a foliar spray or leaves taken from plants receiving a neonicotinoid soil drench (James and Price 2002, Smith et al. 2013, Barati and Hejazi 2015). Although we found greater spider mite densities on treated soybean whole plants, neonicotinoid stimulated reproductive responses of spider mites may be additionally influenced by plant type or neonicotinoid compound. Sclar (1998) found no effect of granular imidacloprid on spider mite populations using an ornamental as whole plants during greenhouse trials. Our results of thiamethoxam effect of increased spider mite densities disappeared when spider mites were spatially confined which may be related to our methodology. Confining spider mites to a defined area may limit feeding and oviposition sites, similar restricted space studies that used sticky ring and clip cages on whole plants also yielded no significant positive reproductive responses (Ako et al. 2004, Ozsisli and Prischmann-Voldseth 2016). However, in our study, when mites were allowed to roam freely over the plant, 80 percent of the population was found on the initial infestation site, although most did not move, these mites had more resource area available than mites in clip cages. Rotem and Agrawal (2003) observed similar patterns of per capita growth rates as density dependent functions of initial spider mite densities related to available leaf area, and plant size. Concentrations of thiamethoxam within seed-treated soybeans have been found to be highest in the oldest leaves (Magalhaes et al. 2009), in our experiments mites were likely exposed to similar concentrations of insecticide, since we used same-age soybean plants and mites were placed on the same location (i.e. middle leaflet of the same trifoliolate).

We found greater spider mite densities on soybean plants grown from thiamethoxam treated seeds vs non-treated and soybean aphid densities were decreased on treated plants. Therefore, we assumed that we successfully provided thrips with soybean leaf tissue that

contained thiamethoxam and spider mite eggs served as poisoned prey for thrips feeding experiments.

In addition to leaf feeding, thrips will supplement their diet with protein sources such as pollen and spider mite eggs (Trichilo and Leigh 1986, Janssen et al. 2003). Resource quality and diet selection are important factors related to omnivorous thrips life history traits (Trichilo and Leigh 1986, Kogel et al. 1999, Janssen et al. 2003), residence time, and migratory behavior (Maris et al. 2004).

Overall most adult thrips, regardless of exposure to neonicotinoid before or during the trial, survived our three day experiments, however we saw more mortality of thrips during preliminary trials which were 96 hours. Cao et al. (2019) has previously demonstrated that lower levels of another neonicotinoid, imidacloprid, can positively influence thrips reproduction, development, and survival. Additionally, we did not measure other thrips life history parameters (e.g., longevity) after treated feeding experiments; neonicotinoid toxicity can affect thrips differently depending on developmental stage and treated host plant (Shan et al. 2019). Our results suggest thrips survival may be related to thiamethoxam dosage, we used lowest rate for soybean, and application method which may be more effective against thrips larval stages compared with adults (Broughton and Herron 2009). Also, for thrips that ingested thiamethoxam compound from either food sources without immediate detrimental effects, may be related to thrips polyphagous attributes, thrips feeding from a broad range of plants allow thrips to detoxify plant allelochemicals and possess enzymes conferring chemical insecticide resistance (Reitz 2009, Gao et al. 2012).

We found that fewer treated eggs were consumed which indicated that thrips predation was deterred suggesting that eggs were contaminated as a result of adult female spider mites

feeding and ovipositing on treated soybean plants. Previous research has demonstrated thrips ability to select between food sources of high and low quality (Agrawal and Klein 2000, Janssen et al. 2003, Magalhaes et al. 2005). Furthermore, Trichilo and Leigh (1986) demonstrated thrips predatory response increases as spider mites egg densities increase. We held prey availability of all eggs constant thereby standardizing encounter probability and treated eggs were less preferred, further indicating that thiamethoxam contaminated eggs deterred thrips predation.

Treatment also impacted thrips herbivory; soybean plants grown from thiamethoxam seed treatment received reduced leaf area damage and feeding scars from both non and pre-exposed thrips. Thrips decreased feeding of treated leaves was more evident when quantifying area damage per feeding scar. Thrips originating from treated colonies averaged a smaller area damage per feeding scar. These results may indicate possible recognition and influenced feeding responses by increased probing and decreased settling time at treated leaf feeding sites. Joost and Riley (2017) showed that another neonicotinoid, imidacloprid may stimulate thrips gustatory nerves when leaf feeding. Additionally, insect herbivores will avoid and or reduce feeding as a behavioral response after ingesting plant or chemical insecticide toxins (Hoy et al. 1998).

## **2.5. Conclusion**

Our results suggest lowest dose thiamethoxam soybean seed treatment positively influenced spider mite reproduction which may contribute to increased spider mite densities on soybean. Additionally, treatment may significantly shift thrips feeding preference when co-occurring with spider mites, toward herbivory despite spider mite egg availability. Thrips role in spider mite population suppression within a treated cropping systems may be minimized as thrips are deterred from mite egg predation. Treatment shifted thrips preference toward feeding on foliage and increased spider mite densities, suggesting co-occurrence may potentially lead to

increased herbivory and damage on shared host plants. We conducted a series of experiments of possible treatment scenarios in order to simulate exposure conditions spider mites and thrips may encounter. Thrips may likely encounter one or a combination of treatment conditions both within or outside a treated system. Because neonicotinoids easily move to nearby non-crop vegetation (Sanchez-Bayo et al. 2007, Bonmatin et al. 2015, Pisa et al. 2015, Botias et al. 2016), crops grown from seed treatment may increase the likelihood of spider mite and thrips populations migrating into treated plots pre-exposed. Potential outcome of this treated tritrophic scenario may be similar to our full treatment bioassays involving plant leaf, spider mite eggs, and thrips.

Implications of neonicotinoid seed treatment use may extend beyond spider mite and thrips interactions. These pests are also prey for several arthropod predators within agroecosystems (Xu et al. 2007) and may potentially serve as sources of toxic prey for natural enemies within treated agroecosystem food webs. Although we did not evaluate thrips as a source of toxic prey, seed treatment may indirectly contribute to spider mite and thrips populations by further increasing the potential of releasing these pest species from predator pressure. As a result, early season insect target pests may be replaced with secondary or minor arthropod pest species such as spider mites and thrips thereby minimizing the value of seed treatment as a pest management strategy.

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