

IDENTIFYING HOST-STRAIN BEHAVIORAL DIFFERENCES OF FALL  
ARMYWORM IN FLORIDA (LEPIDOPTERA: NOCTUIDAE)

By

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by

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To my family: Without them, I would not be the man I am today

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Abstract of Thesis Presented to the Graduate School  
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IDENTIFYING HOST-STRAIN BEHAVIORAL DIFFERENCES OF FALL  
ARMYWORM IN FLORIDA (LEPIDOPTERA: NOCTUIDAE)

By

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Chair: Robert L. Meagher  
Major Department: Entomology and Nematology

Florida is a known overwintering site for the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith). Previous research suggests that this insect comprises two genetically different host-strains: one using large grasses such as corn as a host-plant (corn-strain), and the other using smaller grasses such as rice and forage grasses (rice-strain). My study was conducted with insects collected and identified from various sites throughout Florida. Strain identification was made using the Cytochrome Oxidase subunit I (*COI*) gene as a mitochondrial marker. Once confirmation of strain association was made, corn-strain larvae were fed a corn (*Zea mays* L. 'Truckers Favorite') foliage diet, rice-strain larvae were fed on a type of bermudagrass (stargrass, *Cynodon nlemfuensis* Vanderyst var. *nlemfuensis* 'Florona'). The pure strains were established and colonies reared at the USDA-ARS Gainesville. Successive generations were used in this study. Female ovipositional site selection and larval host choice between corn and stargrass plants were behavioral traits measured. Rice-strain females exhibited a strong

ovipositional preference (95%) for stargrass plants. Corn-strain moths oviposited 53% of egg masses on the test enclosure, rather than on host-plants. Stargrass (30%) and corn plants (17%) also contained egg masses. The unpredictable behavior of corn-strain females contradicts previous studies of the two host-strains; suggesting that the corn-strain is a more generalist feeder than the rice-strain. The conflicting results may be attributed to my documentation of the enclosure as a non-plant-host variable in the experiment.

Larval-choice studies were conducted using multiple bioassays to determine whether there is a strain preference for corn or stargrass. When given a choice of a section of each host-plant in a Petri dish bioassay, neonates of both strains chose corn sections significantly more than stargrass sections. When whole plant material was presented, corn-strain larvae showed a preference for stargrass; while rice-strain larvae were evenly distributed between the two plants.

The ability of the neonate larvae to detect plant volatiles was observed in a Y-tube olfactometer. Corn-strain larvae showed a strong (yet non-significant) preference for corn volatiles. Rice-strain larvae were evenly distributed between the two arms of the Y-tube.

A plastic cage/wind-tunnel bioassay was developed to observe movement of larvae upwind through one host-plant to another. Corn-strain larvae were evenly distributed between the two plants, regardless of plant position. Rice-strain larvae showed a strong trend toward whichever host-plant it first encountered. When corn was the first plant encountered, 70% of the larvae showed a preference for corn; when stargrass was the first encounter, 67% of the larvae showed a preference for stargrass.

## CHAPTER 1 INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) is a migratory pest that makes an annual journey each spring from southern Florida into northern regions of the United States (Mitchell 1979). The first documented observation of FAW feeding on corn, sugarcane, and rice in Florida was by Glover (1856). Quaintance (1897) noted an outbreak of FAW that occurred during late August at the University of Florida campus, Lake City. Large numbers of larvae were seen feeding on crab grass (*Panicum sanguinale*) and he stated that they were “quite eating up the grass on the southern end of the college campus.” The first reported outbreak in Florida crops was in 1899 (Chittenden 1901).

Evidence from early in the last century showed that FAW is a native of tropical and subtropical America (Walton and Luginbill 1916). It has been theorized that management of FAW during overwintering in southern regions could greatly reduce the economic impact this pest causes each year during migration. Populations multiply at overwintering sites in southern Florida and southern Texas, before making their northward spring migration (Tingle and Mitchell 1977, Sparks 1979). Knipling (1980) stated that if overwintering populations in Florida were the primary source of the infestations, a rigid suppression program in the overwintering areas would have a great impact on the FAW population throughout the southeastern and Atlantic coast regions.

Two closely related populations of one species could diverge, allowing them the opportunity to establish new niches in the same environment. Sympatric speciation occurs when one evolutionary lineage splits into two separate species without the occurrence of geographical isolation (Berlocher and Feder 2002). Most work involving speciation in insects has been done with *Drosophila*. Although that work gave valuable insight into insect speciation, the direct cause still remains unclear (McMillian et al. 1997). Sympatric speciation is likely the outcome of competition for resources. Bécerra and Venable (1999) stated that insects have been shifting among hosts that are geographically available; and that a shift to a particular plant species is likely if its geographical range coincides with the geographical distribution of the old host. They stated that host shifts by phytophagous insects might also be attributed to plant chemical similarity.

Previous research into the migration sources of FAW suggests that this insect comprises of two host-strains: the corn-strain that feeds predominantly on corn (*Zea mays* L.), and the rice-strain that feeds on smaller grasses such as rice (*Oryza sativa* L.) and bermudagrass (*Cynodon dactylon* L.) (Pashley et al. 1985, Pashley 1986). Insects collected throughout Florida overwintering areas are of both strains (Meagher and Gallo-Meagher 2003, Meagher and Nagoshi 2004, Nagoshi and Meagher 2004). Molecular data suggest that FAW strains are more likely to be host-associated sibling species in which the strains appear to be sympatric and tend to use different plant hosts (Diehl and Bush 1984, Pashley 1986). In addition to speciation being genetically based, insect behavior plays an important role in the adaptation of FAW to new host-plants.

The FAW larvae will readily feed on at least 60 species of plants, but their ovipositional preference is on members of the Poaceae rather than other plant species (Mitchell 1979, Pitre et al. 1983, Whitford et al. 1988). Some favored crops of agricultural importance that FAW damages include sweet corn, turf grasses, cotton, peanut, cowpea, potato, and sugarcane. FAW is also the most important pest of bermudagrass pastures in the southeast (Pencoe and Martin 1982). When populations are high, FAW can subsist on many types of vegetation it may encounter (Luginbill 1928, Vickery 1929).

Introduction of monoculture corn crops in North America offered FAW a new host on a massive scale. Native Americans first introduced this crop to Florida between 1000 and 1500 A.D. (Leonard 2003). With modern agricultural practices, there are now over 83,000 hectares of corn planted in Florida each year (Nuessly et al. 1999). Florida is the major source of sweet corn during the winter and early spring, in the United States, as harvesting is most active from November to June.

The first bermudagrass variety was introduced to Florida in the early 1880s. By the 1920s, commercial sod was being farmed in the state. After World War II the sod industry began to develop into the business that it is today. Bermudagrass is now grown extensively in Florida for pasture and hay, but commercial sod production has risen due to an increased demand for turf by building contractors and residential homeowners. Bermudagrass is now being used on golf courses, and this plant covers more than 607,000 hectares of Florida (White and Busey 1987). It has been suggested that FAW evolved on native grasses or shifted to bermudagrass as a host from corn when the grass was introduced into the New World (Pashley et al. 1987).

FAW is active year-round in southern Florida, and this area serves as a reservoir for the yearly migration throughout the northeastern United States. It is believed that FAW uses bermudagrass as its primary host, thus increasing the population, which in turn migrates to other food and forage crops (Fuxa 1989, Pitman et al. 2002). However, this observation may be disputed because those observations were made before the existence of two host-strains was discovered. Sweet corn production is also at its peak during the winter months in southern Florida. This may account for the ability of both populations to increase in numbers during winter.

Previous studies on host sensory behavior in moths showed that moths rely on multiple sensory inputs for host location (Ramaswamy 1988). Selection of a suitable oviposition site by the female is initiated by chemical and tactile cues (Rojas et al.). Singer (1984) proposed for Lepidoptera, the host-plant is selected by the adult female. Environmental pressures may account for females selecting a host that is not optimal for larval development. Their limited mobility makes neonate larvae dependent on the adult female to select the most nutritious host, although neonate larvae do use chemoreception in host-plant location (Showler 2001). Many lepidopterous larvae are highly mobile at older instars, and have the ability to seek out a suitable food source (Berdegué et al. 1998). Larvae use olfaction and gustation to provide information for food-plant discrimination (Hanson and Dethier 1973, de Boer and Hanson 1987). Insect feeding behavior is influenced by chemical components of the host-plants that assist in food finding and acceptance (Thorsteinson 1960). Olfaction can induce orientational responses to plant hosts in larvae with prior feeding experiences, although a polyphagous

species may not be equipped with the inherent response to host-plants (Carlsson et al. 1999).

**Research objectives.** These studies were performed to identify behavioral differences between the two FAW host-strains from a Florida perspective. Areas of concentration were ovipositional preference and larval host selection to corn and a type of bermudagrass known as stargrass.

CHAPTER 2  
OVIPOSITIONAL PREFERENCE OF HOST-STRAINS TO CORN AND  
STARGRASS

**Introduction**

Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) is a generalist insect pest that can develop on many host-plant species. Although an economic pest of numerous crops, it has a preference for plants in the family Poaceae (Luginbill 1928). Cotton and soybean can also be injured by FAW feeding, but are usually only attacked when populations are extremely high, or when preferred host-plants are scarce (Pitre et al. 1983).

FAW is thought to be a native of tropical and subtropical America (Walton and Luginbill 1916). Florida and southern Texas are known overwintering sites for FAW from where populations expands into the eastern and central United States during the course of the spring and summer (Mitchell 1979, Sparks 1979). Suppressing overwintering populations in southern Florida before migration has been offered as a possible management strategy to reduce the impact of this pest (Knipling 1980). It is difficult to develop successful management programs due to the fact that this insect is able to sustain life upon a wide variety of food-plants (Pencoe and Martin 1981).

Host selection by a generalist insect may be accomplished by visual, chemical and tactile cues. Adult females use all three senses to find suitable ovipositional sites

(Zacharuk and Shields 1991). In response to plant cues, an orientational movement may initiate the behavioral process leading to host location and acceptance for oviposition and feeding (Jallow et al. 1999).

Host-plant selection in Lepidoptera for larvae is assumed to be the choice of the ovipositing female (Singer 1984). Adult FAW females can be indiscriminate in their selection of oviposition sites. Pitre et al. (1983) observed that females will oviposit on non-plant material despite the presence of a host-plant nearby, and Thomson and All (1984) found eggs laid on objects such as survey flags. FAW females usually place their eggs on the underside of leaves of the food-plant, but eggs have been found on leaves upon which the larvae are not known to feed (Quaintance 1897). Physical stimuli may have a greater impact than close-range chemical cues on ovipositional selection with FAW (Rojas et al. 2003). In order to enhance larval development and survival by providing a suitable diet, many insects prefer to oviposit on certain plant species (Showler 2001). The limited mobility of neonate larvae makes them highly dependent on the female parent's ability to select the most nutritious host (Smits et al. 1987).

In a search for the geographical sources of Louisiana migrants, Pashley et al. (1985) collected specimens in the Caribbean, Florida, Louisiana, Texas, and Mexico and discovered that there were two genetically differentiated host-strains. One host-strain feeds predominantly on corn (*Zea mays* L.) (corn-strain), while the other feeds predominantly on small grasses such as bermudagrass (*Cynodon dactylon* L.) and rice (*Oryza sativa* L.) (rice-strain) (Pashley 1986). Although ovipositional preference is potentially one mechanism that maintains strain fidelity, only one limited study has been

completed (Whitford et al. 1988). My study was designed to determine the ovipositional preference of the two FAW host-strains to corn or to a forage grass.

## **Materials and Methods**

### **Strain Isolation and Plant Growth**

FAW egg masses and larvae of various instars were collected in during 2003 from multiple sites throughout Florida. FAW were collected from the University of Florida Dairy Research Unit, Hague; University of Florida Range Cattle Research and Education Center (REC), Ona; University of Florida, Everglades REC, Belle Glade; and sweet corn fields in Miami-Dade County (Fig. 2-1). Eggs and larvae collected at these locations were reared to pupation on a pinto bean diet (Berger 1963). A single adult male-female pair was placed in an oviposition cage (Fig. 2-2). This cage consisted of a cylindrical inverted 473 mL plastic food container (Solo Cup Co., #Mk16) lined with a 7 cm x 7.6 cm coffee filter (Bunn, BCF). Holes of ~5.0 mm were placed in the top position to allow for airflow. A hole ~1.5 cm was placed in the inverted lid (Solo, ML8) in which a braided cotton roll (Richmond Dental, #200205) cut to a length of 5 cm was inserted. This allowed for absorption of liquid for adult nourishment. The cage was placed over a 177 mL container (Ft. Howard, S306), which held a plastic soufflé cup (Solo, P100) with a 10% honey/sugar solution. Females were allowed to freely deposit eggs on the inner surface of the coffee filter.

Upon death, male and female moths were analyzed separately for strain identification utilizing a PCR technique which amplified the *Cytochrome Oxidase* subunit *I* (*COI*) gene that was used as a mitochondrial marker (Levy et al. 2002, Nagoshi and Meagher 2003a, Nagoshi and Meagher 2003b). Eggs were collected daily, and labeled according to pair mating. Newly emerged larvae were reared on pinto bean diet

until strain identification was verified. Once confirmation of strain association was made, F<sub>2</sub> larvae were placed on either a corn or stargrass (*Cynodon nlemfuensis* Vanderyst var. *nlemfuensis* ‘Florona’) foliage diet, according to their strain host preference. Adults of the corn-strain (Hague, F<sub>3</sub>-F<sub>10</sub>) and rice-strain (Ona and Miami colonies, which were combined, F<sub>3</sub>-F<sub>10</sub>) were used; however, generations of both strains were used concurrently.

Plants were grown in 550 mL pots, in a greenhouse at ambient temperature (~30°C) and were fertilized once weekly with Miracle-Gro® 15-30-15 plant food; no pesticides or fungicides were applied. Plant age during experimentation was approximately three weeks for both field corn (‘Truckers Favorite’) and ‘Florona’ stargrass. ‘Florona’ stargrass is a long-lived, persistent perennial grass similar to bermudagrass types that was observed growing at the Range Cattle REC in Ona in 1973 (Mislevy et al. 1989). Previous research showed that it was an excellent host for FAW (Meagher, unpublished data).

### **Oviposition Bioassay**

Eight pairs of adults from one strain ~48 h old were released in a screen enclosure placed inside a Conviron® plant growth chamber. Each strain was tested separately. The enclosure measuring 178 (L) x 76 (W) x 120 (H) cm was constructed of 1.9 cm PVC pipe and nylon window screen. Five corn and five stargrass plants were placed haphazardly within the enclosure. The chamber’s environment was set at  $23.9 \pm 2^\circ\text{C}$ , ~ 80% RH with a 14/10 day/night cycle. Two plastic soufflé cups (Solo, P100) with a saturated cotton ball containing a 10% honey/sugar solution were placed inside the enclosure for moth nourishment. Females were allowed to freely oviposit within the enclosure. The numbers of egg masses were counted on each host-plant after a period of 72 h. The inner

surface of the enclosure was also inspected as a possible surface for oviposition. Six replicates were performed for each strain.

### **Statistical Analysis**

Analysis of variance (PROC MIXED, Contrasts, Littell et al. 1996) was used to examine variation among oviposition substrates.

### **Results**

PCR analysis of insects collected in the four sites indicated the presence of both corn and rice-strain populations in Florida (Fig. 2-3). This information supports previous findings of populations collected and analyzed in Florida (Meagher and Gallo-Meagher 2003, Meagher and Nagoshi 2004, Nagoshi and Meagher 2004). Insects collected from Ona and Miami were determined to be rice-strain, and those collected from Hague were corn-strain. Insects collected from corn in Belle Glade were a mixture of the two strains, and not utilized in this study.

FAW females oviposited on both host-plants as well as on the top and sides of the enclosure. The two host-strains showed a significant difference in their placement of egg masses (Table 2-1). The greatest amount of egg masses oviposited by rice-strain females was found on stargrass plants (95.4%), as opposed to corn (2%) or the enclosure (2%). Alternately, corn-strain females did not discriminate between host-plants in placement of their eggs. Both host-plants had fewer egg masses than the interior walls of the enclosure on which more than 50% of eggs were laid. Corn-strain females even oviposited on the remains of a dead adult (Fig. 2-4).

### **Discussion**

The results of this study clearly identify a strain behavioral distinction between the two host-strains. The initial scope of this experiment was to identify the ovipositional

preference on two known host-plants. Egg masses rather than eggs per mass were recorded because egg masses deposited provides a better indication of the development of a FAW infestation than eggs per mass due to high neonate mortality (Pitre et al. 1983). Rice-strain females clearly showed a preference for stargrass plants as an ovipositional substrate. However, 53% of the egg masses deposited by corn-strain females were on the enclosure surrounding the host-plants. Previous studies make reference to FAW being indiscriminate in its selection of oviposition sites, depositing eggs on objects as well as plants (Thomson and All 1984). Showler (2001) indicated that *Spodoptera exigua* (Hübner) deposited twice as many egg masses on chamber walls and plant pots than on host-plants. He also indicated that the limited mobility of *S. exigua* neonates made them highly dependent on the female's ability to select the most nutritious host. Prior to the identification of host-strains, FAW was considered a single host-strain polyphagous insect. The indiscriminate ovipositional behavior previously noted may have been that of corn-strain females.

Previous research concerning the ovipositional preference of FAW strains has shown that differences do exist between the two strains. Whitford et al. (1988) presented each strain with corn, sorghum, bermudagrass and centipedegrass. Rice-strain females showed preference for grasses and corn-strain females for corn and sorghum. However, it was not stated in their study whether egg masses were found in locations other than on plants. Also, they used colonies that were reared on an artificial pinto bean diet whereas insects in my study were reared on host-plant material. Test results may be unintentionally altered if insects are reared or collected from various food-plants or artificial diets (Pencoe and Martin 1981). Pashley et al. (1995) stated that the

ovipositional preference of the corn-strain is more specialized, and this strain rarely occurs in pastures. The unpredictable oviposition of corn-strain females in my study contradicts her results. My results suggest that corn-strain females display more generalist ovipositional behavior than rice-strain females.

The indiscriminate behavior of corn-strain females indicates that chemical and tactile cues are of a lesser importance to this strain. The rough surface of the screened enclosure was the most desired ovipositional site. Rojas et al. (2003) stated that host location in FAW is not influenced by plant volatiles but that surface texture alone affects ovipositional behavior. In their study, grooved and pitted surfaces were preferred ovipositional sites rather than smooth surfaces. Unfortunately, it was not stated which strain was used in the experiments, although the colonies used were collected from a corn habitat. The ovipositional preferences that they observed indicate that corn-strain females were probably used in their tests.

An herbivore whose preferred host-plant varies in abundance will utilize a lesser host when the ideal host is not available. Competition or natural enemies at other trophic levels may result in poor performance on a particular host (Price et al. 1980, Thompson 1988). Although FAW are reported to prefer plants in the grass family, it has been shown in many studies that they will readily oviposit and feed on plants of other families. Therefore, it may be chemical stimulants within members of the grass family that influence a female's ovipositional preference (Pitre et al. 1983).

Table 2-1. Number of corn and rice-strain egg masses recovered in the oviposition bioassay

Substrate	Mean number of egg masses <sup>1</sup>	
	Corn-strain	Rice-strain
Corn	1.8 ± 0.6 b	0.2 ± 0.3 b
Stargrass	3.3 ± 0.9 ab	8.3 ± 1.5 a
Enclosure	5.8 ± 1.0 a	0.2 ± 0.14 b

<sup>1</sup> Means ± SEM followed by the same letter within strains were not significantly different ( $P \leq 0.05$ ). ANOVA statistics: n = 6 reps; F = 5.5; df = 2, 10;  $P = 0.0247$  and F = 24.1; df = 2, 10;  $P < 0.0001$  for corn and rice-strain, respectively.

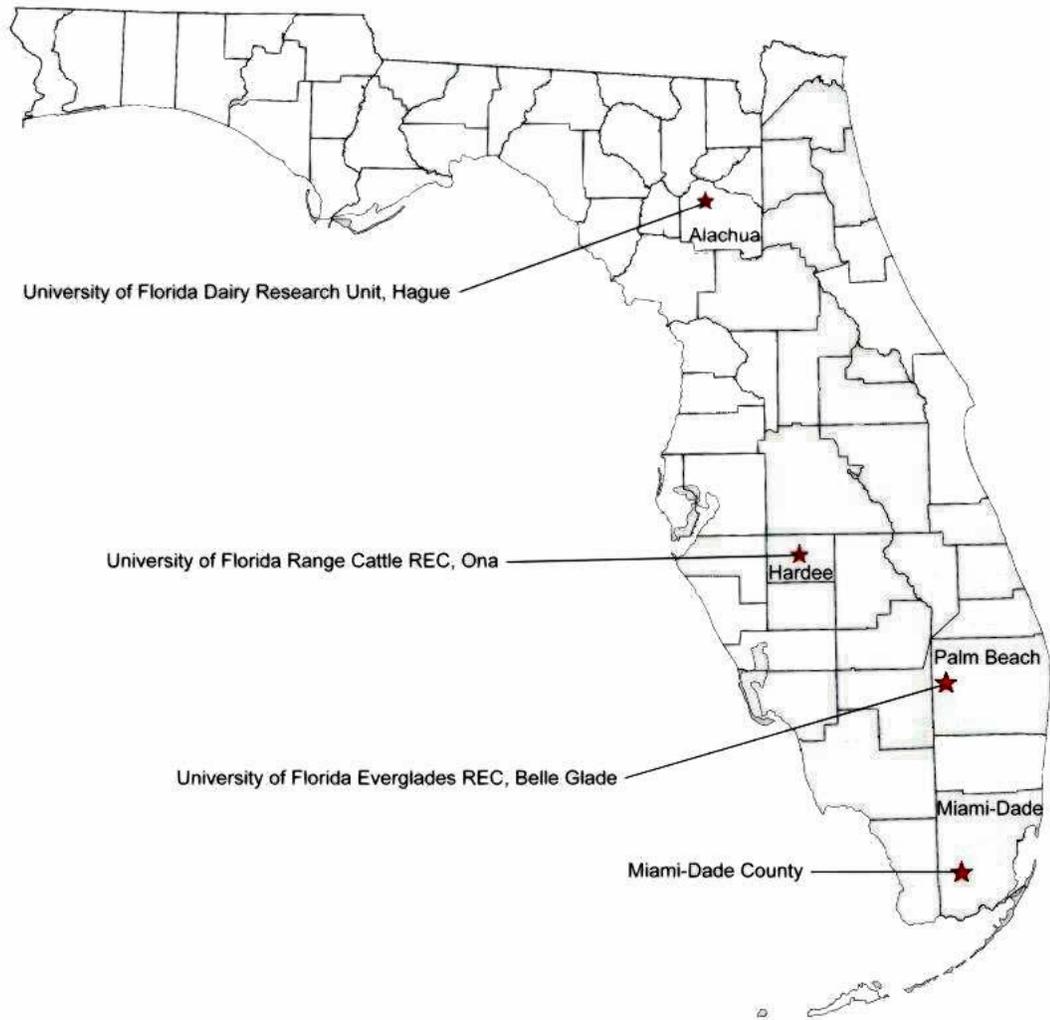


Figure 2-1. Fall armyworm collection sites



Figure 2-2. Strain isolation bioassay container

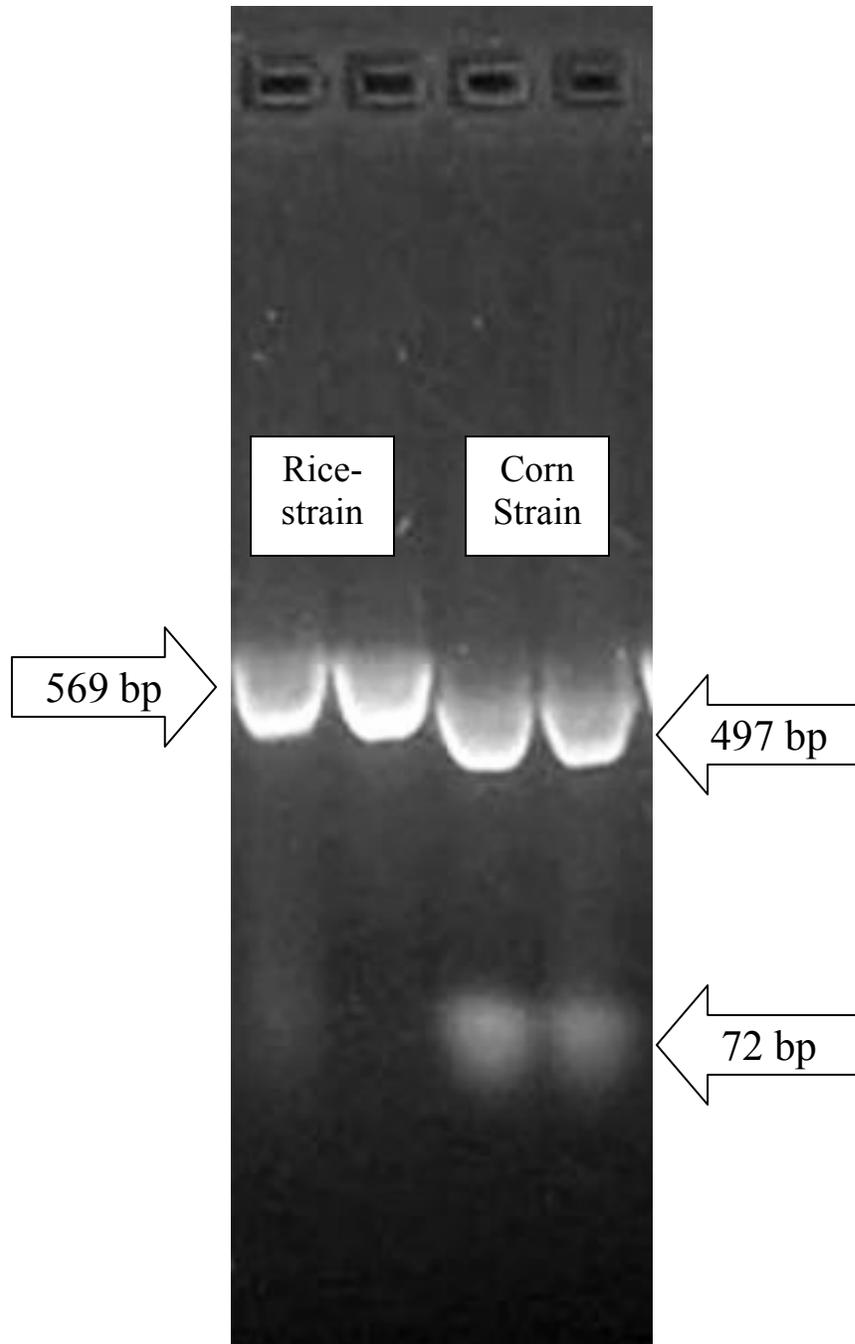


Figure 2-3. Agarose gel showing the rice and corn-strain DNA polymorphism. The rice-strain pattern is a 569 bp PCR band, while the corn-strain fragment is cut by *MspI* to produce two fragments of 497 and 72 bp



Figure 2-4. Dead FAW corn-strain male being used as an oviposition site

## CHAPTER 3 LARVAL PREFERENCE OF HOST-STRAINS TO CORN AND STARGRASS

### **Introduction**

In order for an immature insect to sustain their growth and development, they must be voracious feeders. Food location and feeding behavior of larval herbivores are important attributes of their biology (Zacharuk and Shields 1991). Fall armyworm (FAW) [*Spodoptera frugiperda* (J. E. Smith)] is a polyphagous species that damages a wide range of agricultural crops. This species has two host-strains, one that feeds predominantly on corn (*Zea mays* L.) (corn-strain), and another that feeds predominantly on small grasses such as bermudagrass (*Cynodon dactylon* L.) and rice (*Oryza sativa* L.) (rice-strain) (Pashley et al. 1985, Pashley 1986). As with other moth species, these two strains exhibit differences in physiological characters that may or may not be affected by differences in larval or adult behavior (Pashley 1993, Futuyma and Philippi 1987). Larvae of both strains feed and develop on corn and grasses, although development can be significantly influenced by plant host (Pashley 1988, Whitford et al. 1992, Pashley et al. 1995, Veenstra et al. 1995).

It is not known whether FAW adults or neonate larvae select the host-plant on which development will take place. It has been suggested that female moths in general select the ovipositional substrate that will best sustain their progeny, utilizing visual, chemical and tactile cues in their search. Corn-strain females can be indiscriminate in their selection of oviposition sites, depositing eggs on objects as well as host-plants (Pitre et al. 1983, Thomson and All 1984, Chapter 2).

Therefore, it can also be suggested that it is the newly emerged larva that is making the “choice” of host. Gustatory and tactile cues are of primary importance for food selection to the immature stage (Zacharuk and Shields 1991). Larvae spin threads and descend downward on or near the desired host, and some may be carried a distance by the wind. This may be an adaptive behavior allowing individuals to disperse from the location of the egg mass and thus prevents competition among siblings (Claycomb 1954). Behavioral analysis of larval *Bombyx mori* L., *Manduca sexta* L., and *Pieris brassicae* L., has shown evidence of a high degree of chemosensory specificity at the receptor level (Ishikawa et al. 1969, Schoonhoven 1969). With the diversity of chemicals in green plants, their role in insect feeding behavior has created many theories as to their influence on host selection (Hsiao 1974).

Host selection in immature FAW has received limited study. Pashley et al. (1995) found that both host-strains exhibited a strong preference for corn over bermudagrass in Petri dish bioassays. The current study was conducted using multiple experimental bioassays to determine if the two host-strains demonstrate preference for corn or stargrass (*Cynodon nlemfuensis* Vanderyst var. *nlemfuensis* ‘Florona’), a plant closely related to bermudagrass (Mislevy et al. 1989).

## **Materials and Methods**

### **Strain Isolation and Plant Growth**

Insect culturing and plant growth were conducted using the same colonies and plant-growing techniques as in Chapter 2.

### **Choice Test Bioassays**

These tests were designed to compare preference of neonate larvae of both host-strains for either corn or stargrass. Three separate bioassays were performed. The

first experiments were conducted using 9-cm diameter polystyrene Petri dishes (Thomas Scientific, #3488-B32). New growth leaf sections were taken from each plant type, and trimmed along the top and sides to achieve a uniform size (~ 5 cm x 2 cm). One section of each plant host was placed haphazardly on filter paper discs (Thomas Scientific, #4712B25) moistened with ~ 3 mL deionized water. Sections were placed ~ 2 cm from the center, along the outer edge of the Petri dish (Fig. 3-1). Twenty newly hatched larvae were placed in the center of each dish, and the lid put into place. Ten replicates were performed for each strain. Petri dishes were placed in a Revco™ incubator at  $23.9 \pm 2^\circ\text{C}$  with a 14/10 day/ night cycle, ~80% RH. The number of larvae on or under each leaf section was counted 24 h after introduction.

The second bioassay used a clear acrylic plastic cage measuring 51 (L) x 25 (W) x 28 (H) cm with a testing area of 51 x 25 x 18 cm. This cage allowed for whole plants to be tested rather than plant sections. Potted plants were placed in an elongated recess that was removable, and allowed for the soil/plant interface to be level with the floor surface (Fig. 3-2). During testing, the cage was placed in an environmentally controlled room at  $23.9 \pm 2^\circ\text{C}$  with a 14/10 day/night photoperiod, ~ 80% RH.

The first experiments tested corn or stargrass plants vs. an empty space. The position of the host-plant was alternated within the cage for each replicate. Egg masses containing an unknown number of eggs were placed in the center between the plant and the empty space. The number of neonate larvae on the plant or in the empty space was counted 24 h after introduction. There were three replicates each of corn or rice-strain larvae selecting either corn or stargrass vs. no plant. The second experiment tested corn vs. stargrass plants. Plant location was alternated for each replicate with a total five

replicates completed. Egg masses were placed in the center between plants and counts were made 24 h later. Thus, the choice tests conducted in the plastic cage were plant vs. no plant and corn vs. stargrass.

The third bioassay used was conducted with a Y-tube olfactometer. This unit was constructed of 2.5 cm O.D. clear Plexiglas tubing. The body of the Y-tube measured 58.0 cm, and the arms measured 15.24 cm (Fig. 3-3). Airflow entered the olfactometer by passing through a stainless steel column of activated charcoal. Airflow entering each arm of the Y-tube was set at 0.2 L/min. One-week old plants in 550 mL pots were placed in clear 3.8 l-glass jars. Jar lids were modified to allow airflow to enter and exit the container, thus allowing plant volatiles to be carried into the arms of the olfactometer. Airflow exited the Y-tube by providing a vacuum at 0.40 L/min. The first experiment tested corn or stargrass plants vs. a pot containing moistened soil. The position of the host-plant was alternated for each replicate. Egg masses containing an unknown number of eggs were placed at the midpoint in the body of the Y-tube. A black 9-cm filter paper disk (Thomas Scientific #4740C10) was placed encircling the area outside of the tube above the egg mass. This allowed for larvae to emerge in an area that mimics the underside of a leaf. Larvae were allowed free movement within the olfactometer. The number of larvae in each arm was counted after 24 h. There were three replicates each of corn or rice-strain larvae selecting either corn or stargrass vs. the potted soil. The second experiment tested larval attraction to the volatiles of corn vs. stargrass plants. Egg masses were placed in a similar fashion, and the number of larvae in each arm was counted after 24 h. There were seven replicates each of corn or rice-strain larvae selecting either corn or stargrass plants.

### **Passing-Over Tests**

These tests were conducted to determine if larvae would continue to disperse once they came in contact with a plant source. Two bioassays were conducted. Sections of corn and stargrass leaf material were placed on filter paper discs (Thomas Scientific, #0898V87) moistened with ca. 3 mL deionized water and cut to fit the dimensions of a 140 x 15 mm polystyrene Petri dish (Thomas Scientific, catalog #3488C10). The plant material that was being “passed-over” was cut to dimensions large enough to span the 14 cm diameter of the Petri dish. Another plant section was trimmed to a uniform size (~ 5 cm x 2 cm) and placed ~ 30 mm from the center, and ~ 20 mm along the outer edge of the Petri dish (Fig. 3-4). Twenty neonate larvae were placed in the dish opposite the leaf section and the lid put into place. Petri dishes were placed in a Revco™ incubator at 23.9 ± 2°C with a 14/10 day/ night cycle, ~80% RH. The number of larvae on or under each leaf section was counted 24 h after introduction. This method was performed for ten replicates each of corn-strain passing over corn to stargrass and over stargrass to corn, and rice-strain passing over corn to stargrass and over stargrass to corn.

The second bioassay used a wind tunnel design. The plastic cage used in the choice tests was modified to have inflow and outflow of air (0.25 m/s). Air exiting the cage was vented to the outside to prevent plant volatiles from reentering the cage. Plants were arranged in the cage so that larvae would have to pass through one plant host to reach the other (Figs. 3-5 and 3-6). Newly emerged larvae from an egg mass were placed in the downwind position behind the first plant. There were five replicates each of corn-strain larvae passing through corn to stargrass and through stargrass to corn; and rice-strain

larvae passing through corn to stargrass and through stargrass to corn. The number of larvae on each plant was counted 24 h after introduction.

### **Statistical Analysis**

Nonparametric statistical analysis was performed using the Kruskal-Wallis test (Minitab 14, SAS Institute, 8.0). For the Petri dish test, the number of larvae on each section was compared between host-plants; for the plastic cage tests, the percent larva on each plant was compared.

## **Results**

### **Choice Tests**

Results from the Petri dish bioassay suggested that larvae of both strains showed strong preferences for corn over stargrass, as over 80% of the larvae were found on corn sections (Table 3-1).

In the choice cage bioassay, the first test showed that both strains exhibited a strong preference for whichever host-plant was present as opposed to no plant (Table 3-2). Therefore, no directional bias was observed in this bioassay. The choice test between plants showed that corn-strain larvae exhibited a trend towards selecting stargrass compared to corn, while rice-strain larvae were evenly distributed between the two plants (Table 3-3).

The first experiment with the Y-tube olfactometer demonstrated that larvae would select the arm that contained plant volatiles rather than air from moistened soil (Table 3-4). The second experiment showed that corn-strain larvae displayed a trend towards the volatiles emitted from the corn plant. Although not significant, 68% of the corn-strain larvae collected were present in this arm (Table 3-5). This contrasted with rice-strain larvae, which did not show a significant preference for either plant (Table 3-5).

### **Passing-Over Tests**

Corn-strain larvae demonstrated a preference to select the first plant section they encountered in the Petri dish bioassay, selecting corn when it was first encountered and selecting stargrass when it was first encountered (Table 3-6). Almost 89% of the rice-strain larvae selected stargrass when first exposed to stargrass, however, when first exposed to corn they distributed themselves evenly between the two host-plants (Table 3-7).

The wind tunnel bioassay with whole plants provided slightly different results. Corn-strain larvae were evenly distributed across both plants no matter which host was first encountered (Tables 3-8). Rice-strain larvae selected corn (69.6%) when it was the plant first encountered (Table 3-9). There was a trend for rice-strain larvae to select stargrass (67.4%) when it was first encountered; however the difference was not significant (Table 3-9). Thus, corn-strain larvae showed a preference for the first plant encountered in the Petri dish bioassay, but were evenly distributed between plants in the plastic cage wind tunnel. Rice-strain larvae showed a trend to accept the first plant encountered in the plastic cage; response in the Petri dish was mixed.

### **Discussion**

Previous research conducted in Chapter 2 suggested that there are differences in ovipositional preference between corn-strain and rice-strain moths. However, corn-strain moths were just as likely to oviposit on non-plant materials as host-plants. Therefore, if corn-strain females are not selecting quality host-plants then it is possible that neonates are making the host-plant selection.

Experimental studies that have previously examined the performance of the two host-strains indicated that rice-strain larvae would readily accept corn as a host

(Pashley et al. 1995). The result from the Petri dish experiment supports these findings, in that rice and corn-strain larvae accepted corn sections as their feeding site significantly more often than stargrass sections. When the two strains were presented a choice of whole plants, corn-strain larvae exhibited a trend towards stargrass, while rice-strain larvae were evenly distributed between plants. Perhaps the corn plant sections released large amounts of volatiles in the closed Petri dish that influenced larval behavior in a different manner compared to whole plants.

The olfactometer study was designed to determine if FAW neonates could detect plant volatiles that may guide them to a food source. Results of the first experiment suggested that the neonates have the capacity to detect plant volatiles as part of their food-finding behavior since larvae selected olfactometer arms that contained plant volatiles over those that contained volatiles from moistened soil. Observations suggested that larvae “sampled the air” by raising their heads and swaying back and forth. The comparison between volatiles of each host-plant suggested that corn-strain larvae exhibited a trend for preference for corn over stargrass, while rice-strain larvae were evenly dispersed in both arms of the olfactometer when presented the two plants. Chemical senses have been shown to be important in food choice behavior (Chang 1985, de Boer and Hanson 1987), although olfaction in larvae is usually limited to short range orientation (Zacharuk and Shields 1991).

The olfactometer study was also conducted to determine if neonates exhibited a predisposition to host-plants or was prior feeding required to be attracted to a particular plant host (induction). Several workers have shown with other lepidopteran larvae that neonates generally don't show an orientation response to specific host-plants (Saxena and

Schoonhoven 1978, Saxena and Schoonhoven 1982). The same results were found with *Spodoptera littoralis* (Boisduval), which showed that neonates did not have a preference for host-plants but third instar larvae reared on a host exhibited an increase in orientational response in their attraction to a host-plant (Carlsson et al. 1999). The sensory requirements and development of the immatures are most likely more limited than that of older instars (de Boer and Hanson 1987). Induction of feeding is less evident in a choice test when close relative plant species are used. The more distant relation of the two plants will bring forth a stronger induction of feeding response (de Boer and Hanson 1984).

Fall armyworm neonate larvae have the ability to be mobile upon emergence and disperse from the location of the egg mass (Claycomb 1954). The passing-over study was designed to assess larval ability to choose one host-plant over another after coming in contact with one host. In the Petri dish bioassay, corn-strain larvae readily accepted whichever host-plant section they first encountered. However, when rice-strain larvae first encountered corn, an equal number would pass over the corn to feed upon the stargrass. Conversely, when stargrass was first encountered, it was preferred as a suitable host. The plastic cage wind tunnel was constructed to allow larvae to be mobile over a longer distance than that of a Petri dish and allowed for live plant material to be used rather than plant sections. When corn-strain larvae first encountered either corn or stargrass, they distributed themselves evenly between the two plants. However, rice-strain larvae selected and accepted corn if it was the first plant that they encountered, and displayed a trend to accept stargrass when it was first encountered. This behavior

supports the theory that the corn-strain is the more generalist feeder of the two strains (Whitford et al. 1988).

Significant nutritional and developmental differences have been noted between the two strains when feeding on corn and bermudagrass. Whitford et al. (1988) noted larvae and pupae of the corn-strain were heavier than rice-strain immatures when reared on identical hosts. Also, rice-strain larvae developed faster on bermudagrass than other host grasses presented. Corn-strain pupae were heavier when fed a diet of corn or bermudagrass than on an artificial diet. These developmental differences between the two strains were noted by Pashley (1988), who showed that corn-strain larval development was similar when fed corn or bermudagrass. The rice-strain female's preference for grass in the ovipositional study and larval preference in the wind tunnel indicates strain orientation toward bermudagrass.

Table 3-1. Mean ( $\pm$  SEM) number of corn or rice-strain larvae collected from corn or stargrass sections in the Petri dish choice bioassay

Host-plant	Strain	
	Corn	Rice
Corn	14.4 $\pm$ 1.21 a	16.5 $\pm$ 0.83 a
Stargrass	3.3 $\pm$ 1.17 b	1.1 $\pm$ 0.06 b

Means with same letter are not significantly different, corn-strain  $P < 0.0001$ , rice-strain  $P < 0.0001$ ;  $n = 10$ .

Table 3-2. Percent larvae ( $\pm$  SEM) of both strains selecting either corn or stargrass vs. an empty space in the choice cage bioassay

	Host-plant	% Larvae	<i>P</i> value <sup>1</sup>
Corn-strain	Corn	94.9 $\pm$ 5.1	0.0431
	Empty	5.1 $\pm$ 5.1	
	Stargrass	95.9 $\pm$ 2.2	0.0495
	Empty	4.1 $\pm$ 2.2	
Rice-strain	Corn	99.6 $\pm$ 0.3	0.0431
	Empty	0.4 $\pm$ 0.3	
	Stargrass	85.7 $\pm$ 7.2	0.0495
	Empty	14.2 $\pm$ 7.2	

<sup>1</sup> For each comparison, n = 3, df = 1.

Table 3-3. Percent larvae ( $\pm$  SEM) of both strains selecting either corn or stargrass in the choice cage bioassay

	Host-plant	% Larvae	<i>P</i> value <sup>1</sup>
Corn-strain	Corn	38.2 $\pm$ 7.5	0.1172
	Stargrass	61.8 $\pm$ 7.5	
Rice- strain	Corn	53.2 $\pm$ 8.8	0.6015
	Stargrass	46.8 $\pm$ 8.8	

<sup>1</sup> For each comparison, n = 5, df = 1.

Table 3-4. Percent larvae ( $\pm$  SEM) of both strains selecting either corn or stargrass vs. potted soil in the Y-tube olfactometer bioassay

	Host-plant	% Larvae	<i>P</i> value <sup>1</sup>
Corn-strain	Corn	68.5 $\pm$ 5.1	0.0209
	Soil	31.6 $\pm$ 5.1	
	Stargrass	64.2 $\pm$ 7.1	0.0433
	Soil	34.9 $\pm$ 7.4	
Rice-strain	Corn	81.1 $\pm$ 6.6	0.0209
	Soil	18.9 $\pm$ 6.6	
	Stargrass	67.8 $\pm$ 5.7	0.0209
	Soil	32.1 $\pm$ 5.7	

<sup>1</sup> For each comparison, n = 3, df = 1.

Table 3-5. Percent larvae ( $\pm$  SEM) of both strains selecting either corn or stargrass in the Y-tube olfactometer bioassay

	Host-plant	% Larvae	<i>P</i> value <sup>1</sup>
Corn-strain	Corn	68.2 $\pm$ 15.0	0.1102
	Stargrass	31.8 $\pm$ 15.0	
Rice-strain	Corn	53.8 $\pm$ 9.0	0.5653
	Stargrass	46.2 $\pm$ 9.0	

<sup>1</sup> For each comparison, n = 7, df = 1.

Table 3-6. Mean ( $\pm$  SEM) number of corn-strain larvae that selected either corn or stargrass after first being directionally exposed to corn or stargrass in a Petri dish bioassay

First exposure	Plant section selected	Larvae	<i>P</i> value <sup>1</sup>
Corn to Stargrass	Corn	18.5 $\pm$ 0.47	0.0001
	Stargrass	0.7 $\pm$ 0.21	
Stargrass to Corn	Stargrass	16.6 $\pm$ 0.79	0.0001
	Corn	2.0 $\pm$ 0.72	

<sup>1</sup> For each comparison, n = 10, df = 1.

Table 3-7. Mean ( $\pm$  SEM) number of rice-strain larvae that selected either corn or stargrass after first being directionally exposed to corn or stargrass in a Petri dish bioassay

First exposure	Plant section selected	Larvae	<i>P</i> value <sup>1</sup>
Corn to Stargrass	Corn	9.0 $\pm$ 1.56	0.7035
	Stargrass	8.5 $\pm$ 1.61	
Stargrass to Corn	Stargrass	14.2 $\pm$ 0.94	0.0001
	Corn	1.8 $\pm$ 0.49	

<sup>1</sup> For each comparison, n = 10, df = 1.

Table 3-8. Percent ( $\pm$  SEM) corn-strain larvae that selected either corn or stargrass after first being directionally exposed to corn or stargrass in a wind tunnel bioassay

First exposure	Plant selected	% Larvae	<i>P</i> value <sup>1</sup>
Corn to Stargrass	Corn	58.4 $\pm$ 8.37	0.3472
	Stargrass	41.6 $\pm$ 8.37	
Stargrass to Corn	Stargrass	46.7 $\pm$ 8.13	0.4647
	Corn	56.9 $\pm$ 8.08	

<sup>1</sup> For each comparison, n = 5, df = 1.

Table 3-9. Percent ( $\pm$  SEM) rice-strain larvae that selected either corn or stargrass after first being directionally exposed to corn or stargrass in a wind tunnel bioassay

First exposure	Plant Selected	% Larvae	<i>P</i> value <sup>1</sup>
Corn to Stargrass	Corn	69.6 $\pm$ 7.14	0.0163
	Stargrass	30.4 $\pm$ 6.97	
Stargrass to Corn	Stargrass	67.4 $\pm$ 15.5	0.4647
	Corn	32.6 $\pm$ 15.5	

<sup>1</sup> For each comparison, n = 5, df = 1.



Figure 3-1. Small Petri dish bioassay



Figure 3-2. Choice cage bioassay

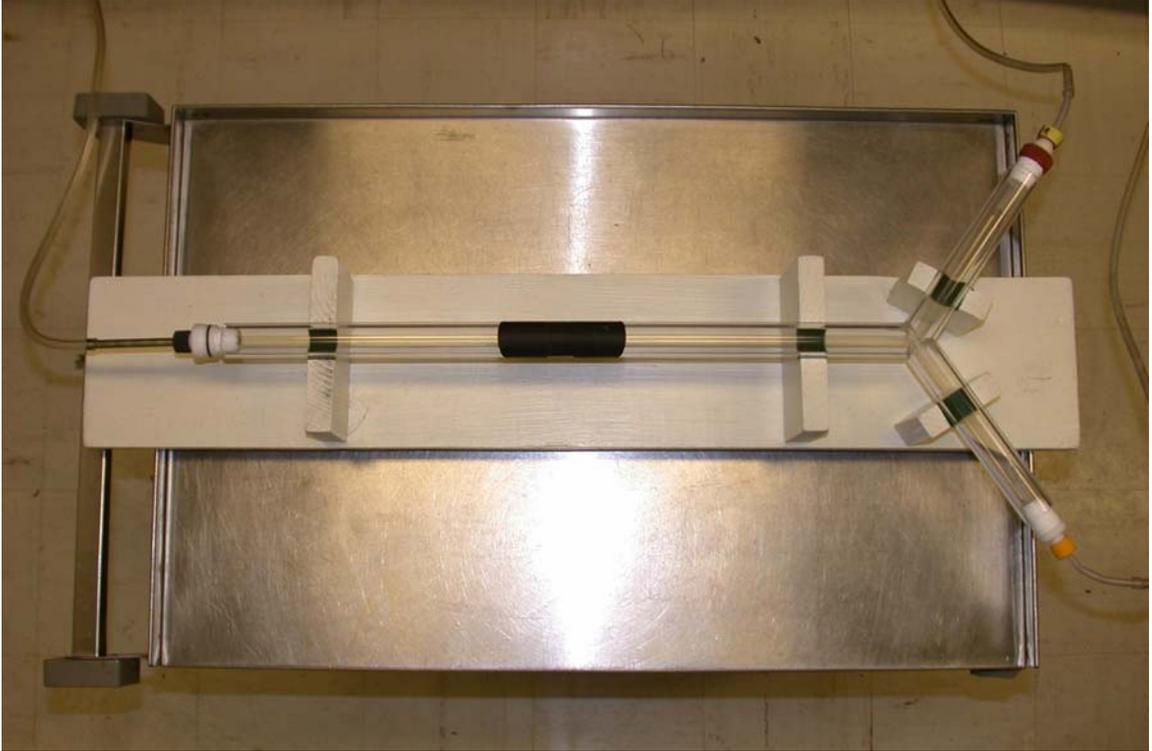


Figure 3-3. Y-tube olfactometer used in volatile study

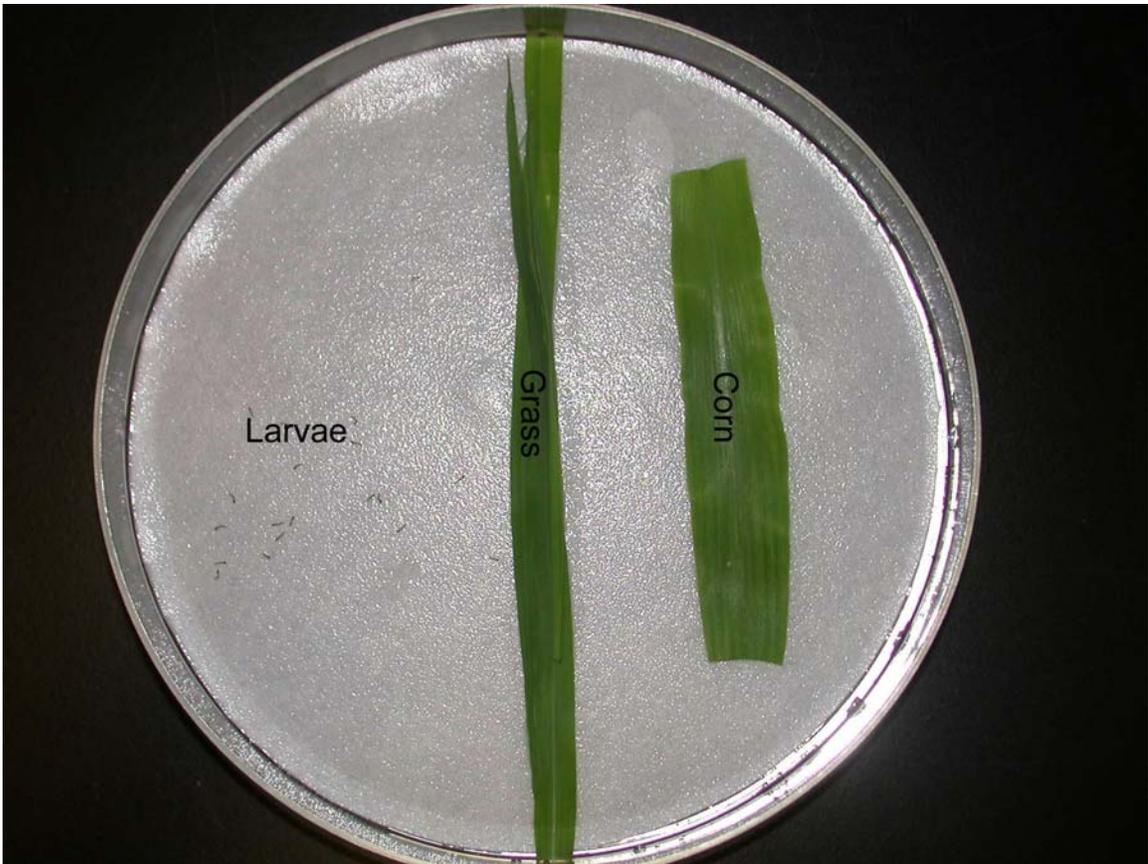


Figure 3-4. Petri dish passing-over study

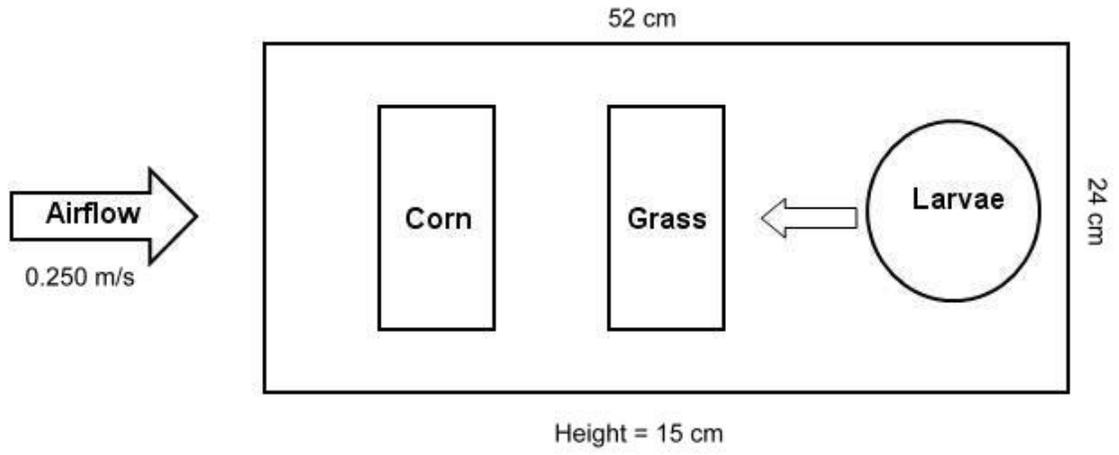


Figure 3-5. Wind tunnel layout

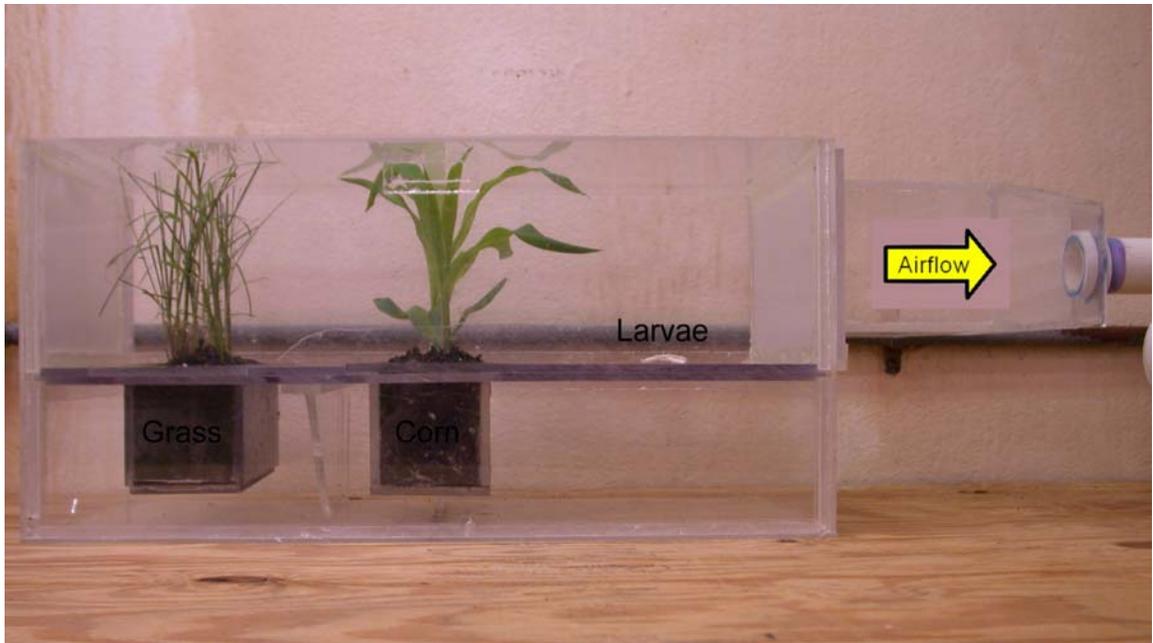


Figure 3-6. Wind tunnel bioassay

## CHAPTER 4 SUMMARY

Results show two FAW host-strains that exhibit host specificity at larval and adult stages. FAW eggs and larvae were collected and identified using PCR techniques and results confirmed that both corn and rice-strain populations reside in Florida corn and grass habitats (Meagher and Gallo-Meagher 2003, Meagher and Nagoshi 2004, Nagoshi and Meagher 2004). Behavioral differences between the two host-strains involving ovipositional site preference and larval host-selection to corn and stargrass were identified.

The rice-strain exhibited a significant preference for utilizing stargrass as its ovipositional substrate. Whitford et al. (1988) indicated this behavioral difference when each strain was presented with corn, sorghum, bermudagrass and centipedegrass. Their study demonstrated the rice-strain's ovipositional preference for grass that indicated a degree of adaptation to bermudagrass. However, the corn-strain indiscriminately placed egg masses within and onto the enclosure. When a host-plant was selected, stargrass was selected over corn. Previous FAW ovipositional studies do not indicate if egg masses were located in other areas than on plants. Pashley et al. (1995) concluded that ovipositional preference in the corn-strain was more specialized, and that this strain rarely occurs in pastures. The ovipositional selection of corn-strain females in my study indicated contradictory results since these females oviposited on PVC, screen, stargrass,

and corn leaves. Corn-strain individuals have been found in grass habitats along with rice-strain (Meagher and Gallo-Meagher 2003). It is not known which strain affects crops other than corn and grass, but my results of my ovipositional study suggest it may be the corn-strain.

My study differs from other research in that moths were reared on natural plant material as opposed to an artificial diet. It is not known what effect that larval feeding on an artificial diet has on the adult's ability to adequately select a suitable host.

When the two host-strains are presented with a choice for feeding, there appears to be certain trends inherent to each host-strain in food selection behavior. The bioassays conducted support previous findings in regards to host-strain behavioral differences. The Petri dish choice study showed a significant preference for corn by both strains. These results support the findings of Pashley et al. (1995) that the two host-strains would accept corn as their host. An even distribution of larvae was noted when the two strains were presented a choice between potted corn and stargrass plants.

The ability for FAW neonates to detect plant volatiles alone to guide them to a food source has yet to be determined. Veenstra et al. (1995) suggested that physiological and biochemical adaptations by the host-strains may account for host-plant use in FAW. It may be that their dispersal is initiated by visual and chemosensory cues. Corn-strain larvae exhibited a preference for corn over stargrass volatiles in the olfactometer. Rice-strain larvae were evenly dispersed in both arms of the olfactometer when presented the two plant hosts. The strong attraction to stargrass exhibited by the rice-strain when visual cues were available may explain the results obtained from the host-plant choice experiments. The olfactometer study also gave an indication that the neonates have

sensilla that assist in their food-finding behavior. Older instars may give a clearer insight to sensory structures that aide in food-finding behavior. As the immature stage develops through several molts, cuticular structures of existing sensilla are replaced with new sensilla (Zacharuk and Shields 1991).

The passing-over experiments were designed to examine the dispersal of both strains. The Petri dish bioassay indicated that corn-strain larvae would readily feed upon whichever host-plant section they first encountered. Rice-strain larvae were evenly distributed upon corn and stargrass when corn was encountered first, but when stargrass was first encountered many of the neonates selected stargrass as a suitable host. Using the plastic cage wind tunnel design simulated a more realistic test since whole plants were used rather than plant sections. Corn-strain larvae evenly dispersed between both plants no matter which was encountered first, however, rice-strain larvae tended to stop and feed at the first plant encountered. An improvement of the bioassay in the future would be to limit the number of larvae used since dispersion to either plant may have been due to crowding rather than plant attraction.

In conclusion, these experiments confirm that there are behavioral differences between the two host-strains of FAW. The studies performed suggest that the adult female determines host selection in the rice-strain by selecting grass plants for oviposition. Corn-strain moths are much less selective in egg mass placement. Neonates of both strains do not have prior feeding experiences on a host-plant and appear to be unbiased in their food-finding behavior. Plant volatiles and visual cues assist the neonates in dispersal, but their ability to seek out a suitable host when not placed in the vicinity is negligible. When food sources are consumed, older larvae become mobile in

search of new hosts. The ovipositional preference and food-seeking behavior observed suggests that the corn-strain is the more generalist feeder.

Strain identification is important for population control and management because previous research has shown toxicological and host genotypic differences between strains. Rice-strain larvae were shown to be more susceptible to various insecticides and more susceptible to transgenic *Bacillus thuringiensis* Berliner (Bt) cotton than corn-strain larvae (Pashley et al. 1987, Adamczyk et al. 1997). Laboratory and field studies have shown distinct differences in feeding of bermudagrass genotypes, with rice-strain larvae generally able to gain more weight and consume more plant material than corn-strain larvae (Quisenberry and Whitford 1988).

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## BIOGRAPHICAL SKETCH

Charles J. Stuhl was born on Long Island, New York on January 13, 1965. A few years after graduation of high school, he enlisted in the US Army. Entering the military in 1986, he spent a three-year tour of duty as a combat medic in Germany. Upon completion of his military duty, he attended Gupton-Jones College of Mortuary Service in Atlanta, Georgia, receiving an A.S. degree in mortuary science. He relocated to Florida to work in the funeral industry. After a few years in his new career, he decided to return to college and pursue a B.S. degree in entomology at the University of Florida, and was awarded his degree in 2000. He began working at the US Department of Agriculture, Agriculture Research Service as an undergraduate, and hopes to make of long career in their employ. He currently resides in Santa Fe, Florida, and plans on pursuing a PhD. in entomology at the University of Florida.