THE EFFECTS OF SILICON ACCUMULATION IN CORN ON THE FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE)

by

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ABSTRACT

The fall armyworm [FAW, *Spodoptera frugiperda* (Smith)] is a major economic pest in the United States and has recently become a significant concern in Africa and Asia. With its acquired resistance to many current control strategies, like pesticide application and transgenic corn, alternative management techniques are becoming a necessity. The use of silicon (Si) as a pest control agent has shown promise in many systems in the past with a variety of plant species due to its ability to alter plant defense responses and mechanically damage herbivorous insects. In this study FAW was used as a model pest species to understand how its growth and ability to survive infection by a parasite are affected when feeding on Si supplemented corn plants. Corn (*Zea mays* L.) was found to accumulate Si, however, this ability varied between cultivars. Feeding damage from FAW larvae caused the release of volatile organic compounds, but Si did not have a significant effect on these. Feeding on Si supplemented plant material did not have a negative effect on the growth or consumption rate of FAW larvae. A significant reduction in live mass was seen only in FAW pupae that had been fed with Si supplemented plant material. FAW larvae had high mortality rates when exposed to the entomopathogenic nematode *Heterorhabditis indica* Poinar, Karunakar, & David. Larvae that consumed a high Si diet had a significantly lower mortality rate than larvae that consumed untreated corn. FAW moths had a greater preference for undamaged plants for oviposition than FAW infested plants. Si had little to no effect on the oviposition preferences of FAW moths. These findings do not support the use of Si as a method of controlling FAW on corn.

Chapter 1

GENERAL INTRODUCTION

Silicon (Si) is one of the most abundant elements in the Earth's crust and, because of this, it is also very abundant in soil (Hans Wedepohl, 1995). While this element may be quite common, it is not always in a bioavailable form. For Si to be available to plants it must be absorbed in the form of silicic acid (Villegas et al., 2017). When this silicic acid is absorbed, it is deposited in the plant tissue as hard structures called phytoliths (Alhousari and Greger, 2018). This Si can end up in cell walls throughout a plant to increase toughness and defend it from external biotic and abiotic factors, but it can also accumulate in specialized structures such as trichomes (Epstein, 2009).

Si concentration in soil is largely determined by the cycling of the mineral into plant tissues and then back into the soil after the death of the plant. The removal of plant tissue from agricultural fields interrupts this cycling process and promotes the sapping of Si from the soil (Haynes, 2014). Due to this break in Si cycling in crop fields, there is a greater need for the manual application of bioavailable Si. This practice can ensure that the Si levels in crop fields are constantly replenished and the element is readily available to the plants.

Application of Si to economically important crops can have several positive effects on the plants. It has been shown to promote vegetative growth in rice (*Oryza sativa* L.) where application of Si to soil resulted in roughly a doubling of plant dry weight and chlorophyll content as well as a significant increase in plant height

(Nascimento et al., 2018). Kuai et al. (2017) conducted a study in which Si application to rapeseed (*Brassica napus* L.) plants increased their total grain yield and the grain weight. They also found that the increase to grain yield depended highly on the variety and life stage of the plants being supplemented. Additionally, Si application can promote greater resistance to abiotic stressors, such as salt-induced osmotic stress (Liu et al., 2015) and drought (Epstein, 2009), as well as resistance to biotic stressors, like insect herbivores (Correa, et al., 2005, Nacimento et al., 2018, Texeira et al., 2017).

In addition to its effects on the physical characteristics of plants, Si can have significant effects on plant chemistry. When plants are attacked by herbivores, they can induce two different types of defenses: direct defenses and indirect defenses. Direct plant defenses are often used to harm or deter the attacker. Indirect defenses, however, are meant to attract natural enemies of the herbivores that induced their production. Volatile organic compounds (VOCs) produced when a plant is attacked by an herbivore can be detected by parasitoids which can then follow the VOCs back to their source (Finidori-Logli et al., 1996, Liu et al., 2017, Steinberg et al., 1993, Turlings et al., 1991). These herbivore-induced plant volatiles (HIPVs) are largely dependent on the jasmonic acid (JA) signaling pathway (Thaler et al., 2002). Ye et al. (2013) found that Si application to rice had an amplifying effect on the JA-mediated defense response of the plants, acting as a primer. This may bolster plant defenses against insect herbivores by amplifying HIPV signals to parasitoids. In addition to its priming abilities, Si can alter the HIPV response by changing the concentrations of constitutive VOCs. In rice, Liu et al. (2017) supplemented plants with Si and then infested them with the rice leaffolder [*Cnaphalocrocis medinalis* (Guenée)]. They found that the Si supplemented plants produced lower concentrations of several VOCs than infested plants that did not receive extra Si. This altered HIPV response proved to be more attractive to *Trathala flavo-orbitalis* Cameron and *Microplitis mediator* (Haliday), natural enemies of the rice leaffolder.

Corn (*Zea mays* L.) is a very important crop worldwide, cultivated on each continent except Antartica. In 2017 in the United States, this crop had an estimated value of almost 50 billion dollars (USDA-NASS, 2017). Since corn is of such economic importance, it is imperative to have effective strategies to protect fields from insect pests. Common pest control strategies are the use of pesticides, transgenic technology, and rotating corn and soybean fields. Unfortunately, over time pests are capable of adapting to these control methods. One such pest is the fall armyworm [FAW, *Spodoptera frugiperda* (Smith)].

Strains of FAW have already developed resistance to the use of pesticides like pyrethroids and organophosphates (Yu, 1991) as well as transgenic Bt corn that has been modified to produce insecticidal toxins from the bacterium *Bascillus thurigensis* (Martinelli et al., 2017). Worse still, FAW has been able to spread to countries that were not prepared for this pest and did not have appropriate management strategies in place to deal with it. FAW has become a significant pest in most of Africa, where it was originally introduced in 2016 and has been able to devastate entire corn fields (Goergen et al., 2016). It has since spread through several countries in Asia (FAO, 2019, IPPC, 2018) and it will likely continue to spread as long as it continues to find favorable environments. Being resistant to several common pest control strategies makes FAW an ideal species for testing alternate strategies for managing this pest on corn, such as Si application.

There are numerous studies that support the effectiveness of using Si application to promote greater resistance in crops against insect herbivores like FAW. In a study on rice, Si application lead to a reduction in larval weight, adult longevity, number of eggs, and egg viability in FAW (Nacimento et al., 2018). Similarly, Teixeira et al., (2017) supplemented collard (*Brassica oleracea* L.) with excess Si which resulted in lower pupal weight and fewer eggs laid by the diamondback moth [*Plutella xylostella* (L.)]. Additionally, the use of Si may work synergistically with the use of other control methods, like the application of entomopathogenic nematodes (EPNs). EPNs are obligate insect parasites typically living in soils and, through mutualistic relationships with bacteria, they are able to kill their host within 24h to 48h after infection (Kaya and Gaugler, 1993). The efficacy of these EPNs can be further increased by simultaneously treating crops with low concentrations of low-toxicity pesticides (Viteri et al., 2018). It is possible that a similar synergistic effect may be seen by treating plants with Si to reduce growth, consumption, and overall health of FAW larvae, making them more vulnerable to EPN attack.

Si application has shown great potential in plants like collard and rice, however, because it is not absorbed the same way by all plants there is still much to be learned about how other species utilize Si. This research was conducted to test several hypotheses about Si with regard to its accumulation in corn and the resulting effects on the health and behavior of FAW. The first hypothesis was that supplementing corn plants with Si would result in a significant increase in Si content in plant tissue. Secondly, this increase would cause changes to the concentrations of certain VOCs emitted by the plants after being damaged by FAW larvae. The next hypothesis was that feeding on plants with higher Si content would lead to a reduction in FAW growth and consumption rate, as well as a greater mortality rate when subjected to EPN attack. Finally, it was hypothesized was that Si accumulation would lead to a change in the oviposition preferences of FAW moths. The data gained by testing these hypotheses will be valuable in determining the efficacy of Si use as a pest control method for dealing with FAW on corn.

Chapter 2

SILICON ACCUMULATION IN CORN AND ITS EFFECTS ON VOLATILE RESPONSE

Introduction

Silicon (Si) is highly abundant in Earth's crust (Hans Wedepohl, 1995), however, with much of it not in bioavailable form, plants are only able to access a fraction. It is possible to artificially supplement plants with Si in a form that they can use, however, not all plants are able to absorb this bioavailable Si to the same extent. Plants that accumulate more Si than most other species are classified as high-accumulators, while species that accumulate less are either medium- or non-accumulators. High Si accumulators are typically wetland grasses like rice (*Oryza sativa* L.) while terrestrial grasses, such as wheat, are medium-accumulators. Non-accumulators are commonly eudicots (Alhousari and Greger, 2018). In past studies, Si application has led to increased plant growth and yield in several important crop species (Kuai et al., 2017, Nascimento et al., 2018). Si can also imbue plants with greater resistance to abiotic stressors, like water stress (Epstein, 2009, Liu et al., 2015) and biotic stressors, like herbivores (Correa, et al., 2005, Nacimento et al., 2018, Texeira et al., 2017).

In addition to its effects on the physical traits of plants, Si application can also alter the induced defense response of plants that have been attacked by insect herbivores. By priming the jasmonic acid pathway, emission of volatile organic compounds (VOCs) by a plant can be amplified (Ye et al., 2013), possibly bringing more parasitoids to the plant's defense. Additionally, by altering the relative concentrations of the component compounds released by a plant, the emissions can be made more attractive to the natural enemies of an herbivore. When Liu et al. (2017)

supplemented rice plants with Si and then infested them with the rice leaffolder [*Cnaphalocrocis medinalis* (Guenée)], the plants produced lower concentrations of α-bergamotene, β-sesquiohellandrene, hexanal 2-ethyl, and cedrol than untreated rice plants. This altered VOC blend proved to be more attractive to parasitoids.

The results of these studies show that Si has potential not only for increasing total growth and yield of crops, but also as a pest control treatment by amplifying or changing the VOC response of plants when they are attacked by herbivores. Little research, however, has been conducted with corn (*Zea mays* L.). With this in mind, corn was chosen as the model plant organism for this research. The study was designed to test, first, the hypothesis that corn would accumulate Si. After this it was designed to test the hypothesis that Si accumulation in corn would result in a change to the VOC profile of plants that had been fed on by fall armyworm [FAW, *Spodoptera frugiperda* (Smith)] larvae.

Methods

Corn Treatments and Growing Conditions

Seeds from three cultivars of corn were selected: Field (35F38, Dupont Pioneer, MI, USA), Organic (51T59, Blue River Organic, IA, USA), and Sweet (Summer Sweet 7902R, Siegers Seed Company, MI, USA). These were washed in a 10% bleach solution for one hour to remove any residual seed treatment before being planted in 25cm diameter pots. Plants were grown in a temperature-controlled greenhouse at 27°C and were watered three times weekly. The plants were split into two groups: Si- and Si+. The Si- group was watered with 250mL of water only while the Si+ group was given 250mL of 500MgL^{-1} Na_2SiO_3 solution (Alfa Aesar, MA, USA), as in Frew et al. (2017). After two weeks of growth, plants were placed individually into mesh rearing cages (0.61m x 0.61m x 91m, Bioquip Products Inc., Rancho Dominguez, CA, USA). The Si- and Si+ groups were further divided into control (Cont) and treatment (Treat) groups resulting in a 2:2 full-factorial design (Si-/Cont, Si-/Treat, Si+/Cont, and Si+/Treat). The Cont plants were left to grow for one week undamaged. A single second instar FAW larvae was placed onto each plant in the Treat groups, and these were allowed to feed for one week. Corn strain FAW were obtained from a lab grown colony (Frontier Scientific Service Inc., DE, USA) and reared in sealed plastic cells with an artificial diet mixture [19g agar, 144g Frontier Scientific general purpose lepidoptera diet (Frontier Scientific Service Inc., DE, USA), 875ml water].

Si Analysis

Three-week, four-week, and six-week-old, Field corn plants from both the Si- and Si+ groups were cut at the base and dried in an oven at 70°C for four days. To evaluate if Si accumulation in above-ground tissue varies across cultivars, plants of the other two cultivars were cut and dried at four weeks as well. Dried plant material was then ground to a fine powder on a ball mill grinder (Mixer Mill MM400, Retsh GmbH, USA) in 25 ml canisters with two 15mm beads. The Si content of the samples was analyzed using a Supermini200 X-ray fluorescence spectrometer (Rigaku Co., TX, USA). Si content was recorded as percent of total mass using carbon as a balance and helium as an atmosphere.

Volatile Analysis

One plant from each of the four treatment groups was placed into a 20cm x 80cm cylindrical sealed glass chamber 24 hours prior to VOC collection. After this period two air pumps were connected to the chambers. The inward pump was set to 0.6psi and the outward pump was set to 1.5psi, pulling air from the chamber through a volatile trap (Absorbent material: HayeSep Q, absorbent quantity: 30mg, absorbent surface area: 17.5m^2 Volatile Assay Systems, NY, USA). The pumps ran for two hours before the traps were removed and extracted with 200µL of dichloromethane (Sigma-Aldrich, MO, USA). Traps were cleaned with 2ml of dichloromethane prior to their next use. VOC samples were stored at -80°C until analyzed via gas chromatography-mass spectrometry (GC-MS). This process was repeated 11 times with new plants each time.

20µL aliquots of the sample were transferred to a separate vial with 5µL of internal standard solution (20ng/µl *n*-octane and *n*-nonyl acetate in dichloromethane) VOC analysis was conducted using a Hewlett Packard HP 6890 series gas chromatograph equipped with an automated column injection system (PAL III, Agilent Technologies Inc., CA, USA) and a mass spectrometer (5973 Network, Agilent Technologies Inc., CA, USA). 3µl of each sample were injected in pulsed splitless mode onto an apolar capillary column (HP-5MS, 30m, 0.25mm ID, 0.25µm film thickness, Alltech Associates, Inc.). Helium was kept at a constant pressure of 1.6psi and was used as carrier gas flow (1.2ml/min). After injection, the column was maintained at 40°C for 2 minutes and then increased to 100°C at 9°/min, then to 200° at 6°/min, and finally a postrun of 2 minutes at 250°C. The resulting mass spectra were analyzed with ChemStation software (Agilent Technologies Inc., CA, USA) and were compared with those in the NIST 05 library. The relative concentrations of the identified VOCs were calculated by comparing the areas of their peaks to the average area of the peaks of the internal standards. A list of compounds of interest were selected from HIPVs found from corn that had been induced by *Spodoptera spp*. in previous studies (Gouinguene et al., 2007, Turlings et al., 1991). These compounds were β-myrcene, (*Z*)-3-hexen-1-ol, linalool, (*E*)-4,8-dimethyl-1,3,7-nonatriene, phenethyl acetate, indole, β-caryophyllene, β-bergamotene, (*E*)-β-farnesene, and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene.

Statistical Analysis

All analyses were conducted in JMP Pro 14 (SAS Institute Inc., NC, USA). Si content among cultivars as well as among corn of different ages was analyzed using two-way ANOVAs followed by an SNK post-hoc test to determine differences in Si accumulation between cultivars and treatments. Relative concentrations of each VOC individually were compared among the four treatment groups using a two-way ANOVA followed an SNK post-hoc test to determine differences in relative concentration between treatments. Total volatile abundance and total number of VOCs of interest were analyzed using two-way ANOVAs followed by SNK post-hoc tests to find the differences between the treatment groups.

Results

Silicon Content in Corn

Significant differences in Si content were found among the three cultivars and their two Si treatments (Fig 1, 2-way ANOVA, $F_{5,34}$=18.534, *P*<0.001). Si treatment had a significant effect (*P*<0.001). Of the plants in the Si+ treatment, the Organic cultivar accumulated the least. The Si content of the Si+ Field and Sweet cultivars were not significantly different from each other (*P*=0.697). Si-/Organic corn did not have a significant difference in Si content from Si-/Field corn (*P*=0.519) or Si-/Sweet corn (*P*=0.752), nor did the Si-/Field corn and Si-/Sweet corn differ from each other (*P*=0.315). Cultivar did, however, have a significant effect on Si content (*P*=0.038).

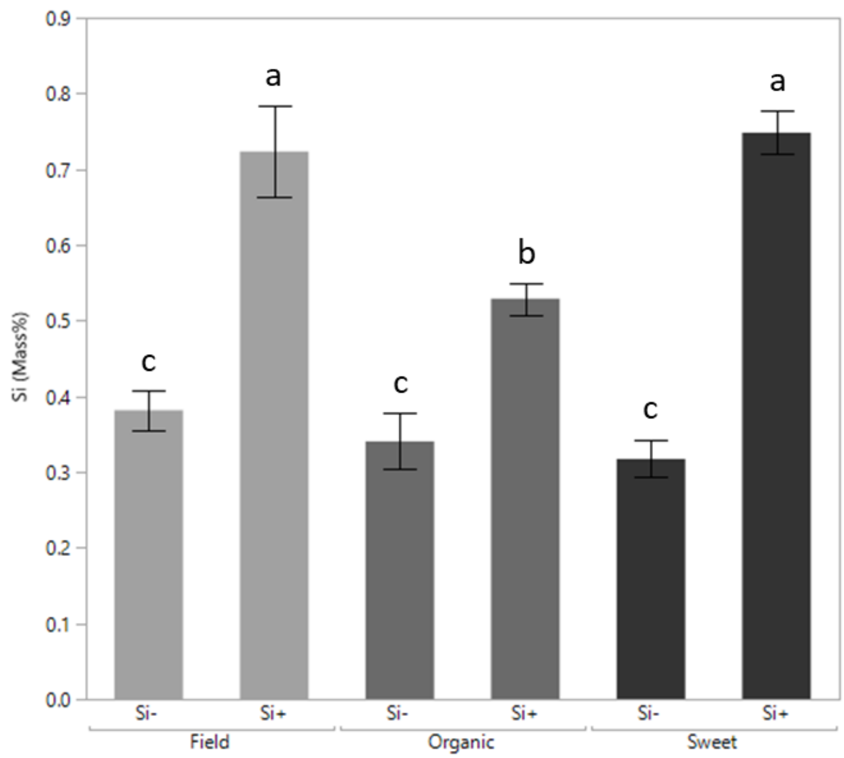

Figure 1 Average mass % silicon from three corn cultivars that had been supplemented with silicon (Si+) or had been left untreated (Si-). Bars indicate standard error. Means labeled with different letters are statistically different (P<0.05, n=5)

While there were significant differences in Si content of corn of different ages (Fig 2, 2-way ANOVA, $F_{5,32}$=13.512, P<0.001), the only variable to have a significant effect on the 3-week, 4-week, and 6-week-old corn was Si treatment (P<0.001). Age did not have an effect (P=0.740) and it had no significant interaction with Si treatment (P=0.148).

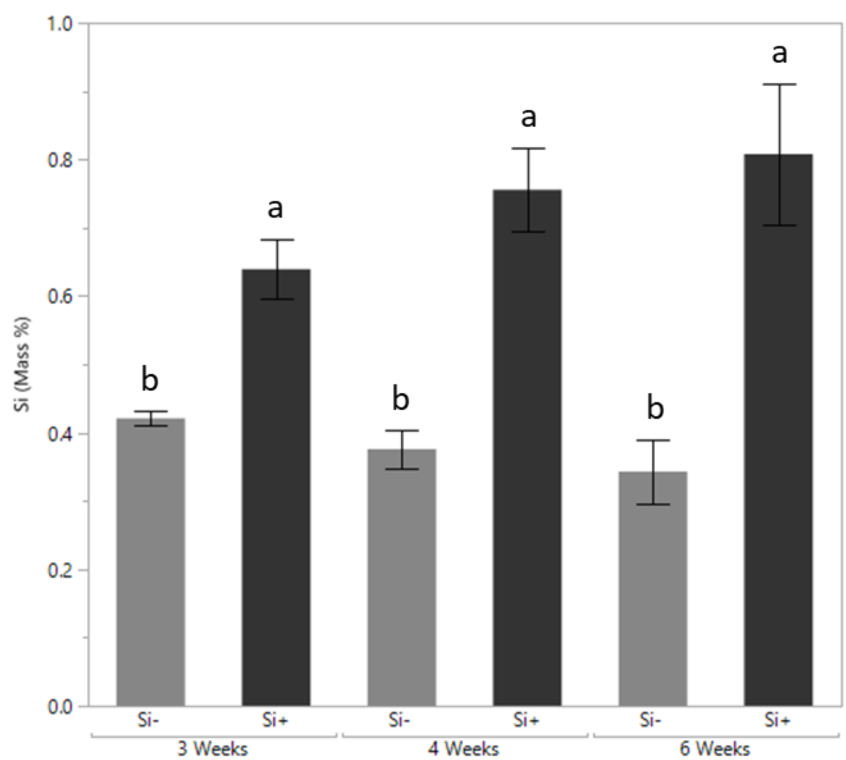

Figure 2 Average mass % Si of field cultivar corn at different ages that had been supplemented with silicon (Si+) or had been left untreated (Si-). Bars indicate standard error. Means labeled with different letters are statistically different (P<0.05, n=5)

Si Effects on Corn VOCs

Herbivore damage had a significant effect on the total abundance of the VOCs of interest with Treat plants having a greater total abundance than Cont plants (Fig 3, 2-way ANOVA, $P=0.002$). Si application did not have a significant effect on total abundance ($P=0.258$) and there was no significant interaction between Si treatment and herbivore treatment ($P=0.429$). Herbivore treatment also had a significant effect on the total number of peaks of interest recorded by GC-MS (Fig 4, 2-way ANOVA, $P=0.004$) while, again, Si treatment had no significant effect ($P=0.834$). There was no statistically significant interaction between Si and herbivore treatment in their effect on the number of peaks ($P=0.653$).

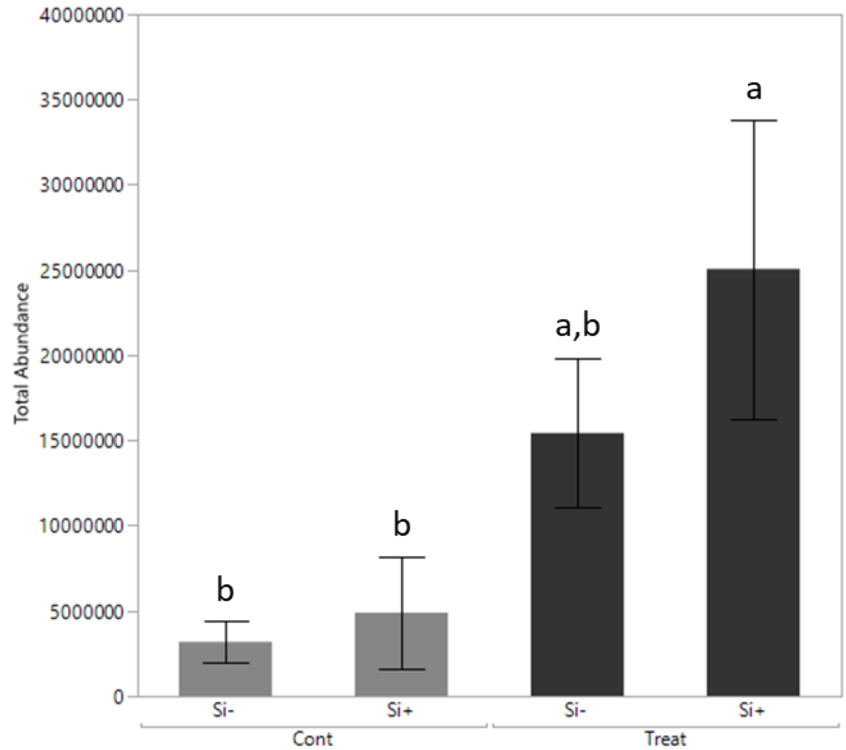

Figure 3 Average total abundance of VOCs of interest given in mass spectra for plants that had been treated with silicon (Si+), had been left unsupplemented (Si-), had been left undamaged (Cont), or had been damaged by herbivore feeding (Treat). Bars indicate standard error. Means labeled with different letters are statistically different (P<0.05, n=5)

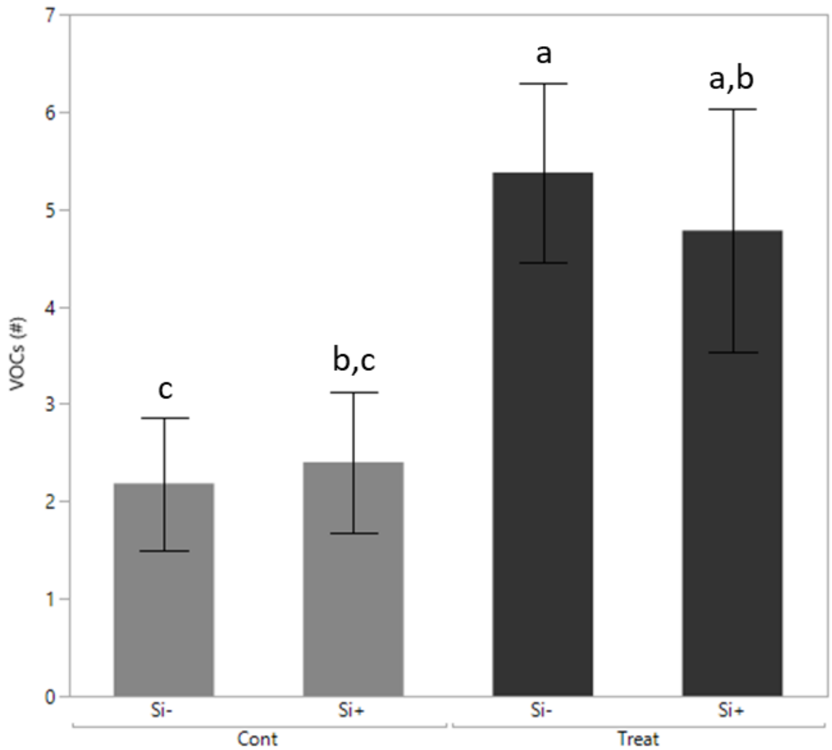

Figure 4 Average number of VOCs of interest emitted by plants that had been treated with silicon (Si+), had been left unsupplemented (Si-), had been left undamaged (Cont), or had been damaged by herbivore feeding (Treat). Bars indicate standard error. Means labeled with different letters are statistically different ($P<0.05$, $n=5$)

No significant differences were found between treatment groups for the relative concentrations of the VOCs β-myrcene (Fig 5, 2-way ANOVA, $F_{3,30}=0.398$, $P=0.756$), β-caryophyllene ($F_{3,30}=0.605$, $P=0.617$), indole ($F_{3,30}=0.687$, $P=0.567$), linalool ($F_{3,30}=1.501$, $P=0.234$), phenethyl acetate ($F_{3,30}=2.762$, $P=0.059$), and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene ($F_{3,30}=2.534$, $P=0.076$). While the results of the ANOVA imply no significant difference between treatments with regard to the release of (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, the Si+/Treat group was the only group in which this VOC was emitted.

Significant differences were found between treatment groups in the relative concentrations of (*Z*)-3-hexen-1-ol (Fig 5, 2-way ANOVA, $F_{3,30}$=3.265, *P*=0.035), (*E*)-4,8-dimethyl-1,3,7-nonatriene ($F_{3,30}$=4.391, *P*=0.011), β-bergamotene ($F_{3,30}$=3.058, *P*=0.043), and (*E*)-β-farnesene ($F_{3,30}$=3.825, *P*=0.020). For all four of these compounds, the significant effect came from the herbivore damage and not the Si treatment. Additionally, there was no significant interaction found between the Si treatment and the herbivore treatment for any of the VOCs. While the effect of Si and it's interaction with herbivore damage were not found to be significant, the relative concentrations of (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, (*E*)-4,8-dimethyl-1,3,7-nonatriene, (*Z*)-3-hexen-1-ol, indole, linalool, and β-myrcene were all higher in the Si+ group than in the Si- group within the same herbivore treatment.

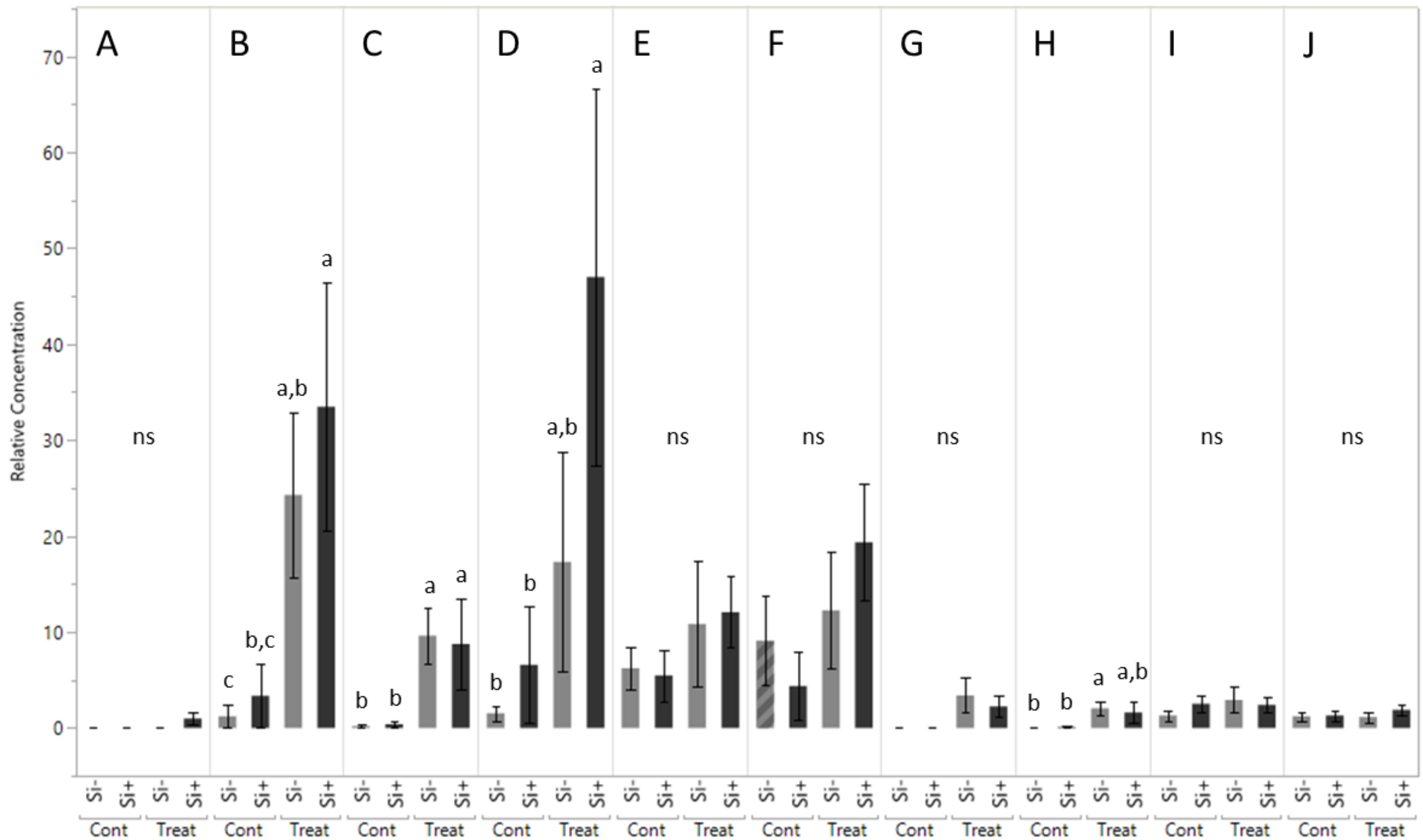

Figure 5 Average relative concentrations of VOCs of interest: A: (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, B: (*E*)-4,8-dimethyl-1,3,7-nonatriene, C: (*E*)-β-farnesene, D: (*Z*)-3-hexen-1-ol, E: indole, F: linalool, G: phenethyl acetate, H: β-bergamotene, I: β-caryophyllene, J: β-myrcene. Bars indicate standard error. Means labeled with different letters are statistically different. "ns" indicates no significant difference ($P<0.05$, $n=11$).

Discussion

All three cultivars used in this study have been shown to be Si accumulators, capable of absorbing Si when applied as a solution of Na_2SiO_3 and water. Interestingly, however, each cultivar was not equal in its ability to uptake Si. This finding means that, if Si application gains widespread use in the future, the cultivar of the corn being treated should be considered. With further testing we may come to understand what causes certain cultivars to accumulate Si to a greater extent than other. This information could be used then to breed the Si accumulation trait into other cultivars.

The length of time that Si application is continued may also be an important factor in determining how much Si is absorbed by the corn. After three weeks of Si supplementation the Si+ corn had significantly greater Si content than the Si- corn. After this point, although the means continued to increase, neither the Si+ corn at four weeks or at six weeks of age had significantly more Si than the 3-week-old plants. The data also suggests that accumulation reaches a plateau after a certain number of weeks. This means that there may be a point at which the level of Si in the plants will not increase at a rate that is fast enough to warrant the expense of the Si application. If this is the case, then the most efficient technique for Si application may be to apply heavily in the first few weeks of growth. Then the concentration of applied Si for future applications can be reduced as long as it remains enough to keep Si levels in the plants from dropping as they increase in mass. Tests should also be performed to evaluate if this late application of Si is necessary as, from a certain age, plants can compensate from insect herbivory by growing new leaves.

Overall greater total abundance of HIPVs produced by Si+ plants and greater relative concentration of (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, (*E*)-4,8-

dimethyl-1,3,7-nonatriene, (*Z*)-3-hexen-1-ol, indole, linalool, and β-myrcene in response to herbivory in Si+ plants, though not statistically significant, still hint at a possible effect of Si. This is also in keeping with the results of Ye et al. (2013) who found that Si can prime the JA pathway, thereby amplifying a plant's indirect defense response. By increasing the amount of Si added to the plants, this change to the VOC profile of corn may become more significant. The next logical step in this research would be to supplement plants with Si, allow herbivores to feed, and then determine how parasitoid preference is affected. If Si application can alter the HIPV response of rice and make it more appealing to the parasitoid enemies of the rice leaffolder (Liu et al., 2017) then it may be possible to see the same effect in corn.

Overall, the results of this study do not support the use of Si as a way to boost or alter VOC production by corn in response to FAW attack. If Si application were to be used for other reasons, such as its ability to boost vegetative growth and total yield in certain crops (Kuai et al., 2017 Nascimento et al., 2018) and to increase drought resistance (Epstein, 2009), the results of this research could be helpful in developing experimental methods to find the most cost efficient application strategies depending on crop age and cultivar.

Chapter 3

EFFECTS OF SI ON FALL ARMYWORM GROWTH AND RESISTANCE TO A PARASITE

Introduction

The fall armyworm [FAW, *Spodoptera frugiperda* (Smith)] is a highly polyphagous species of Noctuidae, feeding on a variety of plants including important crops like rice (*Oryza sativa* L.), corn (*Zea mays* L.), and cotton (*Gossypium spp*) during the larval stage (Luginbill, 1928). FAW larvae go through six to seven instars before pupation, depending on climatic conditions (Luginbill, 1928). Their entire life cycle is temperature dependent and can be completed in as few as four weeks under optimal conditions (Vickery, 1929). This species is also multivoltine, producing new generations constantly throughout the year in many southern states where host plants can grow year-round (Vickery, 1929). This relatively short life cycle, and the ability to produce multiple generations in a year allows FAW to overwhelm undefended crop fields in a short period of time.

While FAW is native to North America, where it is a significant crop pest, is has recently been introduced into South Africa. There, in the absence of FAW-specific pest control techniques and/or natural enemies, it has been able to ravage entire corn fields (Goergen et al., 2016) and has managed to become established on the entire continent within a short time (FAO, 2019). In 2018 *S. frugiperda* was found for the first time in India (Sharanabasappa et al., 2018) and Myanmar (IPPC, 2018), and in early 2019 it was confirmed in Sri Lanka, Bangladesh, Thailand, and China (FAO, 2019). If left unmanaged FAW could pose a considerable threat to the agricultural industry in not only these countries, but surrounding countries as well.

Much of the threat posed by this pest is due to its ability to adapt frequently used pest control methods. Thanks to this plasticity, strains of FAW have developed resistance to many commonly used insecticides such as pyrethroids, organophosphates, and carbamates (Yu 1991, Zhu et al., 2015) as well as Bt corn (Martinelli at al., 2017). Additionally, this pest is a problem because of its preference for agricultural sites over natural ones. Nagoshi and Meager (2004) found that FAW moths in Florida were much more common in developed areas than they were in natural environments. FAW moths are also capable of long-distance migration, making this species a pest not only in temperate regions where winters are mild enough for the insect to survive but also in regions where moths are migrating each year to lay eggs (Luginbill, 1928). Finally, FAW has two strains: the corn strain and the rice strain. In their study, Nagoshi and Meager (2004) found that the corn strain in particular had a high preference for agricultural fields. This preference is the reason that corn strain FAW were selected for use in this study.

The application of silicon (Si) has been shown to reduce the fitness of pests on many agriculturally important crop species including sugarcane, cucumber, collard, and rice (Correa, et al., 2005, Frew et al., 2016, Frew et al., 2017, Nascimento et al., 2018, Teixeira, et al., 2017). Texeira et al. (2017) concluded that this was due to increased mechanical resistance by the plants. This research is supported by a study by Massey and Hartley (2009) showing that a high silicon diet can cause mandibles to wear rapidly in herbivorous insects, likely due to higher concentrations of hard phytoliths in the plant tissue.

In addition to Si application, one pest control technique that has shown some promise is the use of entomopathogenic nematodes (EPNs). While FAW larvae can avoid contact with many EPN species by feeding above-ground and avoiding contact with soil, when larvae are ready to pupate, they must drop down into the soil, where they may come into contact with EPNs. EPN application can also be done by spraying in a similar manner to pesticide application (Garcia et al., 2008) and. Using this method, FAW mortality approached 80% two days after application with 400 infective juveniles (IJs) of the EPN species *Heterorhabditis indica*. Under laboratory conditions, Caccia et al. (2014) found that with just 50 IJs of the EPN *Steinernema diaprepesi* Nguyen & Duncan, 93% of treated FAW larvae were killed within six days. The mortality rate increased to 100% with the application of 100 IJs. The reduction in growth of FAW larvae caused by feeding on high-silicon plant material shown by Nascimento et al. (2018) may also lead to a reduction in the ability of these larvae to fight off infectious nematodes. This would create a synergistic effect between Si and EPN application, potentially leading to more effective and efficient pest management strategies.

With the invasion of FAW across numerous countries and its ability to adapt to many treatment techniques, there is an urgent need for new methods to deal with this agricultural pest. Additionally, these new methods will need to be affordable and sustainable. The use of Si has already shown promise as one such potential pest control chemical, however, to be fully implemented there is still much that needs to be understood about how Si will affect insect-plant systems. With this in mind, this research was designed to test the hypothesis that feeding FAW with Si supplemented corn will result in reduced consumption, growth, and resistance to EPNs.

Methods

Corn Treatments and Growing Conditions

Corn seeds (35F38, Dupont Pioneer, MI, USA) were washed in a 10% bleach solution for one hour to remove any residual seed treatment before being planted in 25cm diameter pots using standard Pro-mix potting soil. Plants were grown in a temperature-controlled greenhouse at 27°C and were watered three times weekly. The plants were split into two groups: Si- and Si+. The Si- group was watered with only 250mL of water while the Si+ group was given 250mL of 500MgL^{-1} Na_2SiO_3 solution (Alfa Aesar, MA, USA).

Impact of Si on FAW Larvae

FAW larvae were fed wither Si- or Si+ plant material to determine how Si concentration in their diet would affect their physical characteristics. After four weeks of growth, corn plants from both the Si- and Si+ groups were cut at the base and the leaves were removed to be cut into smaller sections. Plastic petri dishes (10 cm diameter, Fisher Scientific, Singapore) were filled with 1.5 grams of either Si- or Si+ plant material. A single 1st instar FAW larvae, obtained from a lab colony (Frontier Scientific Service Inc., DE, USA), was placed into each petri dish which were subsequently sealed using parafilm. Larvae were left to feed for 5 days, after which they were removed, and the remaining leaf material was weighed. The petri dishes were refilled with another 1.5g of plant material and the larvae were resealed inside. They were then left to feed for an additional 4 days before being removed, weighed, and placed into 70% alcohol solution until all the necessary measurements could be taken. The remaining plant material was then weighed to assess consumption rate. This process was conducted for ten larvae in both Si treatments with five replicates.

Larvae were removed from alcohol solutions and head capsule width was measured using a Wild Heerbrugg M5A stereomicroscope (Leica Geosystems, Switzerland) with a Dino-Eye USB digital eye-piece microscope camera (Dino-Lite, CA, USA) and DinoCapture 2.0 software (Dino-Lite, CA, USA). They were then placed into a drying oven (Fisher Scientific, Singapore) at 65°C for four days until thoroughly dried so that the dry mass of each larva could be recorded. The dried larvae were then ground into a powder using a ball mill grinder (Mixer Mill MM400, Retsh GmbH, USA) in individual 1.5ml plastic vials with two 6mm metal ball bearings. The Si content of the samples was analyzed using a Rigaku Supermini200 X-ray fluorescence spectrometer (Rigaku Co., TX, USA). Si content was recorded as percent of total mass using carbon as a balance and helium as an atmosphere. To get enough powder for Si analysis the powder from ten larvae was combined to obtain each single sample.

Impact of Si on FAW Pupae

To determine if FAW pupal weight is affected by Si accumulation in their diet larvae were reared in petri dishes as described above. However, rather than being removed after 9 days, larvae were supplied with 1.5g of fresh leaf material every 5 days until pupation. All pupae were then removed, and their live mass was obtained. 14 larvae were treated in this way in two replicates.

Impact of Si on Susceptibility to EPN

To test if the accumulation of Si in FAW tissue impacts mortality caused by EPN infection, last instar larvae were individually placed into wells of 24-well plates (Greiner Bio One, NC, USA) and covered with 2g of moist sand (0.5:9.5 water:sand, wght:wght). A total of 5 *H. indica* IJs, suspended in 50µl of water were pipetted onto the surface of the sand in the treatment (EPN) wells. Water only (50µl) was used in the control (Cont) wells. Each treatment was repeated five times in three replicates. After a period of 48h, dead FAW larvae were removed from the sand and rinsed from any adhering EPNs before being individually laid on White traps (White 1927). Traps were monitored every other day for nematode emergence. After 14 days, larvae from which no EPN emergence was observed were dissected under a Wild Heerbrugg M5A stereomicroscope (Leica Geosystems, Switzerland). FAW survival and number of insects killed by EPN were recorded.

Si Analysis of Midvein and Leaf

Corn plants from both the Si- and Si+ groups were cut at the base and dried in an oven at 70°C for four days. To evaluate if Si accumulation varies between the midvein and the rest of the leaf, these parts were separated. Dried plant material was then ground to a fine powder on a ball mill grinder (Mixer Mill MM400, Retsh GmbH, USA) in 25 ml canisters with two 15mm beads. The Si content of the samples was analyzed using a Rigaku Supermini200 X-ray fluorescence spectrometer (Rigaku Co., TX, USA). Si content was recorded as percent of total mass using carbon as a balance and helium as an atmosphere.

Statistical Analysis

All statistical analyses of FAW growth and consumption were conducted using JMP Pro 14 software (SAS Institute Inc., NC, USA). Comparisons between the Si- group and the Si+ group for each different FAW fitness indicator (live mass, dry mass, head capsule width, pupal live mass, consumption), as well as larval Si data, were made using two-tailed t-tests. Comparisons among all four treatment groups in the nematode susceptibility trials were made using R (RStudio, Inc, MA, USA). The function glm() was used to apply a binomial distribution to the test on survival data. A Tukey post-hoc test was performed to detect differences among treatment groups.

Results

FAW Consumption Rate

Consumption rate of FAW larvae did not significantly different whether feeding on Si- plant material or Si+ material (Fig 6, t-test, P=0.552).

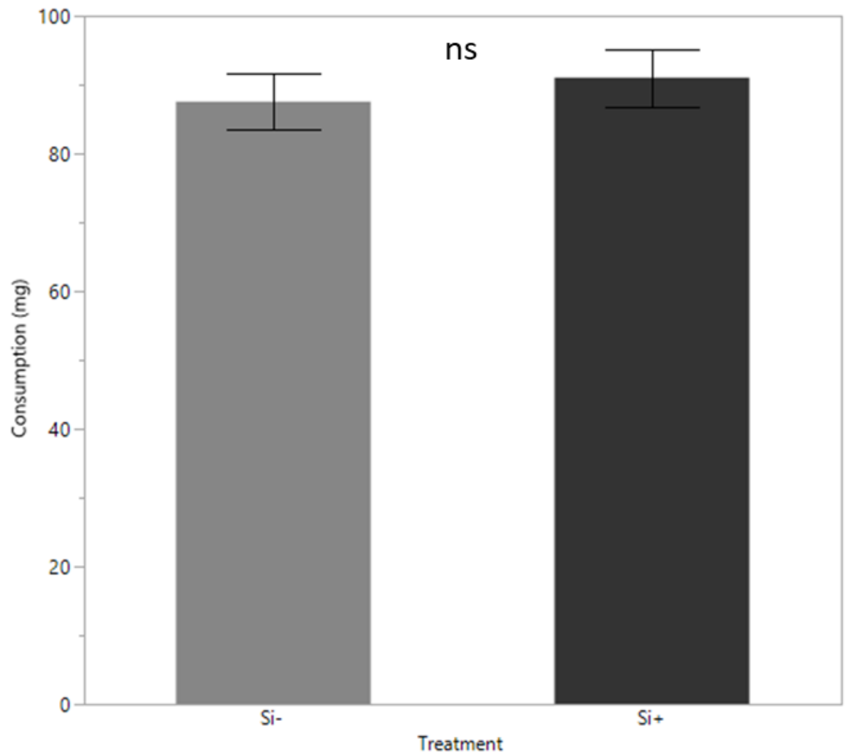

Figure 6 Average leaf consumption of larvae that had been fed silicon supplemented corn (Si+) vs corn that had been untreated (Si-). Bars indicate standard error. “ns” indicates no significant difference. ($P<0.05$, n=51)

FAW Growth

Feeding FAW larvae with Si+ leaf material did not result in a significant reduction in larval mass when compared with larvae fed on Si- leaf material (Fig 7, t-test, P=0.105). Even though the difference is not statistically significant, the mean live mass of Si+ larvae is actually greater than the mean of Si- larvae. Similarly, no significant difference was found for dry mass (Fig 8, t-test, P=0.074) or head capsule width (Fig 9, t-test, P=0.474). The only growth variable that was significantly different between the Si- and Si+ treatments was pupal live mass. The pupae of larvae fed with Si+ leaves had a significantly lower mean mass than the Si- pupae (Fig 10, t-test, *P*=0.031).

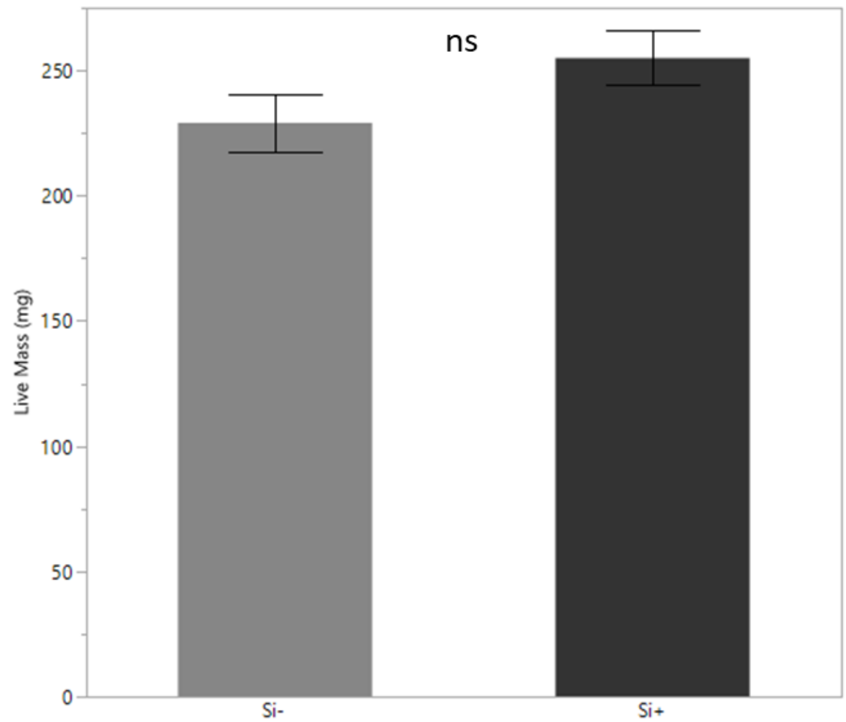

Figure 7 Average live mass of larvae that had been fed silicon supplemented corn (Si+) vs corn that had been untreated (Si-). Bars indicate standard error. “ns” indicates no significant difference. (P<0.05, n=51)

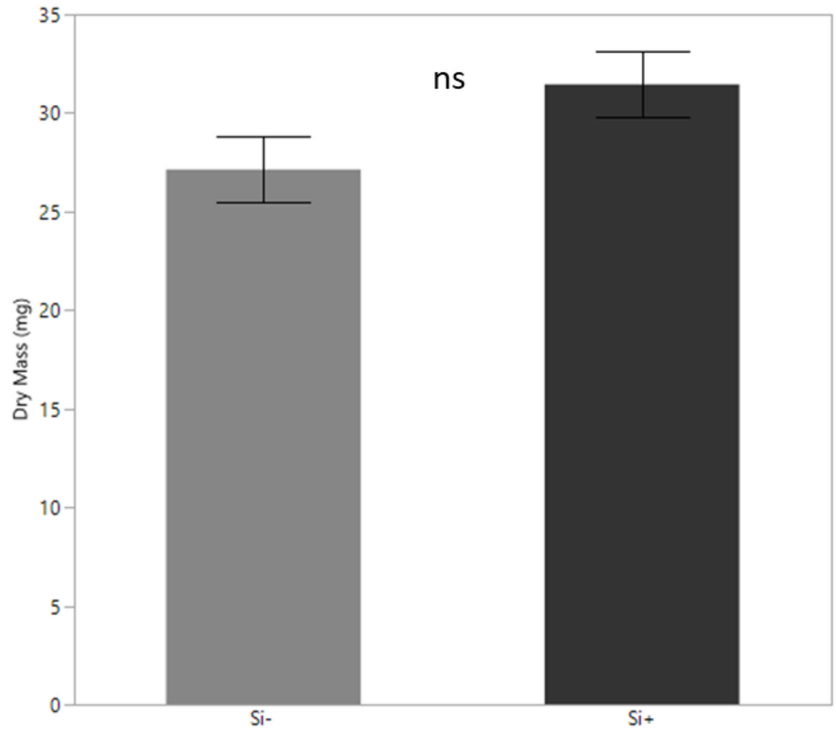

Figure 8 Mean dry mass of larvae that had been fed silicon supplemented corn (Si+) vs corn that had been untreated (Si-). Bars indicate standard error. “ns” indicates no significant difference. (P<0.05, n=51)

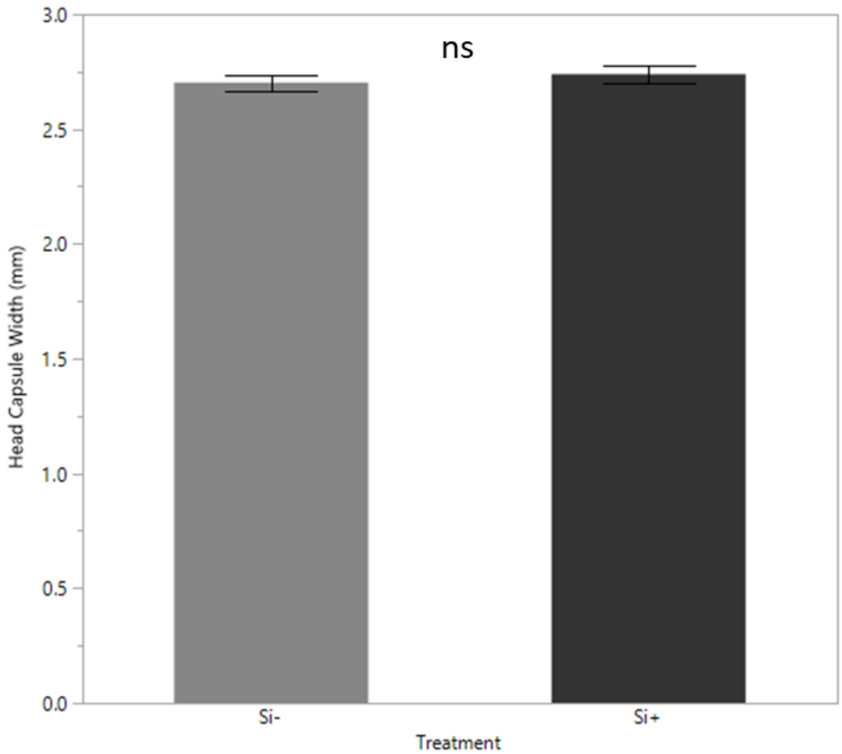

Figure 9 Average head capsule width of larvae that had been fed silicon supplemented corn (Si+) vs corn that had been untreated (Si-). Bars indicate standard error. “ns” indicates no significant difference. (P<0.05, n=51)

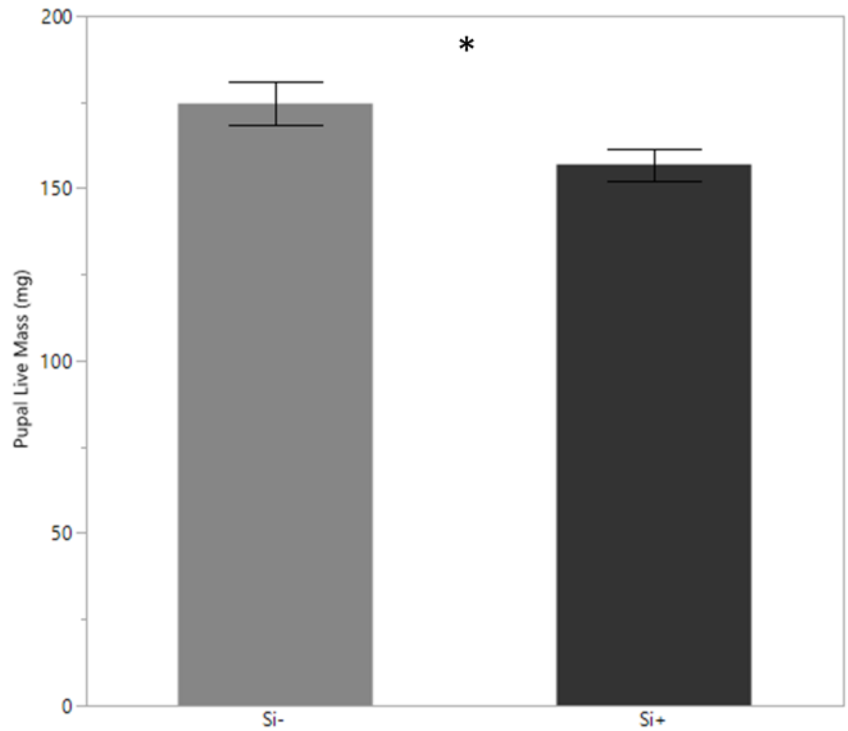

Figure 10 Average live mass of pupae that had been fed silicon supplemented corn (Si+) vs corn that had been untreated (Si-). Bars indicate standard error. An asterisk indicates significant difference (P<0.05, Si-: n=33, Si+: n=32).

FAW Larval Si Content

Larvae that were fed a diet of Si+ plant material had significantly great mass % Si than the larvae fed with Si- plant material (Fig 11, t-test, *P*=0.049).

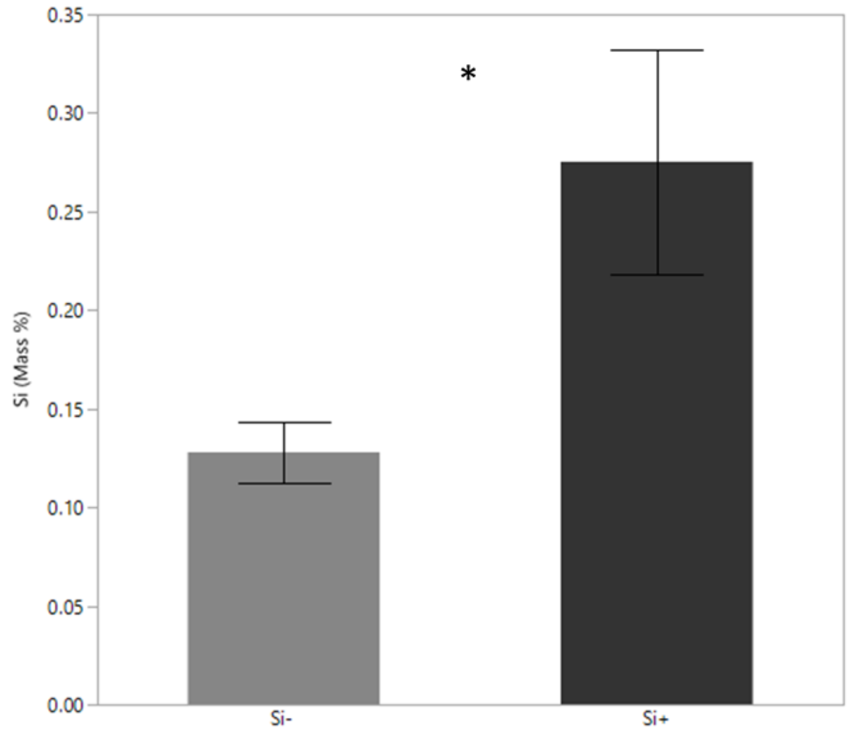

Figure 11 Average mass % silicon (Si) in larvae that had been fed silicon supplemented corn (Si+) vs corn that had been untreated (Si-). Bars indicate standard error. An asterisk indicates significant difference ($P<0.05$, $n=6$).

FAW Susceptibility to Nematodes

There was found to be a statistically significant difference in mortality rates between the four treatment groups (Fig 12, Two-way ANOVA, $F_{3,56}$=, $P<0.001$). No difference in mortality was seen in the Cont larvae, regardless of Si treatment (P=0.931). The Si-/EPN larvae had a significantly greater mortality rate than the Si-/Cont larvae (P=0.0003), the Si+/Cont larvae (P=0.003), and the Si+/EPN larvae (P=0.031). The Si+/EPN larvae did not have significantly higher mortality than either Si-/Cont larvae (P=0.584) or Si+/Cont larvae (P=0.339).

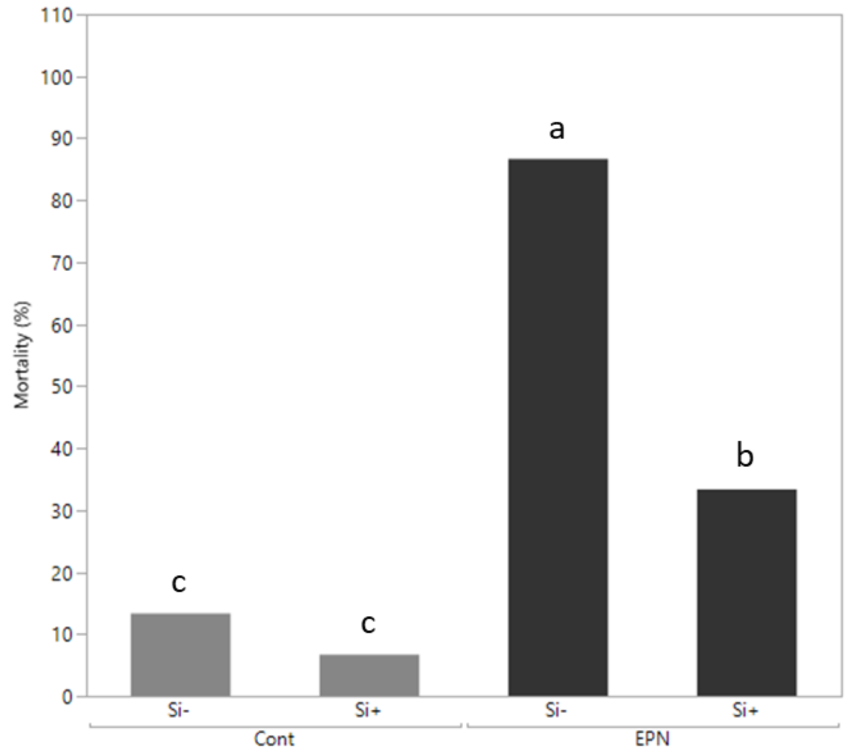

Figure 12 Average % mortality of fall armyworm larvae fed with silicon supplemented corn (Si+) or untreated corn (Si-) that have been treated with entomopathogenic nematodes (EPN) or have been left untreated (Cont). Bars indicate standard error. Means labeled with different letters are statistically different (P<0.05, n=15).

Midvein vs Leaf Si Content

Mass % Si differed significantly among the midvein, leaf, and two Si treatments (Fig 13, Two-way ANOVA, $F_{3,15}$=, P<0.001). However leaf Si content did not differ from midvein Si content in either Si- (P=0.887), or Si+ (P=0.859) plants.

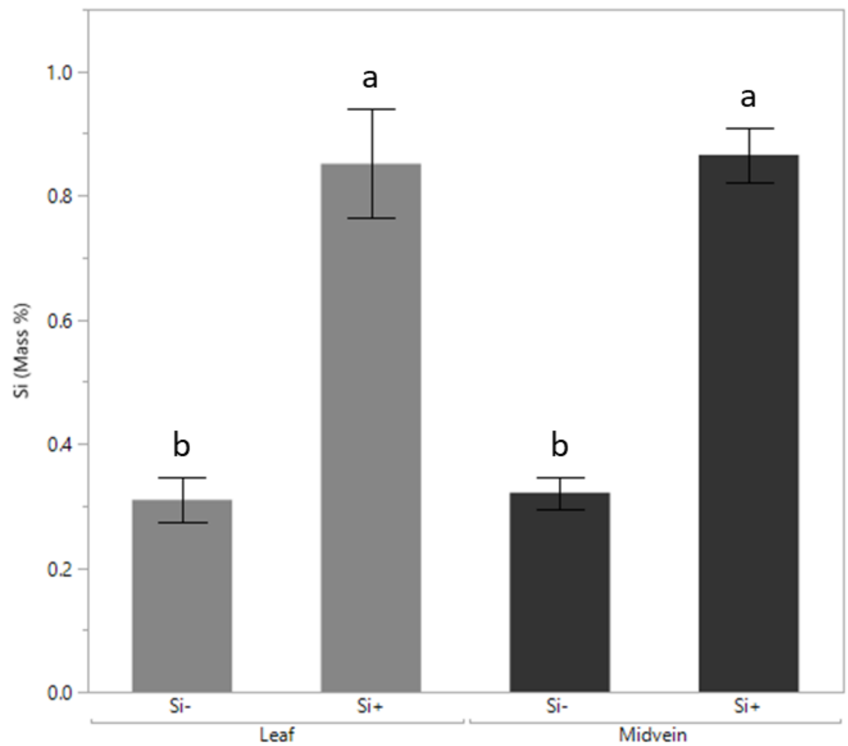

Figure 13 Average mass % silicon of corn leaf and midvein material from plants that have been supplemented with silicon (Si+) or have been left untreated (Si-). Bars indicate standard error. Means labeled with different letters are statistically different ($P<0.05$, $n=5$)

Discussion

Feeding on Si supplemented plant material had no significant effect on the growth or consumption rate of FAW larvae. This was unexpected, as Nascimento, et al. (2018) found that FAW growth was significantly reduced when feeding on Si supplemented rice. To understand whether this inconsistency was due to Si being deposited in the midvein rather than in the rest of the leaf, as has been suggested to happen in some grass species by Johnson et al. (2019), Si content in the leaves and midvein was analyzed. The results of this study show that Si is evenly distributed within the leaf material, meaning that, even if FAW larvae largely avoid feeding on the hardened midvein, they will still be ingesting excess Si, so this does not explain why larvae had no change in growth. Another possible explanation is the form that the Si takes in the corn. If the silicon is deposited in a form that does not interfere with FAW feeding, then no effect on growth would be expected. Alternatively, while there was a significant increase in Si in the plants, it is possible that it simply was not enough to cause a dramatic effect on the larvae.

Sixth instar FAW larvae that were fed on a standard maize diet proved to be highly susceptible to infection by the entomopathogenic nematode *H. indica*, with a mortality rate of 86.7%. What was unexpected however, was that those larvae that were fed a high silica diet appeared to have been conferred a greater resistance this this nematode. The mortality rate of these larvae was only 33.3%. This may be due somehow to the greater amount of Si in the bodies of these FAW larvae. The actual mechanism by which this resistance is conferred is uncertain. To understand this mechanism, it would be important to determine the form and location that Si is deposited inside of the insects' bodies. Al Banna et al. (2018) showed that silicon carbide nanoparticles could be harmful to the nematode species *Caenorhabditis elegans* (Maupas). This was due to internal damage after ingestion of the particles. Perhaps the presence of Si in the bodies of the FAW larvae affected *H. indica* in a similar way. To determine the effects of EPN treatment to control FAW, further testing into the mortality of pupae after EPN exposure is necessary. This is because FAW larvae drop to the ground to pupate in the soil (Luginbill, 1928). This means that they spend more of their life exposed to EPNs when in the pupal stage than in the larval stage.

The results of this study largely contrast with the results of similar research into the effects of Si on pests of crop plants other than corn (Correa, et al., 2005, Frew et al., 2016, Frew et al., 2017, Nascimento et al., 2018, Teixeira, et al., 2017). The difference in the data reported here may be due to corn not accumulating Si in the same way or to the same extent as those other plant species. Si application even seemed to promote greater resistance to EPNs in FAW larvae. Overall, this data does not support the use of Si as a means to harm FAW larvae or reduce damage by their feeding on corn.

Chapter 4

EFFECTS OF SI ON FALL ARMYWORM OVIPOSITION BEHAVIOR

Introduction

Attracting natural predators and parasitoids is one way that plants defend themselves from insect herbivores. They do this by producing and emitting volatile organic compounds (VOCs) when herbivores begin to cause damage which are then detected by parasitoids that will attack the herbivores (Finidori-Logli et al., 1996, Liu et al., 2017, Steinberg et al., 1993, Turlings et al., 1991). Interestingly, the VOCs from herbivore damaged plants have been found to be more attractive to parasitoids than the VOCs from mechanically damaged plants (Steinberg et al., 1993). This is likely due to the plant detecting certain compounds in the saliva of the herbivore and may be an energy saving adaptation to prevent the production of VOCs when leaves are damaged by something other than an herbivore.

Carbon dioxide, a compound that is constantly being released by plants, was demonstrated in a study by Bernklau and Bjostad (1998) to be the primary attractant in corn (*Zea mays* L.) volatiles for the major corn root pest *Diabrotica vergifera vergifera* LeConte. Similarly, fall armyworm [FAW, *Spodoptera frugiperda* (Smith)] moths are able to detect corn volatiles and seek them out to be able to lay their eggs. These moths are even able to differentiate between the herbivore induced plant volatiles (HIPVs) produced by corn in response to FAW feeding and the VOCs produced by undamaged corn. Signoretti et al. (2012) gave FAW moths the choice between VOCs from undamaged corn plants and VOCs from plants that had been fed on by FAW larvae. What they found was that the moths had a greater preference for the VOCs from undamaged plants. This preference is likely an adaptation to reduce the competition that an individual moth's offspring will have to face. By preferentially ovipositing on undamaged plants, the moth's progeny will not have other caterpillars feeding on the same plant that they will have to compete with for food and space and will not be exposed to natural enemies early on.

Silicon (Si) application has the potential to change the volatile responses of plant species, and in doing so, change the way herbivorous insects interact with those plants. Si can have a priming effect on the jasmonate pathway that controls the VOC response of plants (Thaler et al., 2002, Ye et al., 2013). Additionally, Si can change the actual makeup of the HIPVs produced after herbivore attack, and this change can make the HIPVs more attractive to parasitoids (Liu et al., 2017). This alteration to a plant's VOC response has yet to be studied in corn. Similarly missing in the scientific literature is how the changes induced by Si application may affect the herbivores that use VOCs as a way to find their host plants, and once they find them, whether they will still be able to discern previously infested plants from uninfested plants.

This research was conducted to test the hypothesis that Si application will alter the VOC profile of corn, leading to a reduction in FAW preference for undamaged corn. For Si to be implemented successfully as a pest management technique it is important to understand not only how it affects pests that are feeding directly on the plants, but also how it alters the preferences and behavior of those pests.

Methods

Corn Treatments and Growing Conditions

Corn seeds (35F38, Dupont Pioneer, MI, USA) were washed in a 10% bleach solution for one hour to remove any residual seed treatment before being planted in 25cm diameter pots using standard Pro-mix potting soil. Plants were grown in a temperature-controlled greenhouse at 27°C and were watered three times weekly. The plants were split into two groups: Si- and Si+. The Si- group was watered with only 250mL of water while the Si+ group was given 250mL of 500MgL^{-1} Na_2SiO_3 solution (Alfa Aesar, MA, USA).

FAW Rearing

FAW larvae (Frontier Scientific Service Inc., DE, USA) were fed on an artificial diet mixture [19g agar, 144g Frontier Scientific general purpose lepidoptera diet (Frontier Scientific Service Inc., DE, USA)] and were reared in an incubation chamber set to 27°C until pupation. After this, pupae were transferred to damp cotton in a mesh cage to allow adult moths to eclose. Adults were sustained on a liquid diet mixture [12ml beer, 750µl propionic acid, 0.53g ascorbic acid, 0.18g chlortetracycline, 0.9 Frontier Scientific Vanderzant vitamin mix (Frontier Scientific Service Inc., DE, USA)] until they began mating. 24 hours after the first instance of mating was observed, the enclosure was placed in a refrigerator at 6°C for 30 minutes to slow the moths. Females were transferred into individual plastic containers to be transferred to tents.

Two-Choice Designs

Four different two-choice studies were conducted to determine how Si affects the oviposition of FAW moths. For the first study, two Si- plants were placed into a 200cm x 180cm x 150cm pop-up mosquito tent (Datong, Amazon.com Inc, WA, USA) at opposite corners. The control (Cont) was left undamaged while on the treated plant (Treat) was placed a single third instar FAW larva. Larvae at this instar were chosen because they were large enough to cause significant damage to the plants. These larvae were reared to this point on an artificial diet mixture [19g agar, 144g Frontier Scientific general purpose lepidoptera diet (Frontier Scientific Service Inc., DE, USA), 875ml water] in sealed plastic cells in an incubator kept at 27°C. The larva was allowed to feed for 48 hours, after which one gravid adult moth was placed in the tent for a period of 72 hours to allow for oviposition. This resulted in two treatment groups: Si-/Cont and Si-/Treat. The number of egg masses as well as the total number of eggs on each plant was counted after this period. Finally, the moth and plants were removed from the tent and a minimum of 24 hours were allowed to pass before the next trial to allow VOCs to dissipate. This same methodology was also used do test preference for Si+/Cont plants vs Si+/Treat plants, Si-/Cont plants vs Si+/Cont plants, and Si-/Treat plants vs Si+/Treat plants.

Statistical Analysis

Oviposition preference, quantified as number of egg masses or total eggs laid, in each individual two-choice experiment was determined using two-tailed t-tests in JMP Pro 14 (SAS Institute Inc., NC, USA).

Results

Infested vs Uninfested Preference (Si+)

FAW moths showed some preference for Cont plants over Treat plants when both groups were treated with Si, but the statistical significance of this preference was dependent on the variable being used as an indicator. The number of egg masses laid on Si+/Cont plants was significantly higher than the number of egg masses laid on Si+/Treat plants (Fig 14A, t-test, $P = 0.044$). There was not a significant difference, however, in total number of eggs laid on the two treatments (Fig 14B, t-test, $P = 0.089$)., though the mean number of eggs was higher on Si+/Cont plants.

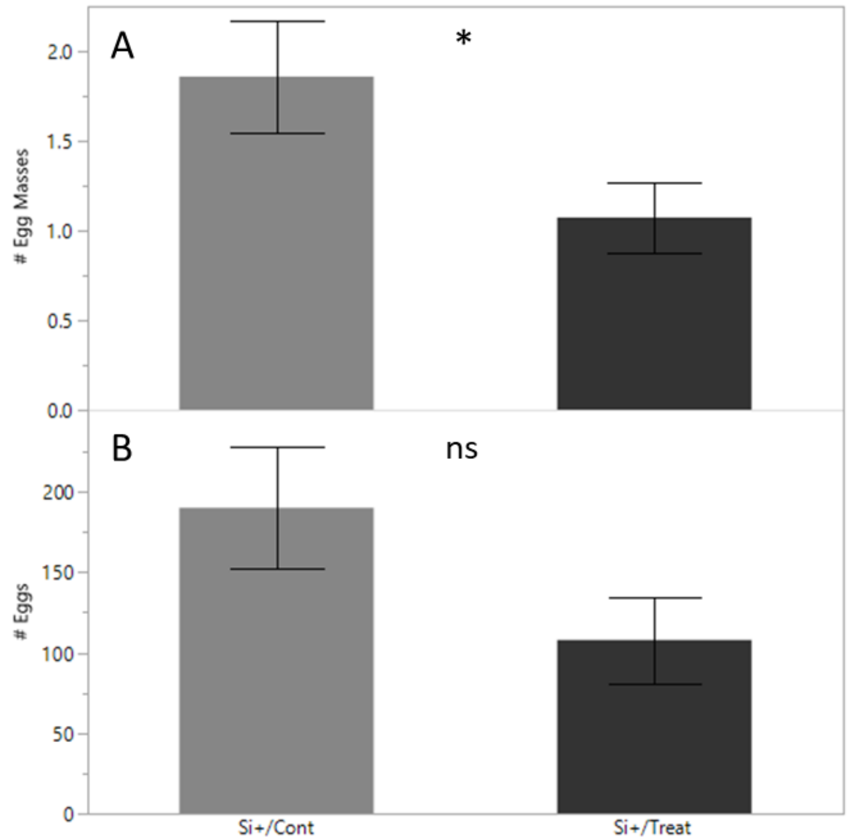

Figure 14 Average number of egg masses (A) and eggs laid (B) on silicon supplemented, undamaged plants (Si+/Cont) plants vs silicon supplemented, infested plants (Si+/Treat). Bars indicate standard error. An asterisk indicates significant difference (P<0.05, n=15).

Si- vs Si+ (Uninfested)

No preference was seen when FAW moths were given a choice between Si-/Cont and Si+/Cont plants. There was no significant difference between these treatments when it came to the number of egg masses (Fig 15A, t-test P = 0.547) or the total number of eggs (Fig 15B, t-test, P = 0.410) laid on the plants.

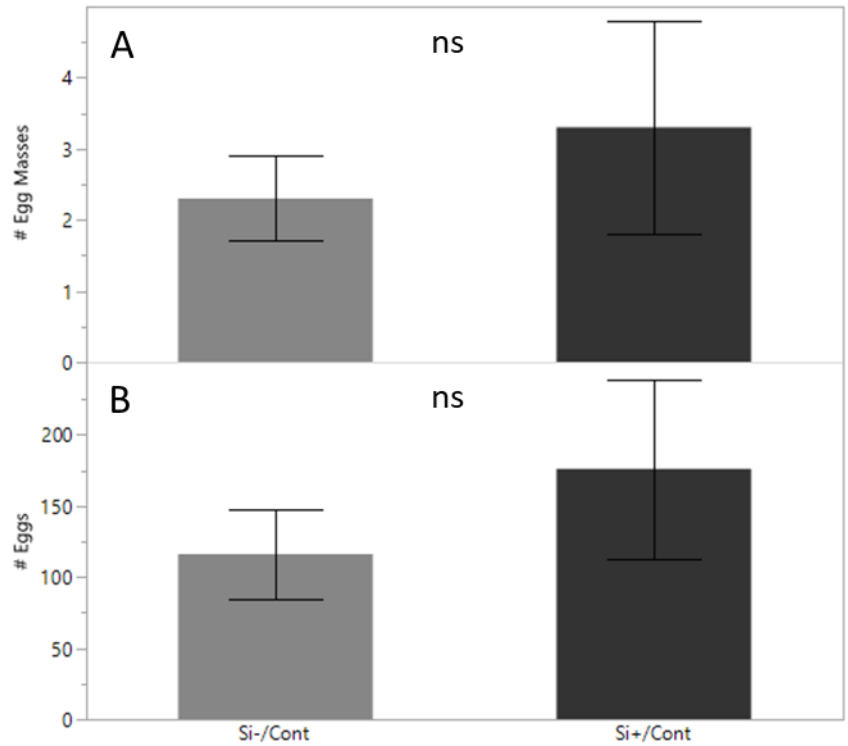

Figure 15 Average number of egg masses (A) and eggs (B) laid on undamaged plants that have not been supplmented with silicon (Si+/Cont) vs silicon supplmented, undamaged plants (Si+/Cont). Bars indicate standard error. “ns” indicates no significant difference. (P<0.05, n=8)

Si- vs Si+ (Infested)

There was no significant difference between the number of egg masses (Fig 16A, t-test, P = 0.612) or the total number of eggs laid (Fig 16B, t-test, P = 0.362) on the Si-/Treat plants vs the Si+/Treat plants. While the difference was not statistically significant, the means for both the number of egg masses and the total number of eggs was higher on the Si+ plants than on the Si- plants.

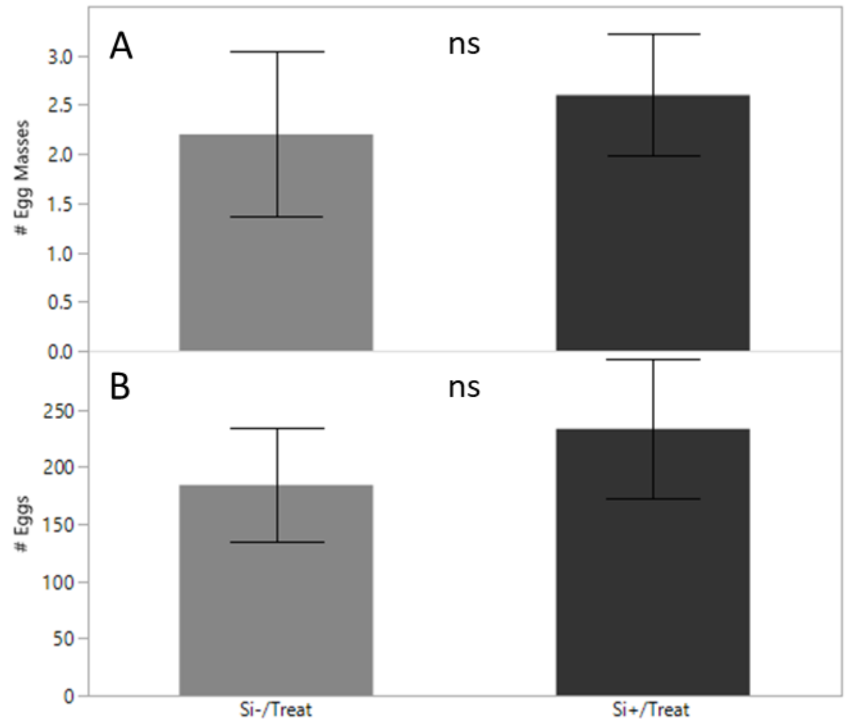

Figure 16 Mean number of egg masses (A) and eggs (B) laid on infested plants that had not been supplemented with silicon (Si-/Treat) vs silicon supplemented, infested plants (Si+/Treat). Bars indicate standard error. "ns" indicates no significant difference. ($P<0.05$, $n=8$)

Discussion

Female FAW moths have already been shown to use corn VOCs when choosing plants for oviposition (Signoretti et al., 2012). These findings have been supported here as, even when plants were supplemented with Si, FAW moths showed a significantly greater preference for undamaged plants for laying their eggs, at least when measure as number of egg masses laid. However, the total number of eggs on the undamaged plants, though greater, was not significantly different than the number of eggs on the FAW damaged plants. This hints that there may be a small effect of Si application altering the HIPV response of the plants that makes the damaged plants slightly less deterrent to FAW moths. If Si had no effect, then the expected results would more closely match with the results of Signoretti et al. (2012) with a much clearer preference for undamaged plants.

While the results of the other choice studies showed no significant preference for one treatment over another, in both studies the FAW laid a greater number of eggs and egg masses on Si supplemented plants. Perhaps if the plants were given greater concentrations of Si this effect could become more significant. To further test the ability of Si on altering FAW host choice, this research should be conducted in other plant species. Liu et al. (2017) demonstrated that Si can change the HIPV profile of rice (*Oryza sativa* L.) and doing so may have an effect on the ability of FAW moths to discern herbivore damaged plants from undamaged plants.

The results of these studies hint at a potential effect of Si on the VOC response of corn, though the differences in oviposition between Si- and Si+ plants were not significant. Further testing, both with different plant species and higher concentrations of Si, will be needed to determine the potential effects of Si application on FAW oviposition preferences. The evidence from this research, however, does not support the use of Si as a way to alter the oviposition behavior of FAW moths on corn.

Chapter 5

OVERALL CONCLUSIONS

It is apparent that corn (*Zea mays* L.) is capable of accumulating silicon (Si) when it is applied in the form of dissolved sodium silicate (Na_2SiO_3), however, there was very little evidence found here to support the use of Si for controlling fall armyworm [FAW, *Spodoptera frugiperda* (Smith)] infestations. Si accumulation had no significant effect on the volatile organic compound (VOC) response of the plants, nor did it have a significant effect on the oviposition preferences of FAW moths. Without significantly modifying or amplifying the VOC signals of the herbivore-damaged plants, it is unlikely that Si application would result in greater attraction of natural enemies or lead to a beneficial change in the oviposition preferences of FAW moths.

FAW growth was also, for the most part, unaffected by the application of Si. Larvae in this study that were fed untreated corn leaves did not consume more leaf material than larvae that were given the treated leaves. Feeding on the Si supplemented plants also had no impact on the growth of the larvae, whether measured in larval mass or width of the head capsule. This was unexpected as it differs from a similar study conducted with rice (*Oryza sativa* L.) by Nascimento et al. (2018), where FAW larvae had reduced consumption and larval mass when fed with Si supplemented plant material. The one variable that was significantly lower when plants were treated with Si was the live mass of the pupae. This could have been because pupation was induced prematurely so the larvae did not have as much time to grow, but the time to pupation was not observed in this study so further testing is needed. Whatever the cause may be, the reduction in pupal mass was the only negative impact that Si was found to have on this pest. While this reduced body size may result in other negative effects, possibly reduced egg laying capacity and shorter flight distances, it likely would not be an efficient method of dealing with FAW infestations.

The most unexpected result of this research was the finding that FAW larvae were actually imbued with greater resistance to EPN attack when they had been fed with Si supplemented corn leaves. This was the opposite of what was predicted, and highlights one more failing of Si as a pest control chemical. If EPN application is already being used to treat FAW in a corn field, then Si use would only serve to reduce the efficacy of the EPN treatment. There may be, however, other applications of this finding. If Si is able to make other species more resistant to other natural enemies, such as parasitoid wasps, then Si application may have uses in conservation of native and beneficial species.

There is little evidence here to support the use of Si as a pest control strategy in corn fields for dealing with FAW. Without amplifying the VOC response of damaged plants to help recruit parasitoids, and without causing any reduction in the growth or consumption rate of FAW larvae, the reduction in pupal mass is likely not enough to negatively impact FAW infestations. There is great potential here, however, for future research to understand the mechanisms by which Si increases FAW resistance to EPNs and whether similar results can be found with other parasites and host species.

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