Tritrophic Responses to Signaling Formulations Sprayed in Wheat Stem Sawfly-Infested Field Plots

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Abstract

The wheat stem sawfly (*Cephus cinctus* Norton) is an economically important pest of wheat in the Northern Great Plains of North America. Producers and researchers are continuing to search for an effective management strategy. A combination of management tactics, such as host-plant resistance and biocontrol, may be the best method to suppress wheat stem sawfly (WSS) populations. My study examines whether direct toxicity, induced host-plant resistance, or the manipulation of tritrophic interactions, can be achieved through the application of signaling molecules in WSS-infested plots. The overall objective was to assess whether signaling molecules could be incorporated as a management tactic for WSS. Field experiments assessed whether aqueous applications of methyl jasmonate (MeJA), methyl salicylate (MeSA), and Actigard® influence WSS fitness, host-plant fitness, or the recruitment of natural enemies. Treatments were applied in WSS-infested plots for 3 consecutive weeks in both 2017 and in a second experiment in 2018. Wheat samples were collected before harvest and processed to assess parameters of infestation and parasitism. Plant growth and yield parameters were also recorded. The field trials suggested that MeJA and Actigard® induced significant changes that can impact tritrophic interactions in winter wheat. No effect was observed from the applications of MeSA. WSS fitness parameters decreased with applications of MeJA. MeJA treated plots had decreased infestation (2017), decreased larval weight (2018), and increased neonate mortality (2018). Actigard® treated plots had decreased larval weight and increased recruitment of clerid beetles (*Phyllobaenus dubius* Wolcott) (2017). Treatments of MeJA decreased stem height and grain weight, while treatments of Actigard® decreased grain weight in 2017. Although many of these findings were not consistent for both growing seasons, this study demonstrates the potential for these signaling molecules to manipulate the interactions between the WSS, or its host plant, and associated natural enemies in winter wheat.

Background

The wheat stem sawfly (*Cephus cinctus* Norton) is a native species of much of the Great Plains grasslands of North America. The species was first collected in 1872 as a specimen from a Colorado wild grass (Ainslie 1929; Lesieur et al. 2016) In 1895, not long after its discovery, the wheat stem sawfly (WSS) was first described as a new pest insect in the production of spring wheat (Ainslie 1929). During the 1900s the cultivation of grain crops, such as wheat (*Triticum aestivum* L.), expanded throughout the Great Plains of North America (Weiss and Morrill 1992). The widespread institution of these grain crops, in areas that were formerly native grasslands, enabled the WSS to adapt and proliferate within this new agroecosystem (Wallace and McNeal 1966). Since a major outbreak in the early 1900s, the WSS has been considered one of the most serious pests of wheat production throughout the Northern Great Plains (Ainslie 1929, Beres et al. 2011a).

The total economic loss associated with the WSS in North America exceeds $350 million annually (Beres et al. 2011a). A 2012 estimate of economic damage found that the WSS cost producers approximately $80 million in Montana alone (Bekkerman 2014). The reduction in crop value occurs from decreases in both the quantity and quality of the grain. Damage from internal feeding by WSS larvae interferes with the photosynthetic potential of the plant (Macedo et al. 2007; Delaney et al. 2010). This feeding also interrupts the transport of water and nutrients, potentially lowering protein content and grain weight (Holmes 1977). The disruption of the wheat primary metabolism accounts for a reduction in yield ranging from 10 to 30%, depending on the cultivar (Beres et al. 2007; Delaney et al. 2010). Additionally, the lodging of stems can result in the total loss of heads that are unable to be collected at harvest by combines (Beres et al. 2009). The amount of yield loss

associated with lodging varies. Windy and rainy conditions before harvest can result in increased lodging; in addition, lodging rates of cut stems vary depending on the cultivar of wheat (Weiss and Morrill 1992).

Current management efforts face both a changing climate for crop production and the dynamic relationships between WSS populations and their host plants. Recently, WSS has been documented in wheat in regions outside of its historical range (Irell and Peairs 2011). This expansion of range, within wheat crops, was first reported by Morrill and Kushnak (1996) in winter wheat in Montana. These authors also predicted that the earlier emergence, allowing WSS to infest winter wheat, would lead to a southern expansion of range. The increase of damaging populations of the WSS in Nebraska, Colorado, and Kansas means that the pest is likely to become of greater economic importance in winter wheat crops further south in the wheat belt (Lesieur et al. 2016). A combined set of management tactics, such as host-plant resistance and biocontrol, could provide producers with strategies that keep damage from WSS below a desired economic injury level, as suggested in Beres et al. (2011b).

Biology of the Wheat Stem Sawfly

The WSS is an oligophagous stem mining species, infesting a variety of hollow-stemmed grasses and it has a single generation per year (Ainslie 1929). The greatest amount of metabolic activity occurs during larval stages within the stem. The adults of the WSS range from 8-13 mm long and are characterized by a shiny black abdomen with yellow striations. It is a weak flying insect, but females can cover several kilometers in search of host plants (Weiss and Morrill 1992; Lesieur et al. 2016). Females are noticeably larger than males and can be distinguished by the shape of their abdomen and the presence of a

black, saw-like ovipositor (Ainslie 1929). Populations of WSS display high genetic variability across growing regions, primarily because of geographic distance, rather than variability due to host plants (Lesieur et al. 2016)

Warming temperatures in the spring end diapause and overwintered late-instar larvae begin metamorphosis to adults. In Montana, WSS emergence and mating typically spans from mid-May to early-July (Fulbright et al. 2017). The lifespan of adults lasts between 5-8 days, depending on environmental conditions (Criddle 1923; Rand et al. 2019). The WSS is an arrhenotokous species, where females lay both unfertilized, haploid, male eggs, as well as fertilized, diploid, female eggs. Female WSS oviposit into the culms of grass hosts. Control of sperm resources after mating determines whether female or male eggs are deposited and female eggs are preferentially deposited into larger stems (Buteler and Weaver 2012). Unmated females can only produce male offspring, like for other haplodiploid Hymenoptera (Criddle, 1917). A higher proportion of female eggs are deposited during the early periods of WSS flight (Morrill et al. 2000). Females usually deposit a single egg per stem, but when WSS population density is high several eggs may be deposited within a single stem (Buteler et al. 2009). Each female produces approximately 33-50 eggs, allowing for population levels of WSS to increase rapidly under ideal conditions (Ainslie 1929; Wallace and McNeal 1966).

Larvae of WSS undergo all development within the stem of their host. They feed on the vascular tissue of the plant while tunneling through nodes and feeding throughout the length of the stem. This disrupts the transportation of plant nutrients, thereby decreasing photosynthetic capacity (Morrill et al. 1994; Delaney et al. 2010). Only one WSS larva survives per stem because of cannibalism (Wallace and McNeal 1966; Peterson et al. 2009).

WSS larvae are likely to cannibalize smaller conspecifics or eggs. Additionally, larvae that are infected, parasitized, and injured are more susceptible to cannibalism (Buteler et al. 2015). Cannibalism within the stem is a limitation on the population density of the WSS (Weaver et al. 2005); however, it increases the fitness of the cannibal and ensures that surviving individuals have full access to the stem. Larval cannibalism of smaller conspecifics and eggs may be one of the natural selection sources resulting in earlier WSS emergence (Morrill and Kushnak 1996).

Management of the Wheat Stem Sawfly

The life history of the WSS has created challenges to the implementation of economically sound management strategies. WSS inhabit a protective niche within the wheat stem, providing a barrier to many chemical tactics. The flight period of the entire WSS population occurs over several weeks, making that life stage difficult to combat as well. Furthermore, due to the homogeneity of the crop landscape and the reluctance for many farmers to abandon a wheat fallow rotation, WSS is likely to re-establish even if populations are suppressed in a single field (Beres et al. 2011a). The goal of most long-term management strategies is aimed at sustaining a reduction of WSS populations, below levels that significantly decrease yield or grain quality. Despite a number of available management tactics, Montana producers and researchers are still working toward a successful and cost-effective management strategy for the WSS (Beres et al. 2011b).

Some management tactics include planting resistant cultivars, insecticide applications, trap crops, and the enhancement of biological control (Table 1). Recent reviews suggest that a collective use of tactics, such as host-plant resistance and biocontrol,

results in the most effective suppression of WSS populations (Beres et al. 2011b; Buteler et al. 2015; Bekkerman and Weaver 2018).

Table 1: Management Tactics to Control WSS

Management Tactic	Examples
Host-plant resistance	Several solid stem varieties were introduced, offering significant improvements to the resistance of the wheat stem sawfly (Ainslie 1929). Stem solidness is highly correlated to water availability and the yield and grain quality of solid-stemmed varieties is often less than that of hollow-stemmed varieties (Saint Pierre et al. 2010; Szczepaniec et al. 2015). Other forms of plant resistance are currently being sought.
Cultural Control	Weaver et al. (2009) found that WSS displays preference to certain cultivars based upon their chemical profile. The alternate year wheat-fallow cropping systems in Montana, and the restriction of low flight among WSS, make border traps crops particularly desirable (Morrill et al. 2001a). Traps crops have shown efficacy for increasing yield and mortality of the WSS, but producers avoid this tactic due to complications involving the planting and harvesting of the wheat (Weaver et al. 2004).
Chemical control	Recently, the systemic insecticide phorate (Thimet®) has become available for WSS control in Montana (Wanner and Tharp 2015). Chemical control is shown to be limited because of incurred costs and varying successes depleting WSS population over large areas.
Biological control	Two congeneric ectoparasitoids, *Bracon cephi* (Gahan) and *B. lissogaster* Muesebeck, have shown promise as biocontrol agents against the WSS. Wheat plants, where sawfly larvae are parasitized, have increased fitness and yield compared to wheat plants where larvae complete development (Buteler et al. 2008). Biocontrol tactics are an effective way to maximize profits as part of a long-term management strategy for WSS (Bekkerman and Weaver 2018). The collection and release of these parasitoids to areas with high WSS populations has been a successful tactic toward establishing biological control (Morrill et al. 1998). Populations of *B. cephi* and *B. lissogaster* provide irreplaceable mortality to WSS (Peterson et al. 2011).

Natural Enemies of the WSS

There are several known natural enemies of the WSS (Ainslie 1929). Two congeneric ectoparasitoids, *Bracon cephi* (Gahan) and *B. lissogaster* Muesebeck, have promise as biocontrol agents against the WSS. Both species are specialists unique to WSS and both are native to the grasslands of Montana. There are several other insect species which have been found to parasitize or predate on the WSS, most notable are two wasps from the family Ichneumonidae and one beetle from the family Cleridae; however, only the two braconid species, *B. cephi* and *B. lissogaster* , have been documented to decrease WSS population density (Morrill 2001; Morrill et al. 1998).

The braconid parasitoids are larval idiobionts, where females paralyze WSS larvae upon attack (Runyon et al. 2001; Weaver et al. 2005; Peterson et al. 2011). Their presence is shown to reduce damage to the wheat plant and provide irreplaceable mortality to WSS populations (Peterson et al. 2011; Buteler et al. 2015). Certain management practices have been linked to the attraction, preservation, and efficacy of these beneficial insects (Runyon et al. 2002; Weaver et al. 2004).

Bracon cephi and *B. lissogaster* exhibit bivoltine life cycles. The female braconids locate and parasitize larvae from the exterior of the stem (Morrill et al. 1998). Eggs are deposited into the stem, on or next to the paralyzed WSS larva. When the eggs hatch, the larvae attach themselves to the integument of their host. They feed on their host until fully developed or until the host is fully consumed. They spin a cocoon, which can be identified by two disc-like plates on each end. The majority of the first generation emerges to complete a second generation, while a portion of the first generation remains dormant. They go into diapause and overwinter, along with the successful individuals from the

second generation. Cocoons from the two generations can be distinguished by morphological differences. Cocoons from the first generation are thin and smooth, appearing light brown, while cocoons from the second generation are tightly woven, white, and cottony (Nelson and Farstad 1953; Somsen and Luginbill 1956). An important difference between these two species is that *B. cephi* is solitary (only a single offspring per host), while *B. lissogaster* can be solitary or gregarious (1-4 offspring on a single host) (Runyon et al. 2001).

The success of parasitoid populations is influenced by several factors. A hot dry summer can result in a faster maturation of the wheat crop, which can be detrimental to the second generation of parasitoids (Holmes et al. 1963; Morrill et al. 1998). This generation may face increased mortality from a lack of active hosts or the inability to oviposit due to the hardening of the wheat stem. The size of the WSS population also influences the success of parasitoids. Increased WSS populations can reach a threshold where parasitism loses efficacy. This occurs because a greater percentage of stems contain multiple sawfly larvae, leading to increased instances where parasitoids succumb to predation by WSS larvae (Weaver et al. 2005). There is continuing research aimed at finding ways to maximize the population levels and efficacy of *B. cephi* and *B. lissogaster*.

Chemical Ecology and Tritrophic Interactions

Plant signaling has been shown to heavily influence the tritrophic relationships between plants, their herbivores, and the natural enemies of herbivores (Karban and Baldwin 1997). Plant secondary metabolites have been shown to function in the direct and indirect defense of plants (Fraenkel 1959; Ozawa et al. 2000; and Raguso et al. 2015). Furthermore, plants release volatile organic compounds (VOCs), also referred to as plant

volatiles (PVs), which serve as signaling molecules, mediating plant-to-plant and plant-insect interactions (Heil and Karban 2010). While it can be difficult for researchers to isolate all compounds that elicit biological activity, the future of using signaling molecules to manipulate tritrophic interactions in agriculture is promising (Raguso et al. 2015).

Researchers have begun to identify new ways in which knowledge of chemical ecology can be adopted in agriculture. Insect pheromones were first described about a century ago and their use in agriculture, to manipulate pest insects, has been occurring for several decades (Kirsch 1988). Ongoing research has been examining how signaling molecules influence host-plant defense and the natural enemies of insects. In a fascinating example, mechanically damaged sagebrush (*Artemisia tridentata*) were shown to induce defense, against *Manduca sexta* caterpillars, in nearby tobacco crops *Nicotiana attenuata* (Kessler et al. 2006). Much research demonstrates that chemical signaling can enhance the resistance of plants to pest insects. James and Price (2004) found that slow-release MeSA significantly increased and maintained populations of beneficial insects in vine crops. Their research, among others, suggests that signaling molecules could improve the management of insect herbivores via the recruitment of their natural enemies. An increased knowledge of how signaling molecules influence biological activity and plant defense could lead to greater commercial application of signaling molecules in agriculture.

Jasmonates

Jasmonates are oxylipins, a diverse group of chemicals generated from the octadecanoid pathway (Prost et al. 2005; Lawrence and Koundal 2002). Jasmonic acid (JA) is synthesized from linolenic acid and is the key component of the jasmonate signaling pathway. Methyl jasmonate (MeJA) is the methyl ester of jasmonic acid. These compounds

are highly volatile and are suggested to play important roles as signaling molecules for both plants and insects (Karban and Baldwin 1997). Jasmonates are especially well-known for their role in wound-induced defense against herbivores; however, jasmonates have also been linked to defenses against pathogens and the attraction of beneficial insects (Prost et al. 2005; Ozawa et al. 2000; Cheong and Choi 2003; and Wasternak 2007).

MeJA has been shown to trigger the buildup of JA in plant tissues. The genesis of JA has been linked to proteinase inhibitor gene expression (Koiwa et al. 1997; Lawrence and Koundal 2002). Proteinase inhibitors (PIs) are small proteins that can decrease herbivore fitness. This is most often achieved through the inhibition of the enzymes that herbivores use to digest plant tissues. The results of increased PIs in plant tissues has had mixed results regarding herbivore population density (Karban and Baldwin 1997). While there are still many questions surrounding the roles of different compounds in the jasmonic acid pathway, it remains of high interest in the management of herbivore pests.

Salicylates

Salicylic acid (SA) is a product of the shikimic acid pathway and has been shown to increase in concentrations in conjunction with infestation from plant pathogens (Mandal et al. 2010, Karban and Baldwin 1997). SA plays important roles in the systemic acquired resistance (SAR) response of plants, while also mediating several plant processes involved with growth and development. SA derived compounds are especially well-known for their direct antibiotic properties against plant pathogens (Mandal et al 2010; Wu et al. 2008; Tang et al. 2015).

Methyl salicylate (MeSA) is the methyl ester of salicylic acid. Many plants naturally synthesize MeSA when the SAR response is activated. MeSA has been linked to the direct

and indirect defense of plants and is also suspected to serve an important role as a signaling molecule (Karban and Baldwin 1997). A substantial amount of research has looked into salicylates for defense against herbivores in agricultural systems, most of which has focused on whether these molecules can induce plant defense systems or whether they can recruit the natural enemies of insect herbivores.

Synthetic Inducers of Defense

Synthetic plant defense elicitors have been used to further explore plant defense signaling pathways in various cropping systems. While research has identified many of the molecules which elicit defense in natural systems, synthetic versions of these signaling molecules are available on a commercial scale; furthermore, synthetic inducers can be more effective and less phytotoxic than their natural counterparts (Bektas and Eulgem 2015).

One of the more successful synthetic inducers of defense is benzo(1,2,3)-thiadiazole-7-carbothioic acid S-methyl-ester, better known as benzothiadiazole (BTH) or acibenzolar-S-methyl (ASM). It became commercially available in the United States in 1990 under the name Actigard®. BTH is a functional analog of salicylic acid and it activates the SAR pathway. While this response is better attributed to defense against plant pathogens, several papers have linked Actigard® and the SAR response to defense against insect herbivores. Inbar et al. (1998) found that Actigard® has an antiherbivore effect on a leaf miner pest of tomato plants. They found that external applications of Actigard® resulted in high levels of pathogenesis-related proteins in the plant tissue. Correa et al. (2005) found that applications of BTH decrease whitefly populations on cucumber, specifically from a reduction in oviposition time and an increase in nymphal mortality. In contrast, several

studies have found negligible effects of BTH on herbivore fitness and damage. Inbar et al. (2001) documented the inability of BTH to provide defense against the cotton bollworm feeding in cotton plants. The efficacy of this synthetic inducer against herbivores appears to be variable between cropping systems. Nevertheless, it has the capacity to be an effective management tool in the defense against herbivore pests.

Introduction

Plant signaling molecules can elicit varying interactions within different agricultural systems and may even evoke differential reactions among genetically distinct cultivars of the same crop (Karban and Baldwin 1997). These molecules have been shown to elicit different biological activity at varying concentrations and they can influence both beneficial insects and the insect herbivores themselves (Heil 2014). Weaver et al. (2009) identified a VOC, (Z)-3-hexenyl acetate, which is released by wheat plants and used for host-location by ovipositing WSS females. This research is an affirmation that chemical signaling plays an important role in the relationship between wheat plants and the WSS. Continueing research aims at improving the understanding of the relationship between VOCS and tritrophic interactions within wheat agricultural systems. An increased understanding of these interactions could allow for application of management tactics as part of an integrated pest management program (Heil 2014). Laboratory research can be an important step toward the identification of signaling molecules that elicit biological activity, but their complex interactions within the agroecosystem can only be studied by examining their effect in-situ. Field studies, examining the tritrophic interactions of signaling molecules should be implemented to determine whether promising compounds can really elicit desirable biological activity.

Researchers have begun exploring how signaling molecules influence WSS, its natural enemies, and the fitness of wheat crops. Bayram and Tonga (2018a) used sticky traps and sweep netting to assess how cis-jasmone influences insect populations in wheat. They found that cis-jasmone attracted a parasitoid (Ichneumonidae) of a related WSS species. Another study from this team examined how the closely related MeJA molecule influenced insect populations in wheat (Bayram and Tonga 2018b). They found that aqueous MeJA applications were a deterrent for aphid, thrips, and hoverfly species, while MeJA treated plots were attractive for a species of WSS in Turkey, as well as a lady beetle species, and a beneficial ichneumonid parasitoid (*Collyria coxator* (Villers)) of the same WSS species. Another study, from the wheat cropping systems in Montana, assessed the impact of Actigard® and cis-jasmone on elicited defenses and potential to recruit beneficial insects in WSS infested wheat plots (Shrestha et al 2018). They found that two applications of Actigard® resulted in increased mortality among WSS larvae. This finding was from wheat plants collected 30 days after the initial treatment. They also found that Actigard® treatments significantly reduced WSS larval body mass. However, these elicitors did not impact the parasitism of WSS (Shrestha et al. 2018). The effects of crop applications of MeSA on WSS have not been well studied; however, several papers, such as Wang et al. (2011), have looked at the effect of external MeSA applications on other insect pests of wheat. These authors found that a slow release application of MeSA lowered mean aphid density, while increasing parasitoid density and grain quality.

Signaling molecules may also possess direct insecticidal properties, allowing for their use as alternatives to synthetic pesticides (Cloyd et al 2009; Prost et al. 2005). Jasmonates and salicylates are molecules associated with induced plant defense. It has

been shown that applications of these compounds may deliver direct toxicity to insects (Ma et al. 2014), while Prost et al. (2005) found that many oxylipins exhibit direct antimicrobial properties against plant pathogenic microorganisms. Whitehill et al. (2014) found that applications of MeJA resulted in mortality of emerald ash borer larvae and a decrease in the emergence of emerald ash borer adults. Salicylic acid was shown to display antibiotic effects when applied exogenously on microbial pathogens (Wu et al. 2008). However, many of these compounds have not been subjected to empirical research in crops and their efficacy as a deterrent or insecticide likely varies by pest and cropping system. Most plant derived compounds are considered safe (GRAS or Generally Recognized As Safe) by the U.S. Environmental Protection Agency and most are exempt from registration under the U.S. Federal Insecticide Fungicide and Rodenticide Act (Cloyd 2009).

There is a potential for MeJA, MeSA, ad Actigard® to induce desirable biological activities within wheat cropping systems. These molecules could provide management to WSS via the induction of direct plant defense (Motallebi et al. 2015). Furthermore, these molecules could provide indirect defense by attracting the natural enemies of the WSS (Aartsma et al. 2017; Bayram and Tonga 2018a). Recent research has examined the potential of exogenous application of jasmonates and salicylates within various cropping systems. The goal of this research is to examine whether spray applications of elicitors (MeJA, MeSA, and Actigard®) can induce direct or indirect defense against WSS in growing winter wheat crops.

Methods

Field Location

In the summers of 2017 and 2018 experimental plots were established in a standing winter wheat fields near Amsterdam, MT (Figure 1). The northeast corner of 2017 plots was at coordinates 45.750995, -111.393715 and the northeast corner of the 2018 plots was at 45.758083, -111.397150. The sites were separated by approximately 800 m. Both fields were managed by the same grower and the same variety was planted each season. The USDA soil survey (Tabular Data 2018 MT622 https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx) classifies the soil as a well-drained silt loam with 4-8% slope. Most of the agricultural land surrounding the site is designated as small grains and fallow cropping system (winter wheat, spring wheat, and barley); however, the Gallatin Valley also produces seed potatoes and a variety of pulse and cover crops. This area is one of the most productive (per acre) winter wheat growing regions in the state of Montana (Montana Grain County Estimates 2016). This site was selected due to the pre-existing knowledge of a large and damaging WSS population.

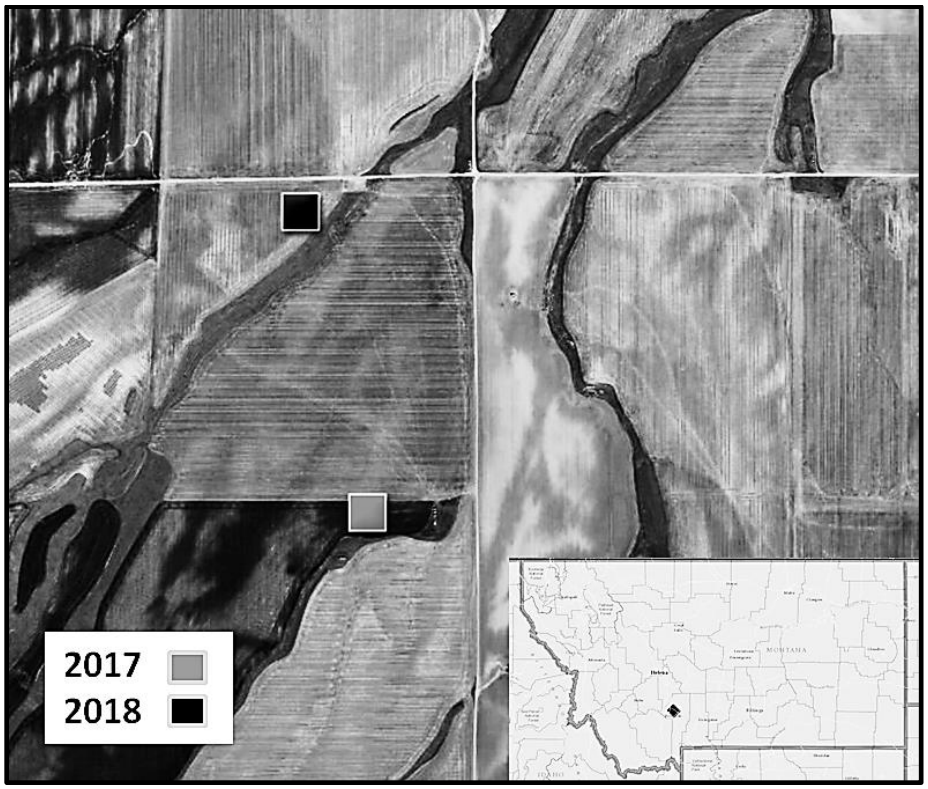

Figure 1: Map of Experimental Areas 2017 and 2018

Experimental design

In 2017, a 511 m^2 area was delineated within a winter wheat field (Figure2). The area was divided into a 5 X 11 grid of 3 m X 3 m plots and 18 plots were designated for treatments (Figure 2). All treatment plots were separated by equivalently sized buffer plots to inhibit incidental drift and contamination. In 2018, this layout was duplicated two times and one plot array was treated with 1:10 dilution application rates (Figure 3). The second plot array, with lower application rates, was added due to both observed phytotoxicity of MeJA in 2017 and to test the effect of two application rates on tritrophic interactions. The winter wheat was Clearstone, a cultivar of wheat used for its resistance to imazamox herbicide, such as Beyond®, in Clearfield® wheat production (Berg et al. 2014). The array of 3 x 3 m plots was established using survey flags. Plots were randomly assigned

to 6 different treatments: MeJA, MeSA, Water Actigard®+, Water+, and Actigard®(Table 2). Both MeJA and MeSA were applied with sticker (Natural Wet® Safer Gro. Aurora, CA) (a product used to adhere chemicals to plants), while water and Actigard® were both applied with and without the addition of sticker. Each treatment was randomly assigned 3 replications.

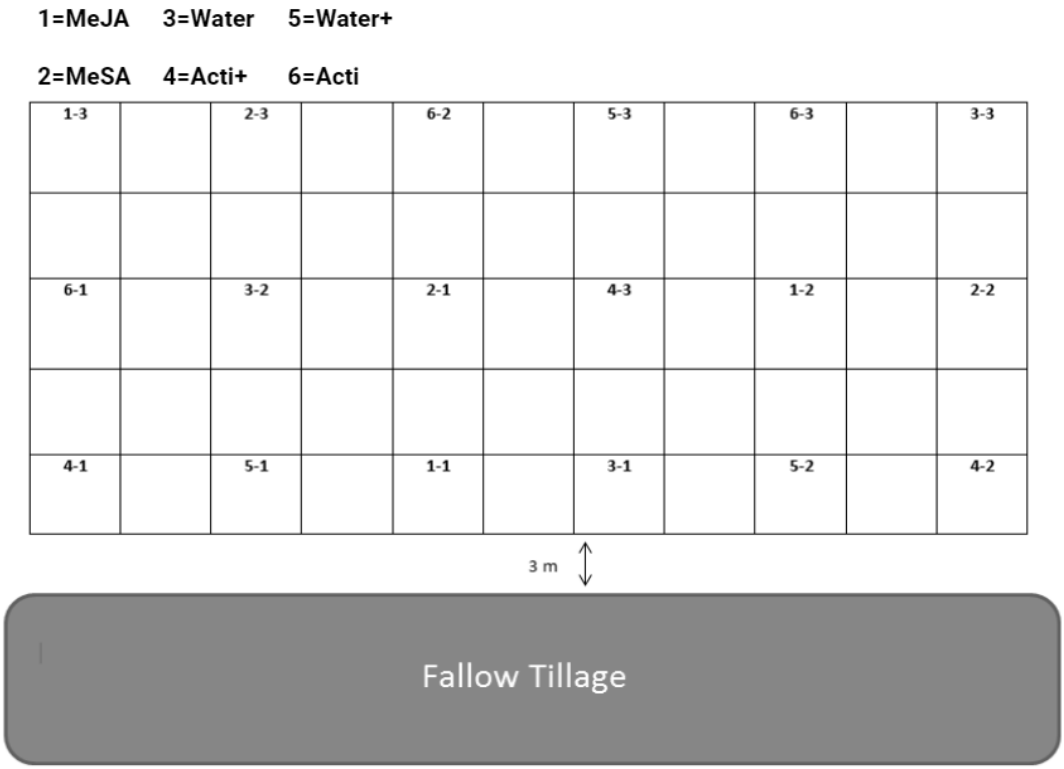

Figure 2: The plot layout of the 2017 experiment. Treated plots are the ones with designated numbers.

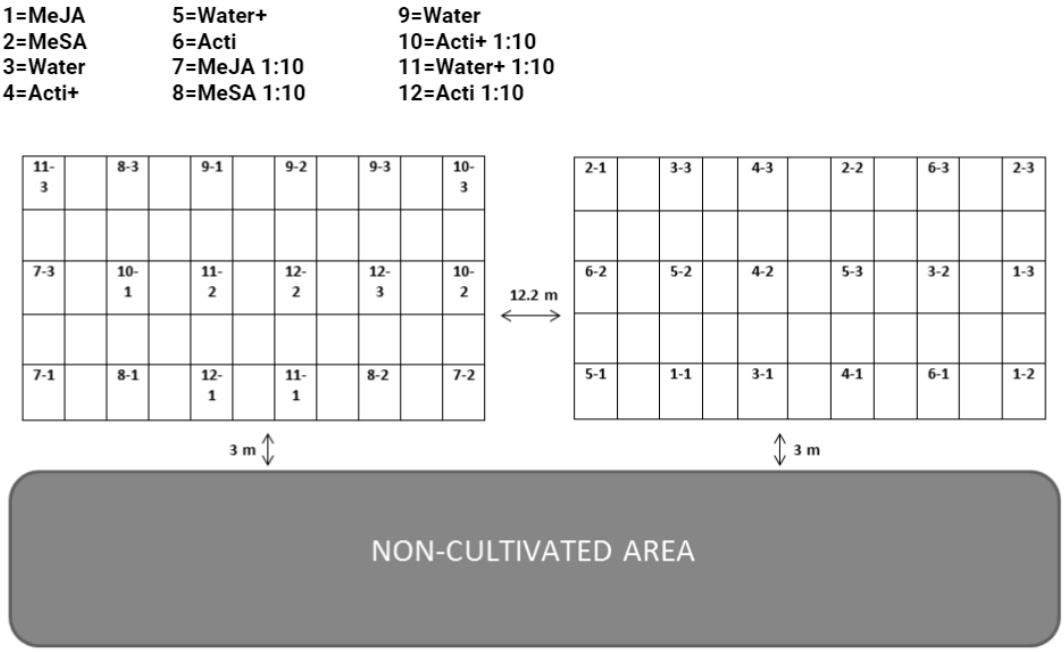

Figure 3: The plot layout of the 2018 experiment. Treated plots are the ones with designated numbers.

Treatments

Treatments were applied using a 3.79 L HDX™ hand sprayer (Home Depot, Atlanta, GA). Each plot received 0.25 liters of an aqueous solution. A steady side-to-side, pendulum spray motion was used to spread the applications as evenly as possible throughout each plot. The arc of the spray nozzle was approximately 153 cm long, the top of which was held about 70 cm from the waist (Figure 4). The nozzle was held approximately 1 m above the ground. A stop watch was used to ensure 10 second walking transects, both maintaining the correct application rate and optimizing the spread of the application throughout the plot.

Calibration

The sprayers were filled with 1.5 liters of tap water and each was pre-marked with 0.25 liter tick marks up to 1.75 liters. The nozzles of the hand sprayers were adjusted to accommodate both the walking-time and the desired application rate. Three 10 second transects (total of 30 seconds) was determined to be the suitable time to evenly cover each plot. The calibration of the HDX™ hand sprayers was checked before each application to ensure a 0.25 L per 30 second flow rate. Once the 0.25 L per 30 second rate was checked, treatments were diluted into 1.5 L and applied (Tables 2, 3).

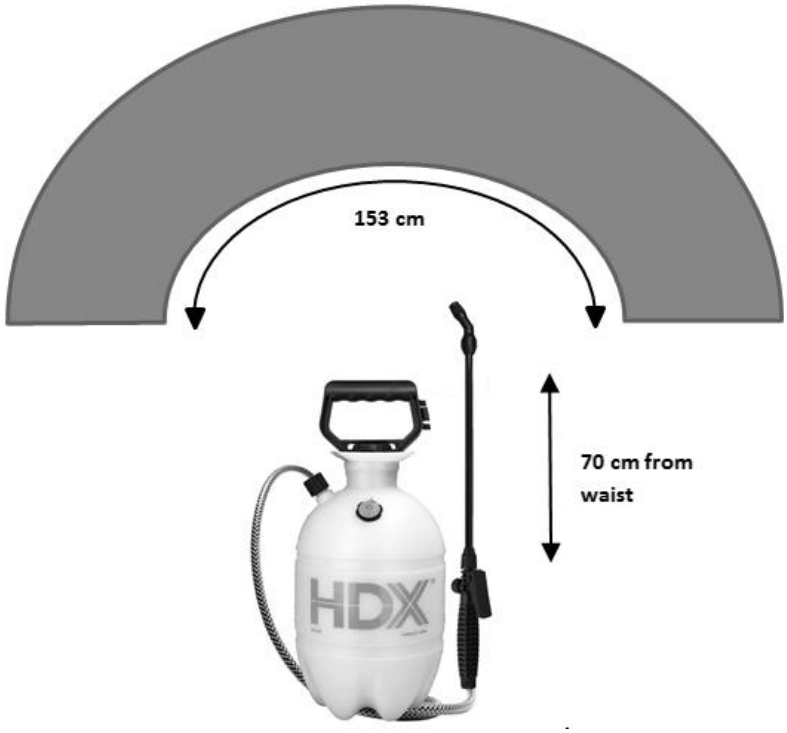

Figure 4: Hand-Sprayer Applications

Methyl jasmonate (95%) was ordered from Sigma-Aldrich (St. Louis, MO). Methyl salicylate (99%) was also acquired from Sigma-Aldrich (St. Louis, MO). Actigard® 50WG was acquired from Syngenta Retail Crop Protection, via a local distributor in Bozeman, MT.

Application rates were as followed:

Table 2: Each treatment in 2017 was applied at the following rate and with the following constituents.

Treatment	Application Rate
MeJA	0.1 molar, about 3.803 g dissolved into 150 ml of water: with 1.95ml of sticker
MeSA	0.1 molar, about 5.608 g dissolved into 150 ml of water: with 1.95ml of sticker
Water	Tap water
Actigard®+	Highest label rate for 3 applications, 0.187 g dissolved into 150 ml of water: with 1.95ml of sticker
Water+	Tap water: with 1.95ml of sticker
Actigard®	Highest label rate for 3 applications, 0.187 g dissolved into 150 ml of water

Table 3: Each treatment in 2018 was applied at the following rate and with the following constituents.

Treatments	Application Rate: full	Application Rate: dilution
MeJA	0.1 molar: with 1.95ml of sticker	0.01 molar: with 1.95ml of sticker
MeSA	0.1 molar: with 1.95ml of sticker	0.01 molar: with 1.95ml of sticker
Water	Tap water	Tap water
Actigard®+	Highest label rate: with 1.95 ml of sticker	1:10 label rate: with 1.95 ml of sticker
Water+	Tap water: with 1.95ml of sticker	Tap water: with 1.95ml of sticker
Actigard®	0.1 molar	0.01 molar

Timing of Applications and Harvesting

Each year, the timing of the first applications took place after WSS flight began. Subsequent applications were made at one and two weeks after the initial treatments. The first treatment was applied before peak oviposition, when a high proportion of the overall WSS infestation was in the egg stage. The proportions of infestation in larval stages were expected to increase for each of the subsequent applications. Applications in 2017 were made on June 16, 22, and 30, while applications in 2018 were made June 28, July 6, and July

12. The applications were applied shortly after dawn in the morning to avoid high temperatures and wind speeds. High temperatures can degrade the compounds, while high wind speed could result in drift, altering the consistency of the application. After the last application, the wheat continued to mature until harvest. Samples were harvested on July 21 in 2017 and August 15 in 2018. A 30-cm measure was used to collect 3 random, 30 cm samples from each plot. Samples were placed in pre-labeled 7.3 kg brown bags (Duro 16 lb., Novolex, Hartsville, SC) and transported and stored in 208-L plastic drum liners (Model: S-12589, ULINE, Pleasant Prairie, WI).

Sample processing

The samples were processed in the Wheat Stem Sawfly Laboratory at Montana State University in Bozeman, MT. The length of each stem was measured from the root crown to the top of the highest internode. The width of the stem was measured using a Mitutoyo™ electronic caliper (Mitutoyo American Corporation, Aurora, IL). The caliper was used to record width of the stem at the lowest internode. Each stem was dissected using a scalpel with a #11 X-acto™ blades (Elmer's Products, Inc., Columbus, OH). They were recorded on a data sheet under parameters of WSS infestation and parasitism. Infestation status of stems was categorized as: uninfested, infested not cut, infested cut, infested neonate mortality, and parasitized. Parasitism status was further recorded as: cocoon(s), emergence hole, ichneumonid, as well as predation by a known clerid species. The head of each stem was threshed and grain kernel count and total grain weight were recorded. Alive larvae from stubs were immediately frozen and later weighed.

Analysis

The data were entered into spreadsheets using Microsoft Excel. RStudio 1.0.136 Desktop was used to analyze the data (RStudio, Boston, MA). The read.csv function was used to import data from excel csv spreadsheets and the boxplot function and the qqnorm function were used to make a visual assessment on the distribution of data. The data was not transformed. A one-way ANOVA was used to assess difference between years and application rates. A one-way ANOVA ('aov' function) and a Tukey HSD test ('TukeyHSD' function) allowed for an assessment of treatment effects (R Core Team 2013).

Results

Year Effects (2017 versus 2018)

We found considerable variability in the data between the 2017 and 2018 field seasons. Mean infestation, mortality, larval weight, neonate mortality, parasitism, and grain weight were all significantly different between the field seasons ($p<0.001$). The discrepancy in infestation and parasitism levels between years made discerning treatment effects on WSS and its natural enemies difficult (Figures 5, 6). The greater grain weight in 2018 may illustrate improved growing conditions that year (Figure 7). The weight of WSS larvae was greater in 2018 (Figure 8). Interestingly, we did not find a difference in stem diameter between 2017 and 2018. The mean height of wheat stems was slightly higher in 2017 ($p<0.05$), which was a surprising finding considering the increased grain weight of wheat in 2018.

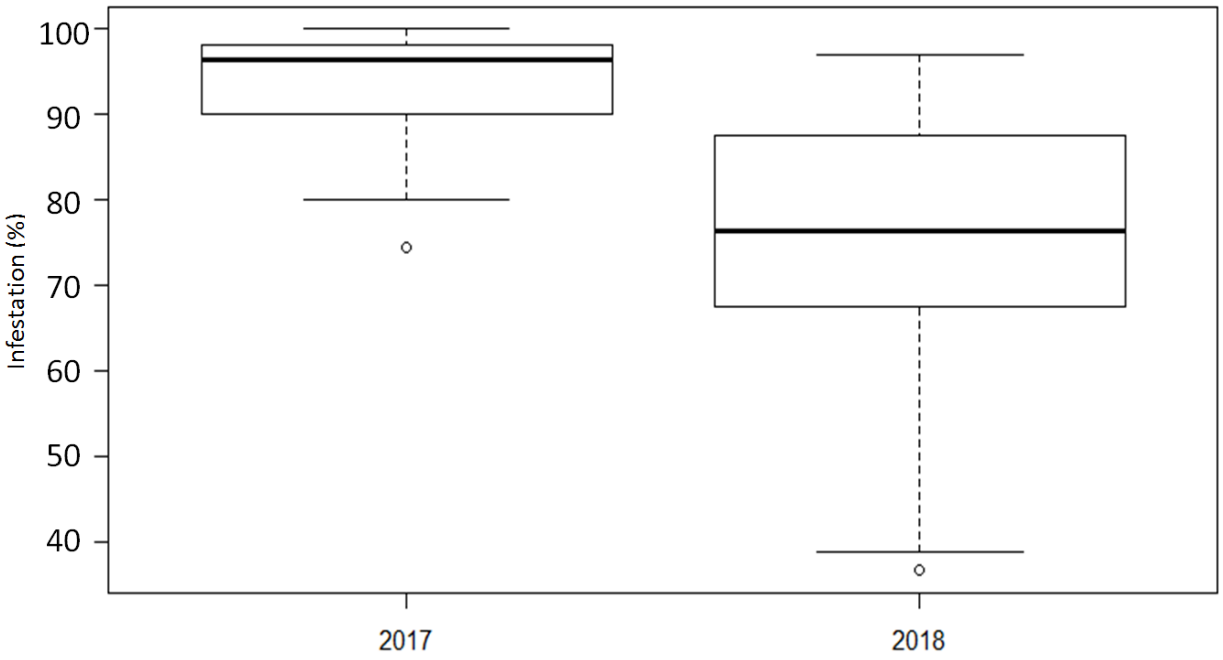

Figure 5: WSS Infestation by Year. Infestation rate was calculated by dividing the total number of infested stems by the total number of stems in a sample. Wheat plots in 2017 experienced significantly greater WSS infestation (p<0.001). Percent infestation was about 95% in 2017 and about 75% in 2018.

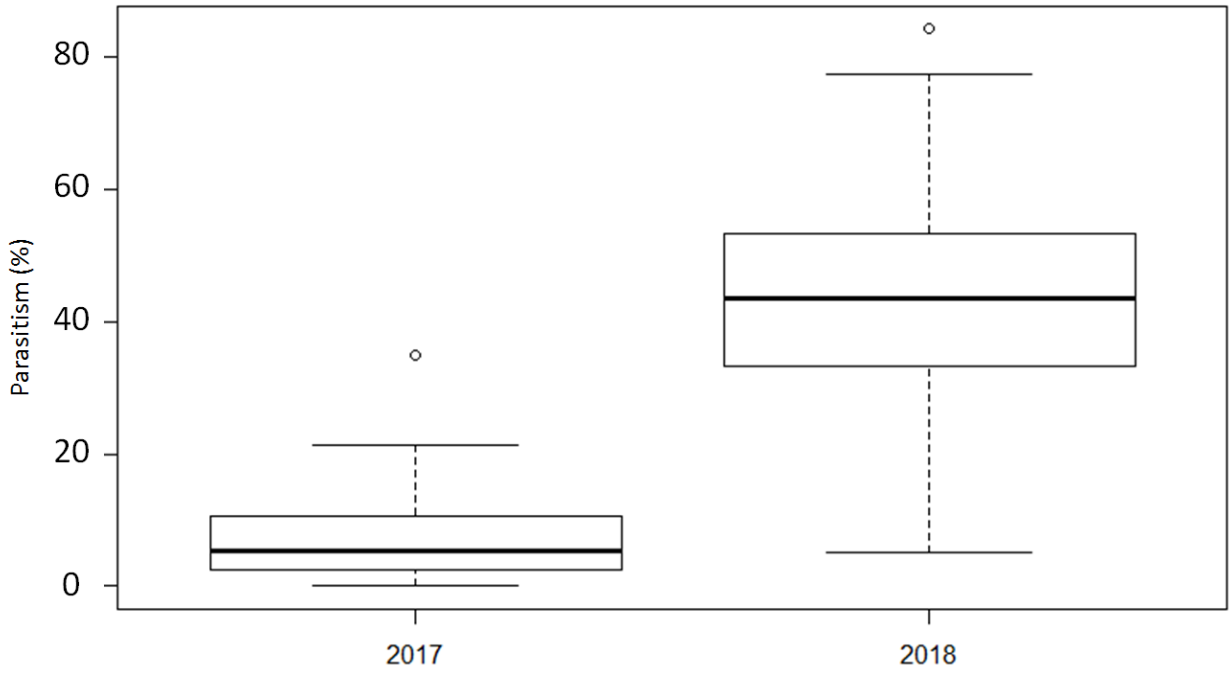

Figure 6: Parasitism of WSS by Year. Parasitism was calculated by the total stems with parasitism events divided by the total number of infested stems. Parasitism levels were significantly greater in 2018 (p<0.001). Percent parasitism was about 7% in 2017 and about 44% in 2018.

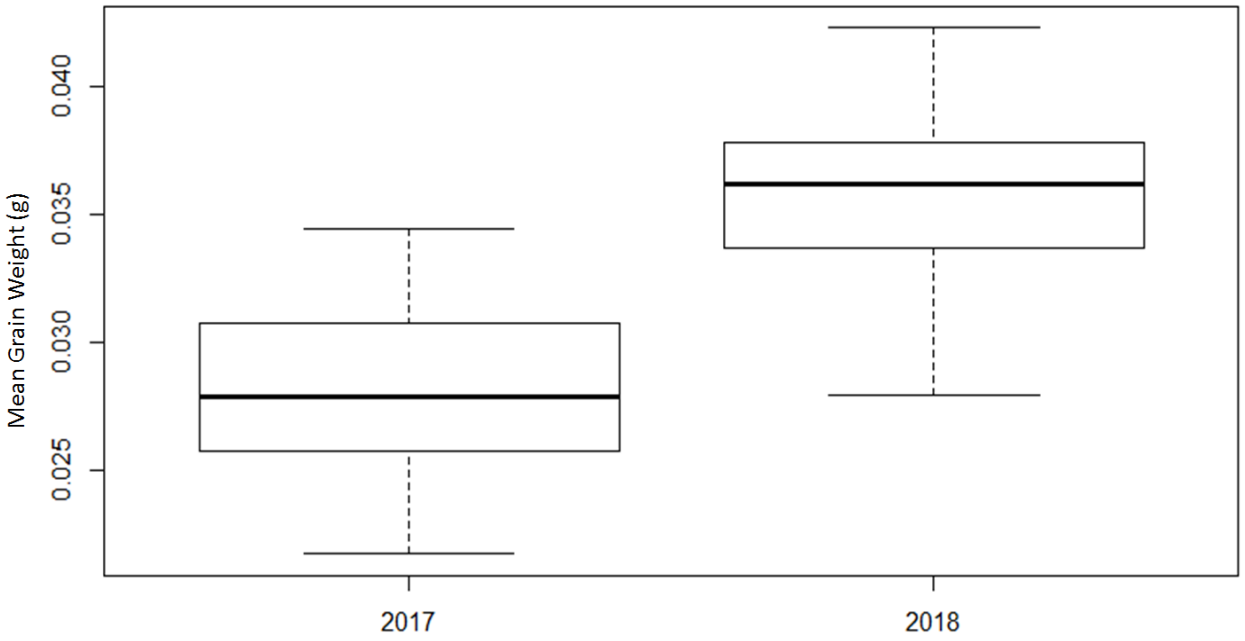

Figure 7: Grain Weight by Year. Mean grain weight is the total recovered kernels divided by total weight of kernels recovered for each head of wheat. Mean grain weight was significantly greater in 2018 ($p<0.001$). Mean grain weight in 2017 was 0.0280, while mean grain weight in 2018 was 0.0356.

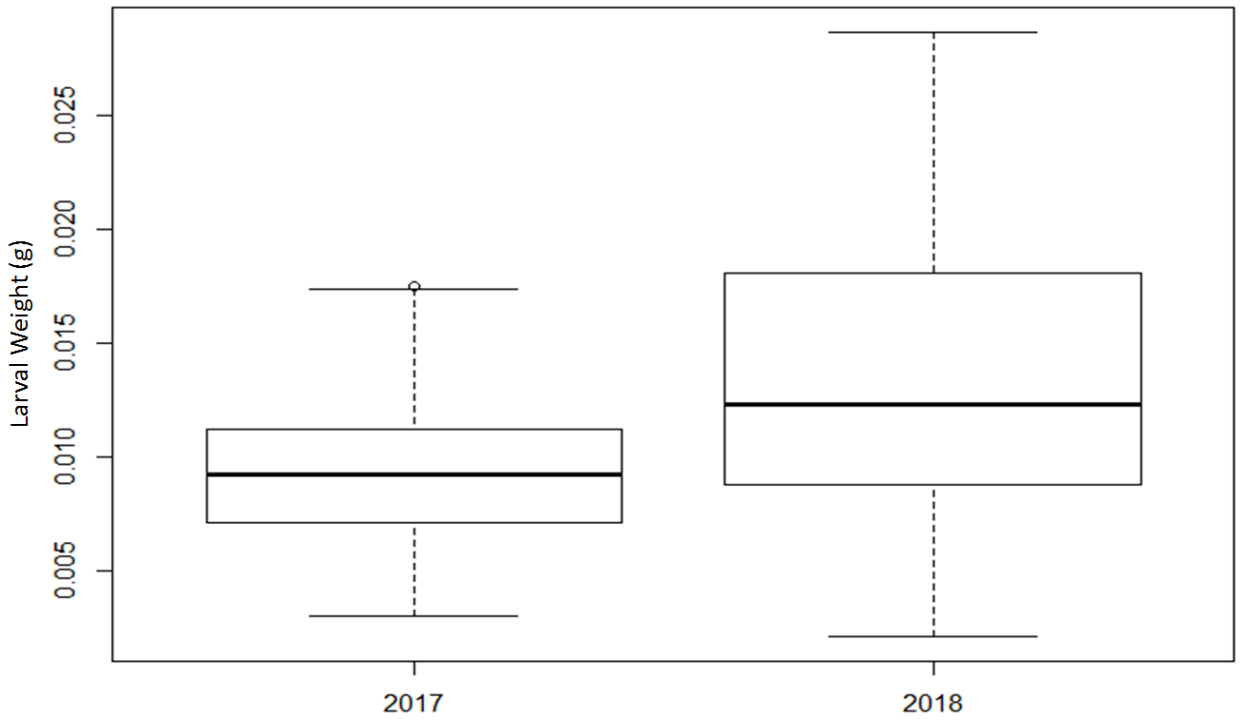

Figure 8: Larval Weight by Year. The larval weight of WSS recovered from cut stubs was significantly greater in 2018 ($p<0.001$). Mean larval weight in 2017 was 0.0093, while mean larval weight in 2018 was 0.0134.

Application Rates 2018

We found considerable variation for several parameters when examining differences between application rates in 2018. Infestation and grain weight were different between the full rate and 1:10 dilution ($p<0.001$), (Figure 9), (Figure 10). Mean percent WSS infestation for 1:10 dilution plots was 50.13%, while mean percent infestation for the full rate plots was 75.34%. We also found considerable variation in mortality and parasitism of WSS between application rates ($p<0.05$). Overall, wheat in the lower application rate plots had a better performance with lower infestation, greater grain weight, greater mortality, and greater parasitism. Several parameters did not show significant differences between application rates. Larval weight and neonate mortality did not vary between the plots treated at different application rates. Additionally, the rate of applications did not illustrate an effect on mean stem height and mean stem diameter.

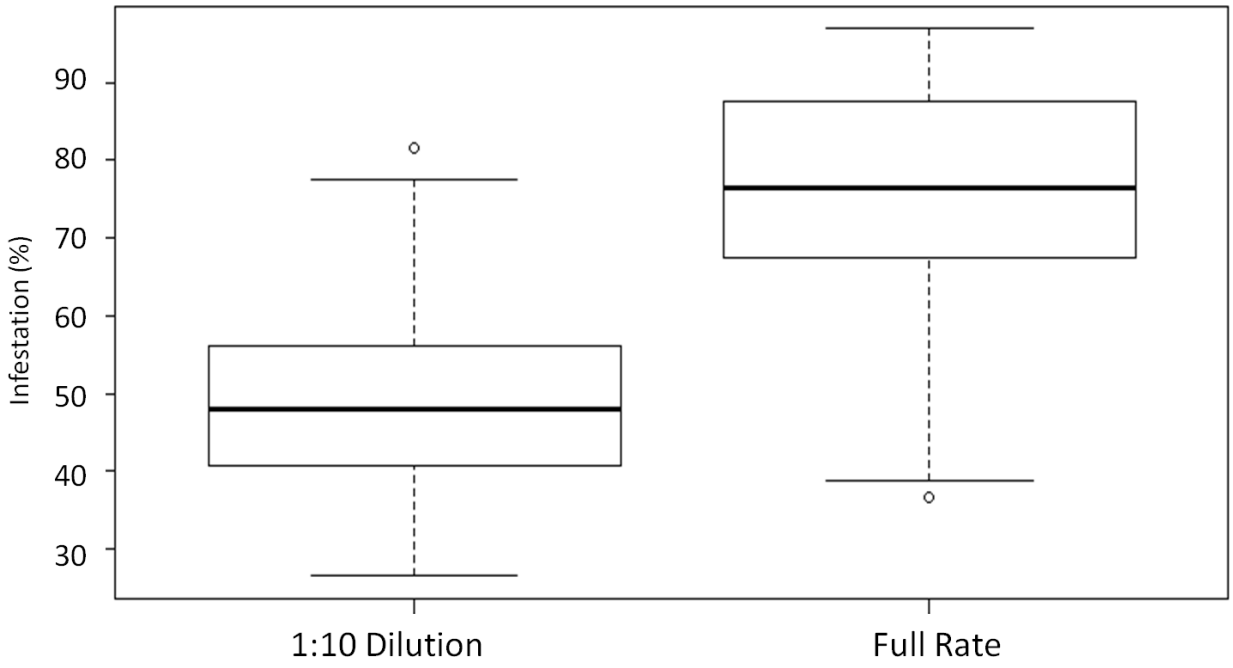

Figure 9: WSS Infestation by Application Rate (2018). Infestation rate was calculated by dividing the total number of infested stems by the total number of stems in a sample. The full rate application plots experienced about 25 % higher infestation (p<0.001)

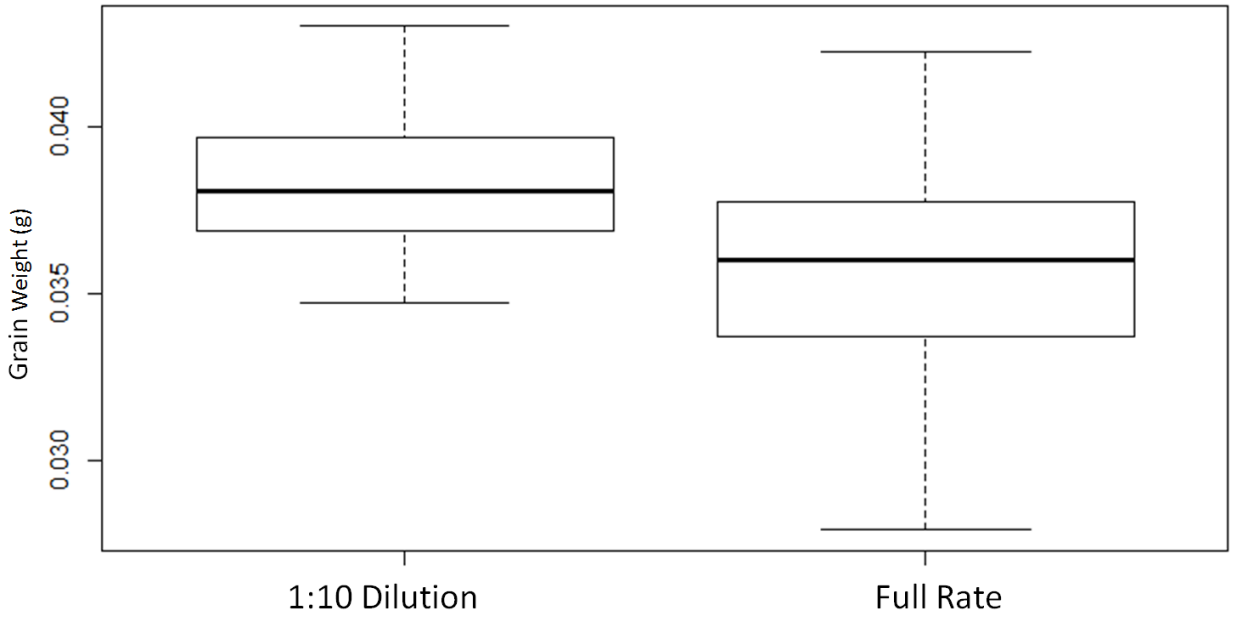

Figure 10: Mean Grain Weight by Application Rate (2018). Mean grain weight is the total recovered kernels divided by total weight of kernels recovered for each stem. Mean grain weight of the 1:10 dilution plots was 0.0383, while mean grain weight of the full rate plots was 0.0355 in 2018 (p<0.001).

Treatments

Overall, we found that several parameters were impacted by the application of signaling molecules. In particular, the MeJA and Actigard® treatment plots had noteworthy differences compared to the Water and Water+ treatment plots for infestation, parasitism, and plant height and yield. MeJA treated plots experienced low infestation compared to Water and Water+ treated plots in 2017 (Figure 11). Acti and Acti+ treated plots had a lower grain weight than plots treated with Water in 2017 (Figure 21), while MeJA treated plots had a lower grain weight compared to plots treated with Water and Water+ in 2018 (Figure 22). WSS larvae recovered from MeJA treated plots weighed significantly less that larvae recovered from Water and Water+ treated plots in 2018 (Figure 15). While these differences were not replicated between growing seasons, they are interesting findings that suggest changes in biological activity from wheat plots treated with MeJA and Actigard®. Neonate mortality of MeJA treated plots was greater in both 2017 and 2018 compared to plots treated with Water+ (Figures 16, 17)). MeJA treated plots also had a significantly lower stem height for both years compared to all other treatment plots (Figures 19, 20).

Infestation

MeJA treated plots had a lower infestation compared to all other treatments in 2017 ($p<0.001$). The Actigard® and MeSA treatments did not show an effect on the infestation of wheat in either 2017 or 2018. The decrease in infestation in MeJA treated plots was not replicated in 2018 and there was no significant difference in infestation among any of the treatment plots in 2018.

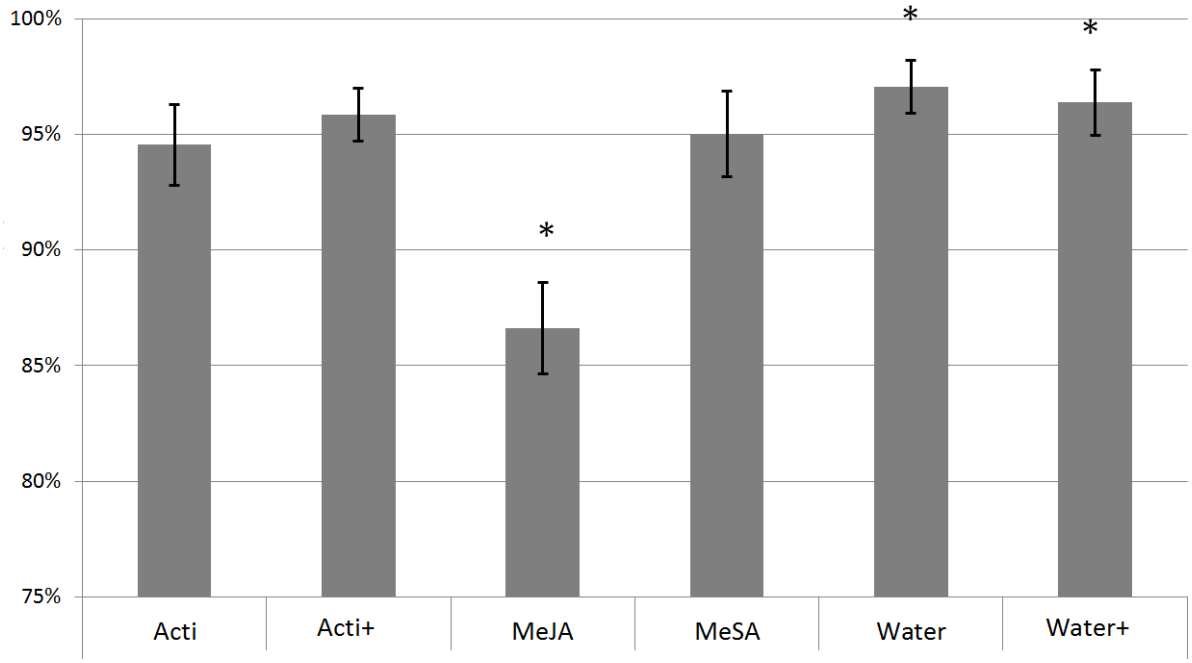

Figure 11: Infestation (2017). Infestation rate was calculated by dividing the total number of infested stems by the total number of stems in a sample. We found that mean infestation was lower in MeJA treated plots compared to Water and Water+ treated plots (MeJA-Water $p<0.001$), (MeJA-Water+ $p<0.001$).

Mean mortality rate

Mortality among the treatments varied significantly in the treated plots in 2017 (Figure 12). MeJA treated plots had greater mortality compared to Water treated plots. However, there was also a significant difference in mortality between the Water and Water + treated plots in 2017. WSS in Water treated plots experienced the lowest mortality, while WSS in Water+ treated plots experienced the greatest mortality in 2017. The Actigard® and MeSA treated plots did not show significant differences in 2017 and treatments did not show an effect on mortality in 2018 (Figure 13).

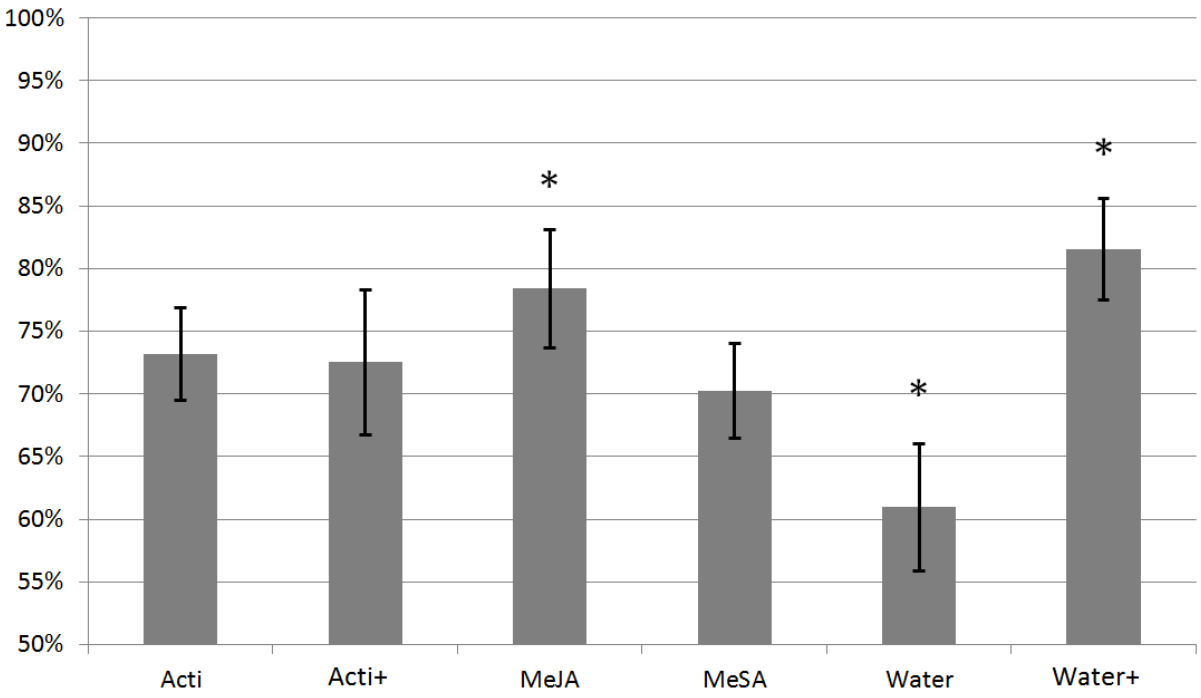

Figure 12: Mortality (2017). Mortality rate is the total number of stems with WSS mortality divided by the total number of infested stems. WSS in wheat treated with Water experienced lower mortality compared to WSS in wheat treated with Water+ ($p<0.05$). WSS in MeJA plots experienced greater mortality compared to WSS in plots treated with Water ($p=0.076$).

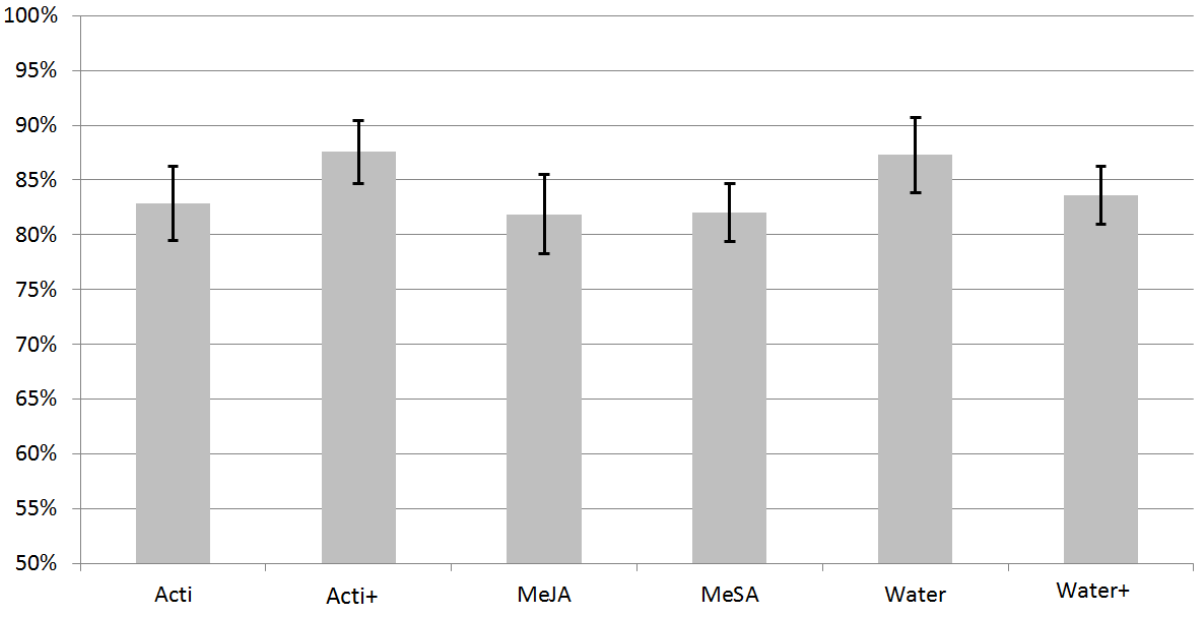

Figure 13: Mortality (2018): full rate. Mortality rate is the total number of stems with WSS mortality divided by the total number of infested stems. We did not find any significance in mortality among treatments in 2018.

Mean larval weight

There was significantly lower larval weight in Acti treated plots versus the Water+ treated plots in 2017 ($p<0.001$) (Figure 14). There was also a significantly greater larval weight in the Acti+ treated plots versus the Water treated plots in 2017 ($p<0.05$). There were no significant differences in larval weight of MeJA and MeSA treated plots when compared to Water or Water+ treated plots in 2017. In 2018, MeJA treated plots had significantly lower larval weight than plots treated with Water+ ($p=0.01$). There were no significant differences in mean larval weight between Acti, Acti+, and MeSA treated plots versus either the Water or Water+ treated plots in 2018.

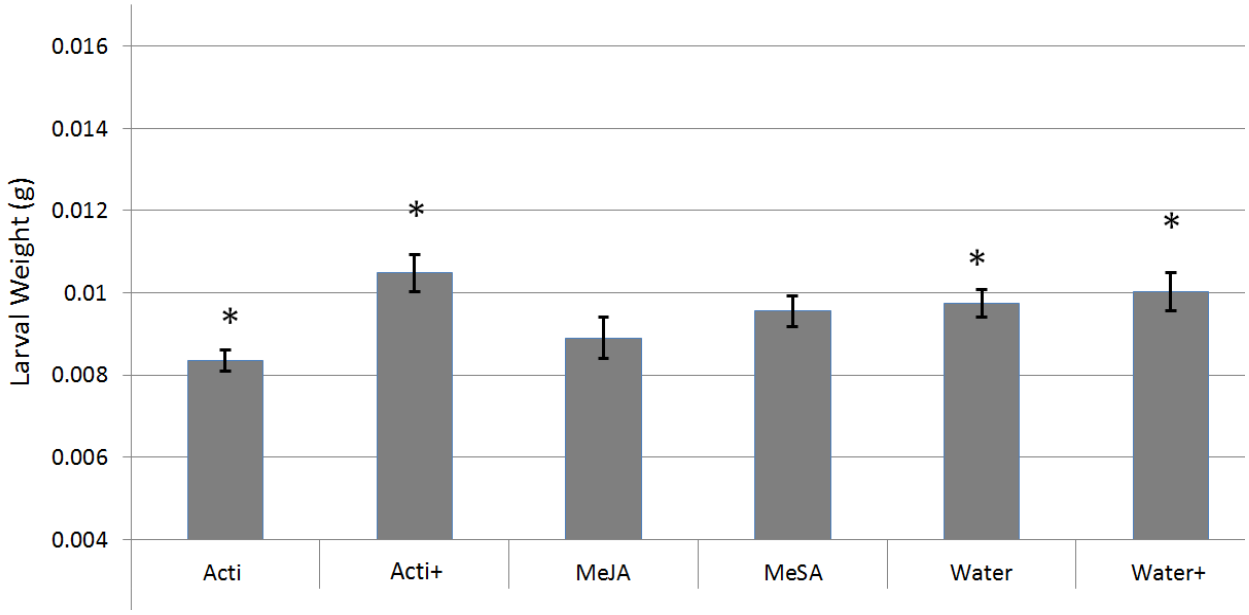

Figure 14: Larval Weight (2017). Acti treated plots had a lower larval weight compared to Water+ treated plots ($p<0.001$), while Acti+ had a greater larval weight compared to plots treated with Water in 2017 ($p<0.05$).

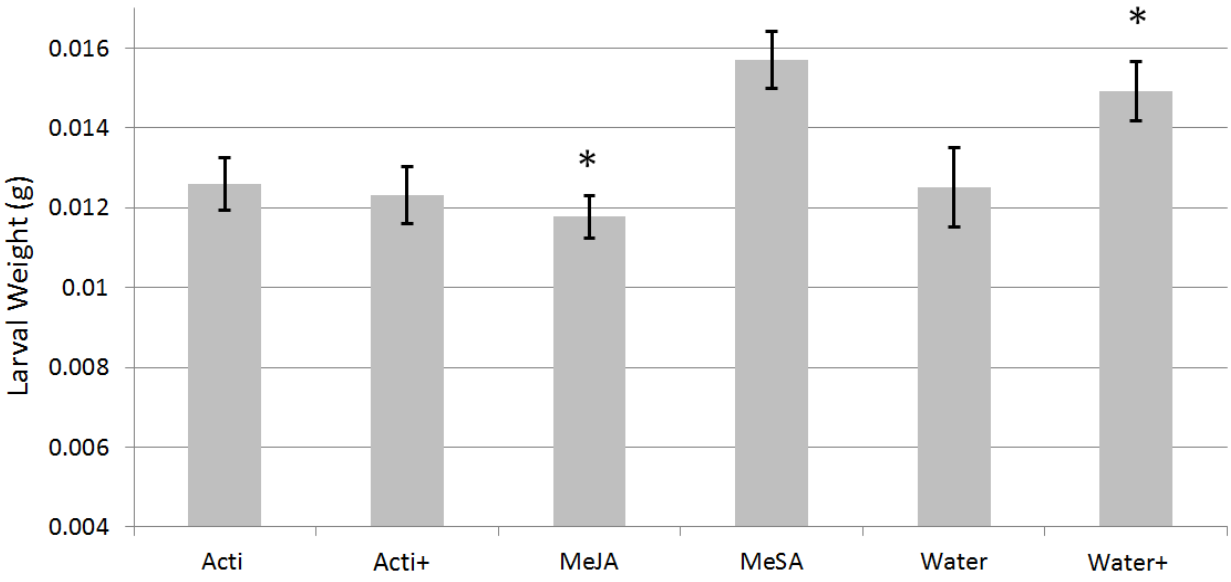

Figure 15: Larval Weight (2018). There was a lower mean larval weight of WSS in MeJA treated plots compared to Water+ treated plots in 2018 (p=0.01).

Mean neonate mortality

In 2017, we found a slightly significant increase in neonate mortality between MeJA treated plots and Water+ treated plots ($p=0.072$). We did not find a significant difference in neonate mortality between any of the other treatment plots in 2017. In 2018, there was a significant increase in neonate mortality in the MeJA treated plots when compared to the Water+ treated plots ($p<0.05$). There was no significant difference in neonate mortality between any of the other treatment plots in 2018.

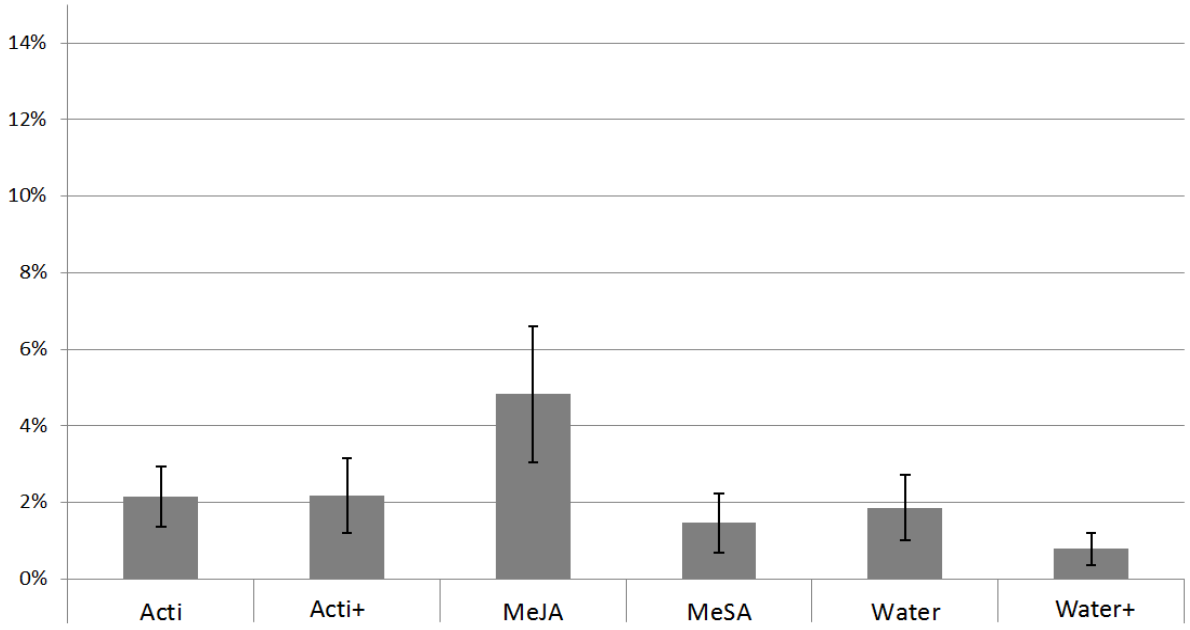

Figure 16: Neonate Mortality (2017). Neonate mortality is a measure of the extent of infestation. It is defined as WSS mortality, where infestation was contained in a single internode. Neonate mortality in MeJA treated plots was greater than Water+ treated plots in 2017 (p= 0.072).

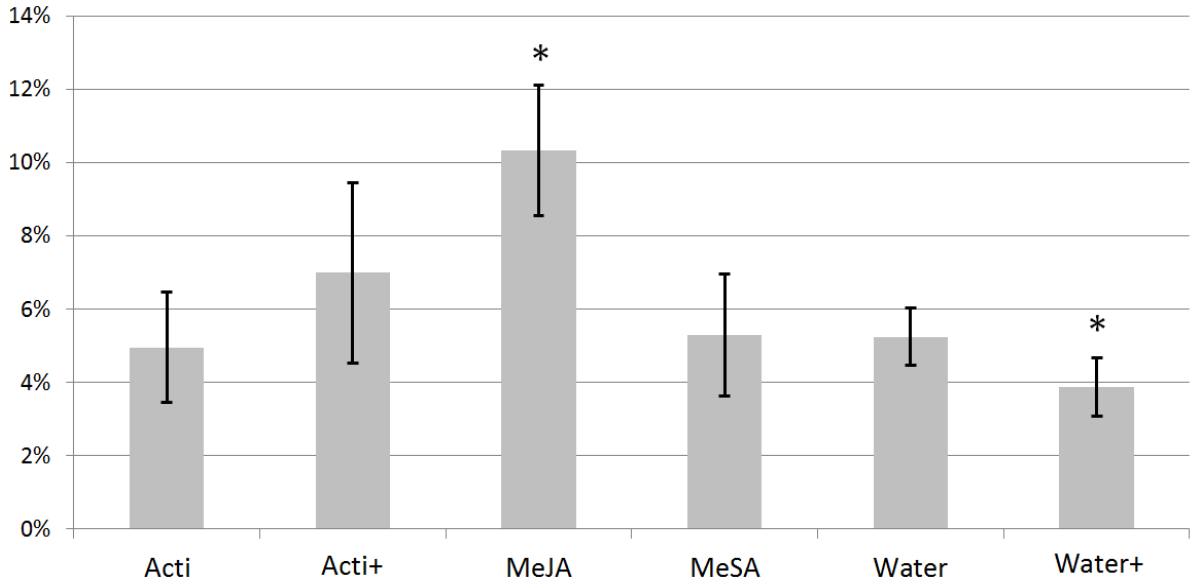

Figure 17: Neonate Mortality (2018). Neonate mortality is a measure of the extent of infestation. It is defined as WSS mortality where infestation was contained in a single internode. Mean neonate mortality was greater in MeJA treated plots compared to Water+ treated plots in 2018. ($p<0.05$)

Parasitism

We did not find a treatment effect on mean parasitism rates in 2017. However, we found that Acti treated plots showed an increase in clerid beetle (*Phyllobaenus dubius*) recruitment in 2017, compared to both the Water and Water+ treated plots ($p<0.01$), (Figure 18). There was no significant difference between the parasitism rates of treatment plots in 2018, at either the full or 1:10 dilution application rates. We did not find the presence of *Phyllobaenus dubius* in 2018. While parasitism levels were significantly different between 2017 and 2018, there was low variation in parasitism levels between treatment plots within each growing season (See Appendix) (Figures 25, 26).

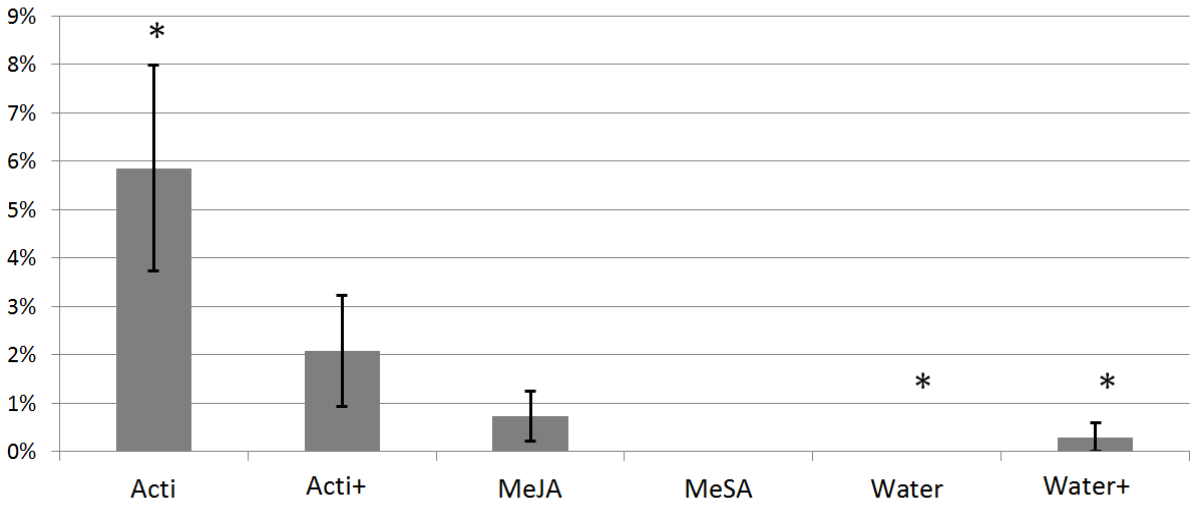

Figure 18: *Phyllobaenus dubius* Predation (2017). Percent predation was calculated from total number of stems with predators divided by the total infested stems per sample. Actigard treated plots experienced higher percent predation levels than either Water or Water + treated plots in 2017. (Acti -Water p<0.01), (Acti-Water+ P<0.01).

Mean stem height

There was a significant decrease in stem height of MeJA treated plots in both 2017 and 2018 versus all other treatment plots ($p<0.001$). There was no significant difference in mean stem height between any of the other treatment plots in either 2017 or 2018. We observed earlier senescence of MeJA treated plots, especially at the full rate applications. The earlier senescence of MeJA did not a correlate to a decrease in grain weight in 2017 or decreases in parasitism either year.

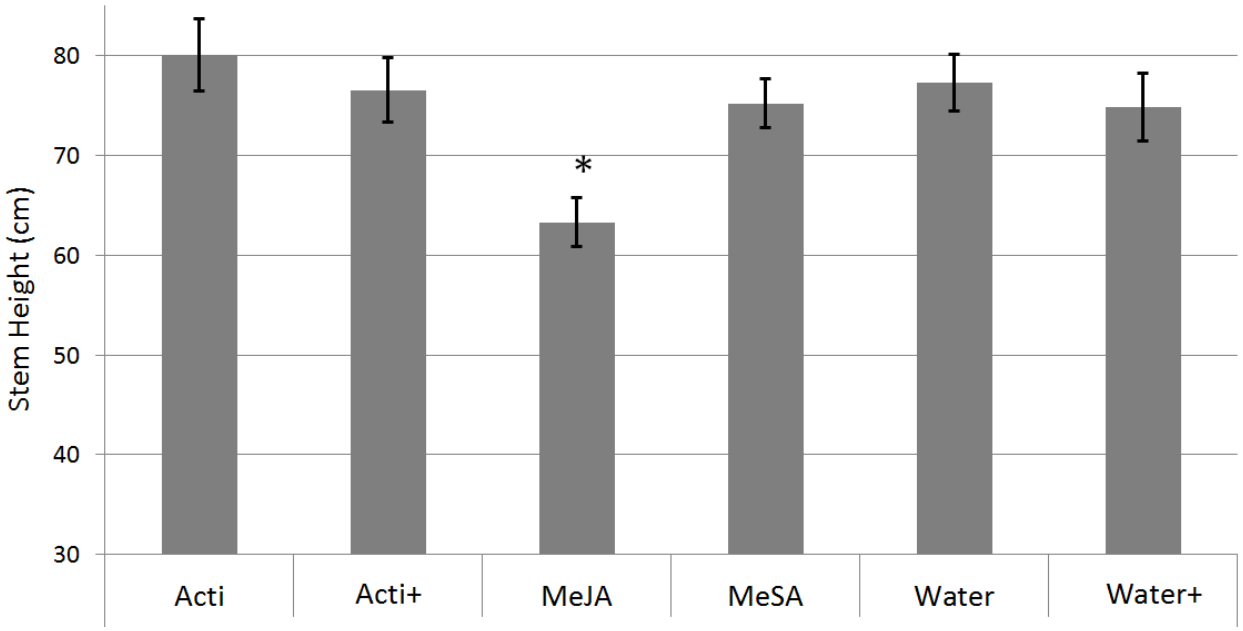

Figure 19: Mean Stem Height (2017). Stem height was measured from the root crown to the top of the highest internode (cm). The MeJA treated wheat had significantly lower stem height compared to all other treatments in 2017 (MeJA p<0.001).

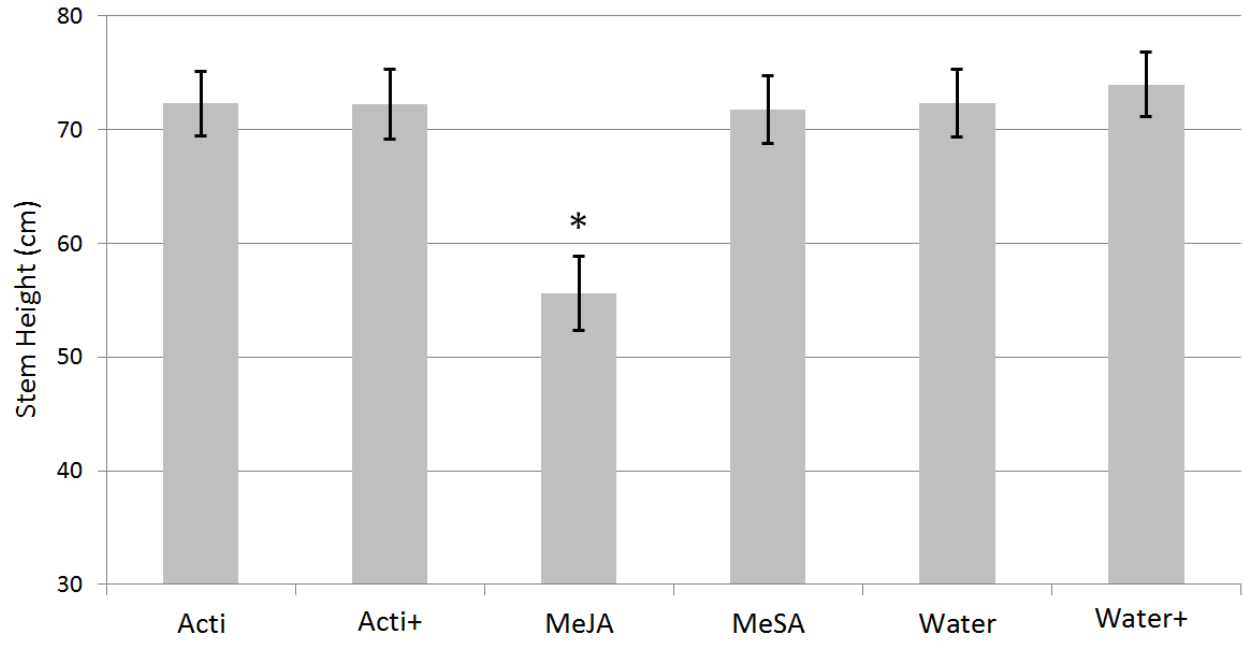

Figure 20: Mean Stem Height (2018). Stem height was measured from the root crown to the top of the highest internode (cm). The MeJA treated wheat had significantly lower stem height compared to all other treatments in 2018 (MeJA p<0.001).

Mean grain weight

Acti and Acti+ treated plots displayed a reduction of mean grain weight versus the Water treated plots in 2017 (Acti $p=0.0017$, Acti+ $p<0.05$). There was not a significant difference in grain weight between the MeJA and MeSA treated plots when compared to either the Water or Water+ treated plots in 2017. However, we found that mean grain weight was significantly lower in MeJA treated plots compared to both the Water and Water+ treated plots in 2018 at the full application rate ($p<0.001$). There was no significant difference between grain weight and any of the treatment plots in 2018 at the 1:10 dilution rate.

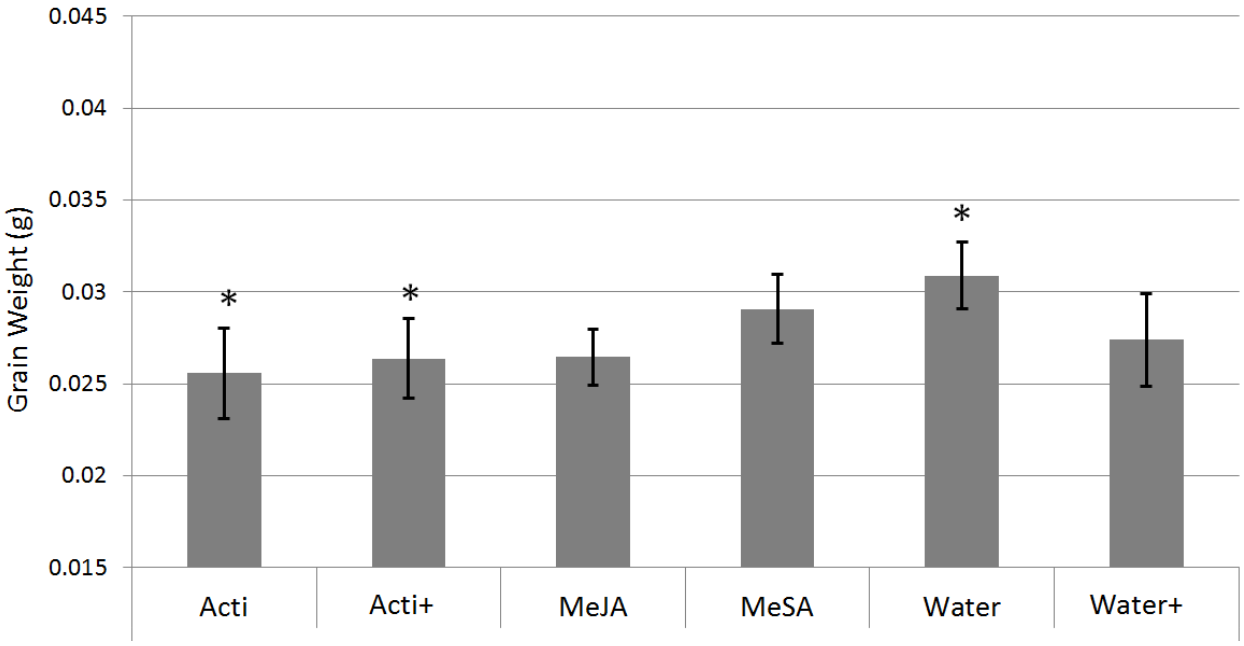

Figure 21: Mean Grain Weight (2017). Mean grain weight is the total recovered kernels divided by total weight of kernels recovered for each stem. The mean grain weight of wheat treated with Acti and Acti+ was 0.0256 and 0.0264, respectively. Wheat treated with Water had a mean grain weight of 0.0309. (Acti-Water $p=0.0017$), (Acti+-Water $p<0.05$)

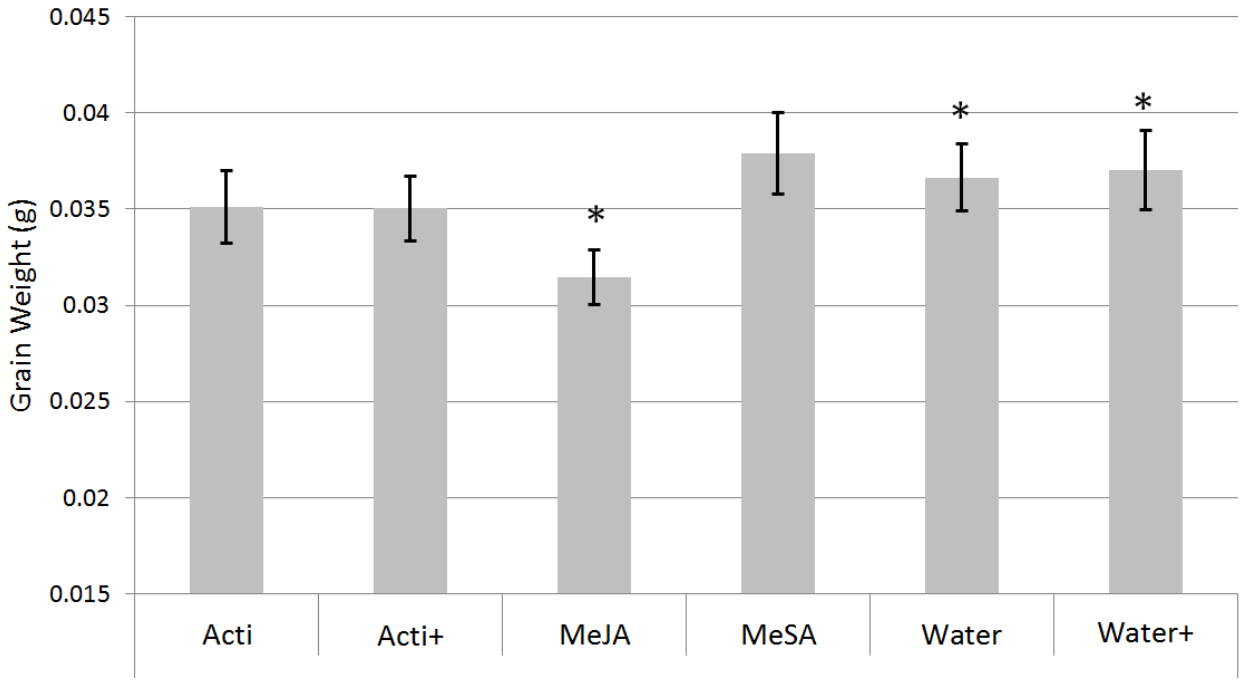

Figure 22: Mean Grain Weight (2018): full rate. Mean grain weight is the number of recovered kernels divided by total weight of kernels recovered for each stem. Mean grain weight for wheat treated with MeJA was 0.0314, while mean grain weight of wheat treated with Water and Water+ was 0.0366 and 0.0370, respectively. (MeJA- Water p<0.001), (MeJA-Water+ p<0.001)

Discussion

The results of our study show that the applications of signaling molecules in winter wheat plots may have several effects on the biological activity of WSS, its natural enemies, and the wheat plants. The variability between year and application rates suggests that several factors are important to control when making an assessment of the effect of signaling molecules through field experimentation. While our results from this preliminary research are variable, evidence suggests that applications of MeJA and Actigard® induce responses in winter wheat. Our data did not suggest any significant changes in plots treated with MeSA. These findings could be used in the planning and design of future field trials.

The significant difference in years may be attributed to the fluctuation in weather between the growing seasons of 2017 and 2018 (Figures 23, 24). Mean monthly temperatures in 2017 were 21.8 °C in July and 19.4 °C August, compared to mean monthly temperatures in 2018 of 19.7 °C in July and 18.1 °C in August. Total precipitation was also greater every month in 2018, including a much wetter end of the growing season in August (NOAA Weather Database; Diamond 2013). Seasonal weather patterns greatly influence the temporal fluctuations of insect populations (Kingsolver 1989). In considering how seasons can vary, it is important to review how Holmes et al. (1963) explained weather influences on the effectiveness of *B. cephi* and *B. lissogaster*. During wet and cool seasons the wheat stems stay succulent longer and the WSS larvae are active longer. These changes significantly improve the success of second generation braconids. Another factor to consider is the separation of the 2017 and 2018 sites (approximately 800 m). Observed differences may be partially linked to spatial fluctuations of WSS (Weaver et al. 2005) that exist within the broader well known "edge effect" distribution. While there may have been some influence of localized spatial effects between plots in 2017 and 2018, we believe that the primarily cause of the profound year effects was due to very different growing seasons.

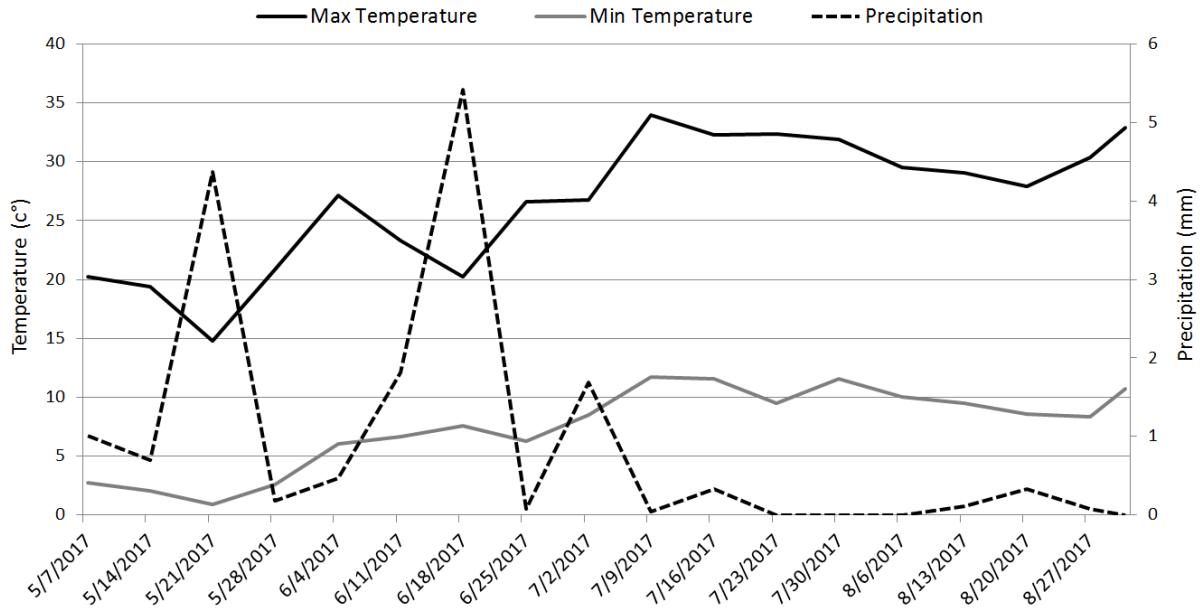

Figure 23: Weather Data (2017). Weekly precipitation and temperature averages for the months of May, June, July, and August.

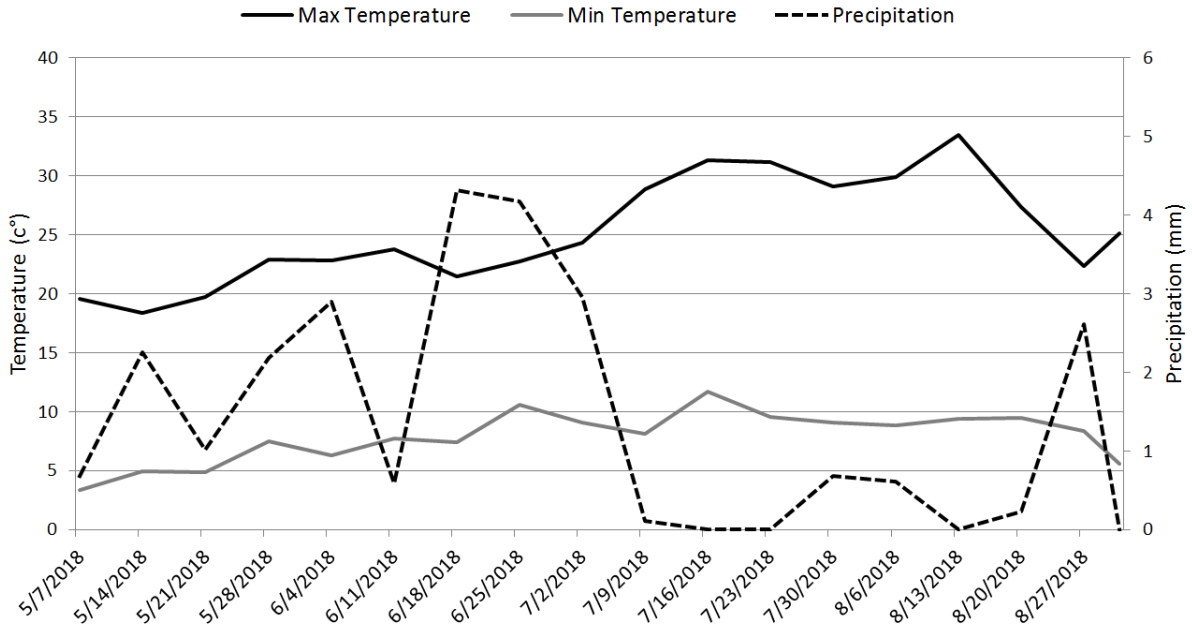

Figure 24: Weather Data (2018). Weekly precipitation and temperature averages for the months of May, June, July, and August.

The plots for the 1:10 dilution rate were spatially separated from those receiving the full rate (Figure 3). The differences reported for data between application rates may be linked to another local difference in the spatial distributions of WSS and parasitoids, as well as spatial variation in wheat performance (Weaver et al. 2005). In 2018, the full application rate plots experienced higher infestation and lower grain weight. This may have occurred from the increased attraction of WSS to the plots where signaling molecules were applied at higher rates. Research on plant signaling is fraught with difficulties when determining the distance at which signals can be perceived, especially under varying environmental conditions (Heil and Karban 2010). Perception limitations of plants result in plant-to-plant signaling occurring over smaller distances than plant-insect signaling (Frost et al. 2008). The full application rate may have recruited WSS at a greater distance compared to the 1:10 application rate by triggering attractants (Weaver et al. 2009). Bayram and Tonga (2018b) found that MeJA was attractive for a WSS in Turkey; however, our data shows that MeJA treated plots actually experience less infestation compared to plots treated with Water and Water+. In 2018 we also found a decrease in grain weight for the full rates of signaling compounds when compared to the 1:10 application rates. This may be attributed to a phytotoxic effect of signaling molecules at high dosages (Bektas and Eulgem 2015; Bayram and Tonga 2018b). A full rate application of Actigard® was shown to decrease grain weight in 2017, while a full rate application of MeJA was shown to decrease grain weight in 2018. The full rate treatment effects on grain weight were not replicated between years. Therefore, more research is needed to determine whether high application rates of signaling molecules have a negative effect on yield in winter wheat. The effect of different

application rates on the recruitment of natural enemies is also inconsistent. In 2018, plots receiving treatments at the 1:10 application rate had greater parasitism than in full application rate plots. More research is needed to determine whether varying application rates influence WSS infestation, the recruitment of natural enemies, or winter wheat growth.

The data from this project do not support MeSA influencing tritrophic interactions in WSS infested wheat plots. We have not found any literature that links MeSA as signaling molecule for WSS or any of its natural enemies. MeSA is associated with induced defense of plants via the SAR response (Karban and Baldwin 1997); however, the cultivar we used in this study (Clearstone) did not demonstrate any changes that suggest biochemical changes (i.e. changes in yield, stem growth, or WSS infestation). Future field work may wish to exclude this treatment or perhaps modify the application strategy. Several studies on this compound have used a slow release mechanism to apply MeSA (James and Price 2004; Wang et al. 2011). A slow release mechanism would have required a different experimental design than a spray application, so it is unclear how a slow release application may differ from 3 repeated applications, even with varying application rates in 2018. Future field work may want to assess slow release applications in WSS infest plots, perhaps with several genetically distinct cultivars to better test for responses to MeSA.

Actigard® treated plots experienced significant differences compared to control plots, suggesting that Actigard® may have effectively induced the SAR response. Several papers, focusing on wheat pathogens, have linked Actigard® applications to an induction of the SAR response. Lu et al. (2005) documents an increase in defense-related proteins and a decrease of bunt disease in wheat treated with Actigard®. Another study found that

Actigard® applications resulted in the reduction of *Fusarium* crown rot of wheat, further demonstrating that this compound activates a SAR response (Moya-Elizondo and Jacobsen 2016). The grain weight of Actigard® treated plots was lower than Water treated plots in 2017; however, no significant difference existed between the grain weight of Actigard® treated plots and the Water or Water+ treated plots in 2018. These changes in yield, while inconsistent, may be related to biochemical changes in the wheat plant. The growth differentiation balance hypothesis (Lorio 1986) explains how plant processes, under limited resources, either allocate these resources toward growth or toward defense, via the production of PSMs. This hypothesis was described when Lorio (1986) found that pine trees were more susceptible to southern pine beetles during periods of growth. The induction of defense in wheat plants may have altered wheat physiology, slowing growth, and increasing the production of defensive secondary metabolites. Another remarkable result of the study occurred in 2017, when Actigard® treated plots had a markedly increased recruitment of clerid beetles (*Phyllobaenus dubious* Wolcott). This species was first documented as a predator of WSS larvae by Morril et al. (2001b). An interesting difference between this predator and the braconid parasitoids is that clerid beetles can cause mortality of WSS larvae in post-harvest stubs. This finding could be evidence of induced indirect defense, where Actigard® treatments may have altered volatile emissions from the wheat plant, better signaling prey availability to this predator of WSS. Future field work assessing signaling molecules in winter wheat could discover techniques that utilize clerid beetles as biocontrol against WSS.

The MeJA treatment plots displayed variability in infestation, grain weight, and stem height compared to Water and Water+ treatment plots. Another noteworthy result was

that MeJA treated plots had greater neonate mortality compared to Water+ treated plot. This relationship was only moderately significant in 2017 ($p=0.072$) compared to in 2018 ($p<0.05$). Dead neonate larvae were categorized by tunneling within a single internode before dying. Likewise, early mortality of WSS, through parasitism, corresponds to increased yield (Buteler et al. 2008; Bekkerman and Weaver 2018). MeJA should be explored further to see if it is a reliable product for the increase in neonate mortality of WSS larvae. MeJA treated plots also experienced a reduction in infestation in 2017. This may be related to direct deterrence of WSS by the MeJA treatments. This effect was not replicated in 2018, so more research is required to delve further into the potential of MeJA as a deterrent for WSS oviposition. The mortality rate was higher in MeJA treated plots relative to Water treated plots in 2017 ($p=0.076$). The 17% increase in mortality suggests that wheat treated with MeJA may negatively impact WSS fitness. The significant decrease in stem height and reduced grain weight are important findings from this research. The jasmonic acid pathway is thought to play an important role in the senescence of plants; therefore, the reduction of stem height and grain weight may have been attributed to the premature activation of the senescence process (Beltrano et al. 1998). Grain weight was not significantly reduced in the 1:10 application of MeJA, suggesting that the decrease in grain weight may have been due to the rate of application of MeJA. Bayram and Tonğa (2018b) observed stunted growth and early maturation of the wheat plots spayed with MeJA. They attributed these effects to phytotoxic activity of MeJA. Future research, especially those which capture yield data, should consider incorporating lower application rates of MeJA. These results suggest that MeJA may have changed the biochemistry of the wheat plant and that it may offer induced defense against WSS.

Future field work, examining signaling molecules in WSS infested wheat, should consider exploring the effects of MeJA and Actigard®. Other treatments could also be introduced to this type of study. Ethylene is another plant compound that has been shown to induce biochemical changes in plants and it is generated at wound sites associated with herbivores and pathogens (Karban and Baldwin 1997). Abscisic acid is a signaling molecule that has been shown to increase at the wound sites of solanaceous plants. It has demonstrated the ability to signal physiological changes in several plant species, one of which being the accumulation of PIs (Karban and Baldwin 1997). The research on the impact of signaling molecules in WSS infested wheat plots is still in its infancy. To our knowledge, this project is only the second study of its kind to take place in the wheat cropping systems of Montana. The other project out of the Western Triangle Research Center in Conrad, MT provides another approach to these questions and an additional source of information (Shrestha et al. 2018). The experimental design of their project was different and they found unique results compared to our project. These researchers examined how spray applications of Actigard®, cis-jasmone, and a botanical insecticide (Azadirachtin) influenced tritrophic interactions in WSS infested plots. Their study closely documents the direct effect of these compounds on insect populations, through laboratory bioassays and the collections of insects using sweep netting. Their data were collected in a single growing season over three sites, compared to our project which collected data in a single site over multiple field seasons. Their results showed that Actigard® applications were effective at decreasing WSS infestation level, whereas the results of ours study suggest MeJA as the most effective compound at decreasing WSS fitness. Interestingly, they, like us, also found an association of Actigard® applications with a decrease in larval weight,

providing more evidence that this treatment may decrease WSS fitness. Both studies only examined a single wheat variety and they used Yellowstone, a closely related variety to the Clearstone cultivar used in our project (Berg et al. 2014). The induction of defense can vary across genetically distinct cultivars within crops (Karban and Baldwin 1997). Future field work may want to examine at least two genetically variable cultivars, possibly a solid-stemmed versus a hollow-stemmed cultivar. This would provide a genetically comprehensive insight on how these compounds impact tritrophic interactions regarding the induction of defense in the wheat plant. Laboratory experimentation on these elicitors could be used to supplement field work. A quantification of VOCs released by treated plants could enhance the understanding of how these compounds influence the biochemistry of wheat. The analysis of plant tissue could help determine whether these compounds cause an increase in concentrations of PSMs.

Future agricultural research should continue investigating the chemical relationships between the WSS, its natural enemies, and its host-plants. The preliminary data in this field have provided new hypotheses on signaling molecules and the possible induction of defense in winter wheat. Actigard® and MeJA are two compounds which may induce defense in winter wheat and interact with tritrophic interactions in WSS infested surroundings. The manipulation of induced defenses in wheat could potentially provide producers with another tactic for the management of WSS, especially in conjunction with applications to suppress crop disease.

Appendix

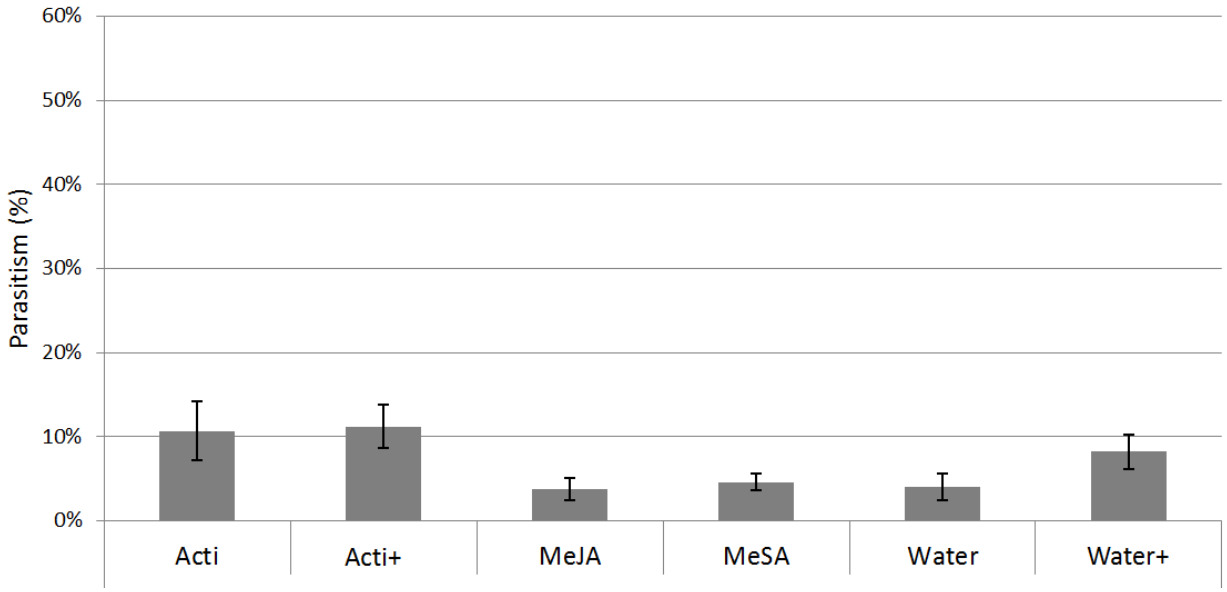

Figure 25: Parasitism of WSS by Treatment (2017). Parasitism rates were determined by dividing the total stems with parasitism by the total number of infested stems of each sample. We found no significance between parasitism and treatment plots in 2017.

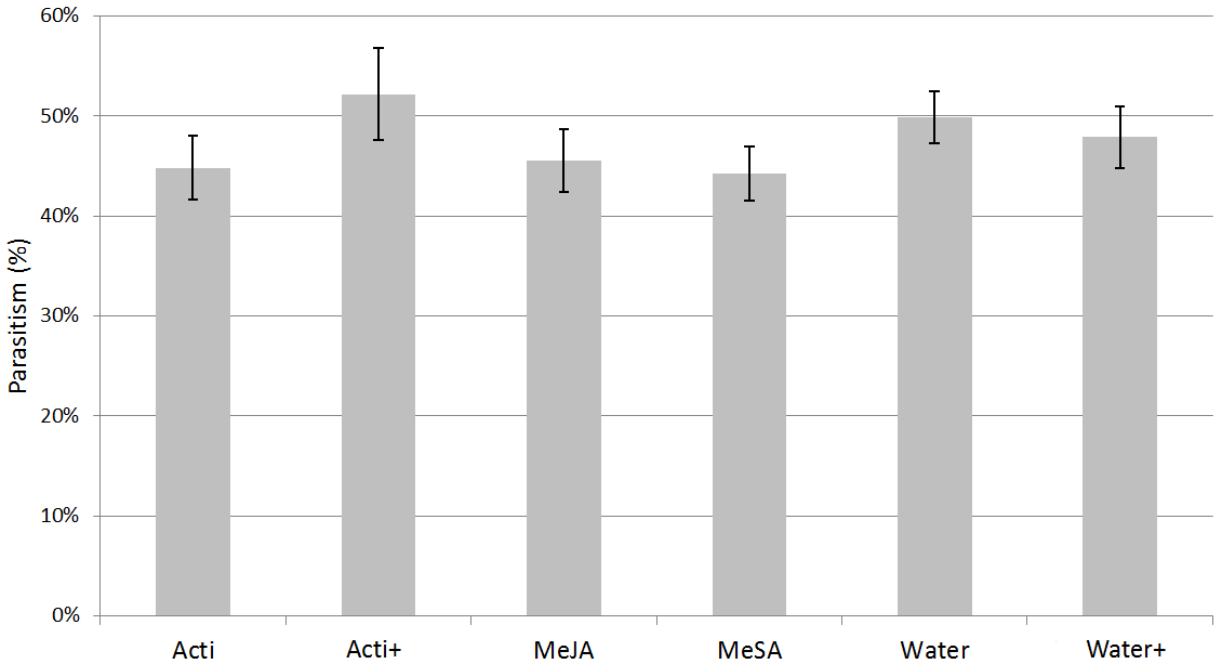

Figure 26: Parasitism of WSS by Treatment (2018). Parasitism rates were determined by dividing the total stems with parasitism by the total number of infested stems of each sample. We found no significance between parasitism and treatment plots in 2018.

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