

**EFFECTS OF BENEFICIAL FUNGI ON OVIPOSITION PREFERENCE
OF THE CABBAGE LOOPER, *TRICHOPLUSIA NI*, ON SOYBEAN,
*GLYCINE MAX***

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ABSTRACT

Effects of Beneficial Fungi on Oviposition Preference of the Cabbage Looper, *Trichoplusia ni*, on Soybean, *Glycine max*

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The soybean plant, *Glycine max*, is a highly cultivated legume with great importance to the current United States economy. One of its major pests is the cabbage looper, *Trichoplusia ni*. The activity of this insect pest is economically significant as it contributes to a large portion of yearly soybean crop loss. Selection of an oviposition site by the adult female looper is crucial to the survival of her offspring. Insect preference and larval performance are considered to be immediate and subsequent consequences, respectively of oviposition. It is crucial to develop successful methods of management for these insect pests. Some common methods of insect management include chemical control, such as pesticides that are often used to combat insect infestation by killing the pest species. Solutions for new Integrated Pest Management (IPM) strategies are needed because of increased insect resistance to pesticides. Endophytic fungi have the ability to associate with plants in ways that may enhance the plants' survival via insect resistance or tolerance. To test the potential of the endophytic fungi *Chaetomium globosum* for use as a biological control method, I analyzed oviposition preferences of adult female cabbage loopers on fungal treated versus untreated soybean plants.

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CHAPTER I

INTRODUCTION

Soybean, *Glycine max*, is an important industrial and agriculture crop in the United States and abroad. Processed soybeans account for the world's largest source of animal feed as well as the second largest source of vegetable oil. The United States is a leading producer and exporter of soybeans with the 2018/2019 forecast projecting 2.08 and 1.9 billion bushels respectively for domestic and exported soybean production (USDA 2018). Soybean is subject to a host of abiotic and biotic stressors such as drought, insect, and weed pressure. Insect pest stress can lead to decreased yield and thus, less production and exportation for soybean farmers (Oerke 2006). Improving methods of pest control are necessary to sustain soybean crops in the United States as 43.8 million bushels of U. S. soybeans were lost due to pests in 2012. Of this, insect pests accounted for 28% of loss due to pests. Results varied by region with the highest loss due to pests being 44%, in the great lakes region (USDA 2014). A key annual soybean pest is the cabbage looper, *Trichoplusia ni*, a generalist moth species whose caterpillars can feed on upwards of 160 different plant species (Sutherland and Greene 1984).

Understanding the life history of *T. ni*, and other generalist pests is integral to developing methods for insect control. Adult *T. ni* moths deposit single, pale green eggs on a chosen host plant. The eggs typically hatch within three to four days post oviposition. Neonate larvae feed on the host plant leaves for a period of two to four weeks (Capinera 1999). The larvae then pupate in cocoons attached to the underside of the host plant's leaves for approximately 10 days. After this, adult moths emerge, disperse and mate to continue the life cycle. *Trichoplusia ni* typically attacks crucifer crops; however, it is a generalist pest and thus feeds on a myriad of

other economically important crops, such as soybeans, clover, cotton, peanuts, sweet potatoes, and tomatoes (Capinera 1999). Initial destruction of host vegetation begins with neonate larvae eating away at the lower leaves of the host with the older and more developed larvae eating away at large portions of the top leaves. Cabbage loopers can eat up to three times their weight per day in plant material (Coapio et al. 2018).

For most of the 20th century, the low cost and high availability of pesticides have made the use of chemical control strategies the primary tool used against insect pests. Reliance on a singular method of insect control, like pesticides, presents issues of diminished efficacy (Oerke 2006). We now know that insects can become resistant to pesticides if their use is left unchecked. An example of this misuse of pesticides is evident in the history of Dichlorodiphenyltrichloroethane (DDT). DDT was used to combat insect pests in the 1950's. Not long after its introduction, scientists began observing insect resistance to the compound in several species such as mosquitos, bed bugs, and body lice (Rebek et al. 2012). Awareness of this resistance developed slowly, yet the onset of resistance came very rapidly. By 1960, at least 137 insect species had begun to display resistance to DDT and similar compounds (Carson 2002). Increased public awareness on the presence of chemical compounds in food and the environment has exacerbated political and economic pressures for exploring new strategies.

Integrated pest management (IPM) strategies exhibit the potential for improved methods of control. According to Barzman et al (2015), the European Union Framework Directive on the Sustainable Use of Pesticides (Directive 2009/128/EC) defines IPM as the protection of plants through methods which discourage the development of harmful organisms that are economically justifiable, ecologically responsible, and minimize hazards to both humans and the environment. This definition can be interpreted in the eight principles of IPM: prevention and suppression,

monitoring, decision making, non-chemical methods, pesticide selection, reduced pesticide use, anti-resistance strategies, and evaluation (Barzman et al. 2015). While all of these principles are of equal importance in attaining maximal efficiency of pest control, our research will focus specifically on prevention and suppression through non-chemical methods. IPM can be applied in four methods of control: biological, cultural, mechanical, physical, and chemical (Barzman et al. 2015). Trap species are a method of cultural control which presents an opportunity for use as a non-chemical method and can be defined as plants which are grown to attract insects, or other organisms, to protect target crops from pest attack (Hokkanen 1991). The idea behind this technique is to plant a weed or non-crop species around a crop that is more attractive to insect pests and encourages association with the trap species (Cameron et al. 2007). It is important to consider the particular biochemicals released by a trap species, as most plants produce secondary metabolites which can be toxic, repellent, or attractive towards herbivorous pests (Mithöfer and Boland 2012). If a trap species emits insect repellent allelochemicals, biochemicals which exert a detrimental physiological effect on another species, then it could potentially provoke infestation of the crops it is intended to protect. A study conducted by Cameron et al. (2007) examined oviposition preference of *T. ni* on broccoli and eight agricultural weeds with the focus of identifying trap species. Results indicated stinkweed and sheep's sorrel contained the most eggs out of the eight weeds tested when compared with broccoli. Data also showed that larval development was severely decreased or halted entirely on both stinkweed and sheep's sorrel (Cameron et al. 2007). It is thought by some that utilizing a fungal endophyte for IPM applications might serve as a more efficient method of pest control while eliminating the caveats associated with maintenance of a trap species (Jaber and Ownley 2017). An endophyte can be defined as any organism, typically fungi or bacteria, which colonize and live within the tissues of

living autotrophs without causing apparent harm (Jaber and Ownley 2017). *Beauveria bassiana*, a species of soil-borne endophytic fungi, has been shown to reduce herbivory of lepidopteran pests of maize and tomato when applied as a fungal biopesticide (Jaber and Ownley 2017).

While the mechanism behind this is still unknown, it is largely accepted that increased allelochemical or secondary metabolite production resulting from endophytic association plays a role (Jaber and Ownley 2017).

Maternal choice of host location is critical for survival of *T. ni* offspring. Understanding the choices that females make can provide insight into host plant preference. The preference-performance hypothesis suggests that females would choose to oviposit eggs onto host plants where their offspring would have the best chance of survival (Levins and McArthur 1969; Coapio et al. 2018). Host plant leaf structure, nutrition, and presence of other organisms can determine where lepidopterans choose to lay their eggs (Cameron et al. 2007). This suggests that the presence of a fungal endophyte on a host plant might affect the plant's affinity for oviposition. Constitutive defenses like mechanical barriers on plant structures are another aspect of plant defense which may factor into oviposition choice. The presence of trichomes, small hairs or outgrowths from the epidermis of a plant, have been found to influence oviposition as well as larval performance. Some glandular trichomes contain structures that secrete compounds toxic to larvae (Mithöfer and Boland 2012). The length and density of trichomes has also been linked to oviposition deterrence. One particular study performed assays with tomato, soybean, and wormseed found that *T. ni* adults laid a greater number of eggs on mature leaves containing low density trichomes rather than young leaves which contained higher density trichomes (Coapio et al. 2018). Trichomes present on soybean leaves in particular secrete a viscous liquid

which hardens when it contacts air. This impedes larval development by restricting movement as they could potentially be trapped in the glue-like substance (Coapio et al. 2018).

Just as it is advantageous to understand the details of the cabbage looper life cycle, it is also important to assess the diversity of these fungi-plant associations to gain a better understanding of potential uses for biocontrol. Naturally occurring fungal endophytes are a non-chemical or biological control method. Association with these endophytes can function to boost the already present defense mechanisms of the host plant and increase stress tolerance, plant vigor, and decrease herbivory. Non-pathogenic endophytic fungi are utilized in production of an array of biologically active secondary metabolites which can be unpalatable, anti-digestive, or toxic (Yan et al. 2014; Mithöfer and Boland 2012). Evidence suggests that symbiosis with mycorrhizal fungi may increase emissions of volatile organic compounds as well. Investigations of the mycorrhizal association of *Rhizophagus irregularis* with *Medicago truncatula*, a legume native to the Mediterranean, found that after noctuid caterpillar feeding a distinguishable difference in emissions was present between mycorrhizal and nonmycorrhizal plants (Leitner et al. 2009; Mithöfer and Boland 2012). These volatile organic compounds or VOCs primarily consist of fatty acid derivatives, termed terpenoids, and additional aromatic compounds which can attract parasitoids. VOCs are triggered for release by the plant upon confronting a mechanical or biological challenge. Additionally, some endophytes can also be insect pathogens, also referred to as entomopathogenic fungi. These fungi exhibit a hemibiotrophic mode of action, colonizing the living tissue of an organism, and may produce compounds which can cause death or major impairment to insects (Shah et al. 2002). Entomophthorales, a subdivision of Zygomycetes, produce protoplasts that circulate in the hemolymph of the insect

and produce toxins through yeast like spores called blastospores. Upon insect death, the fungi surfaces and sporulates through the insect carcass (Shah et al. 2002).

Utilization of the effects of endophytic fungi for pest management depends on the degree of colonization. Infection studies conducted by Impullitti and Maverick (2013) revealed that in soybeans, the same fungal species was found in multiple leaves on the same plant thus indicating the possibility of systemic growth (Yan et al. 2014). *Undifilum oxytropis* is an endophytic fungus found in *Astragalus* and *Oxytropis spp.*, a species of locoweeds. A degree of systemic growth has been confirmed in this locoweed through vertical transmission between generations by first colonizing germinating seeds (Yan et al. 2014). *Chaetomium globosum* is a fungal endophyte that is known to exhibit systemic colonization through seed inoculation in cotton (Zhou et al. 2016). Morphologically, *C. globosum* has ascomata with latent and terminal hairs, perithecia, ascospores, and asci. *C. globosum*, like many endophytic fungi, produces secondary metabolites which likely play a role in host plant disease resistance to seed and soil borne pathogens as well as pest suppression (Zhou et al. 2016). Few studies have examined the relationship between insect pests and *C. globosum*. However, one study conducted by Yan et al. (2011) found that out of 294 fungal isolates assayed, *C. globosum* was 1 of 23 which significantly reduced galls caused by nematodes.

Based on all of these findings, we hypothesize that *C. globosum* symbiosis with *G. max* will have an observable effect on *T. ni* oviposition. We arrived at this hypothesis based on supporting evidence that fungal endophytic association increases the defense mechanisms present in host plants. If the inoculation of the plant with endophytic fungi has an observable effect on oviposition deterrence, its function as a biological method for insect pest control may be preferable to chemical control methods.

CHAPTER II

METHODS

Oviposition assays

To elucidate whether *C. globosum* affects oviposition preference of the adult female *T. ni*, we performed oviposition assays by counting the eggs laid by adult moths on fungal endophyte treated and control soybean plants. These assays were conducted with plants grown from *C. globosum* treated *G. max* seeds. Cabbage loopers were purchased from Benzon Inc. as eggs and reared on a wheat germ-soy flour artificial diet produced by Southland Products Inc. Soybean seeds were treated with a concentrated solution of fungal spores from three different isolates of *C. globosum* that had been previously isolated from cotton in Texas (Ek-Ramos et al. 2013). For simplification, we will refer to the three isolates of *C. globosum* as: A, B, and C. Isolate cultures were kept on a tomato-based agar (V8) media in 100 x 15mm petri dishes (Figure 1). The resulting fungal spores were then harvested when mature (Figure 1). Conidia were harvested by adding 2mL of 0.1% Triton X-100 solution with a sterile metal spatula into 15mL falcon tubes. The concentration of spores was mixed on a vortex and subsequently diluted with

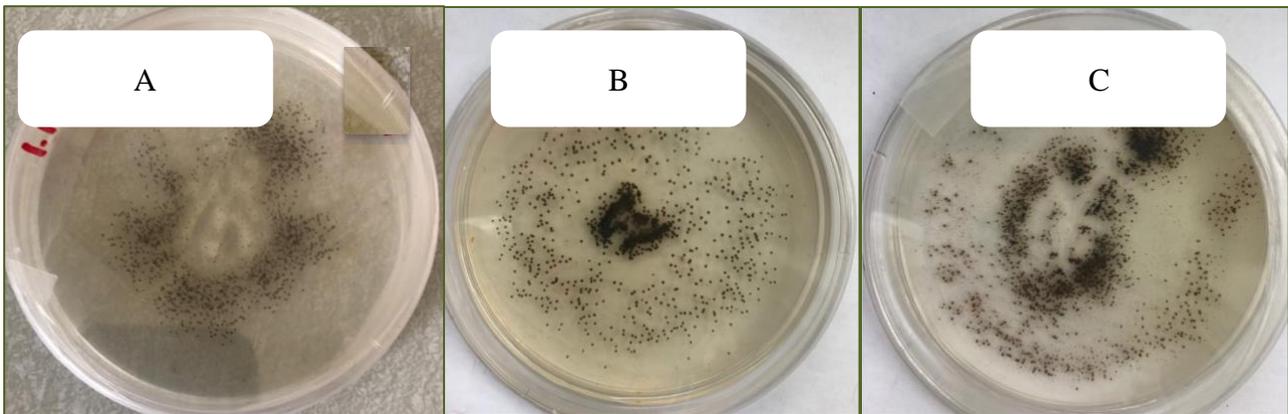


Figure 1. Fungal isolates cultures A, B, and C of *Chaetomium globosum*.

sterile water to attain a concentration of 1×10^6 plus 60 μ L FloRite. FloRite is a plantability polymer that acts as an adhesive for the spores to the seeds. One hundred seeds were treated with 1mL of the solution. Seeds were dried for 60 minutes before planting in Professional Growing Mix soil obtained from Sungro Horticulture. This soil consisted of Canadian Sphagnum peat moss, bark, perlite, vermiculite, dolomitic limestone, and a long-lasting wetting agent. The same procedures were followed for each of three subsequent trials. Soybean seeds were grown in the greenhouse under optimal conditions and allowed to reach the 3rd trifoliolate stage before being utilized in oviposition preference assays. Concurrently, a colony of *T. ni* was sustained in the laboratory until pupation. Insect cages, 28cm (width) x 28cm (length) x 61cm (height), were used in the greenhouse to contain the adult moths during the oviposition assay. The oviposition assays were set up with a control soybean plant and an endophyte treated soybean plant placed inside the same cage, approximately 15cm apart, at opposite corners (Figure 2). Six pupae were placed in a plastic tray on the floor of the cage in the middle of treated and control plants. A cotton ball soaked in 1:10 honey-water solution was also placed in the cages to sustain the adult moths when they pupated. Once 40% of pupae had eclosed, oviposition assays commenced and ran for 48 hours. Oviposition data consisted of counting eggs and noting the trifoliolate on which eggs were observed. Photographs of adaxial (upper) and abaxial (lower) domains of leaves were taken to confirm the location of eggs and for future reference (Figure 3). Data were collected at the 24-hour and 48-hour marks. This experiment was replicated in three replicate trials.



Figure 2. Oviposition assay set up with control (left) and treated (right) soybean plants.

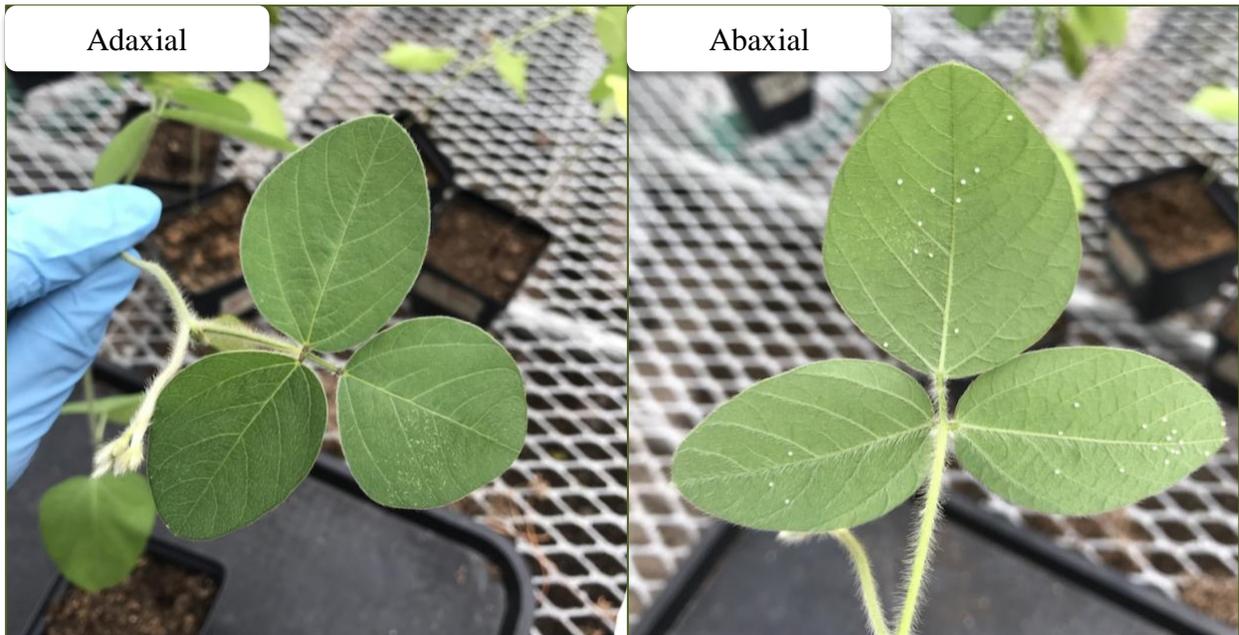


Figure 3. Oviposited eggs on adaxial and abaxial surfaces of soybean leaves.

Colonization assays

Colonization assays were also performed to determine efficacy of the fungal endophyte inoculation of the soybean seeds. Plants were harvested and surface sterilized by immersing the whole plant in 70% ethanol for 1 minute and in 3% sodium hypochlorite for 1 minute before being rinsed in sterile water. Tissue fragments of leaves, roots, stem, pods, and beans were sampled and plated on V8 media under sterile conditions. All tissue samples were also imprinted onto V8 media to verify successful surface sterilization. Plates containing tissue samples were incubated in the dark at room temperature for a period of two weeks. These plates were examined every three days for evidence of *C. globosum* growth and were evaluated for analysis at the end of the two-week incubation period.

Statistical analysis

Statistical queries were conducted using R studio statistical software and Excel data analysis toolPak software. Several variables were considered such as: oviposition preference between control and endophyte treated plants, the effect on oviposition preference between endophyte isolates, and location of eggs laid, adaxial versus abaxial leaf surfaces. Preference to oviposit eggs on endophyte treated versus control soybeans was tested using a mixed model ANOVA with p-values being obtained using the Satterthwaite's method. Linear mixed model fit by residual maximum likelihood (REML) using fixed effects and random effects models determined if there was a difference in the effect on oviposition preference for treated or control plants between isolates A, B and C. We explained variation in the response variable, total number of eggs laid, through fitting treatment type and timing of data collection as fixed effects. Random effects took into account the choice aspect, residual or unexplained variation of eggs laid between the three endophyte isolates. Due to the small nature of our sample size, we used

the Satterthwaite's method to obtain p-values for the linear mixed model. The Student's t-test was used to calculate the difference between the average eggs oviposited on adaxial and abaxial surfaces of leaves. When calculating fungal colonization, we observed 25 V8 agar plates per treatment with five plates each of soybean tissue types, roots, stems, leaves, pods, and beans for each replicate trial. An average of the soybean fragments that were colonized by *C. globosum* was noted for each plant of each treatment type to determine the extent of fungal colonization. An average of the mean colonization per plant was obtained for each treatment to determine if any of the endophyte isolates exhibited a higher frequency of colonization when compared collectively. For both trials 1 and 2, we considered a particular tissue type of either roots, stem, leaves, pods, or beans, positively colonized if just one of the fragments was colonized by *C. globosum*. We considered individual plants to be positively colonized if just one of the tissue types exhibited some degree of *C. globosum* colonization. Colonization data from trial 3 was not presented in this analysis as additional time is need for development of fungal growth.

CHAPTER III

RESULTS

Oviposition assays

Oviposition preference: treatment versus control

Data analyzed by the mixed model ANOVA for individual endophyte isolates did not reveal consistent significant p-values for variation of oviposition preference for endophyte treated or control plants. The linear mixed model did not show consistent significant p-values when comparing the effects on oviposition preference for treated or control plants between isolates A, B, and C. There was also a significant trial effect observed indicating that the treatment effects varied across the different replicate trials. Therefore, the data is reported separately below for each trial.

For trial 1, variation of the mean eggs laid by *T. ni* on isolates A, B, C, and their respective controls was not significant. Thusly, we did not observe any variation in the effect on oviposition preference for treated or control plants between isolates A, B, and C (Figure 4).

Analysis of trial 2 showed that in cages containing control and isolate A treated soybeans, *T. ni* laid significantly more eggs on soybeans treated with isolate A compared to the control (df=6, f=7.74, p=0.03) (Figure 4). We did not observe a significant oviposition preference in cages containing control and isolate B treated soybeans or in cages containing control and isolate C treated soybeans, as roughly the same average number of eggs were laid on control and treated plants. When comparing the degree of effect on oviposition preference between isolates A, B, and C in trial 2, we found that isolate A was the only strain of *C. globosum* to significantly affect

the oviposition preference of *T. ni* compared to isolates B and C ($df=6$, $t=2.78$, $p=0.03$) (Figure 4).

In trial 3, we did not observe any significant variance in the mean eggs laid by *T. ni* on control and treated soybeans in cages containing isolates A, B, C, and their respective control pairs. As such, we did not observe a significant difference in the effect on *T. ni* oviposition between isolates A, B, and C.

Overall, we found that there was not a significant difference between the average number of eggs oviposited on endophyte treated versus control plants. This was determined through a mixed model ANOVA which did not support the alternative hypothesis, that a greater number of eggs would be oviposited on control plants; and failed to reject the null hypothesis, that there would be no difference in the number of eggs oviposited on endophyte treated versus control plants. We also did not observe a consistent difference in the magnitude of effect on *T. ni* oviposition between endophyte treatments A, B, and C. This was determined by a linear mixed model which failed to reject the null hypothesis, that there would be no difference in the effect on oviposition between endophyte isolates A, B, and C. A graphical representation of the data can be found in Figure 4.

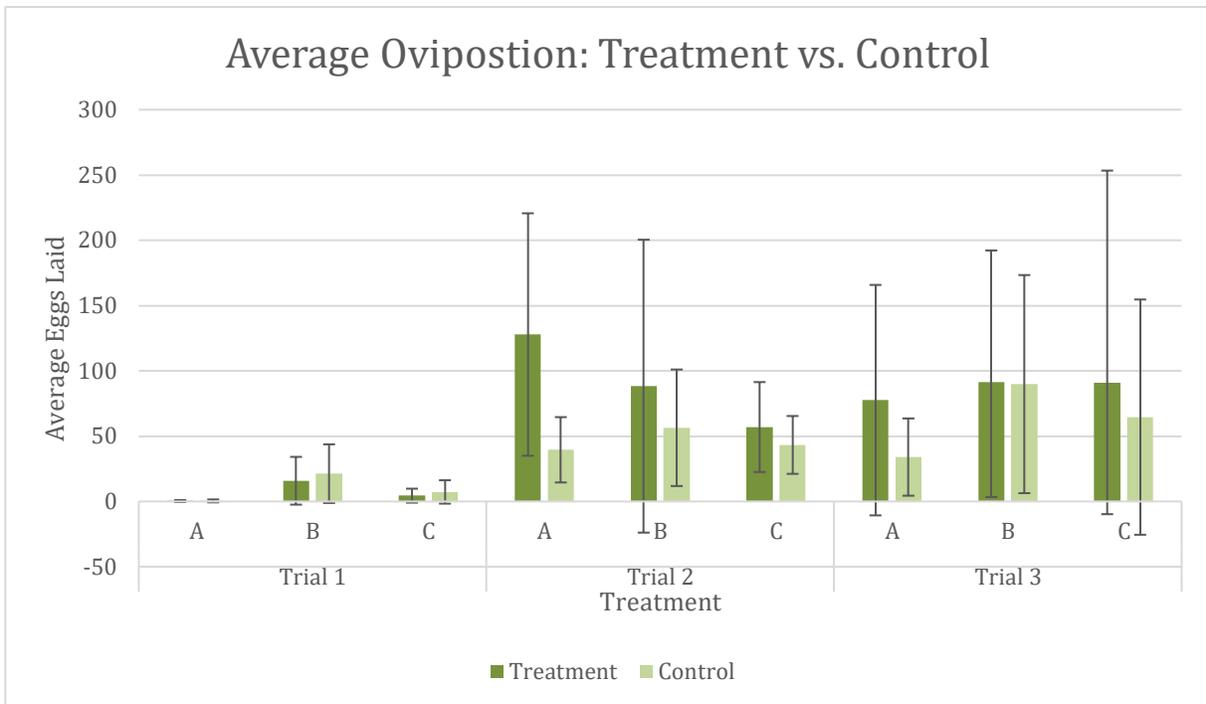


Figure 4. Bar graph depiction of mean \pm SE oviposition per cage on treated and control plants not specific to tissue type for all three trials sampled at the 48-hour mark. A, B, and C across the axis represent the different isolates of *C. globosum* used as fungal treatments in this study.

Oviposition preference: adaxial versus abaxial surface

Trial 1 yielded a significant relationship between a higher quantity of eggs oviposited on the abaxial surface of leaves and plants treated with isolates B ($p=0.033$, one-tail) and C ($p=0.058$, one-tail), but not for plants treated with isolate A ($p=0.178$, one-tail). Controls paired with isolates B ($p=0.045$, one-tail) and C ($p=0.040$, one-tail) exhibited a significant relationship for a greater number of abaxial eggs laid as well. Significant p -values were not observed for controls paired with isolate A ($p=0.178$, one-tail) (Figure 5).

In trial 2, significant p -values were obtained for a higher number of abaxial eggs oviposited on endophyte treated plants of isolates A ($p=0.023$, two-tail), B ($p=0.04$, one-tail), and C ($p=0.008$, two-tail). We observed significant p -values for a higher number of eggs laid on the abaxial surface compared to the adaxial surface in controls paired with isolates A ($p=0.008$, two-tail), B ($p=0.007$, two-tail), and C ($p=0.011$, two-tail) (Figure 6).

For trial 3, a greater number of abaxial eggs were oviposited on plants treated with endophyte isolates A ($p=0.038$, one-tail) and B ($p=0.056$, two-tail). Plants treated with endophyte isolate C ($p=0.094$, one-tail) did not have a significant difference in the location eggs were laid. All of the control plants paired with isolates A ($p=0.034$, two-tail), B ($p=0.059$, one-tail), and C ($p=0.051$, one-tail), yielded significant numbers for a higher quantity of abaxial eggs laid for this trial. Across all trials, we observed a trend for *T. ni* to oviposit on the abaxial surface of leaves for both control and treated plants (Figure 7).

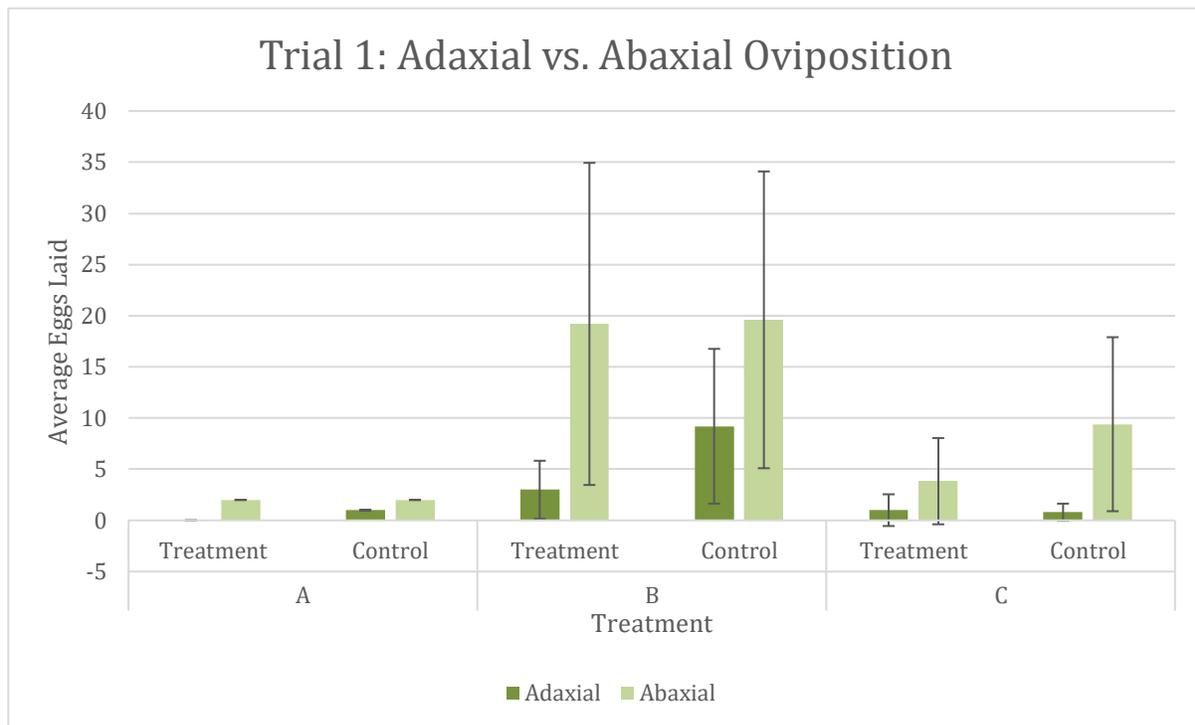


Figure 5. Bar graph depiction of mean \pm SE oviposition on upper side (adaxial) and underside (abaxial) of leaves at the 48-hour mark for trial 1. A, B, and C across the axis represent the different isolates of *C. globosum* used as fungal treatments in this study.

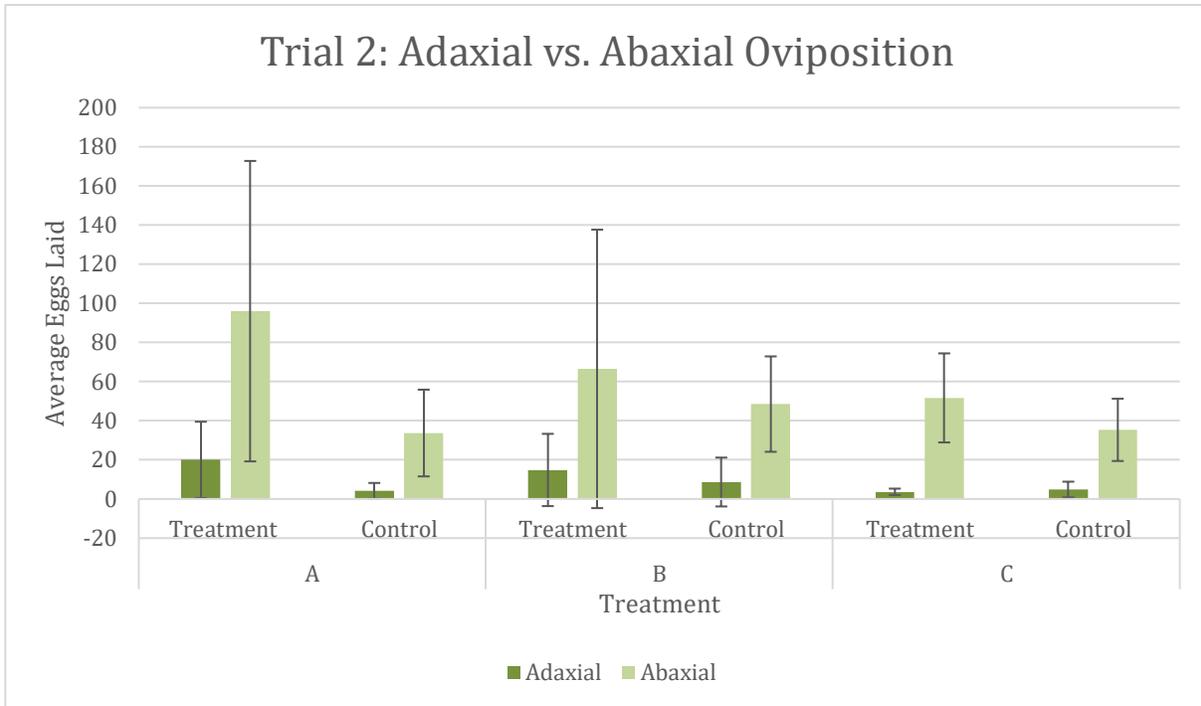


Figure 6. Bar graph depiction of mean \pm SE oviposition on upper side (adaxial) and underside (abaxial) of leaves at the 48hr mark for trial 2. A, B, and C across the axis represent the different isolates of *C. globosum* used as fungal treatments in this study.

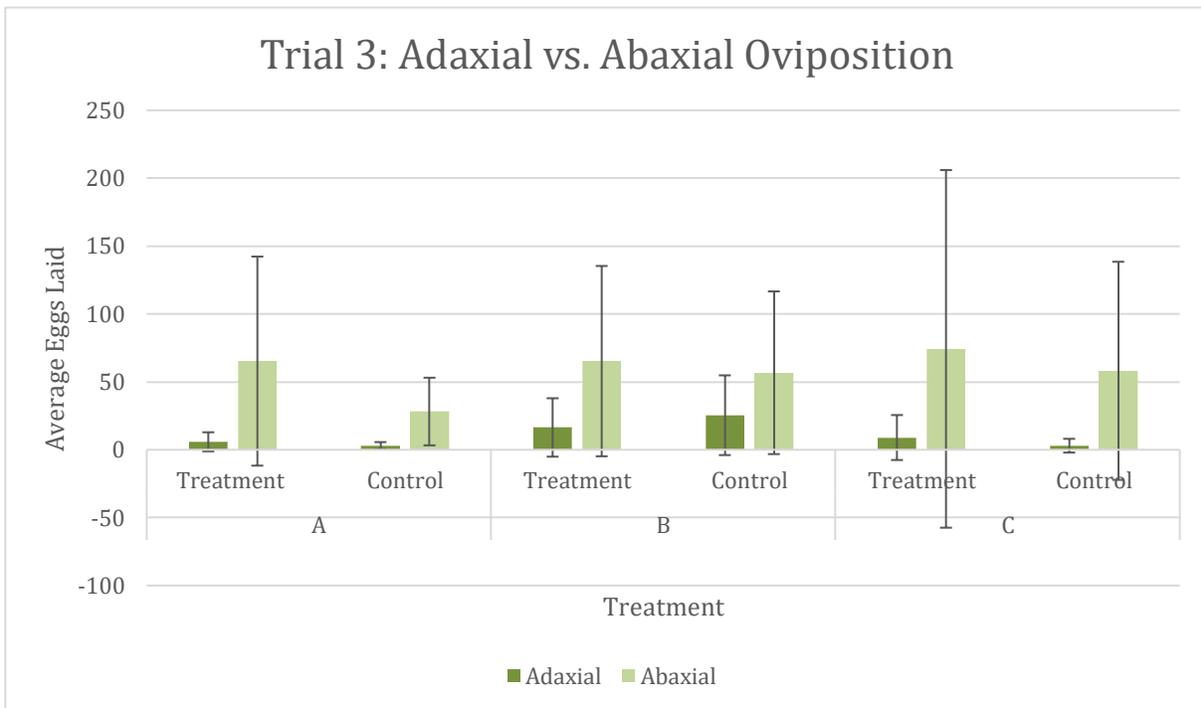


Figure 7. Bar graph depiction of mean \pm SE oviposition on upper side (adaxial) and underside (abaxial) of leaves at the 48-hour mark for trial 3. A, B, and C across the axis represent the different isolates of *C. globosum* used as fungal treatments in this study.

Colonization assays

In trial 1, the five plants selected that were treated with isolate C showed *C. globosum* colonization of soybean tissue fragments at an average of 100%, 80%, 80%, 80%, and 80%, respectively for each plant. Isolate C trended to show the highest frequency to colonize host tissue fragments and positively colonized 100% of plants selected in trial 1 (Figure 8). Isolate A colonized host tissue fragments in each plant selected at average of 100%, 100%, 80%, 60%, and 60%, respectively for each plant. Isolate A showed the second highest frequency to colonize soybean tissue fragments in trial 1 and showed some degree of *C. globosum* colonization in 100% of treated plants selected (Figure 8). The five sampled plants treated with isolate B in trial 1 colonized host tissue fragments at an average of 80%, 60%, 60%, 80%, and 20%, respectively for each plant, and also showed positive *C. globosum* colonization in 100% of plants selected with this treatment (Figure 8). All of the 15 selected treated plants from trial 1 showed some degree of *C. globosum* colonization in soybean tissues, thus all treated plants for this trial were positively colonized. Controls for trial 1 exhibited a singular instance of fungal colonization on one of the five bean tissue plates. As such, 4% of the all control soybean tissue fragments exhibited *C. globosum* colonization and showed positive colonization in 20% of selected control plants.

For trial 2, the five plants selected that were treated with isolate A showed endophyte colonization in soybean tissue fragments at an average of 40%, 40%, 60%, 40%, and 80%, respectively for each plant. Isolate A showed the highest frequency to colonize soybean tissues for trial 2 and positively colonized 100% of plants selected (Figure 8). The five plants treated with isolate B in trial 2 colonized soybean tissue fragments at an average of 0%, 20%, 20%, 20%, 40%, and 60%, respectively for each plant. Isolate B showed positive colonization in 80%

of soybean plants selected with this treatment in trial 2 (Figure 8). The five plants treated with isolate C in trial 2 colonized soybean tissue fragments at an average of 60%, 0%, 0%, 20%, and 40%, respectively for each plant, and thus showed positive colonization of *C. globosum* in 60% of selected soybeans (Figure 8). Both the frequency and the extent that *C. globosum* colonized treated plants was lower in trial 2 when compared to trial 1. Isolate A was the only treatment to colonize all of the five plants selected in trial 2 with isolates B and C colonizing four and three of the treated soybeans, respectively. Trial 2 control soybeans exhibited a singular instance of fungal colonization on one of the five leaf tissue plates. This trial showed *C. globosum* colonization in 4% of all control tissue fragments plated and positively colonized 20% of control plants sampled (Figure 8).

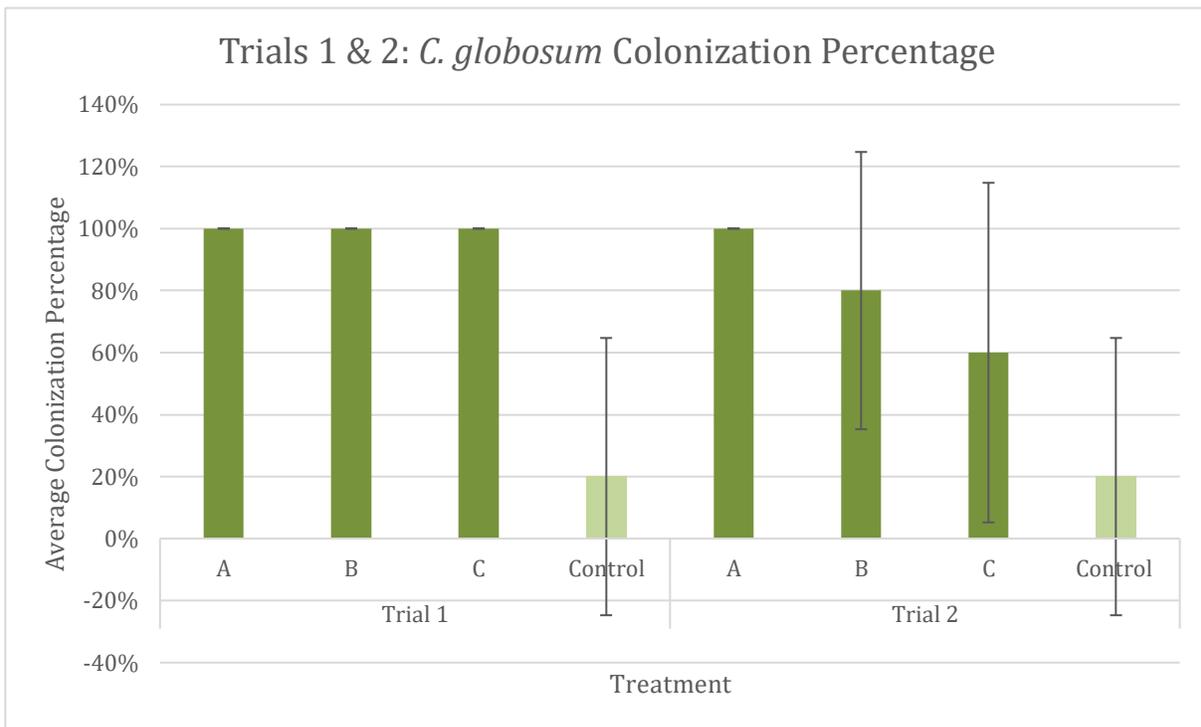


Figure 8. Bar graph depiction of the mean \pm SE of *C. globosum* colonization of soybean plants per treatment for trials 1 & 2. A, B, and C across the axis represent the different isolates of *C. globosum* used as fungal treatments in this study.

CHAPTER IV

DISCUSSION

We were unable to statistically support the hypothesis that adult female cabbage loopers would display preference of oviposition for control soybean plants rather than endophytically treated soybean plants. It is important to note that there was a trial effect observed for trial 1. Referring back to Figure 4, one can see that there was a significantly lower quantity of eggs laid on all three isolate and control pairs for this trial. This could be explained by the fact that trial 1 was conducted in November and coincided with a cold front. Pupal eclosion rates were much slower for trial 1, taking a total of 21 days to reach 40% eclosion, when compared with trials 2 and 3 which were conducted in March and took only six and three days, respectively, to reach 40% eclosion. This colder temperature might have translated to overall decreased activity of the adult cabbage looper moths and resulted in decreased oviposition, as this species is known to display intermittent activity during winter months (Capinera 1999).

Within individual trials of the oviposition assay, we did observe there to be a difference in the mean eggs laid between control and isolate pairs. In general, when a difference did occur, adult moths tended to oviposit on endophyte treated versus untreated control plants, particularly in trials 2 and 3 (Figure 4). Thus, these results do not support the alternative hypothesis of an effect of endophyte treatment on adult oviposition. However, the effect was variable, with more eggs laid on plants treated with isolates A and C, but not B across both trial 2 and 3. When treated with strain B, there were greater numbers of eggs on treated plants relative to the control plants only in trial 2 and roughly equal numbers on both treated and control plants in trial 3 (Figure 4).

Variation in the number of eggs laid over 48-hours within and across trials could be due to a multitude of environmental factors, such as light variation and temperature spikes (Uyi, Zacharides, & Heshula 2018; Saethre & Hofsvang 2002). However, these factors were unlikely to have an effect on the number of eggs observed on control and isolate pairs within the individual trials, as optimal conditions were maintained in the greenhouse. Internal physiological factors such as egg load, the number of mature oocytes available for oviposition, may also have a role in the variation of oviposition rates. One study of egg load as it relates to oviposition behavior found that *Battus philenor* females with higher egg loads displayed greater search intensity for an oviposition site than individuals with lower egg loads (Minkenberg, Tatar, & Rosenheim 1992).

We found that for all three trials, adult female moths consistently exhibited a preference to oviposit on the abaxial leaf surface of both control and treated soybeans. These findings were consistent with a study regarding the role of trichomes in *T. ni* female oviposition on soybean which revealed that female moths preferred to oviposit on the abaxial side of both mature and young leaves (Coapio et al. 2018). Rational for this could be that the abaxial surface offers added protection from predators, as eggs laid on the adaxial surface are much easier to detect. Further, the density of trichomes is greater on the adaxial surface, compared to the abaxial surface. Thusly, *T. ni* neonates which begin their lives on the abaxial surface could exhibit increased mobility and have easier access to host vegetation, increasing their overall performance. Future work will employ the application of endophytic foliar sprays to the abaxial surface of leaves to examine whether this affects oviposition location preference.

The primary take-away from the colonization assay is that seed inoculation is a valid method to achieve fungal endophytic colonization, but the efficacy is variable. Multiple studies

in cotton, onion, cucumber, and other plant species have confirmed this fact (Yan et al. 2011; Ek-Ramos et al. 2013; Muvea et al. 2014,). The colonization observed in control plants can be attributed to human error in the surface sterilization process. Once plates from trial 3 have incubated for a sufficient amount of time, all trials will collectively be analyzed with the Chi Square test to achieve a more functional statistical analysis.

CHAPTER V

CONCLUSION

In conclusion, the endophytic fungi *C. globosum* when applied to soybean via seed inoculation did not significantly affect oviposition preference of the adult female *T. ni*. However, there was significant variation in oviposition across trials, and a trend was observed for more oviposition on untreated control plants paired with isolate B treated plants in two out of three trials. Other methods of fungal inoculation, such as endophytic foliar sprays and leaf surface inoculation, may result in a higher degree of fungal colonization in tissues which are more directly correlated with oviposition location (Muvea et al. 2014).

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