BT RESISTANCE MANAGEMENT OF

FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE)

HOST STRAINS

by

David A. Ingber

A dissertation submitted to the Faculty of the University of Delaware in partial
fulfillment of the requirements for the degree of Doctor of Philosophy in Entomology and
Wildlife Ecology

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ABSTRACT

The fall armyworm, *Spodoptera frugiperda* (Smith), is a highly damaging, multivoltine pest of commercial crops including corn (*Zea mays* L.), cotton (*Gossypium Spp.* L.), sorghum (*Sorghum bicolor* L.), rice (*Oryza sativa* L.), and pasture grasses. Fall armyworm have become a growing concern in agricultural communities across the Americas as field populations in many locales have evolved resistance to several Cry1 toxins derived from the bacterium *Bacillus thuringiensis* Berliner (Bt). An often overlooked aspect of fall armyworm biology is the existence of two host strains, the "rice" and "corn" strains. Despite knowledge of fall armyworm host strains for thirty years, there has been little research devoted to their characterization. Here the Bt tolerances, oviposition preferences, and host plant tissue feeding of one rice strain, two corn strain, and one rice-corn hybrid population fall armyworm were compared. Bt tolerance was assessed using diet-based bioassays were conducted for each population to Cry1Ab, Cry1Ac, and Cry1F, and Cry1F again using tissue-based bioassays. Oviposition preferences of one rice strain and one corn strain population were compared using no-choice and two-choice greenhouse experiments, and host plant tissue feeding was compared between one rice strain, two corn strains, and one rice-corn hybrid population. Results from diet-based bioassays indicated that the corn strain and hybrid populations were more tolerant to the Bt toxins, especially to Cry1F, than the rice strain population. This was corroborated by the tissue-based bioassays, though the results were narrowly non-significant. The greenhouse oviposition experiments indicated that corn was a preferred oviposition host for both rice and corn strain fall armyworm; this was supported by the host plant tissue feeding experiments that indicated that corn is suitable larval host plant for each of the four fall armyworm populations tested. The overall results of this study indicate that the

host strain should be taken into account when preparing insect resistance management plans for fall armyworm. Regions whose fall armyworm populations have larger proportions of corn strain or hybrid insects may be identified using pheromone traps and established methods of host strain identification. These identified regions should be monitored more closely for the evolution of Bt resistance.

Chapter 1

REVIEW OF RELEVANT LITERATRE

Fall Armyworm Biology. The fall armyworm, *Spodoptera frugiperda* (Smith),

(Lepidoptera: Noctuidae) is a native, multivoltine pest of the Americas found on pasture,

turf, and several agricultural crops including corn (*Zea mays* L.), cotton (*Gossypium Spp.* L.),

sorghum (*Sorghum bicolor* L.), rice (*Oryza sativa* L.), pasture grasses, and Bermuda grass

(*Cynodon dactylon.* L.) (Luginbill 1928, Young 1979, Nagoshi and Meagher 2008). Fall

armyworm outbreaks have been recorded in the United States since the late 1700's

(Luginbill 1928, Sparks et al. 1979). Larvae are highly polyphagous and have been

documented on over 80 species of host plants that span 23 families (Pashley 1988),

though there is an apparent preference towards grasses (Luginbill 1928, Johnson 1987,

Pashley 1988). Larval feeding damage is especially severe in corn, where yield losses can

range from 17 to 72% depending on the locality and conditions (Cruz and Turpin 1983,

Barros et al. 2010, Murúa et al. 2015). Feeding on host plants occurs throughout the

entire larval stage of the insect, where the majority of tissue consumption occurs during

the 5th and 6th instars (Luginbill 1928).

In the presence of preferred host plants, female fall armyworm will oviposit

during the evening, typically on the abaxial (underside) portion of leaves (Luginbill 1928,

Sparks et al. 1979). There exists a general preference towards oviposition on plants in the

mid-to-late growth stage (Pitre et al. 1983, Ching'oma and Pitre 1999), possibly due to

the presence of volatile oviposition stimulants (Wearing et al. 1973). There is also evidence that undamaged plants are preferred over plants with injury caused by insect feeding (Téllez-Rodriguez et al. 2014). In the absence of preferred host plants, or at high population densities, female fall armyworm will oviposit indiscriminately on any available vegetation or non-vegetative surfaces (Luginbill 1928, Sparks et al. 1979).

Unlike many other lepidopteran agricultural pests, the fall armyworm cannot diapause, and subsequently cannot survive the winter in colder latitudes (Luginbill 1928, Sparks et al. 1979, Johnson 1987). A yearly migration occurs over the course of several generations where the species inhabits more northern climates in North America and more southern and higher elevation habitats in South and Central America. The exact course and timing of this movement varies annually and is driven by weather patterns and the availability of host plants (Hogg et al. 1982, Sparks 1986, Mitchell et al. 1991). In most years, migrating fall armyworms eventually reach their northernmost range in the United States in the late summer to early fall (Luginbill 1928, Johnson 1987, Sparks et al. 1979). A generation can take as little as 4 weeks, or as long as 13 weeks depending on temperature, which often corresponds with a population's latitude (Vickery 1929, Sparks et al. 1979). Fall armyworm survive year-round in the tropical regions of South and Central America and Mexico, as well as south Florida, Texas, and Louisiana in the United States (Sparks et al. 1979, Pair et al. 1986, Johnson 1987, Mitchell et al. 1991). There is also some evidence for populations in Israel that were likely imported from the United States Virgin Islands (Wiltshire 1977), as well as recent, severe outbreaks in West and Central Africa (Georgen et al. 2016). In addition, recent research has made it possible to map the migration patterns of certain populations of the species (Pashley1986) using

mitochondrial CO1 haplotyping (Nagoshi and Meagher 2008, Nagoshi et al. 2009, Nagoshi et al. 2012, Nagoshi et al. 2015).

Bt Resistance. Genetically modified crops producing insecticidal toxins derived from the bacterium *Bacillus thuringiensis* Berliner (Bt) have played a key role in the control of many agricultural pests. Currently, the most commonly used Bt toxins are the crystalline (Cry) proteins (Rang et al. 2005) that are produced during the sporulation growth phase (Schnepf et al. 1998). The Cry protein mode of action is complex and involves multiple biochemical steps including ingestion, activation in the gut, and binding of the protein to receptors in the target insect's midgut. These bound proteins will form pores lysing the cells and causing deterioration of the gut, which eventually results in the death of the insect (Schnepf et al. 1998). The vegetative insecticidal proteins (Vip) compose another form of Bt toxins (Estruch et al. 1996). Vip proteins are produced during the vegetative growth phase of Bt (Estruch et al. 1996, Lee et al. 2003). The Vip mode of action may be similar to that of Cry proteins (Rang et al. 2005) but may involve different receptor proteins in the midgut epithelium (Lee et al. 2003). Additionally, Vip proteins do not share any sequence homology with any known Cry proteins, which makes them potential candidates for combining with non-cross resistant cry proteins in-planta (pyramiding) to promote insect resistance management and extend plant durability (Estruch et al. 1996, Lee et al. 2003).

Transgenic corn expressing the Cry1F Bt toxin for the management of European corn borer (*Ostrinia nubilalis* [Hübner]) and southwestern corn borer (*Diatraea grandiosella* Dyar) was registered with the United States Environmental Protection Agency (USEPA) in 2001 (USEPA 2001a) and commercialized in 2003. In addition to

the two original target species, Cry1F corn varieties are also effective for the management of several additional Lepidopteran pests, including the fall armyworm (Siebert et al. 2008a). Transgenic cotton expressing Cry1F with Cry1Ac was also registered by the USEPA in 2005 (USEPA 2005) and commercialized in the same year. Most recently, transgenic cotton and corn producing modified Vip proteins (Vip3Aa19 and Vip3Aa20 respectively) for the management of several lepidopteran pests, including fall armyworm, were registered in 2008 (USEPA 2008a, b) and commercialized in 2009 and 2010 respectively. In 2016 it was reported that ~79% of corn acreage and ~84% of cotton acreage in the United States were planted with Bt-varieties for the management of damaging insect pests (NASS 2016).

Plant varieties that produce Bt toxins must be carefully managed to delay and mitigate the development of Bt resistance. Resistance is defined as a genetically based decrease in susceptibility of an insect population to an insecticide (Tabashnik 1994). Fields planted with Bt-varieties pose a strong selective pressure that favors insects capable of tolerating the toxins. Tolerance adaptations may include: decreased sensitivity to toxins, decreased membrane permeability, conversion of toxins to less toxic or inert compounds, behavioral avoidance, or any combination thereof (Mallet 1989, McKenzie and Batterham 1994).

There are several mechanisms from which Bt resistance can arise. One of the best characterized mechanisms of resistance is the inactivation or reduced activity of Bt receptors in the insect gut (Ma et al. 2005). These midgut receptors include: cadherin-like proteins, glycolipid receptors, aminopeptidases, and alkaline phosphatases (Ma et al. 2005). Mutations that alter the function of receptors may result in the development of

"Mode 1" resistance, which is characterized by generally high levels of Bt tolerance compared to susceptible insects, recessive effective inheritance (the fitness of heterozygote insects on Bt plants compared to that of resistant homozygotes given the conditions present in the environment [Gould 1998, Tabashnik et al. 2004]), and little to no cross-resistance to other Bt toxins (Tabashnik et al. 1998). This type of resistance is present in several Lepidopteran pest species found in the families Noctuidae, Plutellidae, and Gelechiidae (Heckel et al. 2007).

Inducible tolerance mechanisms may also result in the development of Bt resistance in insect pests. Increased immune responses such as gut protease activity (Loseva et al. 2002), gut cell regeneration rates (to recover from the effects of Bt toxins [Loeb et al. 2001]), or coagulation responses are all categorized as inducible tolerance mechanisms (Gould 1998, Ma et al. 2005). In general, inducible tolerance mechanisms tend to result in lower overall levels of Bt resistance that are accompanied by non-recessive inheritance and cross-resistance (Heckel 1994, Gould 1998, Tabashnik et al. 1998). Inducible tolerance mechanisms are not observed in the field as often as cases of Mode 1 resistance (Ma et al. 2005), possibly due to the increased prevalence of accompanying fitness costs (negative pleotropic effects of Bt resistance that affect a variety of life history traits [Carrière and Tabashnik 2001, Gassmann et al. 2009]).

Cry1F Resistance in Fall Armyworm. Beginning in late 2006 in Puerto Rico, commercial farms and seed company field stations began to submit reports of unexpectedly high damage to Cry1F corn by fall armyworm. This was later confirmed as Cry1F resistance by Storer et al. (2010). Cry1F resistance in fall armyworm appears to follow the trends of Mode 1 resistance being characterized by generally high levels of

resistance, recessive inheritance, few fitness costs, and low degrees of cross-resistance to other Bt toxins (Storer et al. 2010, Niu et al. 2013, Velez et al. 2013, Niu et al. 2014, Velez et al. 2014).

Several factors likely contributed to the development of Cry1F resistance in Puerto Rican fall armyworm populations. Though the commercial planting of Cry1F corn did not begin until 2003, Cry1F varieties of corn had been planted in Puerto Rico since 1996 in experimental plots for variety development on seed company farms (Storer et al. 2010). Therefore, there had been at least some selection pressure from Cry1F on fall armyworm populations in Puerto Rico prior to its commercial planting in 2003. Additionally, after its commercialization, Cry1F corn quickly became the preferred corn variety in the region, and was planted broadly and continually promoting selection for resistance (USEPA 2007, Niu et al. 2013, Niu et al. 2014).

In the United States, Australia, India, and several other countries where Bt crop varieties are registered it is required as a condition of registration that a proportion of each field planted with a Bt crop variety be planted with non-Bt plants. This area is referred to as a refuge, and promotes the development of susceptible insects (USEPA 2010, Downes and Mahon 2012, Mohan and Oufattole 2015). The insects that develop in the refuge will have a high likelihood of mating with any resistant insects that come from the Bt portion of the field. Offspring from susceptible by resistant insect matings will consist of heterozygote progeny. The degree to which heterozygote insects have lower fitness when feeding on Bt plants compared to homozygous resistant insects will result in delays in the evolution of resistance (Gould 1998, Tabashnik et al. 2003, Tabashnik et al. 2013). There are no mandatory refuge requirements for Cry1F corn in Puerto Rico and

Brazil. This promotes increased matings between resistant individuals and the subsequent production of homozygous resistant progeny.

In the mainland United States the refuge requirement for a field planted with a Bt corn variety may be in a single contiguous section (block refuge), in strips required to be a minimum of 4 rows wide (strip refuge), or dispersed throughout the field via a seed blend (blended refuge). For Lepidoptera a field planted with a Bt corn variety that expresses a single Bt trait must be planted with a 20% block or strip refuge; whereas a field planted with a Bt corn variety that expresses a pyramid of two or more Bt traits can be planted with a 5% block, strip or blended refuge (USEPA 2010). All refuges for Bt corn varieties grown in southern regions where cotton is also grown must be planted as a block refuge.

A potentially major contributing factor to Cry1F resistance in fall armyworm is their susceptibility to the toxin in relationship to the dose being expressed in the plant. Binning et al. (2014) found that susceptible fall armyworm that were not killed by Cry1F were still able to grow and gain weight. This may be due to Cry1F corn producing its toxin in insufficient concentrations to be considered "high-dose" (Hardke et al. 2011, Binning et al. 2014). In order for a toxin to be considered high dose it must be present in concentrations at least 25 times greater than that which is required to kill a susceptible insect, or results in at least 99.99% mortality (USEPA 1998). The purpose of a high-dose toxin is two-fold. The first function is to cause 100% mortality in heterozygote resistant insects, which forces the inheritance of resistance to become functionally recessive due to an increased fitness differential between heterozygote resistant insects that are killed by the toxin, and homozygote resistant insects that are not (Taylor and Georghiou 1979,

Tabashnik and Croft 1982, Roush and McKenzie 1987, Denholm and Rowland 1992). Another requirement of a high-dose Bt toxin is to cause nearly 100% mortality in susceptible homozygote insects (Tabashnik et al. 2013). This ensures that the only insects that will survive on the Bt plant variety will be those possessing the homozygous resistant genotype (Tabashnik et al. 2004, Carrière et al. 2010). Matings between surviving resistant insects and susceptible insects from the refuge will produce heterozygous progeny that will be subsequently killed by the Bt toxins (Gould 1998). A lack of high-dose in Cry1F corn will result in higher proportions of heterozygote survival, which limits the effectiveness of the refuge strategy to delay the evolution of resistance.

Certain behavioral mechanisms may also have bolstered the evolution of Bt resistance in populations of fall armyworm. In a short-term feeding experiment, Binning et al. (2014) demonstrated that the initial response of third-instar fall armyworm to Cry1F corn was avoidance. Larvae exposed to Cry1F corn leaves fed only for a short tasting duration, then rejected the leaf as a food source. In a blended refuge scenario, this could result in fall armyworm moving from Bt to non-Bt plants (Pan et al. 2011, Zukoff et al. 2012, IRAC 2013), preventing the ingestion of a lethal dose of Bt and increasing the rate of resistance evolution (Mallet and Porter 1992). However, an alternative interpretation of this phenomenon is the reduction of selection for resistance due to decreased mortality, and therefore decreased selective pressure (Binning et al. 2014). Additionally, fall armyworm females exhibit a preference towards oviposition on plants with little insect feeding damage (assumedly to ensure an adequate food supply for offspring) (Téllez-Rodriguez et al. 2014). Bt plants often suffer lower degrees of insect feeding due to the activity of their toxins, and there is evidence to suggest that the presence of Bt does not

appear to deter fall armyworm oviposition (Hardke et al. 2012). Simulations have determined that this damage-avoiding oviposition of fall armyworm on Bt plants results in increased selective pressures for Bt resistance and subsequently increases the rate of resistance evolution, as larvae are more consistently exposed to low-dose Bt toxins (Téllez-Rodriguez et al. 2014).

Host-Associated Insect Strains. The terms "host strain," "host race," and "biotype" all refer to divisions of a species that possess adaptive differentiation to different host plants (Drès and Mallet 2002). Host-associated insect strains are generally defined by four criteria: 1) association or fidelity to certain host species; 2) sympatric occurrence; 3) some degree of detectable genetic differentiation from one another; and 4) some degree of gene flow between strains associated with different hosts (Bush 1969, Jaenike 1981, Diehl and Bush 1984, Drès and Mallet 2002). These strains have been well documented in a variety of herbivorous insects (Drès and Mallet 2002) including: soapberry bug (*Jadera haematoloma*) [Herrich-Schäffer]) on soapberry (*Sapindus saponaria* L.), *Serjania brachycarpa* Gray, and balloon vine (*Cardiospermum spp.* L.) versus golden rain trees (*Koelreuteria spp*. Laxm) (Carroll et al. 1997); pea aphid (*Acyrthosiphon pisum* [Harris]) on alfalfa (*Medicago sativa* L.) versus red clover (*Trifolium pretense* L.) (Via 1999, Caillaud and Via 2000); willow leaf beetle (*Lochmaea capreae* L.) on birch (*Betula pubescens* Ehrh) versus willow (*Salix caprea* L.) (Mikheev and Kreslavsky 1980); larch budmoth (*Zeiraphera diniana* [Hübner]) on European larch (*Larix decidua* Mill) versus cembran pine (*Pinus cembra* L.) (Baltensweiler et al. 1977, Emelianov et al. 1995); and apple maggot fly (*Rhagoletis pomonella* [Walsh]) on apple (*Malus pumila* Mill) versus hawthorn (*Crataegus spp.* L.) (Bush 1969, Feder et al. 1994).

There is some evidence of insect host strains exhibiting differing tolerances to insecticides. Carletto et al. (2009) examined the response of four host races (eggplant (*Solanum melongena* L.), pepper (*Capsicum annum* [Dierbach]), cotton (*Gossypium spp.* L., and Cucurbitaceae) of the cotton-melon aphid (*Aphis gossypii* Glover) to seven insecticides using leaf-disk bioassays coupled with mutation detection and enzyme assays. The eggplant, pepper, and one of the two cotton races tested carried a mutation that conferred a moderate resistance to the organophosphates profenofos and monocrotophos. The same eggplant and cotton races also were strongly resistant to the pyrethroid cypermehtrin. Studies on the whitefly, *Bemisia tabaci* (Gennadius), have indicated that the species' "B" biotype may exhibit a greater tolerance to pyrethroid insecticides than other biotypes (Erdogan et al. 2008), and the "Q" biotype are more tolerant than the B biotype to neonicotinoids and the juvenile hormone analog, pyriproxyfen (Horowitz et al. 2005). There is also concern for differing Bt tolerances between the "E" and "Z" pheromone-races of the European corn borer (*Ostrinia nubilalis* [Hübner]) due to their divergent host-use patterns; E-race moths are more often associated with non-corn hosts than the Z-race (Sorenson et al. 2005). Increased exposure of the Z-race to corn could subsequently be associated with increased Bt selection, resulting in an increased rate of resistance evolution (Bontemps et al. 2003, O-Rourke et al. 2010).

Host Strains of Fall Armyworm. Fall armyworm consist of two host strains that are referred to as the "corn" and "rice" strains due to the host plants they were originally collected from (Pashley et al. 1985, Pashley 1986). Although there is evidence for differentiation via wing morphometrics (Cañas-Hoyos et al. 2014), the two stains are

nearly identical as larvae and adults and therefore; primarily distinguished through the use of genetic markers (Cano-Calle et al. 2015, Pashley 1986, McMichael and Prowell 1999, Prowell et al. 2004, Nagoshi et al. 2006a, Nagoshi et al. 2007). In general, corn strain larvae exhibit a preference towards feeding on larger grasses, such as corn or sorghum, whereas the rice strain prefer pasture and turf grasses such as Bermuda grass or some non-grasses such as alfalfa (*Medicago sativa* L.) (Pashley 1986, Pashley et al. 1987, Groot et al. 2010, Murúa et al. 2015). The host plant fidelities of the two host races do not appear to be absolute. For example, the rice strain will occasionally be collected feeding on corn (Prowell et al. 2004, Groot et al. 2010), and corn strain larvae have been documented feeding on late stage cotton (Adamczyk et al. 1997, Luttrell and Mink 1999). The corn strain generally occurs in the Western hemisphere, in corn-rich areas; there is less known about the distribution of the rice strain due to little sampling on non-corn hosts (Pashley et al. 1985, Pashley 1986, Pashley 1988). However, the rice strain tends to occur sympatrically with the corn strain (Pashley 1988).

Pheromone composition differs somewhat between corn and rice strain fall armyworm. Significant differences in dodecenyl acetate, (Z)-7-dodecenyl acetate, (Z)-9-dodecenyl acetate, and (Z)-11-hexadecenyl acetate concentrations have been detected between corn and rice strain females (Groot et al. 2008, Unbenhend et al. 2013). In field trials conducted in a corn field more males of both strains were attracted to a synthetic corn-strain pheromone than to that of the rice strain. However, in a rice field, both strains were equally attracted to each synthetic pheromone (Unbenhend et al. 2013). In general, corn strain males respond more readily to commercially available pheromone lures than rice strain males (Meagher et al. 2013). Because males of each strain readily respond to

the pheromones of the opposite strain, female behavior may be of greater significance in the reproductive isolation of the two strains (Pashley et al. 1992).

There is also evidence for mate selection preferences in host strains of fall armyworm. Pashley and Martin (1987) observed in a laboratory study that no spermatophores were transferred in matings between corn strain females and rice strain males. In contrast, rice strain females successfully mated with corn strain males, but fitness deficits were present in F1 progeny. Hybrid females would only mate with their brothers, but with reduced fertility. Hybrid males were capable of fertilizing females of any strain, but again exhibited reduced fertility. These results should be approached with caution as subsequent studies and experiments on mate selection have not been able to identify similar patterns of mate selection (Whitford et al. 1988, Quisenberry 1991, Nagoshi and Meagher 2004).

Temporal isolation may also play a key role in breeding isolation; however, results are ambiguous (Groot et al. 2010). Pashley et al. (1992) found that corn strain females begin calling for males significantly earlier during scotophase than rice strain females, with no overlap of mating times. Schölf et al. (2009) conducted a follow up study and obtained similar results, but with overlap between the mating times of the two strains. A later study by Vélasquez-Vélez et al. (2011) was not able to identify any significant differences between mating times.

Lastly, there is some evidence to suggest that female corn and rice strain fall armyworm exhibit significant oviposition preferences. Whitford et al. (1988) reported that corn strain females showed a strong preference towards oviposition on corn or

sorghum; whereas, rice strain females preferred to oviposit on bermudagrass. Contrastingly, an earlier study by Pitre et al. (1983) obtained results suggesting that female fall armyworm collected from corn plants (suspected to be corn strain [Meagher et al. 2011]) will oviposit readily on a variety of host plants; including corn, sorghum, wheat, Bermuda grass, and rye. Meagher et al. (2011) later reported that populations of rice strain fall armyworm exhibited a strong fidelity towards oviposition on grasses, whereas females from corn strain populations oviposited indiscriminately on host plants as well as on artificial structures.

A complicating matter for host strain identity is the existence of rice-corn hybrids (Nagoshi and Meagher 2003, Prowell et al. 2004, Saldamando and Vélez-Arango 2010). Though there is ample laboratory evidence for host strain reproductive isolation (Groot et al. 2010), and it has been predicted that inter-strain matings would occur very rarely in the field (Nagoshi et al. 2006ab), Saldamando and Vélez-Arango (2010) found that 24% of their field-collected fall armyworm were, in fact, hybrids of the two host strains. The majority of hybrid fall armyworm were collected from corn fields (Prowell et al. 2004), where 32% of collected larvae fit the genotype (Saldamando and Vélez-Arango 2010). This finding supports previous data, as the rice strain is known to utilize corn as a host, but the corn strain does not typically utilize hosts normally associated with the rice strain. Increased inter-strain mating opportunities associated with corn fields would of course result in increased hybridization (Prowell et al. 2004). The existence of hybrids is currently somewhat of a suprise, as there is ample evidence for reproductive isolation mechanisms between corn and rice strain fall armyworm (Nagoshi and Meagher 2003, Groot et al. 2010); yet there must be at least some gene flow between the two host strains

for hybridization to occur (Prowell et al. 2004), which is congruent with the general criteria for host-associated strains (Bush 1969, Jaenike 1981, Diehl and Bush 1984, Drès and Mallet 2002).

The two host strains of fall armyworm may differ in their tolerances to select insecticides and Bt traits, though results are mixed. There is evidence that corn and rice strain fall armyworm differ in their tolerances to several chemicals in the pyrethroid, organophosphate, and carbamate families (Adamczyk et al. 1997, Ríos-Díez and Saldamando-Benjumea 2011). Of particular association with this dissertation is evidence that the rice strain may be less tolerant to the Cry1Ab and Cry1Ac Bt toxins than the corn strain (Adamczyk et al. 1997, Ríos-Díez et al. 2012). An additional study found that two populations of rice strain fall armyworm could survive significantly longer periods of exposure to artificial diet treated with Cry1F and Cry1Ab than all other corn and rice strain populations tested, though a third rice strain population survived for significantly shorter periods (Virla et al. 2008). Both host-strains appear to be equally susceptible to a pyramided cotton variety that produces Cry1Ac and Cry1F (Siebert et al. 2008b). There are currently no studies that have examined the Bt tolerance of rice-corn hybrid fall armyworm to the individual toxins.

Justification of Research. The USEPA requires resistance monitoring programs as part of its insect resistance management (IRM) plans for Bt corn and cotton products. These programs must include bioassay evaluations of resistance, and the encouragement of growers to report cases of unexpected insect damage to Bt crops (USEPA 2001b). The purpose of monitoring programs is the early identification of resistance in order to quickly implement remedial actions to delay its further development (Devos et al. 2013).

Effective programs should also include data on a pest species' baseline susceptibility to Bt toxins to allow for monitoring of changes in the pest species' tolerance to Bt (Devos et al. 2013). Data from resistance monitoring programs provides evidence for the effectiveness of the IRM programs they support, as well as evidence for or against the model parameters used in designing associated IRM plans (Glaser and Matten 2003).

Even though the existence of host strains of fall armyworm has been known for 30 years, there is a dearth of data comparing their biology, and there are many contradictory results in the data that is available. This is especially prevalent in the host strains' tolerance to Bt toxins, where there have been only four studies to make such comparisons to date (Adamczyk et al. 1997, Siebert et al. 2008b, Virla et al. 2008, Ríos-Díez et al. 2012). Several Cry proteins have been evaluated with the notable absence of Cry1F, the most commonly employed Bt toxin used in fall armyworm management (Storer et al. 2010). There have been three studies for oviposition preferences (Pitre et al. 1983, Whitford et al. 1988, Meagher et al. 2011). Considering that the fall armyworm has become an increasingly severe agricultural pest resulting in significant yield loss in many systems, this lack of characterization is a large oversight and directly contradicts the purpose of resistance monitoring programs. Data on differential Bt susceptibility between the host strains of fall armyworm, when combined with already established methods of strain identification (Nagoshi et al. 2006a, Nagoshi et al. 2007, Cano-Calle et al. 2015), could aid in the development of regional pest and resistance management strategies in locations where the host-strains of fall armyworm are known. This could allow for a more targeted approach toward fall armyworm IRM.

REFERENCES

Adamczyk Jr., J. J., J. W. Holloway, B. R. Leonard, and J. B. Graves. 1997. Susceptibility of fall armyworm collected from different plant hosts to selected insecticides and transgenic Bt cotton. J. Cotton Sci. 1: 21-28.

Baltensweiler, W., G. Benz, P. Bovey, and V. Delucchi. 1977. Dynamics of larch bud moth populations. Ann. Rev. Entomol. 22: 7-100.

Barros, E. M., J. B. Torres, and A. F. Bueno. 2010. Oviposition, development, and reproduction of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) fed on different hosts of economic importance. Neotrop. Entomol. 39: 996-1001.

Binning, R. R., J. Coats, X. Kong, and R. L. Helmich. 2014. Susceptibility and aversion of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Cry1F Bt maize and considerations for insect resistance management. J. Econ. Entomol. 107: 368-374.

Bontemps, A., D. Bourguet, L. Pélozuelo, M.-T. Bethenod, and S. Ponsard. 2003. Managing the evolution of *Bacillus thuringiensis* resistance in natural populations of the European corn borer, *Ostrinia nubilalis*: host plant, host race, and pherotype of adult males at aggregation sites. Proc. R. Soc. Lond. B. 271: 2179-2185.

Bush, G. L. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). Evolution 23: 237–251.

Caillaud, M. C. and S. Via. 2000. Specialized feeding behavior influences both ecological specialization and assortative mating in sympatric host races of pea aphids. Am. Nat. 156: 606–621.

Cañas-Hoyos, N., E. J. Márquez, and C. I. Saldamando-Benjumea. 2014. Differentiation of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) corn and rice strains from central Colombia: a wing morphometric approach. Ann. Entomol. Soc. Am. 107: 575-581

Cano-Calle, D., R. E. Arango-Isaza, and C. I. Saldamando-Benjumea. 2015. Molecular identification of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) corn and rice strains in Colombia by using a PCR-RDLP of the mitochondrial gene cytochrome oxidase I (COI) and a PCR of the gene FR (for rice). Ann. Entomol. Soc. Am.: 1-9.

Carrière, Y., and B. E. Tabashnik. 2001. Reversing insect adaptation to transgenic insecticidal plants. Proc. R. Soc. Lond. B. 268: 1475-1480.

Carrière, Y., D. W. Crowder, and B. E. Tabashnik. 2010. Evolutionary ecology of insect adaptation to Bt crops. Evol. Appl. 3: 561-573.

Carletto, J., T. Martin, F. Vanlerberghe-Masutti, and T. Brévault. 2009. Insecticide resistance traits differ among and within host races in *Aphis gossypii*. Pest Manag. Sci. 66: 301-307.

Carroll, S. P.,H. Dingle. and S. P. Klassen. 1997. Genetic differentiation of fitness-associated traits among rapidly evolving populations of the soapberry bug. Evolution 51: 1182–1188.

Ching'oma, G. P. and H. N. Pitre. 1999. Ovipsotion by fall armyworm, *Spodoptera frugiperda* (J. E. Smith): effects of moth age and sorghum maturation stage. Ceiba 40: 57-62.

Cruz, L. and F. T. Turpin. 1983. Yield impact of larval infestations of the fall armyworm (Lepidoptera: Noctuidae) to midwhorl growth stages of corn. J. Econ. Entomol. 76: 1052-1054.

Da Silva, C. S. B. and J. R. P. Para. 2013. New method for rearing *Spodoptera frugiperda* in laboratory shows that larval cannibalism is not obligatory. Rev. Bras. Entomol. 57: 347-349.

Denholm, I. and M. W. Rowland. 1992. Tactics for managing pesticide resistance in arthropods: theory and practice. Ann. Rev. Entomol. 37: 91-112.

Devos, Y., L. N. Meihls, J. Kiss, and B. E. Hibbard. 2013. Resistance evolution of the first generation of genetically modified *Diabrotica*-active Bt maize events by western corn rootworm: management and monitoring considerations. Transgenic Res. 22: 269-299.

Diehl, S. R., and G. L. Bush. 1984. An evolutionary and applied perspective of insect biotypes. Ann. Rev. Entomol. 29: 471-504.

Downes, S. & R.Mahon. 2012. Evolution, ecology and management of resistance in *Helicoverpa* spp. to Bt cotton in Australia. J. Invertebr. Pathol. 110, 281–286.

Drès, M., and J. Mallet. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. Phil. Trans. R. Soc. Lond. B. 357: 471-492.

Emelianov, I., Mallet, J. and Baltensweiler, W. 1995. Genetic differentiation in the larch budmoth *Zeiraphera diniana* (Lepidoptera: Tortricidae): polymorphism, host races or sibling species? Heredity 75: 416–424.

Erdogan, C., G. D. Moores, M. O. Gurkan, K. J. Gorman, and I. Denholm. 2008. Insecticide resistance and biotype status of populations of the tobacco whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Turkey. Crop. Prot. 27: 600-605.

Estruch, J. T., G. W. Warren, M. A. Mullins, G. T. Nye, J. A. Craig, and M. G. Koziel. 1996. Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against Lepidopteran insects. Proc. Nat. Acad. Sci. USA 93: 5389-5394.

Feder, J. L., S. B. Opp, B. Wlazlo, K. Reynolds, W. Go. and S. Spisak. 1994. Host fidelity is an effective premating barrier between sympatric races of the apple maggot fly. Proc. Natl Acad. Sci. USA 91: 7990–7994.

Gassmann, A. J., Y. Carrière, and B. E. Tabashnik. 2009. Fitness costs of insect resistance to *Bacillus thuringiensis*. Annu. Rev. Entomol. 54: 147-163.

Georgen, G., P. L. Kumar, S. B. Sankungh, A. Togola, and M. Tamò. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. PLoS One 11(10).

Glaser, J.A. and S. R. Matten. 2003. Sustainability of insect resistance management strategies for transgenic Bt corn. Biotechnol. Adv. 22: 45-69.

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu. Rev. Entomol. 43: 701-726.

Groot, A. T., M. Marr, G. Schöfl, S. Lorenz, A. Svatos, and D. G. Heckel. 2008. Host strain specific sex pheromone variation in *Spodoptera frugiperda*. Front. Zool. 5: 20.

Groot, A. T., M. Marr, D. G. Heckel, and G. Schöfl. 2010. The roles and interactions of reproductive isolation mechanisms in fall armyworm (Lepidoptera: Noctuidae) host strains. Ecol. Entomol. 55: 105-118.

Hardke, J. T., B. R. Leonard, F. Huang, and R. E. Jackson. 2011. Damage and survivorship of fall armyworm (Lepidoptera: Noctuidae) on transgenic field corn expressing *Bacillus thuringiensis* Cry proteins. Crop Prot. 30: 168-172.

Hardke, J. T., B. R. Leonard, and J. H. Temple. 2012. Fall armyworm oviposition on cotton plants expressing WideStrike™, Bollgard®, and Bollgard II® Cry proteins. Southwest. Entomol. 37: 295-303.

Heckel, D. G. L. J. Gahan, S. W. Baxter, J.-Z. Zhao, A. M. Shelton, F. Gould, and B. E. Tabashnik. 2007. The diversity of Bt resistance genes in species of Lepidoptera. J. Invertebr. Pathol. 95: 192-197.

Hogg, D. B. H. N. Pitre, and R. A. Anderson. 1982. Assessment of early-season phenology of the fall armyworm (Lepidoptera: Noctuidae) in Mississippi. Environ. Entomol. 11: 705-710.

Horowitz, A. R., S. Kontsedalov, V. Khasdan, and I. Ishaaya. 2005. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. Arch. Insect Biochem. Physiol. 58: 216-225.

(IRAC) Insecticide Resistance Action Committee. 2013. IRAC position on seed blends for IRM. (https://croplife.org/wp-content/uploads/pdf_files/IRAC-Position-on-Seed-Blends-for-IRM.pdf) [Accessed 23 February 2017].

Jaenike, J. 1981. Criteria for ascertaining the existence of host races. Am. Nat. 117: 830–834.

Johnson, S. J. 1987. Migration and the life history strategy of the fall armyworm, *Spodoptera frugiperda* in the western hemisphere. Insect Sci. Its Appl. 8: 543-549.

Lee, M. K., F. S. Walters, H. Hart, N. Palekar, and J.-S. Chen. 2003. The mode of action of the *Bacillus thuringiensis* vegetative insecticidal protein Vip3A differs from that of Cry1Ab δ-endotoxin. Appl. Environ. Microbiol. 69: 4648-4657.

Loeb, M. J. P. A. W. Martin, R. S. Hakim, S. Goto, and M. Takeda. 2001. Regeneration of cultured midgut cells after exposure to sublethal doses of toxin from two strains of *Bacillus thuringiensis*. J. Insect Physiol. 47: 599-606.

Loseva, O., M. Ibrahim, M. Candas, C. N. Koller, L. S. Bauer, and L. A. Bulla Jr. 2002. Changes in protease activity and Cry3Aa toxin binding in the Colorado potato beetle: implications for insect resistance to *Bacillus thuringiensis* toxins. Insect Biochem. Molec. 32: 567-577.

Luginbill, P. 1928. The fall armyworm. USDA Tech. Bull. 34. 92 pp.

Luttrell, R. G., and J. S. Mink. 1999. Damage to cotton fruiting structures by the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Cotton Sci. 3: 35-44.

Ma, G., M. Sarjan, C. Preston, S. Asgari, and O. Schmidt. 2005. Mechanics of inducible resistance against *Bacillus thuringiensis* endotoxins in invertebrates. Insect Sci. 12: 319-330.

Mallet. J. 1989. The evolution of insecticide resistance: have the insects won? Trends Ecol. Evol. 4: 336-339.

Mallet, J. and P. Porter. 1992. Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? P. Roy. Soc. B-Biol. Sci. 250: 165-169.

McKenzie, J. A. and P. Batterham. 1994. The genetic, molecular, and phenotypic consequences of selection for insecticide resistance. Trends Ecol. Evol. 9: 166-169.

McMichael, M., and D. P. Prowell. 1999. Differences in amplified fragment-length polymorphisms in fall armyworm (Lepidoptera: Noctuidae) host strains. Ann. Entomol. Soc. Am. 92: 175-181.

Meagher, R. L., R. N. Nagoshi, and C. J. Stuhl. 2011. Oviposition choice of two fall armyworm (Lepidoptera: Noctuidae) host strains. J. Insect Behav. 24: 337-347.

Meagher, R. L., R. N. Nagoshi, R. S. Armstrong, J. Niogret, N. D. Epsky, and K. L. Flanders. 2013. Captures and host strains of fall armyworm (Lepidoptera: Noctuidae) males in traps baited with different commercial pheromone blends. Fla. Entomol. 96: 729-740.

Mikheev, A. V. and Kreslavsky, A. G. 1980. Interrelations of the willow and birch races of *Lochmaea capreae* L. (Coleoptera, Chrysomelidae) with food plants. Russ. J. Zool. 59: 705–714.

Mitchell, E. R., J. N. McNeil, J. K. Westbrook, J. F. Silvain, B. Lalanne-Cassou, R. B. Chalfant, S. D. Pair, V. H. Waddill, A. Sotomayor-Rios, and F. I. Proshold. 1991. Seasonal periodicity of fall armyworm (Lepidoptera: Noctuidae) in the Caribbean basin and northward to Canada. J. Entomol. Sci. 26: 39-50.

Mohan, K. S., and M. Oufattole. 2015. Genetically Engineered Crops in Developing Countries. Ch 5: A decade of Bt cotton technology in India – what has it delivered and how can it be sustained? Stadium Press LLC., Houston, TX, USA.

Murúa, M. G., R. N. Nagoshi, D. A. Dos Santos, M. M. Hay-Roe, R. K. Meagher, and J. C. Vilardi. 2015. Demonstration using field collections that Argentina fall armyworm populations exhibit strain-specific host plant preferences. J. Econ. Entomol. 108: 2305-2315.

Nagoshi, R. N., and R. Meagher. 2003. Fall armyworm FR sequences map to sex chromosomes and their distribution in the world indicate limitations in interstrain mating. Insect. Mol. Biol. 12: 453-456.

Nagoshi, R. N., and R. Meagher. 2004. Behavior and distribution of the two fall armyworm host strains in Florida. Fla. Entomol. 87: 440-449.

Nagoshi, R. N., and R. Meagher. 2008. Review of fall armyworm (Lepidoptera: Noctuidae) genetic complexity and migration. Fla. Entomol. 91: 546-554.

Nagoshi, R. N., R. L. Meagher, J. J. Adamczyk, S. K. Braman, R. L. Brandenburg, and G. Nuessly. 2006. New restriction fragment length polymorphisms in the cytrochrome oxidase I gene facilitate host strain identification of fall armyworm (Lepidoptera: Noctuidae) populations in the southeastern United States. J. Econ. Entomol. 99: 671-677.

Nagoshi, R. N., R. L. Meagher, G. Nuessly, and D. G. Hall. 2006b. Effects of fall armyworm (Lepidoptera: Noctuidae) interstrain mating in wild populations. Environ. Entomol. 35: 561-568.

Nagoshi, R. N., P. Silvie, and R. L. Meagher. 2007. Comparison of haplotype frequencies differentiate fall armyworm (Lepidoptera: Noctuidae) corn-strain populations from Florida and Brazil. J. Econ. Entomol. 100: 654-961.

Nagoshi, R. N., S. Fleisher, and R. L. Meagher. 2009. Texas is the overwintering source of fall armyworm in central Pennsylvania: implications for migration in the Northeastern United States. Environ. Entomol. 38: 1546-1554.

Nagoshi, R. N., R. Meagher, and M. Hay-Roe. 2012. Inferring the annual migration patterns of fall armyworm (Lepidoptera: Noctuidae) in the United States from mitochondrial haplotypes. Ecol. Evol. 2: 1458-1467.

Nagoshi, R. N., N. M. Rosas-García, R. L. Meagher, S. J. Fleisher, J. K. Westbrook, T. W. Sappington, M. Hay-Roe, J. M. G. Thomas, and G. M. Murúa. 2015. Haplotype profile comparisons between *Spodoptera frugiperda* (Lepidoptera: Noctuidae) populations form Mexico with those from Puerto Rico, South America, and the United States and their implications to migratory behavior. J. Econ. Entomol. 108: 135-144.

(NASS) National Agricultural Statistics Service. 2016. Acreage. (http://usda.mannlib. cornell.edu/usda/current/Acre/Acre-06-30-2016.pdf) [Accessed 23 February 2017].

Niu, Y., R. L. Meagher, F. Yang, and F. Huang. 2013. Susceptibility of field populations of the fall armyworm (Lepidoptera: Noctuidae) from Florida and Puerto Rico to purified Cry1F protein and corn leaf tissue containing single and pyramided Bt genes. Fla. Entomol. 69: 701-713.

Niu, Y., F. Yang, V. Dangal, and F. Huang. 2014. Larval survival and plant injury of Cry1F-susceptible, -resistant, and –heterozygous fall armyworm (Lepidoptera: Noctuidae) on non-Bt and Bt corn containing single or pyramided genes. Crop Prot. 59: 22-28.

O'Rourke, M. E., T. W. Sappington, and S. J. Fleischer. 2010. Managing resistance to Bt crops in a genetically variable insect herbivore, *Ostrinia nubilalis*. Ecol. Appl. 20: 1228-1236.

Pair, S. D., J. R. Raulston, A. N. Sparks, J. K. Westbrook, and G. K. Douce. 1986. Fall armyworm distribution and population dynamics in the southeastern States. Fla. Entomol. 69: 468-487.

Pan, S. W., D. W. Onstad, T. M. Nowatzki, B. H. Stanley, L. J. Meinke, and J. L. Flexner. 2011. Western corn rootowrm (Coleoptera: Chrysomelidae) dispersal and adaptation to single-toxin transgenic corn deployed with block or blended refuge. Environ. Entomol. 40: 964-978.

Pashley, D. 1986. Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): a sibling species complex? Ann. Entomol. Soc. Am. 79: 898-904.

Pashley, D. 1988. Current status of fall armyworm host strains. Fla. Entomol. 71: 227-234.

Pashley, D., and J. A. Martin. 1987. Reproductive incompatibility between host strains of the fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 80: 731-733.

Pashley, D., S. J. Johnson, and A. N. Sparks. 1985. Genetic population structure of migratory moths: the fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 78: 756-762.

Pashley, D., T. C. Sparks, S. W. Quisenberry, T. Jamjanya, and P. F. Dowd. 1987. Two fall armyworm strains feed on corn, rice and Bermuda grass. Louisiana Agric. 30: 8-9.

Pashley, D., A. M. Hammond, and T. N. Hardy. 1992. Reproductive isolating mechanisms in fall army worm host strains (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 84: 400-405.

Pitre, H. N., J. E. Mulrooney, and D. B. Hogg. 1983. Fall armyworm (Lepidoptera: Noctuidae) oviposition: crop preferences and egg distribution on plants. J. Econ. Entomol. 76: 463-466.

Prowell, D. P. M. McMichael, and J. –F. Silvain. 2004. Multilocus genetic analysis of host use, introgression, and speciation in host strains of fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 97: 1034-1044.

Quisenberry, S. S. 1991. Fall armyworm (Lepidoptera: Noctuidae) host strain reproductive compatibility. Fla. Entomol. 74: 194-199.

Rang, C., P. Gil, N. Neisner, J. V. Rie, and R. Frutos. 2005. Novel Vip3-related protein from *Bacillus thuringiensis*. Appl. Environ. Microbiol. 71: 6276-6281.

Ríos-Díez, J. D. and C. I. Saldamando-Benjumea. 2011. Susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains from central Colombia to two insecticides, methomyl and lamda-cyhalothrin: a study of the genetic basis of resistance. J. Econ. Entomol. 104: 1698-1705.

Ríos-Díez, J. D., B. Siegfried, and C. I. Saldamando-Benjumea. 2012. Susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains from central Colombia to Cry1Ab and Cry1Ac entotoxins of *Bacillus thuringiensis*. Southwest. Entomol. 37: 281-293.

Roush, R. T., and J. A. McKenzie. 1987. Ecological genetics of insecticide and acaricide resistance. Annu. Rev. Entomol. 32: 361-380.

Saldamando, C. I., and A. M. Vélez-Arango. 2010. Host plant association and genetic differentiation of corn and rice strains of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) in Colombia. Neotrop. Entomol. 39: 921-929.

Schnepf, E., N. Crickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson, D. R. Zeigler, and D. H. Dean. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. 62: 775-806.

Schöfl, G., D. C. Heckel, and A. T. Groot. 2009. Time-shifted reproductive behaviors among fall armyworm (Noctuidae: *Spodoptera frugiperda*) host strains: evidence for differing modes of inheritance. J. Evol. Biol. 22: 1447-1459.

Siebert, M. W., K. V. Tindall, B. R. Leonard, J. W. Van Duyn, and J. M. Babcock. 2008a. Evaluation of corn hybrids expressing Cry1F (Herculex® I Insect Protection) against fall armyworm (Lepidoptera: Noctuidae) in the southern United States. J. Entomol. Sci. 43: 41-51.

Siebert, M. W., J. M. Babcock, S. Nolting, A. C. Santos, J. J. Adamczyk Jr., P. A. Neese, J. E. King, J. N. Jenkins, J. McCarty, G. M. Lorenz, D. D. Fromme, and R. B. Lassiter. 2008b. Efficacy of Cry1F insecticidal protein in maize and cotton for control of fall armyworm (Lepidoptera: Noctuidae). Fla. Entomol. 91: 555-565.

Sorenson, C. E., G. G. Kennedy, C. Schal, and J. F. Walgenbach. 2005. Geographical variation in pheromone response of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae), in North Carolina: a 20-Y perspective. Environ. Entomol. 34: 1057-1062.

Sparks, A. N. 1979. A review of the biology of the fall armyworm. Fla. Entomol. 62: 82-87.

Sparks, A. N. 1986. Fall armyworm (Lepidoptera: Noctuidae) potential for area-wide management. Fla. Entomol. 69: 603-614.

Storer, N. P., J. M. Babcock, M. Schlenz, T. Meade, G. T. Thompson, J. W. Bing, and R. M. Huckaba. 2010. Discovery and characterization of field resistance to Bt maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico. J. Econ. Entomol. 103: 1031-1038.

Tabashnik, B. E., and B. A. Croft. 1982. Managing pesticide resistance in crop-arthropod complexes: interactions between biological and operational factors. Environ. Entomol. 6: 1137-1144.

Tabashnik, B. E. 1994. Evolution of resistance to *Bacillus thuringiensis*. Ann. Rev. Entomol. 39: 47-79.

Tabashnik, B. E., Y. B. Liu, T. Malvar, G. G. Heckel, and L Masson. 1998. Insect resistance to *Bacillus thuringiensis*: uniform or diverse. Phil. T. R. Soc. B. 353: 1751-1756.

Tabashnik, B. E., Y. Carrière, T. J. Dennehy, S. Morin, M. S. Sisterson, R. T. Roush, A. M. Shelton, and J. Z. Zhao. 2003. Insect resistance to transgenic Bt crops: lessons from the laboratory and field. J. Econ. Entomol. 96: 1031-1038.

Tabashnik, B. E., F. Gould, and Y. Carrière. 2004. Delaying evolution of insect resistance to transgenic crops by decreasing dominance and heritability. J. Evol. Biol. 17: 904-912.

Tabashnik, B. E., T. Brevault, and Y. Carrière. 2013. Insect resistance to Bt crops: lessons from the first billion acres. Nat. Biotechnol. 31: 510-521.

Taylor, C. E., and G. P. Georghiou. 1979. Suppression of insecticide resistance by alteration of gene dominance and migration. J. Econ. Entomol. 72: 105-109.

Téllez-Rodriguez, P., B. Raymond, I. Morán-Bertot, L. Rodríguez-Cabreara, D. J. Wright, C. G. Borroto, and C. Ayra-Pardo. 2014. Strong oviposition preference for Bt over non-Bt maize in *Spodoptera frugiperda* and its implications for the evolution of resistance. BMC Biol. 12:48.

Unbenhend, M., S. Hänniger, R. L. Meagher, D. G. Heckel, and A. T. Groot. 2013. Pheromonal divergence between two strains of *Spodoptera frugiperda*. J. Chem. Ecol. 39: 364-376.

(USEPA) United States Environmental Protection Agency. 1998. Final report of the FIFRA scientific advisory panel subpanel on *Bacillus thuringienses* (Bt) plant-pesticides and resistance management. (http://archive.EPA.gov/scipoly/sap/meetings/web/pdf/finalfeb.pdf) [Accessed 23 February 2017].

(USEPA) United States Environmental Protection Agency. 2001a. Biopesticides registration action document: Cry1Ab and Cry1F *Bacillus thuringiensis* (Bt) corn plant-incorporated protectants. (http://www3.EPA.gov/pesticides/chem_search/reg_actions/pip/cry1f-cry1ab-brad.pdf) [Accessed 23 February 2017].

(USEPA) United States Environmental Protection Agency. 2001b. Biopesticides registration action document: Bt plant plant-incorporated protectants: insect resistance management. (http://www3.EPA.gov/pesticides/chem_search/reg_actions/pip/bt_brad2/4-irm.pdf) [Accessed 23 February 2017].

(USEPA) United States Environmental Protection Agency. 2005. Biopesticides registration action document: *Bacillus thuringiensis* var. Aizawai Cry1F and the genetic material (from the insert of plasmid PGMA281) necessary for its production in cotton and *Bacillus thuringiensis* var. Kurstaki Cry1Ac and the genetic material (from insert of plasmid PMYC3006) necessary for its production in cotton. (http://www3.EPA.gov/pesticides/chem_search/reg_actions/registration/decision_PC-006512_1-Sep-05.pdf) [Accessed 23 February 2017].

(USEPA) United States Environmental Protection Agency. 2007. Review of Dow Agroscience's (and Pioneer HiBred's) submission (dated July 12, 2007) regarding fall armyworm resistance to the Cry1F protein expressed in TC1507 Herculex® I insect protection maize in Puerto Rico (EPA registrations 68467-2 and 29964-3). DP barcode: 342635. Decision: 381550. MRID#: 471760-01. (http://www3.EPA.gov/pesticides/chem_search/cleared_reviews/csr_PC-006481_24-Aug-07_a.pdf) [Accessed 23 February 2017).

(USEPA) United States Environmental Protection Agency. 2008a. Biopesticides regisration action document: *Bacillus thuringiensis* modified Cry1Ab (SUN-IR67B-1) and Vip3Aa19 (Syn-IR102-7) insecticidal proteins and the genetic material necessary for their production in COT02 X COT67B cotton. (http://www3.EPA.gov/pesticides/chem_search/reg_actions/registration/decision_PC-006529_12-Aug-08.pdf) [Accessed 23 February 2017].

(USEPA) United States Environmental Protection Agency 2008b. Biopesticides regisration action document: *Bacillus thuringiensis* Vip3Aa20 insecticidal protein and the genetic material necessary for its production (via elements of vector pNOV1300) in event MIR162 maize (OECD unique identifier: SYN-IR162-4). (http://www3.EPA.gov/pesticides/chem_search/reg_actions/registration/decision_PC-006599_3-Apr-09.pdf) [Accessed 23 February 2017].

(USEPA). United States Environmental Protection Agency. 2010. Biopesticides registration action document: Optimum AcreMax Bt corn seed blends. (http://www3.EPA.gov/pesticides/chem_search/reg_actions/pip/bt-seed-blends.pdf) [Accessed 23 February 2017].

Vélasquez-Vélez, M. I., C. I. Saldamando-Benjumea, and J. D. Ríos-Diez. 2011. Reproductive isolation between two populations of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) collected in corn and rice fields from central Colombia. Ann. Entomol. Soc. Am. 104: 826-833.

Vélez, A. M., T. A. Spencer, A. P. Alves, D. Moellenbeck, R. L. Meagher, H. Chirakkal, and B. D. Siegfried. 2013. Inheritance of Cry1F resistance, cross-resistance and frequency of resistant alleles in *Spodoptera frugiperda* (Lepidoptera: Noctuidae). B. Entomol. Res. 103: 700-713.

Vélez, A. M., T. A. Spencer, A. P. Alves, L. B. Crespo, and B. D. Siegfried. 2014. Fitness costs of Cry1F resistance in fall armyworm *Spodoptera frugiperda*. J. Appl. Entomol. 138: 315-325.

Vickery, R. A. 1929. Studies of the fall armyworm in the Gulf Coast district of Texas. USDA Tech. Bull. 134. 64 pp.

Via, S. 1999. Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice. Evolution 53: 1446–1457.

Virla, E. G., A. Álvares, F. Loto, L. M. Pera, and M. Baigorí. 2008. Fall armyworm strains (Lepidoptera: Noctuidae) in Argentina, their associate host plants and response to different mortality factors in laboratory. Fla. Entomol. 9: 63-69.

Wearing, C. H., P. J. Connor, and K. D. Ambler. 1973. Olfactory stimulation of oviposition and flight activity of codling moth *Laspeyresia pomonella* using apples in an automated olfactometer. New Zeal. J. Sci. 16: 697-710.

Whitford, F., S. S. Quisenberry, T. J. Riley, and J. W. Lee. 1988. Oviposition preference, mating compatibility, and development of two fall armyworm strains. Fla. Entomol. 71: 234-243.

Wiltshire, E. P. 1977. Middle East Lepidoptera, XXXVII: notes on the *Spodoptera litura* (F.) group (Noctuidae Trifinae). Proc. Brit. Ent. Nat. Hist. Soc. 9: 92-96.

Xie, W., S. Wang, Q. Wu, Y. Feng, H. Pan, X. Jiao, L. Zhou, X. Yang, W. Fu, H. Teng, B. Xu, and Y. Zhang. 2010. Induction effects of host plants on insecticide susceptibility and detoxification enzymes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). Pest Manag. Sci. 67: 87-93.

Young, J. R. 1979. Fall armyworm: control with insecticides. Fla. Entomol. 62: 130-133.

Zukoff, S. N., W. C. Bailey, M. R. Ellersieck, and B. E. Hibbard. 2012. Western corn rootworm larval movement in SmartStax seed blend scenarios. J. Econ. Entomol. 105: 1245-1260.

Chapter 2

BT SUSCEPTIBILITIES OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) HOST STRAINS

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Abstract

The fall armyworm, *Spodoptera frugiperda* (Smith), is a highly polyphagous, multivoltine pest of commercial crops including corn (*Zea mays* L.), cotton (*Gossypium Spp.* L.), rice (*Oryza sativa* L.), and pasture grasses. Fall armyworm have become a growing concern in agricultural communities across the Americas as field populations in many locales have evolved resistance to several Cry1 toxins derived from the bacterium *Bacillus thuringiensis* Berliner (Bt). An often overlooked aspect of fall armyworm biology is the existence of two host strains, the "rice" and "corn" strains. Thas been little research devoted to the characterization of fall armyworm host strains, though there is evidence that the rice and corn strains may differ in their tolerances to Bt toxins expressed by transgenic plants. In this study, diet-based bioassays were conducted to compare the susceptibilities of one rice strain, two corn strain, and one rice-corn hybrid

population to Cry1Ab, Cry1Ac, and Cry1F. Results indicate that the corn strain and hybrid populations are more tolerant to the Bt toxins, especially to Cry1F, than the rice strain population. Results from this study, when combined with existing techniques for host strain identification, may aid in the development of regional insect resistance management programs for fall armyworm.

Introduction

The fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), is a multivoltine pest of several agricultural, pasture, and turf crops including corn (*Zea mays* L.), cotton (*Gossypium Spp.* L.), sorghum (*Sorghum bicolor* L.), rice (*Oryza sativa* L.), and several grasses (Luginbill 1928, Young 1979). Larvae are highly polyphagous, and have been documented on over 80 host plant species spanning 23 families (Pashley 1988), though there is an apparent preference towards monocots, particularly grasses (Luginbill 1928, Johnson 1987, Pashley 1988).). Larval feeding damage is severe, especially to corn, where yield losses can range from 17 to 72% depending on the locality and conditions (Cruz and Turpin 1983, Barros et al. 2010, Murúa et al. 2015). Fall armyworm cannot diapause, and subsequently are not capable of surviving the winter in cold latitudes (Luginbill 1928, Sparks et al. 1979, Johnson 1987). Instead, a yearly migration occurs over the course of several generations; branching out from tropical overwintering grounds in South and Central America, Mexico, and the Southern United States (Sparks

et al. 1979, Pair et al. 1986, Johnson 1987, Mitchell et al. 1991, Nagoshi and Meagher 2008). Their course depends on weather patterns and the availability of host plants (Sparks et al. 1979, Pair et al. 1986, Johnson 1987, Mitchell et al. 1991).

Genetically modified crops producing insecticidal toxins derived from the bacterium *Bacillus thuringiensis* Berliner (Bt) became a useful tool for the management of fall armyworm in beginning with Cry1F corn (USEPA 2001). In 2005 Cry1F along with Cry1Ac were expressed in an additional FAW host plant, cotton (USEPA 2005). Unfortunately, the efficacy of these technologies is under threat due to field-evolved Cry1F resistance in populations of fall armyworm, first identified in Puerto Rico (Storer et al. 2010), and later in Brazil (Farias et al. 2014). Cry1F resistance in fall armyworm appears to follow the patterns of "Mode 1" resistance (Tabashnik et al. 1998), being characterized by generally high levels of resistance, recessive inheritance, few fitness costs, and low degrees of cross-resistance to other Bt toxins (Storer et al. 2010, Niu et al. 2013, Vélez et al. 2013, Jakka et al. 2014, Niu et al. 2014, Vélez et al. 2014). There is a plethora of factors that may have contributed to the evolution of Cry1F resistance in fall armyworm populations, including a lack of mandatory refuge requirements in Puerto Rico, a lack of "high-dose" (Hardke et al. 2011, Binning et al. 2014, Vélez et al. 2016), possible behavioral avoidance of Bt plants (Binning et al. 2014), and a possible preference towards oviposition on Bt plants (Téllez-Rodriguez et al. 2014).

An often overlooked aspect of fall armyworm biology is the existence of two host strains referred to as the "rice" and "corn" strains due to the host plants they were originally collected from (Pashley et al. 1985, Pashley 1986). Although there is evidence for differentiation of the strains via their wing morphometrics (Cañas-Hoyos et al. 2014),

they are primarily distinguished through the use of genetic markers (Cano-Calle et al. 2015, Pashley 1986, McMichael and Prowell 1999, Prowell et al. 2004, Nagoshi et al. 2006a, Nagoshi et al. 2007). In general, corn strain fall armyworm larvae exhibit a preference towards feeding on larger grasses, such as corn or sorghum, whereas the rice strain prefers pasture and turf grasses such as Bermuda grass or non-grasses such as alfalfa (Pashley 1986, Pashley et al. 1987a, Groot et al. 2010, Murúa et al. 2015), though host plant fidelities of the two strains do not appear to be absolute. For example, the rice strain will occasionally be collected feeding on corn and corn strain larvae have been documented feeding on late stage cotton (Adamczyk et al. 1997, Luttrell and Mink 1999).

Despite knowledge of their existence for over 30 years, there is little comparative data pertaining to corn and rice strain fall armyworm life history and biology. To further complicate the matter, data from the available published studies are often contradictory; so some results must be approached with caution. Generally, corn and rice strain fall armyworm appear to be reproductively isolated via host plant preferences (Groot et al. 2010), mate selection preferences (Pashley and Martin 1987), female calling times (Pashley et al. 1992, Schöfl et al. 2009), and oviposition preferences (Whitford et al. 1988, Meagher et al. 2011). There is, however, some evidence for field hybridization (Prowell et al. 2004, Saldamando and Vélez-Arango 2010).

In addition, there is evidence that corn and rice strain fall armyworm may differ in their tolerances to certain insecticides. Several studies have identified differential tolerances to several chemicals in the pyrethroid, organophosphate, and carbamate families (Pashley et al. 1987b, Adamczyk et al. 1997, Ríos-Díez and Saldamando-Benjumea 2011)., There is also a line of evidence suggesting that corn strain fall

armyworm may be more tolerant to Bt toxins than the rice strain (Adamczyk et al. 1997, Virla et al. 2008, Ríos-Díez et al. 2012).

Due to growing concerns regarding the spread of Cry1F resistance in field populations of fall armyworm, additional data on differential Bt tolerances of host strains may provide important baseline information for the development of more effective insect resistance management (IRM) programs (Glaser and Matten 2003). Here we measure the Bt tolerance of one rice strain, two corn strains, and one hybrid population of fall armyworm to three Bt toxins, Cry1Ab, Cry1Ac, and Cry1F, using diet-based bioassays. Data from this study will provide a better understanding of fall armyworm populations that may be at risk for the evolution of Bt resistance.

Materials and Methods

Study Insects. Colonies of corn and rice strain fall armyworm were reared from egg masses originally obtained on 21 April, 2015 from colonies maintained at USDA-ARS in Gainesville, Florida. Strains were identified using the protocols described in Nagoshi et al (2007). The rice strain population was collected from pasture grass in Duval County, FL in 2014. The corn strain population, hereby referred to as $Corn_{(1)}$, was collected from sweet corn in Hendry County, FL in 2013. A field of Bt corn that produced Cry1F was adjacent to the sweet corn field that the corn strain was collected from. Subsequently, a second corn strain population, hereby referred to as $Corn_{(2)}$ that was laboratory reared at Frontier Agricultural Sciences in Newark, DE that had no prior exposure to Bt, was also tested as a comparison to assess the Bt susceptibility of $Corn_{(1)}$.

Corn$_{(2)}$ had been previously identified as corn strain using the same methods applied to the rice strain population and Corn$_{(1)}$ by Vélez et al. (2013). A fourth population originally collected from the Mississippi delta region from soybean plants was provided by DuPont Pioneer (Johnston, IA). The population was selected in early 2016 with the intention of rearing a pure rice-strain colony; however, molecular typing determined that the population is a rice-corn hybrid, displaying markers for both host strains.

Colony Rearing. Fall armyworm rearing protocol was adapted from that of Perkins (1979) and Vélez et al. (2013). The protocol was used to maintain populations of the rice strain and Corn$_{(1)}$. Colonies of Corn$_{(2)}$ and the hybrid population were not maintained in the laboratory, instead, eggs were obtained from Frontier Agricultural Sciences and DuPont Pioneer as needed for bioassays. Fall armyworm egg masses were held in zip-top plastic bags until neonate larvae emerged. Neonates were then transferred into pre-prepared 56-cell trays (Frontier Agricultural Sciences, Newark, DE), that contained general purpose Lepidoptera diet (Frontier Agricultural Sciences, Newark, DE). Diet trays with larvae were then stored in a growth chamber (Model E-54U, Pervical Scientific, Perry, IA) set to 25° C and 16:8 L:D photoperiod. Large trays filled with water were placed at the bottom of the growth chamber in order to increase the relative humidity, which was maintained between 50% to 70%.

Larval fall armyworm remained in the diet trays until pupation, at which time pupae were removed and placed on top of damp cotton pads (Richmond Dental, Richmond, NC) in petri dishes (diameter = 100 mm) (Thermo Fisher Scientific Inc., Waltham, MA) that were then placed in 20.3 x 20.3 x 20.3 cm, 6 mm wire mesh hermit crab cages (Florida Marine Research, Sarasota, FL). Cotton was wetted daily. Cages were

fitted with 1 mm wire mesh covers to prevent adult escape. No more than 120 pupae were placed in a single cage, and cages were stored in a growth chamber set to 28°C and 16:8 L:D photoperiod, with water trays as before. Cages were monitored daily for adult emergence. After emergence was detected a 50 ml Erlenmeyer flask containing a liquid adult diet and fitted with a braided cotton wick (Richmond Dental, Richmond, NC) was placed in each cage. Adult diet was comprised of flat beer, ascorbic acid, propionic acid, aureomycin, and a vitamin mixture (Vanderzant vitamin mix, Frontier Agricultural Sciences, Newark, DE) following Perkins (1979) and Vélez et al. (2013). The adult population for each strain was maintained at approximately 200 insects. Waxed paper was provided for an oviposition substrate, and was placed in between the cage and outer mesh covering. Eggs were collected daily and incubated in the 28° C growth chamber.

   Diet-Based Bioassays. Diet-based bioassays were conducted based on the methods of Storer et al. (2010) to compare the susceptibilities of corn and rice strain fall armyworm to Cry1Ab, Cry1Ac, and Cry1F proteins. Frontier Agricultural Sciences general Lepidoptera diet was prepared and dispensed into 128-well bioassay trays (Frontier Agricultural Sciences, Newark, DE), 1 ml per well, and left to set. Truncated Cry1Ab and Cry1Ac protein solutions along with corresponding buffer solutions for overlay preparation were obtained from the Monsanto Company (Monsanto Company, St. Louis, MO). Lypholized, Cry1F protein was obtained from Dow AgroSciences (Dow AgroSciences, Indianapolis, IN). Cry1F solutions were prepared by adding lypholized protein to 10 mmol of 3-cyclohexylamino propanesulfonic acid (CAPS), pH 10.5, to generate a 1000 ng/ml stock solution that was subsequently used to prepare the highest overlay concentration. Cry1Ab and Cry1Ac solutions were prepaby adding protein in

solution to the corresponding buffer to prepare the highest overlay concentration. The buffer solution for Cry1Ab was comprised of 20 mmol of Tris(hydroxymethyl)aminomethane (Tris) and 1 mmol ethelyne-diamine-tetraacetic acid (EDTA), pH 8.6. The buffer solution for Cry1Ac was comprised of 50 mmol of CAPS, 1 mmol of EDTA, 2.5 mmol of dithiothreitol (DTT), and 1 mmol of benzamidine-hydrochloric acid, pH 10.25. Toxin overlays of 2000 ng/cm^2, 1500 ng/cm^2, 1100 ng/cm^2, 800 ng/cm^2, 500 ng/cm^2, 250 ng/cm^2, and 62.5 ng/cm^2 were prepared via serial dilutions for all Bt toxins tested. A no-toxin (0 ng/cm^2) control that consisted solely of buffer solution was also prepared. The 128-well trays consist of eight sections, each containing 16 wells. Toxin overlays were applied to 16-well tray sections using a Thermo Scientific Finnpipette F2 (Thermo Fisher Scientific Inc., Waltham, MA).

Newly hatched first instar fall armyworm larvae were placed, one per well, onto the toxin overlaid diet using a soft tipped paint brush, and the wells were covered with 16-well polyethylene tray seals (Bioserv, Flemington, NJ). Infested diet trays were maintained in a growth chamber (Model I-66 LL, Percival Scientific, Inc., Boone, IA) at constant 28°C, 50% RH, and16:8 L:D photoperiod for 7 d, after which they were scored for survival. Larvae that did not grow past the first instar were considered dead (Marçon et al. 1999). Surviving larvae were weighted with an electronic balance (Mettler Toledo AL54, Mettler Toledo LLC, Bristol, PA), and the average weight for surviving larvae on each toxin concentration was calculated. A replicate was deemed "successful" for statistical analysis if it resulted in at least 75% larval survival in the control group (at least 12 surviving larvae), and six successful replicates were required for each population by toxin treatment group for statistical analyses.

Negative Controls. Negative controls were conducted in order to assess each population's response to being reared on artificial diet. Trays were prepared with artificial diet in the same manner as in the diet-based bioassays, though no toxin or buffer solutions were overlaid atop the diet. Negative control trays were infested, sealed, and maintained in a growth chamber for 7 d in the same manner as in the diet-based bioassays. The survival of 256 individual larvae were measured in two 128 well bioassay trays. Larvae were transferred onto artificial diet and trays were sealed with polyethylene seals in the same manner as in the diet-based bioassays. After the 7 d testing period the trays were removed from the growth chamber and scored for survival and weighed again following the same methods as the diet-based bioassays.

Data Analyses. Abbot's correction was applied to larval mortality data to account for mortality in the 0 ng/cm^2 control groups (Abbot 1925). Data on corrected larval mortality from the diet-based bioassays were then used in probit analyses in SAS 9.4 (PROC PROBIT, SAS Institute Inc., Cary, NC) to calculate lethal concentrations of 50% mortality (LC$_{50}$) for corn and rice strain fall armyworm populations to each Bt toxin. Goodness of fit tests based on a χ^2 distribution were also conducted in PROC PROBIT to assess the linearity of log$_{10}$ transformed data. In cases where the goodness of fit tests yielded P > 0.05 the variance was multiplied by a heterogeneity factor (χ^2/df) the subsequent 95% fiducial limits were determined and the data in question were reanalyzed using the modified variance assuming a t distribution (t = 2.02) as opposed to a z distribution (z = 1.96) to compensate for divergence from the probit model (Lacey 1997).

The mean weights of surviving larvae in the diet-based bioassays on each toxin concentration were transformed into growth inhibition values via the following equation:

[inhibition = (weight$_{control}$ − weight$_{test}$) / weight$_{control}$], where weight$_{control}$ is the mean weight of surviving larvae in the 0 ng/cm^2 group, and weight$_{test}$ is the mean weight of surviving larvae tested at the desired toxin overlay concentration. Logistic regression analyses based on a four-parameter logistic model (Hill 1910) using a Gauss-Newton refinement criterion were conducted using JMP PRO 13 (SAS Institute Inc., Cary, NC) to determine effective concentrations of 50% growth inhibition (EC$_{50}$). LC$_{50}$ or EC$_{50}$ values of corn and rice strain fall armyworm were considered significantly different if their 95% fiducial limits (LC$_{50}$) or 95% confidence intervals (EC$_{50}$) did not overlap (Savin et al. 1977).

Proportion survival and mean weight of surviving larvae data relative to the negative controls were each analyzed using separate one-way ANOVA in SAS 9.4 (PROC GLM). Pairwise comparisons were made based on least square means (LSMEANS statement with PDIFF option in PROC GLM) with a Bonferroni correction based on six pairwise comparisons applied to the level of significance (P < 0.0083) (Sokal and Rohlf 2012).

Results

Cry1Ab. The mortality values of Corn$_{(1)}$ and Corn$_{(2)}$ did not reach 50% at the concentrations of Cry1Ab tested (Fig. 1a), which made it impossible to accurately calculate LC$_{50}$ values for the two populations (Table 1). The LC$_{50}$ values for the rice strain populaiton (1354.0 ng/cm^2; 95% FL: 785.8 ng/cm^2 to 2555.0 ng/cm^2) and the hybrid population (1912.0 ng/cm^2; 95% FL: 1235.0 ng/cm^2 to 6691.0 ng/cm^2) were not significantly different due to variable mortality data resulting in wide 95% fiducial limits

(Table 1). The EC_{50} values for the rice strain (108.6 ng/cm^2; 95% CL: 25.7 ng/cm^2 to

219.2 ng/cm^2), Corn$_{(1)}$ (190.9 ng/cm^2; 95% CL 155.8 ng/cm^2 to 242.3 ng/cm^2), and

Corn$_{(2)}$ (128.3 ng/cm^2; 95% CL: 50.8 ng/cm^2 to 205.8 ng/cm^2) were not significantly

different from one another (Table 1). Hybrid population larvae reached 50% growth

inhibition short of the lowest concentration of Cry1Ab in the logistic model, thus it was

not possible to accurately calculate an EC_{50} value (Table 1).

Cry1Ac. The mortality of Corn$_{(2)}$ did not reach 50% at the concentrations of

Cry1Ac tested (Fig. 1b), and thus it was impossible to accurately calculate an LC_{50} value

(Table 1). The LC_{50} value for the hybrid population (1471.0 ng/cm^2; 95% FL: 1278.0

ng/cm^2 to 1632.0 ng/cm^2) was significantly greater than both Corn$_{(1)}$ (927.4 ng/cm^2; 95%

FL: 552.3 ng/cm^2 to 1207.0 ng/cm^2) and the rice strain population (472.6 ng/cm^2; 95%

FL: 21.0 ng/cm^2 to 861.3 ng/cm^2), which were not significantly different from one

another (Table 1). The EC_{50} value for Corn$_{(2)}$ was 123.1 ng/cm^2 (95% CL: 38.3 ng/cm^2 to

207.9ng/cm^2) (Table 1). It was not possible to accurately calculate EC_{50} values for the

rice strain population, Corn$_{(1)}$, and the hybrid population due to their reaching 50%

growth inhibition prior to the lowest concentration of Cry1Ac in the logistic model

(Table 1).

Cry1F. The LC_{50} values for Corn$_{(1)}$ (565.0 ng/cm^2; 95% FL: 409.6 ng/cm^2 to

696.6 ng/cm^2), Corn$_{(2)}$ (544.7 ng/cm^2; 95% FL: 409.9 ng/cm^2 to 654.4 ng/cm^2), and the

hybrid population (547.9 ng/cm^2; 95% FL: 321.4 ng/cm^2 to 753.8 ng/cm^2) on Cry1F were

not significantly different from one another, and all were significantly greater than that of

the rice strain population (211.3 ng/cm^2; 95% FL: 158.7 ng/cm^2 to 264.4 ng/cm^2) (Table

1). It was not possible to accurately calculate EC_{50} values for any of the four populations examined on Cry1F (Table 1).

Negative Controls. On artificial diet with no toxin or buffer overlay the hybrid population had significantly decreased proportions of survival compared to each of the three remaining populations, whose survival proportions were not significantly different from one another (df = 3,63, F = 24.10, P < 0.0001) (Fig. 2). The mean weights of surviving larvae for each population were significantly different from each other (df = 3,63, F = 754.74, P < 0.0001); with surviving $Corn_{(2)}$ larvae weighing the most, followed by $Corn_{(1)}$, the rice strain population, and the hybrid population weighing the least (Fig. 2).

Discussion

In this study we measured the tolerance of one rice strain fall armyworm population, two corn strain populations ($Corn_{(1)}$ and $Corn_{(2)}$), and one rice-corn hybrid population to the Bt toxins, Cry1Ab, Cry1Ac, and Cry1F using diet-based bioassays. This is the first study to compare the Bt tolerance of a hybrid fall armyworm population to that of pure corn and rice strain populations. The existence of rice-corn hybrid fall armyworm is somewhat of a suprise, as there is a fair degree of evidence for reproductive isolation between the two established host strains (Nagoshi and Meagher 2003, Groot et al. 2010), though results in the available literature are often mixed or contradictory (Groot et al. 2010). In general corn strain larvae exhibit feeding preferences towards larger grasses such as corn and sorghum, whereas the rice strain prefers pasture and turf grasses such as Bermuda grass or non-grasses such as alfalfa (Pashley 1986, Pashley et al. 1987, Groot et

al. 2010, Murúa et al. 2015). However, these fidelities are not absolute. The rice strain has been documented feeding on corn (Prowell et al. 2004, Groot et al. 2010), and the corn strain has been documented feeding on cotton (Adamczyk et al. 1997, Luttrell and Mink 1999). There is some evidence for mate selection preferences where corn strain females will not mate with rice strain males, but rice strain females will mate with corn strain males (Pashley and Martin 1987), though subsequent studies have not been able to identify a similar pattern (Whitford et al. 1988, Quisenberry 1991, Nagoshi and Meagher 2004). Several studies have determined that female, corn strain fall armyworm call for males significantly earlier than rice strain females (Pashley et al. 1992, Schölf et al. 2009), resulting in temporal isolation between the host strains. However, a later study was able to identify significant differences between mating times (Vélasquez-Vélez et al. 2011). Lastly, there is mixed evidence pertaining to the oviposition preferences of corn and rice strain fall armyworm, where general trends are in support of corn strain oviposition being largely indiscriminate, and rice strain oviposition strongly tied to grasses (Pitre et al. 1983, Whitford et al. 1988, Meagher et al. 2011).

Despite laboratory evidence for host strain reproductive isolation (Groot et al. 2010), and predictions of interstrain matings occurring rarely in a field setting (Nagoshi et al. 2006b), Saldamando and Vélez-Arango (2010) have found that 24% of their field-collected fall armyworm were hybrids. The majority of hybrid fall armyworm are collected from corn fields (Prowell et al. 2004), where 32% of collected larvae fit the genotype (Saldamando and Vélez-Arango 2010). This makes intuitive sense, as the rice strain is known to utilize corn as a host, but the corn strain does not typically utilize hosts normally associated with the rice strain. Increased interstrain mating opportunities

associated with corn fields would of course result in increased hybridization (Prowell et al. 2004). This evidence suggests that there must be at least some degree of gene flow between the two host strains, which is congruent with the general criteria for host-associated strains (Bush 1969, Jaenike 1981, Diehl and Bush 1984, Drès and Mallet 2002). More research on the distribution and comparative biology of rice-corn hybrid fall armyworm is required to better predict and understand rates of hybridization (Nagoshi et al. 2006b).

Based on evidence from $Corn_{(1)}$'s apparent lower tolerance to Cry1Ab and Cry1Ac when compared with $Corn_{(2)}$, and near-identical response to Cry1F (Table 1, Fig. 1), it does not appear that $Corn_{(1)}$'s possible exposure to Cry1F in the field has resulted in increased tolerance of the population to Cry1 Bt toxins. The decreased mortality exhibited by $Corn_{(2)}$ in the diet-based bioassays may be due to the population's extensive history of laboratory rearing on an artificial growth medium. $Corn_{(2)}$ has been in continuous laboratory culture since 1998, during which time it has been through over 300 generations, with only occasional introgressions of field stock to increase its genetic variability. It is possible for the fitness of laboratory colonies of insects to change over time due to adaptation to laboratory settings (Rossler 1975), which is evident in the greater weight of surviving $Corn_{(2)}$ larvae than the three other populations in the negative controls (Fig. 2). It has been determined that neither Cry1Ab, Cry1Ac, nor Cry1F pose a high-dose level of effectiveness for the management of fall armyworm (Adamczyk and Sumerford 2000, USEPA 2001, Storer et al. 2010). However, Cry1F is more efficacious than Cry1A toxins for the reduction of fall armyworm feeding injury (Hardke et al. 2011). Thus, it is possible that the extensive laboratory rearing of $Corn_{(2)}$, coupled with

the low toxicity of Cry1A toxins towards fall armyworm, allowed the $Corn_{(2)}$ population to mostly escape the effects of Cry1Ab and Cry1Ac (Fig. 1a,b), but not overcome the effects of Cry1F due to its increased toxicity (Fig. 1c).

It was only possible to calculate EC_{50} values for four of the twelve population by toxin treatment groups due to growth inhibition values exceeding 50% short of the first toxin concentration (62.5 ng/cm^2) in the logistic models, thus preventing comparisons (Table 1). Though it was not possible to accurately calculate LC_{50} values for $Corn_{(1)}$ and $Corn_{(2)}$ on Cry1Ab and $Corn_{(2)}$ on Cry1Ac due to them never reaching 50% mortality (Table 1), and non-significance between the rice strain and hybrid populations Cry1Ab (Table 1, Fig. 1a) and the rice strain and $Corn_{(1)}$ on Cry1Ac (Table 1, Fig. 1b) the data generally support the conclusion that the two corn strain and one hybrid fall armyworm populations were more tolerant to each of the three Bt toxins examined than the rice strain population; especially in Cry1F where the rice strain population's LC_{50} was significantly lower than that of the three remaining populations (Table 1, Fig. 1c). The calculated LC_{50} values for the hybrid population in actuality may be greater than what is reported, as larvae exhibited decreased survival when reared on artificial diet in the negative controls (Fig. 2). The hybrid population's increased tolerance to Bt toxins over that of the rice strain may suggest a degree of dominance associated with the natural Bt tolerance possessed by corn strain populations of fall armyworm, that is then passed on to hybrid progeny in mixed strain matings. Unfortunately, due to a lack of literature on the subject at this time it is difficult to draw confident conclusions pertaining to rice-corn hybrid fall armyworm overall.

The general trend presented here is in accordance with those present in the available literature on fall armyworm host strains on Cry1Ab and Cry1Ac, where rice strain populations exhibited decreased tolerance to the Bt toxins as well (Adamczyk et al. 1997, Ríos-Díez et al. 2012). The corn strain and rice-corn hybrid populations tested were all significantly more tolerant to Cry1F than the rice strain population (Fig. 1c). The only other study to date that has examined host strain tolerances to Cry1F was conducted by Virla et al. (2008), and reported mixed results; where some rice strain populations exhibited decreased tolerance to Cry1F when compared with corn strain populations, but some also exhibited increased tolerance. There is little evidence for differing Bt tolerances among insect host strains in other insect species, though there is some concern that the "E" and "Z" pheromone-races of the European corn borer (*Ostrinia nubilalis* [Hübner]) may develop Bt resistance at different rates due to their divergent host-use patterns (Bontemps et al. 2003, Sorenson et al. 2005, O'Rourke et al. 2010). E-race moths are more often associated with non-corn hosts than the Z-race (Sorenson et al. 2005). Increased exposure of the Z-race to corn could subsequently be associated with increased Bt selection, resulting in an increased rate of resistance evolution (Bontemps et al. 2003, O'Rourke et al. 2010).

There is some additional evidence in the literature that fall armyworm host strains may differ in their tolerances to select insecticides as well (Adamczyk et al. 1997, Ríos-Díez and Saldamando-Benjumea 2011). Adamczyk et al. (1997) determined that fall armyworm collected from forage grasses were significantly more susceptible to cypermethrin (pyrethroid), methyl parathion (organophosphate), and methomyl (carbamate). Ríos-Díez and Saldamando-Benjumea (2011) did not find the same result

for methomyl, but determined that corn strain fall armyworm evolved tolerance to lambda-cyhalothrin (pyrethroid) faster than rice strain. There is similar evidence for differential host strain insecticidal tolerances in other insect pest species. Carletto et al. (2009) examined the response of four host races (eggplant, pepper, cotton, and Cucurbitaceae) of the cotton-melon aphid (*Aphis gossypii* Glover) to seven insecticides using leaf-disk bioassays coupled with mutation detection and enzyme assays. The eggplant, pepper, and one of the two cotton races tested carried a mutation that conferred a moderate resistance to the profenofos and monocrotophos (organophosphates). The same eggplant and cotton races also were strongly resistant to cypermehtrin. Studies on the whitefly, *Bemisia tabaci*, have also indicated that the species' "B" biotype may exhibit a greater tolerance to pyrethroid insecticides than other biotypes (Erdogan et al. 2008), and the "Q" biotype are more tolerant than the B biotype to neonicotinoids and the juvenile hormone analog, pyriproxyfen (Horowitz et al. 2005).

The generally increased Bt tolerances of the corn strain and rice-corn hybrid populations over that of the rice strain population could be significant for fall armyworm IRM in that the host strain composition of a region may play a role in predicting the risk of evolution to Bt toxins. A region with a greater proportion of corn strain and hybrid insects may be at greater risk for Bt resistance than a region with generally higher proportions of rice strain insects. It may be possible to combine established methods of strain identification (Cano-Calle et al. 2015, Nagoshi et al. 2006a, Nagoshi et al. 2007) with current IRM practices to develop more targeted, regional strategies for fall armyworm Bt resistance management. However, there is currently a staggering dearth of data pertaining to the characterization of the fall armyworm host strains, making

confident recommendations nearly impossible at this time. More studies comparing the

Bt tolerances of corn strain, rice strain, and hybrid fall armyworm populations are

required. In addition, future studies on the interactions of fall armyworm populations with

Bt toxins should make note of the host strains of their employed insect populations.

TABLE

Table 1: Probit goodness of fit, lethal concentration of 50% larval mortality (LC_{50}), and effective concentration of 50% larval growth inhibition (EC_{50}), for diet-based bioassays comparing the Bt tolerances of fall armyworm host strains

Toxin	Population	df	χ^2	P	LC_{50}[a]	95% FL[a]	EC_{50}[a]	95% CL[a]
Cry1Ab	Rice	40	63.6	0.0101	1341.0	785.8 to 2555.0	108.6	25.7 to 219.2
	Corn(1)	40	-	-	-	-	190.9	155.8 to 242.3
	Corn(2)	40	-	-	-	-	128.3	50.8 to 205.8
	Hybrid	40	31.1	0.8439	1912.0	1235.0 to 6691.0	-	-
Cry1Ac	Rice	40	152.2	<0.0001	472.6	21.0 to 861.3	-	-
	Corn(1)	40	43.9	0.3111	927.4	552.3 to 1207.0	-	-
	Corn(2)	40	-	-	-	-	123.1	38.3 to 207.9
	Hybrid	40	51.4	0.1077	1471.0	1278.0 to 1932.0	-	-
Cry1F	Rice	40	48.7	0.1626	211.3	158.7 to 264.4	-	-
	Corn(1)	40	115.3	<0.0001	565.0	409.6 to 696.6	-	-
	Corn(2)	40	63.6	0.0101	544.7	409.9 to 654.4	-	-
	Hybrid	40	49.9	0.1351	547.9	321.4 to 753.8	-	-

[a] Measured in ng/cm^2

FIGURES

a)

b)

46

c)
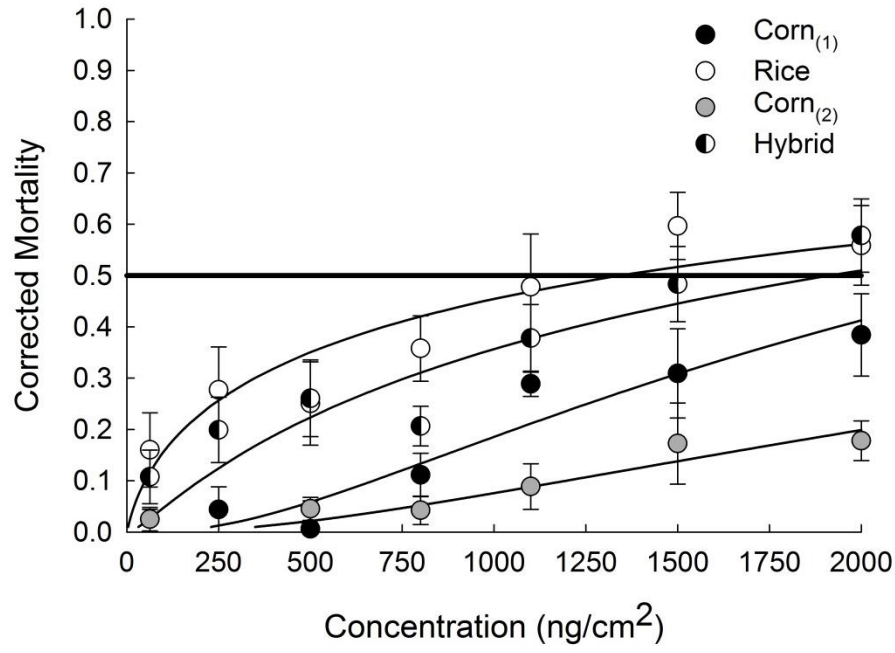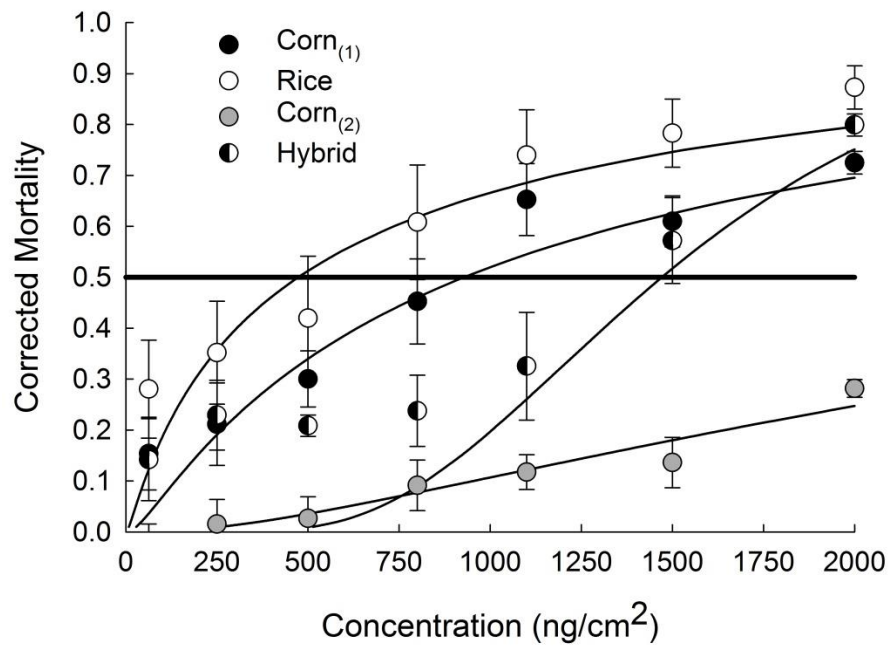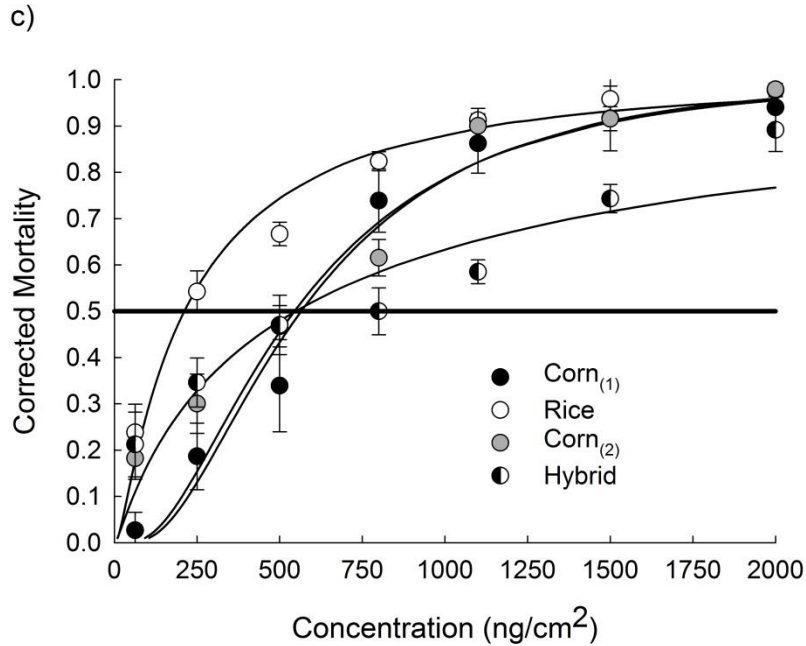
Fig. 1: Corrected mortality of fall armyworm larvae in diet-based bioassays. Points represent means and error bars are the standard error of the mean. The curves plot probits and the horizontal line denotes the LC_{50}. Data are presented for fall armyworm populations tested on a) Cry1Ab, b) Cry1Ac, and c) Cry1F.
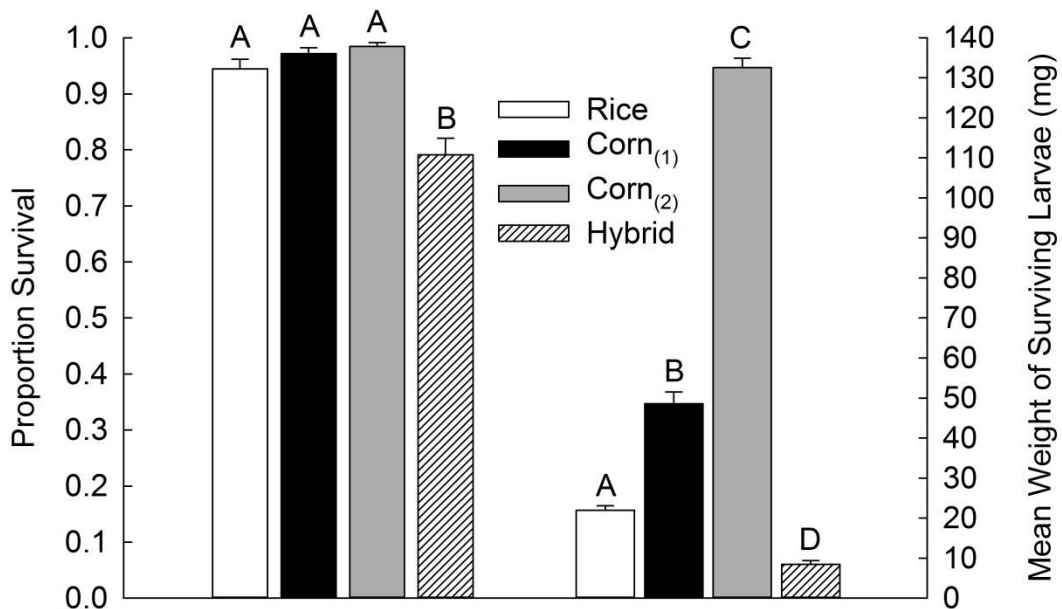
Fig. 2: Proportion survival and mean weight of surviving fall armyworm larvae in negative controls. Bars represent mean values and error bars are the standard errors of the means. Letters represent pairwise comparisons between strains.

REFERENCES

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267

Adamczyk Jr., J. J., J. W. Holloway, B. R. Leonard, and J. B. Graves. 1997. Susceptibility of fall armyworm collected from different plant hosts to selected insecticides and transgenic Bt cotton. J. Cotton Sci. 1: 21-28.

Adamczyk Jr., J.J., and D. V. Sumerford. 2000. Increased tolerance of fall armyworms (Lepidoptera: Noctuidae) to Cry1Ac δ-endotoxin when fed transgenic *Bacillus thuringiensis* cotton: impact on the development of subsequent generations. Fla. Entomol. 84: 1-6.

Barros, E. M., J. B. Torres, and A. F. Bueno. 2010. Oviposition, development, and reproduction of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) fed on different hosts of economic importance. Neotrop. Entomol. 39: 996-1001

Binning, R. R., J. Coats, X. Kong, and R. L. Helmich. 2014. Susceptibility and aversion of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Cry1F Bt maize and considerations for insect resistance management. J. Econ. Entomol. 107: 368-374.

Bontemps, A., D. Bourguet, L. Pélozuelo, M.-T. Bethenod, and S. Ponsard. 2003. Managing the evolution of *Bacillus thuringiensis* resistance in natural populations of the European corn borer, *Ostrinia nubilalis*: host plant, host race, and pherotype of adult males at aggregation sites. Proc. R. Soc. Lond. B. 271: 2179-2185.

Bush, G. L. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). Evolution 23: 237–251.

**Cañas-Hoyos, N., E. J. Márquez, and C. I. Saldamando-Benjumea. 2014.** Differentiation of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) corn and rice strains from central Colombia: a wing morphometric approach. Ann. Entomol. Soc. Am. 107: 575-581.

Cano-Calle, D., R. E. Arango-Isaza, and C. I. Saldamando-Benjumea. 2015. Molecular identification of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) corn and rice strains in Colombia by using a PCR-RDLP of the mitochondrial gene cytochrome oxidase I (COI) and a PCR of the gene FR (for rice). Ann. Entomol. Soc. Am.: 1-9.

Carletto, J., T. Martin, F. Vanlerberghe-Masutti, and T. Brévault. 2009. Insecticide resistance traits differ among and within host races in *Aphis gossypii*. Pest Manag. Sci. 66: 301-307.

Cruz, L. and F. T. Turpin. 1983. Yield impact of larval infestations of the fall armyworm (Lepidoptera: Noctuidae) to midwhorl growth stages of corn. J. Econ. Entomol. 76: 1052-1054.

Diehl, S. R., and G. L. Bush. 1984. An evolutionary and applied perspective of insect biotypes. Ann. Rev. Entomol. 29: 471-504.

Drès, M., and J. Mallet. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. Phil. Trans. R. Soc. Lond. B. 357: 471-492.

Farias, J. R., D. A. Andow, R. J. Horikoshi, R. J. Sorgatto, P. Fresia, A. C. Santos, and C. Omoto. 2014. Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: Nocutidae) in Brazil. Crop Prot. 64: 150-158.

Glaser, J.A. and S. R. Matten. 2003. Sustainability of insect resistance management strategies for transgenic Bt corn. Biotechnol. Adv. 22: 45-69.

Groot, A. T., M. Marr, D. G. Heckel, and G. Schöfl. 2010. The roles and interactions of reproductive isolation mechanisms in fall armyworm (Lepidoptera: Noctuidae) host strains. Ecol. Enomol. 55: 105-118.

Hardke, J. T., B. R. Leonard, F. Huang, and R. E. Jackson. 2011. Damage and survivorship of fall armyworm (Lepidoptera: Noctuidae) on transgenic field corn expressing *Bacillus thuringiensis* Cry proteins. Crop Prot. 30: 168-172.

Hill, A. V. 1910. The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. J. Physiol. 40: *iv-vii*.

Horowitz, A. R., S. Kontsedalov, V. Khasdan, and I. Ishaaya. 2005. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. Arch. Insect Biochem. Physiol. 58: 216-225.

Jaenike, J. 1981. Criteria for ascertaining the existence of host races. Am. Nat. 117: 830–834.

Jakka, S. R. K., V. R. Knight, and J. L. Jurat-Fuentes. 2014. Fitness costs associated with field-evolved resistance to bt maize in *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Econ. Entomol. 107: 342-351.

Johnson, S. J. 1987. Migration and the life history strategy of the fall armyworm, *Spodoptera frugiperda* in the western hemisphere. Insect Sci. Its Appl. 8: 543-549.

Lacey, L. A., 1997. Manual of techniques in insect pathology. Academic Press, San Diego, CA.

Luginbill, P. 1928. The fall armyworm. USDA Tech. Bull. 34.

Luttrell, R. G., and J. S. Mink. 1999. Damage to cotton fruiting structures by the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Cotton Sci. 3: 35-44.

Marçon, P. C. R. G., L. J. Young, K. L. Steffey, and B. D. Siegfried. 1999. Baseline susceptibility of European corn borer (Lepidoptera: Crambidae) to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 93: 925-930.

McMichael, M., and D. P. Prowell. 1999. Differences in amplified fragment-length polymorphisms in fall armyworm (Lepidoptera: Noctuidae) host strains. Ann. Entomol. Soc. Am. 92: 175-181.

Meagher, R. L., R. N. Nagoshi, and C. J. Stuhl. 2011. Oviposition choice of two fall armyworm (Lepidoptera: Noctuidae) host strains. J. Insect Behav. 24: 337-347.

Mitchell, E. R., J. N. McNeil, J. K. Westbrook, J. F. Silvain, B. Lalanne-Cassou, R. B. Chalfant, S. D. Pair, V. H. Waddill, A. Sotomayor-Rios, and F. I. Proshold. 1991. Seasonal periodicity of fall armyworm (Lepidoptera: Noctuidae) in the Caribbean basin and northward to Canada. J. Entomol. Sci. 26: 39-50.

Murúa, M. G., R. N. Nagoshi, D. A. Dos Santos, M. M. Hay-Roe, R. K. Meagher, and J. C. Vilardi. 2015. Demonstration using field collections that Argentina fall armyworm populations exhibit strain-specific host plant preferences. J. Econ. Entomol. 108: 2305-2315.

Nagoshi, R. N., R. L. Meagher, J. J. Adamczyk, S. K. Braman, R. L. Brandenburg, and G. Nuessly. 2006a. New restriction fragment length polymorphisms in the cytochrome oxidase I gene facilitate host strain identification of fall armyworm (Lepidoptera: Noctuidae) populations in the southeastern United States. J. Econ. Entomol. 99: 671-677.

Nagoshi, R. N., R. L. Meagher, G. Nuessly, and D. G. Hall. 2006b. Effects of fall armyworm (Lepidoptera: Noctuidae) interstrain mating in wild populations. Environ. Entomol. 35: 561-568.

Nagoshi, R. N., P. Silvie, and R. L. Meagher. 2007. Comparison of haplotype frequencies differentiate fall armyworm (Lepidoptera: Noctuidae) corn-strain populations from Florida and Brazil. J. Econ. Entomol. 100: 654-961.

Nagoshi, R. N., and R. L. Meagher. 2003. Fall armyworm FR sequences map to sex chromosomes and their distribution in the world indicate limitations in interstrain mating. Insect. Mol. Biol. 12: 453-456.

Nagoshi, R. N., and R. L. Meagher. 2004. Behavior and distribution of the two fall armyworm host strains in Florida. Fla. Entomol. 87: 440-449.

Nagoshi, R. N., and R. L. Meagher. 2008. Review of fall armyworm (Lepidoptera: Noctuidae) genetic complexity and migration. Fla. Entomol. 91: 546-554.

Niu, Y., R. L. Meagher, F. Yang, and F. Huang. 2013. Susceptibility of field populations of the fall armyworm (Lepidoptera: Noctuidae) from Florida and Puerto Rico to purified Cry1F protein and corn leaf tissue containing single and pyramided Bt genes. Fla. Entomol. 69: 701-713.

Niu, Y., F. Yang, V. Dangal, and F. Huang. 2014. Larval survival and plant injury of Cry1F-susceptible, -resistant, and –heterozygous fall armyworm (Lepidoptera: Noctuidae) on non-Bt and Bt corn containing single or pyramided genes. Crop Prot. 59: 22-28.

O'Rourke, M. E., T. W. Sappington, and S. J. Fleischer. 2010. Managing resistance to Bt crops in a genetically variable insect herbivore, *Ostrinia nubilalis*. Ecol. Appl. 20: 1228-1236.

Pair, S. D., J. R. Raulston, A. N. Sparks, J. K. Westbrook, and G. K. Douce. 1986. Fall armyworm distribution and population dynamics in the southeastern States. Fla. Entomol. 69: 468-487.

Pashley, D. 1986. Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): a sibling species complex? Ann. Entomol. Soc. Am. 79: 898-904.

Pashley, D. 1988. Current status of fall armyworm host strains. Fla. Entomol. 71: 227-234.

Pashley, D., and J. A. Martin. 1987. Reproductive incompatibility between host strains of the fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 80: 731-733.

Pashley, D., S. J. Johnson, and A. N. Sparks. 1985. Genetic population structure of migratory moths: the fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 78: 756-762.

Pashley, D., T. C. Sparks, S. W. Quisenberry, T. Jamjanya, and P. F. Dowd. 1987a. Two fall armyworm strains feed on corn, rice and Bermuda grass. Louisiana Agric. 30: 8-9.

Pashley, D., T. C. Sparks, S. W. Quisenberry, T. Jamjanya, and P. F. Dowd. 1987b. Two fall armyworm strains feed on corn, rice, and Bermuda grass. Louisiana Agric. 30: 8-9.

Pashley, D., A. M. Hammond, and T. N. Hardy. 1992. Reproductive isolating mechanisms in fall army worm host strains (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 84: 400-405.

Perkins, W. D. 1979. Laboratory rearing of the fall armyworm. Fla. Entomol. 62: 87-91.

Pitre, H. N., J. E. Mulrooney, and D. B. Hogg. 1983. Fall armyworm (Lepidoptera: Noctuidae) oviposition: crop preferences and egg distribution on plants. J. Econ. Entomol. 76: 463-466.

Prowell, D. P. M. McMichael, and J. –F. Silvain. 2004. Multilocus genetic analysis of host use, introgression, and speciation in host strains of fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 97: 1034-1044.

Quisenberry, S. S. 1991. Fall armyworm (Lepidoptera: Noctuidae) host strain reproductive compatibility. Fla. Entomol. 74: 194-199.

Ríos-Díez, J. D. and C. I. Saldamando-Benjumea. 2011. Susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains from central Colombia to two insecticides, methomyl and lamda-cyhalothrin: a study of the genetic basis of resistance. J. Econ. Entomol. 104: 1698-1705.

Ríos-Díez, J. D., B. Siegfried, and C. I. Saldamando-Benjumea. 2012. Susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains from central Colombia to Cry1Ab and Cry1Ac entotoxins of *Bacillus thuringiensis*. 2012. Southwest. Entomol. 37: 28-293.

Rossler, Y. 1975. Ability to inseminate: comparison between laboratory-reared and field populations of mediterranean fruit fly (*Ceratitis capitata*). Entomol. Exp. Appl. 18: 255-257.

Saldamando, C. I., and A. M. Vélez-Arango. 2010. Host plant association and genetic differentiation of corn and rice strains of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) in Colombia. Neotrop. Entomol. 39: 921-929.

Savin, N. E., J. L. Robertson, and R. M. Russell. 1977. A critical evaluation of bioassays in insecticide research: likelihood ratio tests of dose-mortality regression. Bull. Entomol. Soc. Am. 23: 257-266.

Schöfl, G., D. C. Heckel, and A. T. Groot. 2009. Time-shifted reproductive behaviors among fall armyworm (Noctuidae: *Spodoptera frugiperda*) host strains: evidence for differing modes of inheritance. J. Evol. Biol. 22: 1447-1459.

Sokal, R. R. and F. J. Rohlf. 2012. Biometry: the principles and practice of statistics in biological research. 4th ed. W. H. Freeman and Company, New York, NY.

Sorenson, C. E., G. G. Kennedy, C. Schal, and J. F. Walgenbach. 2005. Geographical variation in pheromone response of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae), in North Carolina: a 20-Y perspective. Environ. Entomol. 34: 1057-1062.

Sparks, A. N. 1979. A review of the biology of the fall armyworm. Fla. Entomol. 62: 82-87.

Storer, N. P., J. M. Babcock, M. Schlenz, T. Meade, G. T. Thompson, J. W. Bing, and R. M. Huckaba. 2010. Discovery and characterization of field resistance to Bt maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico. J. Econ. Entomol. 103: 1031-1038.

Tabashnik, B. E., Y. B. Liu, T. Malvar, G. G. Heckel, and L Masson. 1998. Insect resistance to *Bacillus thuringiensis*: uniform or diverse. Phil. Trans. Roy. Soc. B. 353: 1751-1756.

Téllez-Rodriguez, P., B. Raymond, I. Morán-Bertot, L. Rodríguez-Cabreara, D. J. Wright, C. G. Borroto, and C. Ayra-Pardo. 2014. Strong oviposition preference for Bt over non-Bt maize in Spodoptera frugiperda and its implications for the evolution of resistance. BMC Biol. 12:48.

(USEPA) United States Environmental Protection Agency. 2001. Biopesticides registration action document: Cry1Ab and Cry1F *Bacillus thuringiensis* (Bt) corn plant-incorporated protectants. (http://www3.EPA.gov/pesticides/chem_search/ reg_actions/pip/cry1f-cry1ab-brad.pdf) [Accessed 8 March 2017].

(USEPA) United States Environmental Protection Agency. 2005. Biopesticides registration action document: *Bacillus thuringiensis* var. Aizawai Cry1F and the genetic material (from the insert of plasmid PGMA281) necessary for its production in cotton and *Bacillus thuringiensis* var. Kurstaki Cry1Ac and the genetic material (from insert of plasmid PMYC3006) necessary for its production in cotton. (http://www3.EPA.gov/pesticides/chem_search/reg_actions/ registration/decision_PC-006512_1-Sep-05.pdf) [Accessed 8 March 2017].

Vélasquez-Vélez, M. I., C. I. Saldamando-Benjumea, and J. D. Ríos-Diez. 2011. Reproductive isolation between two populations of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) collected in corn and rice fields from central Colombia. Ann. Entomol. Soc. Am. 104: 826-833.

Vélez, A. M., T. A. Spencer, A. P. Alves, D. Moellenbeck, R. L. Meagher, H. Chirakkal, and B. D. Siegfried. 2013. Inheritance of Cry1F resistance, cross-resistance and frequency of resistant alleles in *Spodoptera frugiperda* (Lepidoptera: Noctuidae). B. Entomol. Res. 103: 700-713.

Vélez, A. M., T. A. Spencer, A. P. Alves, L. B. Crespo, and B. D. Siegfried. 2014. Fitness costs of Cry1F resistance in fall armyworm *Spodoptera frugiperda*. J. Appl. Entomol. 138: 315-325.

Vélez, A. M., N. N. Vellichirammal, J. L. Jurat-Fuentes, and B. D. Siegfried. 2016. Cry1F resistance among lepidopteran pests: a model for improved resistance management? Curr. Opin. Insect. Sci. 15: 116-124.

Virla, E. G., A. Álvares, F. Loto, L. M. Pera, and M. Baigorí. 2008. Fall armyworm strains (Lepidoptera: Noctuidae) in Argentina, their associate host plants and response to different mortality factors in laboratory. Fla. Entomol. 9: 63-69.

Whitford, F., S. S. Quisenberry, T. J. Riley, and J. W. Lee. 1988. Oviposition preference, mating compatibility, and development of two fall armyworm strains. Fla. Entomol. 71: 234-243.

Chapter 3

OVIPOSITION PREFERENCES AND TISSUE FEEDING OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) HOST STRAINS

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Abstract

The fall armyworm, *Spodoptera frugiperda* (Smith) is a highly damaging pest of many

commercial crops across the Americas. Reports of field-derived resistance to several

insecticidal toxins derived from the bacterium *Bacillus thuringiensis* Berliner (Bt) have

caused growing concern in agricultural communities over the species' continued

management. The existence of two host strains referred to as the "rice" and "corn" strains

is a complicating and under-researched factor of fall armyworm biology and

management, when considering evidence for differential Bt tolerances between them.

Here the oviposition preferences of one rice strain and one corn strain population are

compared in light of the bioassay and feeding differences found among one rice strain,

two corn strains, and one rice-corn hybrid population exposed to Cry1F. Although

results were narrowly non-significant, the rice strain population appears to be less

tolerant to Cry1F corn tissue than the corn and hybrid populations. Results indicate that corn is a preferred oviposition host for both rice and corn strain fall armyworm and a suitable larval host plant for each of the four populations tested. Data on fall armyworm host strain biology and Bt tolerance may aid in the development of more effective insect resistance management (IRM) practices for this pest.

Keywords: Cry1F Bt corn, *Spodoptera frugiperda*, corn strain, rice strain, resistance management

Introduction

The noctuid moth, fall armyworm (*Spodoptera frugiperda* [Smith]) is a damaging pest of a variety of agricultural, pasture, and turf plant species. Larval feeding has been reported on a plethora of hosts spanning 23 families and over 80 species (Pashley 1988) with an apparent affinity towards grasses (Luginbill 1928, Johnson 1987, Pashley 1988). Significant damage to economically important crops such as corn (*Zea mays* L.), cotton (*Gossypium spp.* L), sorghum (*Sorghum bicolor* L.), and several turf and pasture grasses has been documented for over a century (Luginbill 1928, Young 1979, Johnson 1987). Larval feeding damage is often severe, with reported yield losses ranging between 17 and 72% depending on the locality and conditions (Cruz and Turpin 1983, Barros et al. 2010, Murúa et al. 2015). Fall armyworm are multivoltine and do not diapause (Luginbill 1928, Sparks et al. 1979, Johnson 1987). Populations migrate into eastern and central North America from overwintering grounds in South and Central America, Mexico, and the Southern United States over the course of several generations (Sparks et al. 1979, Pair et

al. 1986, Johnson 1987, Mitchell et al. 1991, Nagoshi and Meagher 2008). The exact migration course is somewhat variable, depending on the availability of host plants and the current year's weather patterns (Sparks et al. 1979, Pair et al. 1986, Johnson 1987, Mitchell et al. 1991); but general routes taken by populations can be tracked via the combined use of pheromone traps (Meagher 2001, Fleischer et al. 2005) and mitochondrial haplotyping (Nagoshi and Meagher 2008, Nagoshi et al. 2009, 2012, 2015).

Fall armyworm are divided into two host strains referred to as the "rice" and "corn" strains (Pashley et al. 1985, Pashley 1986). The strains were originally named for the host plants they were identified on (Pashley et al. 1985, Pashley 1986), Rice strain fall armyworm generally prefer to feed on pasture and turf grasses such as Bermuda grass (*Cynodon dactylon.* L.) or alfalfa (*Medicago sativa* L.), where the corn strain prefers larger grasses such as corn or sorghum (Pashley 1986, Pashley et al. 1987, Groot et al. 2010, Murúa et al. 2015); however, host fidelities are not absolute. Examples include corn strain feeding on late stage cotton (Adamczyk et al. 1997, Luttrell and Mink 1999) and collections of rice strain fall armyworm feeding on corn (Prowell et al. 2004, Groot et al. 2010). The corn strain generally occurs in the Western hemisphere, in corn-rich areas; there is less known about the distribution of the rice strain due to little sampling on non-corn hosts (Pashley et al. 1985, Pashley 1986, Pashley 1988). However, the rice strain tends to occur sympatrically with the corn strain (Pashley 1988).

Rice and corn strain fall armyworm are generally differentiated from one another via the use of genetic markers (Cano-Calle et al. 2015, Pashley 1986, McMichael and Prowell 1999, Prowell et al. 2004, Nagoshi et al. 2006a, Nagoshi et al. 2007), though

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there is some evidence for differentiation via wing morphometrics (Cañas-Hoyos et al. 2014). The availability of data comparing the biology of rice and corn strain fall armyworm is limited despite knowledge of their existence for over 30 years. What is present in the published literature must be approached cautiously as it is often contradictory between studies. General trends support that the host strains are reproductively isolated from one another through a variety of mechanisms (Pashley and Martin 1987, Whitford et al. 1988, Pashley et al. 1992, Schöfl et al. 2009, Groot et al. 2010, Meagher et al. 2011). However, hybridization does occur in the field at low to moderate percentages (Prowell et al. 2004, Nagoshi et al. 2006b, Saldamando and Vélez-Arango 2010).

There are a variety of studies pertaining to the larval fitness of rice and corn strain fall armyworm when reared on different host plants (Pashley et al. 1995, Whitford et al. 1988, Meagher et al. 2004, Stuhl et al. 2008, Pashley et al.1992, Groot et al. 2010) though results are inconsistent. Among the possible reproductive isolation mechanisms of fall armyworm, host strains oviposition preferences have received the least attention, with only three published studies (Pitre et al. 1983, Whitford et al. 1998, Meagher et al. 2011). This area seems understudied, as information on host utilization is important in constructing integrated pest management (IPM) and insect resistance management (IRM) plans for this pest, considering the use of transgenic crops expressing toxins derived from the bacterium *Bacillus thuringiensis* Berliner (Bt). Currently, the efficacy of Bt corn and cotton used in fall armyworm pest management is at risk due to field-evolved resistance in populations to the Cry1F Bt toxin (Storer et al. 2010, Farias et al. 2014). Among the plethora of factors that have possibly contributed to Cry1F resistance evolution (Storer et

al. 2010, Hardke et al. 2011, Niu et al. 2013, Vélez et al. 2013, Binning et al. 2014, Jakka et al. 2014, Niu et al. 2014, Téllez-Rodriguez et al. 2014, Vélez et al. 2014) there is some evidence that corn strain fall armyworm are generally more tolerant to Bt toxins than the rice strain (Adamczyk et al. 1997, Virla et al. 2008, Ríos-Díez et al. 2012). Thus, the characterization of host strain biology becomes important for providing accurate baseline information for the development of more effective IRM policies for the species (Glaser and Matten 2003). Here we measure and compare the oviposition preferences, Bt tolerance, and host plant tissue feeding of four populations of fall armyworm using no-choice and two-choice greenhouse experiments, a Cry1F tissue-based bioassay, and a series of tissue-based feeding experiments. Data from this study will provide better insight into the biology of fall armyworm host strains that may contribute to the evolution of Bt resistance.

Materials and Methods

Study Insects. Four populations of fall armyworm were used in experiments and bioassays. Rice and corn strain fall armyworm eggs were obtained from USDA-ARS in Gainesville, Florida and used to establish laboratory colonies of each population. Host strain identities for the two populations were previously characterized using the methods of Nagoshi et al (2007). The rice strain population was originally collected from pasture grasses in Duval County, Florida, in 2014. The corn strain population was collected in 2013, from sweet corn in Hendry County Florida, and will hereby be referred to as Corn$_{(1)}$. Due to the reported presence of a Cry1F corn field planted directly adjacent to

the collection site for Corn$_{(1)}$, a second corn strain population that had no prior exposure to Bt was included in the study to serve as a control. This population has been in continuous culture since 1998, and is currently reared by Frontier Agricultural Sciences in Newark, Delaware. The population was previously identified as corn strain by Vélez et al. (2013), and will hereby be referred to as Corn$_{(2)}$. The fourth fall armyworm population used was collected by DuPont Pioneer (Johnston, IA, USA) from soybean plants in the Mississippi delta region in 2016. Typing using genetic markers by DuPont Pioneer has determined that the population is a rice-corn hybrid.

Colony Rearing. Colonies of the rice strain population and Corn$_{(1)}$ were reared in the laboratory following the methods of Perkins (1979) and Vélez et al. (2013). Corn$_{(2)}$ and hybrid populations were not maintained in the laboratory, and eggs were obtained from Frontier Agricultural Sciences (Newark, DE, USA) and DuPont Pioneer (Johnston, IA, USA) as needed for experiments. Rice strain or Corn$_{(1)}$ egg masses were placed in zip-top plastic bags and incubated in a growth chamber (Model E-54U, Pervical Scientific, Perry, IA, USA) set to 25° C and a 16:8 L:D photoperiod until larval emergence. Relative humidity in the growth chamber was maintained at 50% to 70% via large, water filled containers placed at the bottom of the chamber. Emerging neonates were transferred into pre-prepared 56-cell trays (Frontier Agricultural Sciences, Newark, DE, USA), that contained general purpose Lepidoptera diet (Frontier Agricultural Sciences, Newark, DE, USA), and incubated in the same growth chamber as the egg masses until pupation.

Pupae were removed and placed on cotton pads (Richmond Dental, Richmond, NC, USA) wet with deionized water in petri dishes (diameter = 100 mm) (Thermo Fisher

Scientific Inc., Waltham, MA, USA). Petri dishes were placed in 20.3 x 20.3 x 20.3 cm, 6 mm wire mesh hermit crab cages (Florida Marine Research, Sarasota, FL, USA) in a second growth chamber set to 28° C and 16:8 L:D photoperiod. A maximum of 120 pupae were placed in a single cage. Cages were fitted with 1 mm wire mesh covers to prevent adult escape, and cotton was moistened daily. Emergence of adults was monitored daily, and once detected, a liquid adult diet was introduced into the cages. Diet was placed in 50 ml Erlenmeyer flask fitted with a braided cotton wick (Richmond Dental, Richmond, NC, USA). The liquid diet consisted of flat beer, ascorbic acid, propionic acid, aureomycin, and a vitamin mixture (Vanderzant vitamin mix, Frontier Agricultural Sciences, Newark, DE, USA) following Perkins (1979) and Vélez et al. (2013). A sheet of waxed paper (Reynolds Consumer Products, Lake Forest, IL, USA) was placed in between the cage wall and outer mesh cover to serve as an oviposition substrate. Waxed paper was replaced every three days.

Plant Varieties. Potted plants were grown in a greenhouse in 3.8 L plastic nursery pots with drainage (C-400 Grip Lip, Nursery Supplies Inc., Chambersburg, PA, USA). Cry1F expressing corn (Pioneer N3DON11048-N1 PDF with ApronXL (active ingredient mefenoxam), Cruiser250 (thiamethoxam), Dynasty (axoxystrobin), and Maxim (fludioxonil); DuPont-Pioneer, Johnston, IA, USA), non-Bt near isoline corn (Pioneer J3DON11000-N PDF; DuPont-Pioneer, Johnston, IA, USA), and an additional non-Bt corn variety (35F38 with FDMM Apron XL and Celest XL (metalaxyl), DuPont-Pioneer, Wilmington, DE, USA) were planted two seeds per pot in Metro-Mix 855 growing mix (SunGro Horticulture, Vancouver, British Columbia, Canada). Non-Bt cotton (Delta Pine DP1441 GENRRF, Monsanto Company, St. Louis, MO, USA) was planted two seeds per

pot in Sunshine LC1 Professional Growing Mix (SunGro Horticulture, Vancouver, British Columbia, Canada). The fungicides present on the corn seeds used pose no known effects on fall armyworm survival, feeding, or oviposition; and it has been demonstrated that thiamethoxam does not exert any significant effect on fall armyworm larval survival on early or middle vegetative stage corn compared to untreated seed (Thrash et al. 2013). Thus, the effects of seed treatments were deemed negligible for this study.

Corn and cotton plants were culled to one plant per pot at the V-2 growth stage (corn) (Abendroth et al. 2011) and when the first true leaf was fully formed (cotton), leaving the healthier of the two plants to grow. Bermuda-grass (Princess 77, unhulled, 47.51% coating, Hancock Seed Company, Dade City, FL, USA) was planted at ca. 0.25 ml of seed per pot in Sunshine LC1 Professional Growing Mix. Rice seeds (#167-Koshihikari rice, unhulled and untreated, Kitazawa Seed Company, Oakland, CA, USA) were pre-soaked in tap water for 24 h prior to planting. Rice seeds were planted six per pot in Cornell Rice Mix (Harrington 2010) consisting of 0.16 m^3 peat, 0.34 m^3 vermiculite, 2.27 kg lime, and 0.45 kg Peters Professional Uni-Mix Plus III (10-5-10, Scotts Company, Baulkham Hills, New South Wales, Australia). Potted rice plants were placed three to a tray in plastic growing trays without drainage holes (1020 trays, Hydrofarm East, Fairless Hills, PA, USA) that were filled with water. Plants used in any given experimental trial were all planted and grown in cohorts for the same period of time.

No-Choice Oviposition Experiments. Fall armyworm pupae were sexed following Butt and Cantu (1962) (Santos et al. 2002) and placed in separate cages, following the rearing procedure, in a 2:1 female to male ratio. After emergence, male fall

armyworm adults were chilled in a refrigerator for 2.5 min then marked with a red marker on their right forewing. Following their marking, the males were introduced into the female cage. Adult fall armyworm in mixed sex cages were allowed to mass mate for 3 d after which they were again chilled in a refrigerator for 2.5 mins. Females were removed and placed in individual 44.4 ml plastic cups, and males were disposed of.

Potted plants of each species (the corn variety used: 35F38) were fitted with mesh cages constructed from 83.8 cm galvanized steel wire round tomato cages (Lowe's Company, Mooresville, NC, USA) that were covered in tulle fabric (matte tulle RN35055, Jo-Ann Fabric and Craft Stores, Hudson, OH, USA). Corn plants were at approximately the V5 to V6 growth stage, and were cut at the top in order for them to fit in the cages. After fitting with fabric, a single gravid female fall armyworm of either the rice strain population or Corn$_{(1)}$ was released onto each caged plant, and the cages were fitted to the pots with rubber bands (Advantage file bands #117B, Alliance Rubber Company, Alliance, OH, USA). The females were left in the plant cages for 3 d to oviposit, and then removed. After female removal, each plant was examined for the presence of egg masses (egg masses deposited on the cage or pot were not counted). The number of egg masses on each plant was recorded and the number of eggs per egg mass were counted using a Wild M5A stereo-microscope (Wild-Heerbrugg, Heerbrugg, Switzerland) and a click counter. Trials were conducted for each strain on each of the four plant species; ten replications were performed for each plant species by insect population treatment group prior to statistical analyses.

Two-Choice Oviposition Experiments. Fall armyworm adults were sexed and separated following the methods of the no-choice experiments. Potted plants were placed

inside 60 cm x 60 cm x 75 cm insect rearing tents (Bugdorm, MegaView Science Co. Ltd., Taichung, Taiwan) in one of six possible combinations of the four plant varieties planted, corn plants were not cut. A single female fall armyworm of the rice strain population or Corn$_{(1)}$ was released into each tent, left for a 3 d oviposition period, and then removed. After female removal, each plant was examined for the presence of egg masses in the same manner as in the no-choice experiments. Six replications were conducted for each plant combination by population treatment group prior to statistical analyses.

In order to test if the cutting of the corn plants in the no-choice experiments had an effect on fall armyworm oviposition, an additional two-choice experiment was conducted using only cut and uncut corn plants of the same corn variety (35F38 with FDMM Apron XL and Celest XL) used in the previous oviposition experiments. Cut plants were cut to approximately the height that was needed in the no-choice experiments. One cut and one uncut corn plant were placed in each insect rearing tent. Further methods follow those of the two-choice experiments using solely the rice strain population. Six trials were conducted.

Cry1F Tissue-Based Bioassays. Cry1F and near isoline corn plants were grown to the V7 to V8 growth stage prior to use, and tissue-based bioassays were conducted following the methods of Hardke et al. (2011). Leaves were removed from the plants and cut into rectangles approximately 2.5 cm X 10 cm. Tissue rectangles were placed into individual 44.4 ml plastic cups (Solo T125-0090 plastic soufflé portion cups, Solo Cup Company, Hampstead, MD, USA), two per cup. Cups with corn tissue were lightly misted with deionized water to prevent tissue desiccation. A single neonate fall

armyworm (less than 24 h old) was placed in each cup with corn tissue, and lids were affixed (Solo PL1-0090, Solo Cup Company, Hampstead, MD, USA). Sixty replicates (cups) were conducted for each corn variety (Cry1F and non-Bt isoline), for each population examined (eight total treatment groups). Cups with corn tissue and larvae were stored in a growth chamber (E-54U, Pervical Scientific, Perry, IA, USA) set to maintain an environment of 28°C, 50% RH, and 16:8 L:D photoperiod for 7 d. After the 7 d period had expired, cups were checked for larval survival. Surviving larvae were weighed using an electronic balance (Mettler Toledo AL54, Mettler Toledo LLC, Bristol, PA, USA).

Tissue-Based Feeding Experiments. The second set of tissue experiments was conducted using the additional non-Bt corn variety (35F38), rice, and Bermuda grass to determine feeding preferences of the fall armyworm populations. Corn tissue was prepared in the same manner as in the Cry1F tissue-based bioassays. Cups with rice and Bermuda grass tissue were prepared by inserting enough plant tissue to fill the cups approximately three quarters full. Cups with plant tissue were misted, and larvae were placed and prepared cups were incubated following the methods of the Cry1F tissue-based bioassays. Twenty replicates (cups) were conducted for each plant for each population tested (12 total treatment groups). Surviving larvae were weighed and capsule widths (HCW) were also measured using a Wild M5A stereo-microscope.

Data Analyses. Data from no-choice experiments were analyzed using one-way ANOVA in SAS 9.4 (PROC GLM, SAS Institute Inc., Cary, NC). Separate one-way ANOVA were conducted for the number of egg masses per plant and number of eggs per egg mass for each of the two fall armyworm populations tested. Two-choice experiment

data (including the additional cut versus uncut experiment) were initially analyzed in SAS 9.4 with Fisher's exact test (PROC FREQ) against an expected 1:1 ratio for each plant species combination to determine if the ratio of egg masses on each plant differed between replications. The information from the individual two-choice experiments for each host-strain was then summarized on a single scale by calculating preference indices for each host plant (Csiszár and Tusnády 1984; McDonald et al. 1999). A minimization procedure was used to calculate preference indices that reduced the weighted sum of independence (D) between the expected proportion of egg masses on each host expected by the procedure and the actual observed values in order to ensure the best possible fit (Csiszár and Tusnády 1984; McDonald et al. 1999).

In the Cry1F tissue-based bioassay, survival and weight data for larval fall armyworm of each population on each corn variety were compared using two-way ANOVA in SAS 9.4 (PROC GLM). Fixed effects tested were the fall armyworm population, the corn variety, and their interaction. Pairwise comparisons were made using an LSMEANS statement and examined against a Bonferroni adjusted level of significance of 0.0031 for 16 comparisons (Sokal and Rohlf 1995). In the second set of tissue experiments survival, weight, and head capsule width data were also analyzed with two-way ANOVA. Fixed effects tested were the fall armyworm population, the plant species, and their interaction (12 total treatment groups). Pairwise comparisons were made in the same manner as in the Cry1F tissue-based bioassay against a Bonferroni adjusted level of significance of 0.0018 for 28 comparisons.

RESULTS

No-Choice Oviposition Experiments. There was a near significant (P = 0.0524) difference in the number of egg masses deposited by rice strain females (Table 2), with rice and Bermuda grass plants receiving more eggs than corn or cotton (Fig. 3a). There was no significant difference in the number of egg masses deposited by $Corn_{(1)}$ females on any of the four host plants tested (Table 2, Fig. 3a). There were no significant differences detected for the mean number of eggs per egg mass for either the rice strain population or $Corn_{(1)}$ (Table 2, Fig. 3b).

Two-Choice Oviposition Experiments. There was one significant comparison for the rice strain population, corn vs. cotton (P = 0.0039), and two significant host comparisons in the two-choice oviposition experiments for $Corn_{(1)}$, corn vs Bermuda grass (P = 0.0005) and corn vs. cotton (P = 0.0042), all with corn as the preferred oviposition host (Table 3). The preference indices for the rice strain population calculated by the minimization procedure fit the observed proportions of oviposition moderately (D = 6.396); rice, corn, and Bermuda grass were all preferred similarly to one another, but significantly more so than cotton (Fig. 4a). The preference indices for $Corn_{(1)}$ calculated by the minimization procedure fit the observed proportions of oviposition very well (D = 0.637); corn was significantly preferred for oviposition over all other tested host plants in $Corn_{(1)}$ (Fig. 4b). In the additional two-choice oviposition experiment between cut and uncut corn plants there were significantly more egg masses deposited on uncut corn plants (11) than on cut corn plants (1) (P = 0.0063).

Cry1F Tissue-Based Bioassays. The interaction between fall armyworm population and corn variety for proportion survival was nearly non-significant (P = 0.0558. Table 4), though the individual effects of fall armyworm population and corn

variety were both significant (P < 0.0001, Table 4). Proportions of survival were lower for all fall armyworm populations tested when reared on Cry1F corn tissue than on the non-Bt near isoline (Fig. 5a). All effects tested for the mean weight of surviving larvae were significant (P < 0.0001, Table 4). Mean weights were significantly lower for surviving larvae reared on Cry1F corn tissue than on the non-Bt near isoline (Fig. 5b). Surviving larvae of $Corn_{(1)}$ weighed significantly more than all other populations tested, with $Corn_{(2)}$intermediate, and surviving rice strain and hybrid larvae weighing the least (Fig. 5b).

   Tissue-Based Feeding Experiments. All effects tested were statistically significant for the analysis of proportion survival, weight, and head capsule width data (Table 5). There were no significant differences in proportion survival on corn for any of the four populations tested (Fig. 6a). On rice tissue, $Corn_{(1)}$ and the hybrid population exhibited significantly reduced proportions of survival compared to $Corn_{(2)}$ and the rice strain population; there were no significant differences in the proportions of survival between $Corn_{(1)}$ and the hybrid population, and $Corn_{(2)}$ and the rice strain population respectively (Fig. 6a). $Corn_{(1)}$ exhibited 100% survival on Bermuda grass, which was not significantly greater than that of the rice strain population, but was significantly greater than $Corn_{(2)}$ and the hybrid population; the proportions of survival of $Corn_{(1)}$, the rice strain population, and the hybrid population were not significantly different from one another (Fig. 6a). $Corn_{(1)}$ exhibited significantly reduced survival on rice tissue in comparison to corn and Bermuda grass; in contrast, there were no significant differences in the survival of $Corn_{(2)}$, the rice strain population, or the hybrid population between the three host plants tested (Fig. 6a).

The mean weight of surviving $Corn_{(2)}$ larvae was significantly greater than any of the remaining three populations when fed corn or rice tissue; there were no significant differences in the mean weights of surviving larvae that were fed Bermuda grass tissue (Fig. 6b). The mean weights of surviving $Corn_{(1)}$ and rice strain larvae were not significantly different between any of the three host plants tested (Fig. 6b). Surviving $Corn_{(2)}$ larvae weighted significantly less when fed Bermuda grass tissue compared to corn and rice (Fig. 6b). Hybrid larvae fed corn tissue weighted significantly greater than those fed Bermuda grass, but not rice (Fig. 6b).

The mean head capsule width (HCW) of surviving $Corn_{(2)}$ larvae fed corn tissue was significantly greater than that of the rice strain and hybrid populations, but not $Corn_{(1)}$; mean HCW's of $Corn_{(1)}$, rice strain population, and hybrid population larvae fed corn tissue were not significantly different from one another (Fig. 6c). On rice, surviving $Corn_{(2)}$ larvae had significantly greater mean HCW compared to the remaining three populations tested (Fig. 6c). $Corn_{(1)}$ and $Corn_{(2)}$ had significantly greater mean HCW's compared to the rice strain and hybrid populations when fed Bermuda grass tissue (Fig. 6c). Surviving rice strain and $Corn_{(2)}$ larvae had significantly larger HCW's when reared on either corn or rice compared to Bermuda grass (Fig. 6c). The HCW's of surviving $Corn_{(1)}$ larvae were significantly greater when on corn than on either rice or Bermuda grass, which were not significantly different from one another (Fig. 6c). Surviving hybrid larvae had significantly greater HCW's when reared on corn tissue compared to Bermuda grass, but not when reared on rice which was not significantly different from either corn or Bermuda grass (Fig. 6c).

Discussion

In this study we compared the oviposition preferences, Cry1F Bt tolerance, and host plant tissue feeding of host strain populations of fall armyworm. Oviposition preferences were measured using no-choice and two-choice oviposition experiments in a greenhouse using one rice strain fall armyworm population and one corn strain population, Corn$_{(1)}$. Cry1F Bt tolerances were measured using tissue-based bioassays, and host plant tissue feeding was measured using tissue-based feeding experiments, each comparing one rice strain fall armyworm population, two corn strain populations (Corn$_{(1)}$ and Corn$_{(2)}$), and one rice-corn hybrid population.

This is the first study to compare the Bt tolerance and host plant tissue feeding of a hybrid fall armyworm population to that of pure corn and rice strain populations. The existence of rice-corn hybrid fall armyworm in nature is somewhat uncertain; as there is a distinct divide between laboratory evidence for reproductive isolation and field collections of hybrid insects. In addition to possible isolating effects of their general host plant preferences (Pashley 1986, Pashley et al. 1987, Adamczyk et al. 1997, Luttrell and Mink 1999, Prowell et al. 2004, Groot et al. 2010, Murúa et al. 2015), rice and corn strain fall armyworm are reproductively isolated from one another through mate selection preferences (Pashley and Martin 1987), female calling times (Pashley et al. 1992, Schöfl et al. 2009), and oviposition preferences (Pitre et al. 1983, Whitford et al. 1988, Meagher et al. 2011); though results between studies are often contradictory (Groote et al. 2010). Inter-strain matings are predicted to occur very rarely in the field (Nagoshi et al. 2006b); however, rice-corn hybrid fall armyworm have been collected in field settings in proportions as high as 24% (Saldamando and Vélez-Arango 2010). The majority of these

hybrid populations have been collected from corn fields (Prowell et al. 2010, Saldamando and Vélez-Arango 2010). Host-associated insect strains are generally defined by four criteria: 1) association with, or fidelity to certain host plant species; 2) sympatric occurrence; 3) some degree of genetic differentiation from one another; and 4) some degree of gene flow between strains associated with different hosts (Bush 1969, Jaenike 1981, Diehl and Bush 1984, Drès and Mallet 2002). Rice and corn strain fall armyworm fit into each of the first three criteria, and the existence of rice-corn hybrids suggests that there must be some gene flow between them, making the fall armyworm host strains congruent with the above definition as a whole. More information pertaining to the distribution of rice-corn hybrid fall armyworm in the field is required to better estimate the frequency of hybridization (Nagoshi et al. 2006b).

Though the data for proportion of survival from the Cry1F tissue-based bioassays were narrowly non-significant (Table 4), the proportions of survival for the $Corn_{(1)}$ and $Corn_{(2)}$ populations were very similar when reared on Cry1F corn tissue (Fig. 5a), and the mean weights of surviving larvae for all four fall armyworm populations tested were not significantly different from one another (Fig. 5b). From this evidence, it does not appear that possible exposure of $Corn_{(1)}$ to Cry1F corn due to the field adjacent from its collection site resulted in increased tolerance to Cry1F. The proportion of survival results in the tissue-based feeding experiments are mixed, with no significant difference in the proportions of survival between $Corn_{(1)}$ and $Corn_{(2)}$ on corn, $Corn_{(2)}$ having a significantly greater proportion of survival than $Corn_{(1)}$ on rice, and $Corn_{(1)}$ having a significantly greater proportion of survival than $Corn_{(2)}$ on Bermuda grass (Fig. 5a). Surviving $Corn_{(2)}$ larvae were also significantly heavier than those of $Corn_{(1)}$ when reared on corn and rice

tissue (Fig. 5b), and had significantly larger head capsule widths than $Corn_{(1)}$ when reared on rice tissue (Fig. 5c).

Although there were no significant effects in the no-choice oviposition experiments (Table 2) it is evident that rice and Bermuda grass were preferred oviposition hosts compared to corn and cotton for the rice strain population (Fig. 5a). There are contrasting results between the no-choice and two-choice oviposition experiments, where, for both the rice strain population and $Corn_{(1)}$, corn appears to be a preferred oviposition host in the two-choice experiments (Fig. 4), but not in the no choice experiments (Fig. 3). This apparent divide is explained through the results of the additional two-choice oviposition experiment comparing cut vs. uncut corn plants, where there were significantly more egg masses deposited on uncut corn plants than on cut plants. There is evidence that female fall armyworm prefer to oviposit on corn plants free of feeding damage (Téllez-Rodriguez et al. 2014), thus the cutting of the corn plants in the no-choice oviposition experiments likely depressed oviposition. These data facilitate agreement between the two oviposition experiments for the rice strain population, where corn, rice, and Bermuda grass were preferred oviposition hosts over cotton (Fig. 3a, Fig. 3a). There is still a divide between the no-choice and two-choice oviposition experiments results for $Corn_{(1)}$ even with the results of the cut vs. uncut experiment. Bermuda grass appears to be a preferred oviposition host for $Corn_{(1)}$ in the no-choice experiments (Fig. 3a). However, in the two-choice experiments, corn appears to be a strongly preferred oviposition host over each of the three other host plants (Fig. 4b). The number of eggs deposited in each egg mass was also the greatest on corn plants in both the rice strain population and $Corn_{(1)}$, though not significantly so (Fig. 3b).

The few studies in the existing literature pertaining to the oviposition preferences of rice and corn strain fall armyworm exhibit somewhat mixed results. Whitford et al. (1988) reported that rice strain females preferred to oviposit on Bermuda grass over corn, sorghum, and centipede grass; whereas corn strain females showed a strong preference towards corn and sorghum. This contrasts an earlier study by Pitre et al. (1983) that suggested that female fall armyworm collected from corn plants (suspected to be corn strain females [Meagher et al. 2011]) will readily oviposit on several possible host plants including corn, sorghum, wheat, Bermuda grass, and rye. Meagher et al. (2011) would later report that populations of rice strain fall armyworm exhibit a strong fidelity towards oviposition on grasses, where corn strain females oviposited indiscriminately on tested host plants as well as on artificial structures.

Though the population by variety interaction for proportion survival in the Cry1F tissue-based bioassays was narrowly non-significant (Table 4), there is a clear indication that the rice strain population was more susceptible to the Bt toxin than either $Corn_{(1)}$, $Corn_{(2)}$, or the hybrid population as indicated by its greatly decreased proportion of survival (Fig. 5a). This result is in agreement with the general trend present in the available literature that rice strain fall armyworm are more susceptible to Bt toxins than corn strain (Adamczyk et al. 1997, Ríos-Díez et al. 2012). The rice strain population also had a lower proportion of survival than each of the other three fall armyworm populations tested when reared on the non-Bt near isoline corn variety (Fig. 5a). This may indicate that the rice strain population exhibits generally reduced fitness when reared on corn. However, the proportion of survival for the rice strain population was not significantly different from either of the three remaining populations when reared on corn in the tissue-

based feeding experiments (Fig. 6a); nor did the weight or head capsule widths of surviving rice strain larvae significantly differ from Corn$_{(1)}$ or the hybrid population (Fig. 6b,c). Thus, the rice strain population used may have responded adversely to the non-Bt near isoline corn variety used in the tissue-based bioassays. An alternative explanation is that rice strain fall armyworm exhibit poorer performance on corn tissue when compared to the corn strain. Veenstra et al. (1995) conducted comparative corn tissue consumption and utilization studies coupled with assays to measure enzymatic activity in the gut. It was determined that the rice strain population tested exhibited lower consumption rates and efficiency of converted digested food into biomass than the tested corn strain population; the rice strain population also had overall lower levels of enzymatic activity in the gut (Veenstra et al. 1995). However, this again does not account for the performance of the rice strain population on corn tissue in the tissue-based feeding experiments (Fig. 6). The only existing study in the current literature that has examined fall armyworm host strain tolerances to Cry1F was conducted by Virla et al. (2008). A diet-based bioassay was employed, resulting in mixed results where some rice strain populations exhibited decreased tolerance to Cry1F when compared to corn strain populations, and others exhibited increased tolerance (Virla et al. 2008).

The proportions of survival for the hybrid population reared on Cry1F and non-Bt near isoline corn tissue in the tissue-based bioassays were similar to those of the two corn strain populations (Fig. 5a). Following the general trend that corn strain fall armyworm are more tolerant to Bt toxins than rice strain fall armyworm (Adamczyk et al. 1997, Ríos-Díez et al. 2012), there may be a certain degree of dominance associated with the corn strain's Bt tolerance that is passed on to hybrid offspring. However, the current lack

of literature pertaining to rice-corn hybrid fall armyworm makes it very difficult to draw conclusions at this time. Surviving hybrid larvae reared on non-Bt near isoline corn tissue weighed significantly less than either corn strain population, but not the corn strain population (Fig. 5b). Similar to the proportions of survival of the rice strain, the decreased weight of surviving hybrid larvae reared on the non-Bt near isoline corn variety is not reflected in the tissue-based feeding experiments, suggesting a possible adverse effect of the non-Bt near isoline corn variety used in the tissue-based bioassays.

The tissue-based feeding experiments were performed using one host plant commonly associated with corn strain fall armyworm (corn) and two commonly associated with the rice strain (rice and Bermuda grass). Each of the four fall armyworm populations performed nearly equally when reared on corn tissue, (the exception being Corn$_{(2)}$'s greater weight and HCW [Fig 6]). Corn appears to be a suitable host plant capable of supporting larval fall armyworm from each of the four populations examined. This is not surprising given numerous reports of larval fall armyworm feeding on corn plants throughout its history of study worldwide (Luginbill 1928, Sparks et al. 1979, Raulston et al. 1986, Sparks 1986, Prowell et al. 2004, Storer et al. 2010, Ríos-Díez et al. 2012, Murúa et al. 2015, Georgen et al. 2016), and congruent with rice strain collections from corn fields (Prowell et al. 2004, Nagoshi et al. 2006b, Groot et al. 2010) as well as the tendency for the rice strain to occur sympatrically with the corn strain (Pashley 1988). Corn$_{(1)}$ performed poorly when reared on rice tissue, but very well when reared on Bermuda grass (Fig. 6), an apparent contradiction considering that both of these hosts are associated more closely with rice strain fall armyworm than corn strain. Corn$_{(2)}$'s apparent vigor when reared on rice tissue is similarly confusing, exhibiting significantly

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increased weight and HCW when compared to each of the three remaining populations (Fig. 6). It is possible that Corn$_{(2)}$'s long removal from the field is the root cause of this effect; however, its extreme vigor was not apparent when reared on Bermuda grass (Fig. 6). It may be possible that corn and rice tissue are more nutritionally similar to the artificial diet Corn$_{(2)}$ was reared on than Bermuda grass tissue, explaining its increased performance on corn and rice tissue, but not Bermuda grass (Fig. 6). Additionally, the rice strain population performed equally well when reared on corn and rice tissue, but had decreased mean weights of surviving larvae (though not significant) and HCW when larvae were reared on Bermuda grass (Fig. 6b,c); this is inconsistent with the rice strain population's original collection from a pasture grass field. It cannot be discounted at this time that the experimental methods used in the tissue-based feeding experiments generally favored corn and rice tissue over Bermuda grass; which would explain the results for Corn$_{(2)}$ being more in-line with the remaining three populations, and the rice strain population's reduced fitness; though not the difference in Corn$_{(1)}$'s fitness on rice versus Bermuda grass.

Previous studies on the effects of host plant on the survival and fitness of rice and corn strain fall armyworm larvae have reported highly contradictory results (Groot et al. 2010). Whitford et al. (1988) reported decreased survival, larval weights, and pupal weights, and increased pupal duration in rice strain larvae reared on corn when compared to the corn strain. Pashley et al. (1995) would later corroborate these results by reporting decreased rice strain larval and pupal weights compared to the corn strain when reared on corn tissue. However, Meagher et al (2004) published contrasting results indicating that rice strain larvae had significantly greater survival and larval weight, and decreased

development times, when reared on corn tissue; though decreased pupal weights were also found. Groot et al. (2010) did not find any differences in larval weight, but did report that rice strain larvae developed faster on corn tissue. Additionally, larval fitness of corn and rice strain larvae on host plants does not appear to affect adult fecundity (Pashley et al. 1995). The lack of consistency in reported results may be due to variability in the conditions present in collection sites, or possibly overall genotypic or phenotypic variation in field-collected populations (Stuhl et al. 2008, Groot et al. 2010). Considering that fall armyworm migrate great distances yearly, on a somewhat variable route where a variety of host plants are encountered (Hogg et al. 1982, Sparks 1986, Mitchell et al. 1991) it is unlikely that the differences in host plant utilization associated with corn and rice strain fall armyworm are due to larval host adaptation. Similar to the Cry1F tissue-based bioassays, the lack of available benchmarks for rice-corn hybrid fall armyworm makes it difficult to draw confident conclusions regarding the host plant tissue feeding of the hybrid population at this time. It is pertinent to note that the current literature suggests that hybridization events are more likely to occur in corn rich environments (Pashley 1988, Nagoshi et al. 2006b, Groot et al. 2010, Prowell et al. 2010, Saldamando and Vélez-Arango 2010), and the results for the hybrid population generally support that corn is a suitable host for the population's larval development (Figs. 5,6).

The overall results of this study indicate that corn is a preferred oviposition substrate for both the rice and corn strains, and a suitable host plant for the larval development of rice strain, corn strain, and rice-corn hybrid fall armyworm. Considering evidence of differential Bt susceptibilities between rice and corn strain fall armyworm (Adamczyk et al. 1997, Virla et al. 2008, Ríos-Díez et al. 2012), with corn strain fall

armyworm being generally more tolerant to Bt toxins than rice strain; a propensity for both host strains to utilize corn as a primary host could affect a field population's overall Bt susceptibility depending on the proportions of each host strain present. A fall armyworm population comprised predominantly of corn strain individuals may be more tolerant to Bt overall than a population with a significant proportion of rice strain insects. It is also possible that the production of rice-corn hybrid offspring in a mixed population could offset the effects of rice strain presence in lowering the population's overall Bt susceptibility, given the similarity of corn strain and rice-corn hybrid tolerance to Cry1F corn tissue reported here. Hybridization events would only serve to increase the rate of resistance evolution. The current lack of information pertaining to fall armyworm host strains makes management recommendations difficult. More studies comparing the host utilization, Bt tolerances, and distribution of fall armyworm host strains are currently required, and future studies should make note of the host strains of any insect populations used. Such information could be vital to the construction of more effective IPM and IRM strategies for this damaging pest.

Table 2: One-way ANOVA analyses for no-choice experiments comparing fall armyworm host strain oviposition preferences

Analysis	Population	df[a]	F	P
Masses	Rice	3,39	2.82	0.0524
	Corn$_{(1)}$	3,39	2.05	0.1248
Eggs per mass	Rice	3,41	0.19	0.9015
	Corn$_{(1)}$	3,53	0.94	0.4273

[a] numerator and denominator degrees of freedom

Table 3: Exact tests of goodness-of-fit for two-choice experiments comparing fall armyworm host strain oviposition preferences

Population	Rice		Corn$_{(1)}$	
Comparison	Masses[a]	P[b]	Masses	P
Corn / Rice	6 / 1	0.1250	8 / 2	0.1094
Corn / BG[c]	1 / 6	0.1250	15 / 1	0.0005
Corn / Cotton	9 / 0	0.0039	14 / 2	0.0042
Rice / BG	3 / 2	1.0000	2 / 1	1.0000
Rice / Cotton	3 / 0	0.2500	4 / 4	1.0000
BG / Cotton	2 / 0	0.5000	3 / 3	1.0000

[a] Number position corresponds to the host plant, e.g.: for corn / rice, eight egg masses were deposited on corn, and two were oviposited on rice.
[b] Compared to an expected 1 / 1 ratio.
[c] BG = Bermuda grass

Table 4: Two-way ANOVA analyses for Cry1F tissue-based bioassays comparing fall armyworm host strain Bt tolerances

Analysis	Effect	df[a]	*F*	*P*
Survival	Population	3,479	32.15	<0.0001
	Variety	1,479	66.16	<0.0001
	Population*Variety	3,479	2.54	0.0558
Weight	Population	3,232	43.20	<0.0001
	Variety	1,232	141.43	<0.0001
	Population*Variety	3,232	37.20	<0.0001

[a] numerator and denominator degrees of freedom

Table 5: Two-way ANOVA analyses for host strains of fall armyworm feeding on three host plants: corn, rice, and Bermuda grass.

Analysis	Effect	df[a]	*F*	*P*
Survival	Population	3,239	4.18	0.0066
	Host	2,239	6.50	0.0018
	Population*Host	6,239	7.63	<0.0001
Weight	Population	3,150	27.98	<0.0001
	Host	2,150	38.90	<0.0001
	Population*Host	6,150	5.26	<0.0001
HCW[b]	Population	3,150	34.48	<0.0001
	Host	2,150	44.41	<0.0001
	Population*Host	6,150	3.72	0.0018

[a] Numerator and denominator degrees of freedom
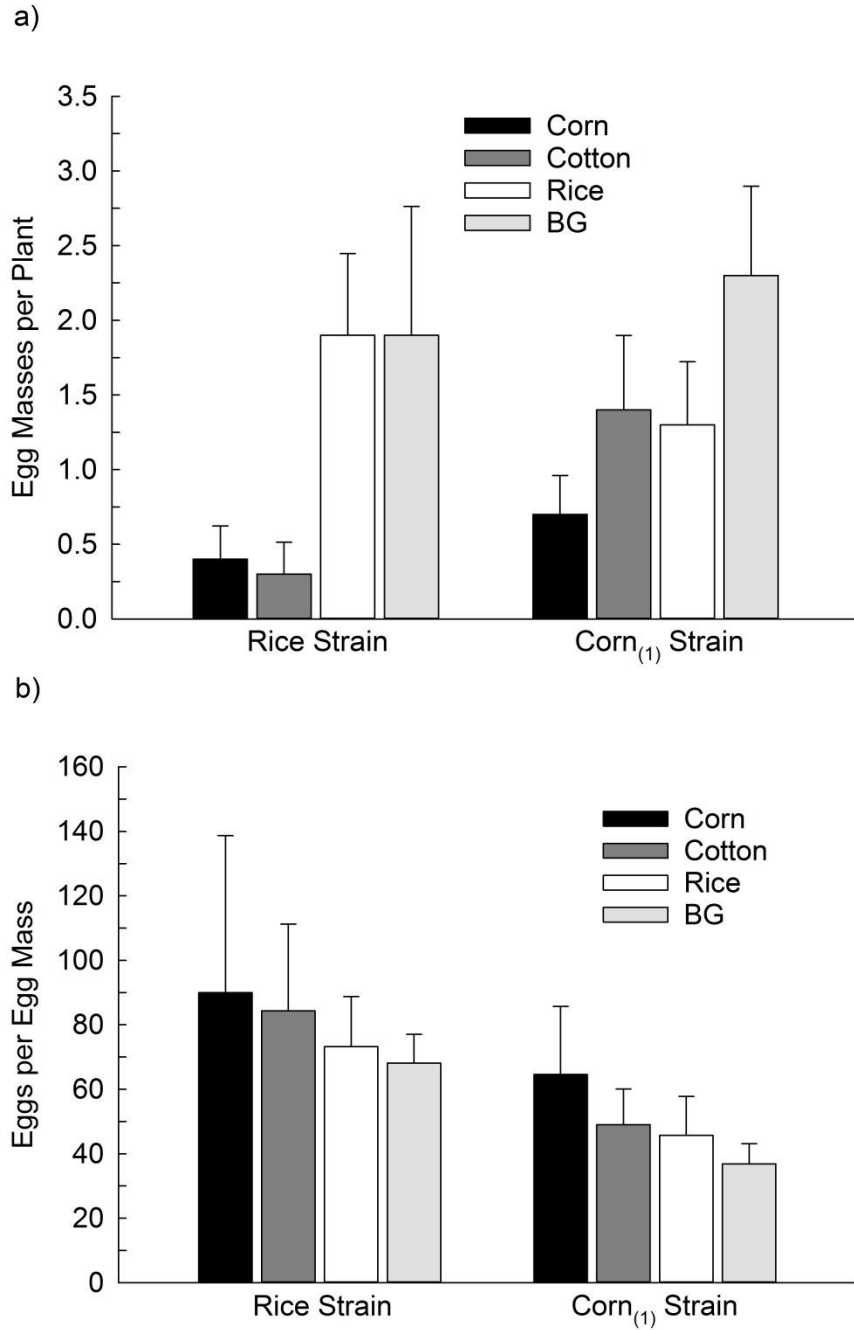[b] Head capsule width

a)

b)

Fig. 3: No-choice host oviposition by rice and corn strains of fall armyworm. a) Mean egg masses per plant. b) Mean eggs per collected egg mass. Bermuda grass is abbreviated as "BG." Bars represent the mean number of egg masses per plant, and error bars are the standard errors of the means.
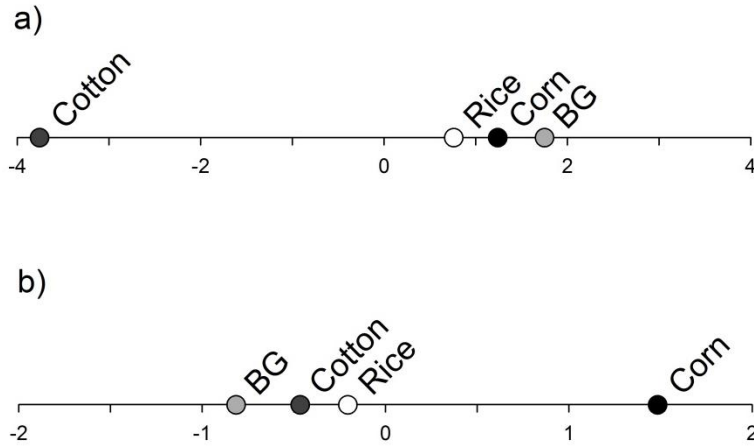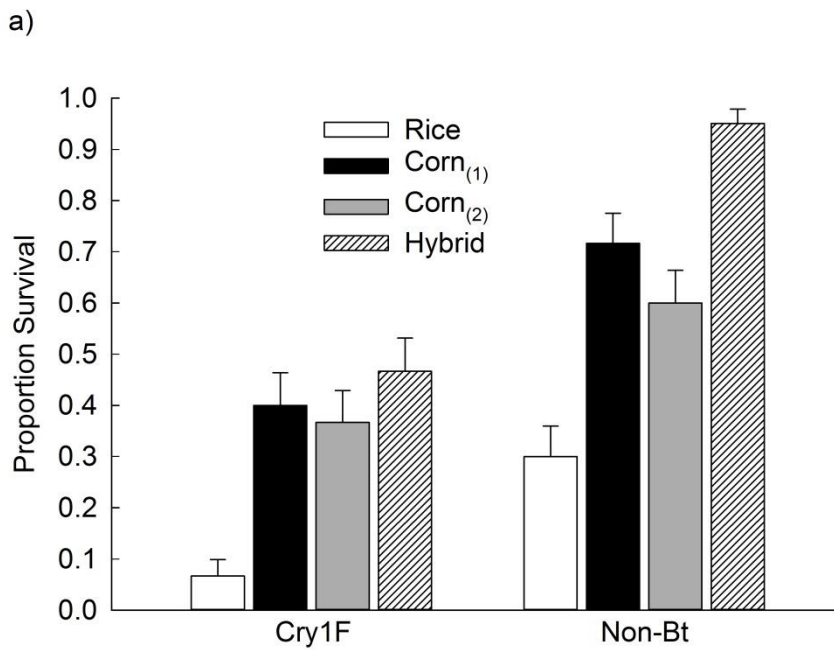
Fig. 4: Fall armyworm oviposition preference indices for a) Rice strain and b) Corn$_{(1)}$ strain. Greater distance between points determines preference, e.g.: in a) corn is significantly preferred over cotton, but not significantly preferred over rice or Bermuda grass. Bermuda grass is abbreviated as "BG."
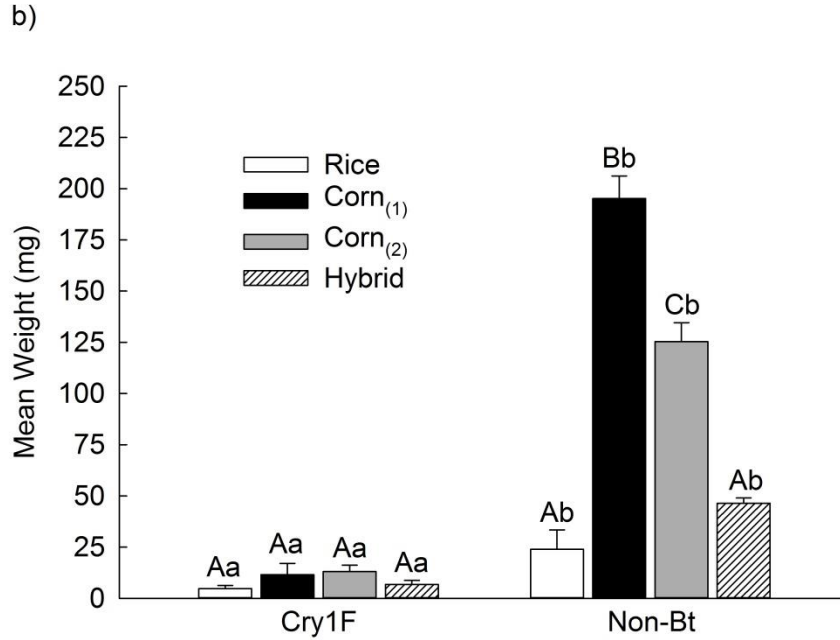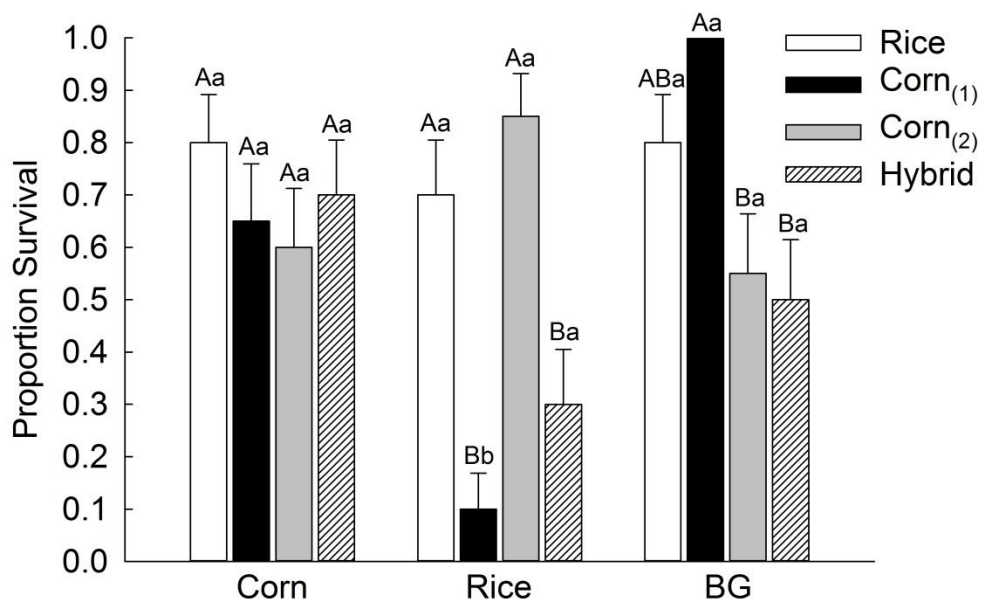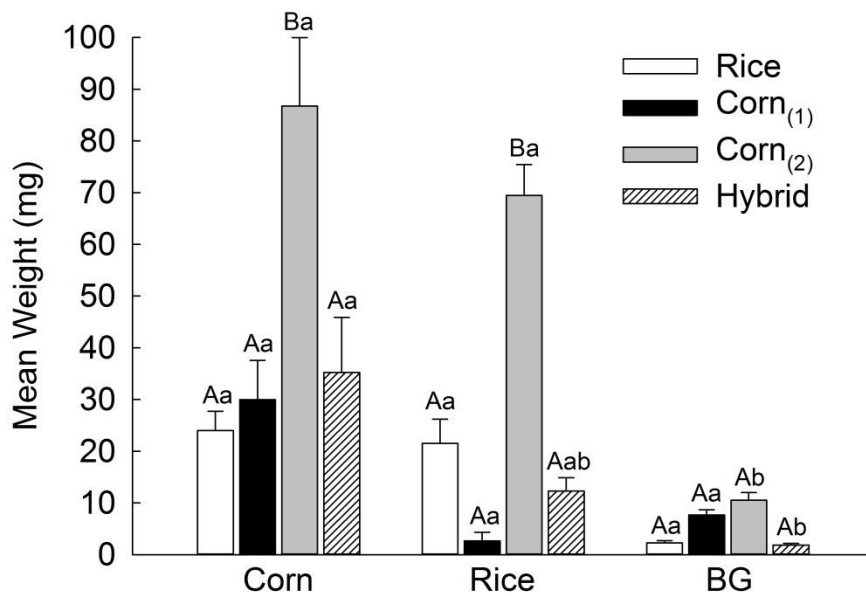
a)

b)

Fig. 5: Cry1F tissue-based bioassays for four fall armyworm host strain populations. a) Proportion of survival. b) Mean weight in mg of surviving larvae. Bars represent mean values, and error bars are the standard errors of the means. Uppercase letters represent pairwise comparisons of fall armyworm populations within each corn variety. Lowercase letters represent pairwise comparisons of each fall armyworm population between corn varieties.
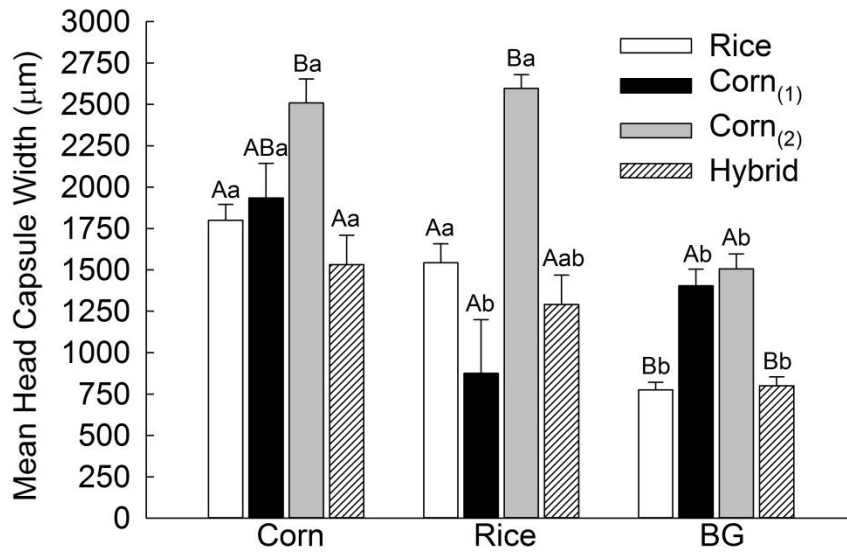
c)

Fig. 6: Larval growth and development on host plant tissues for four fall armyworm host strain populations. a) Proportion survival. b) Mean weight in mg of surviving larvae. c) Mean head capsule width in μm of surviving larvae. Bermuda grass is abbreviated to "BG." Bars represent mean values, and error bars are the standard errors of the means. Capital letters represent pairwise comparisons within each host plant, and lowercase letters represent pairwise comparisons of each fall armyworm population between host plants.

REFERENCES

Abendroth, L. J., R. W. Elmore, M. J. boyer, and S. K. Marlay. 2011. Corn Growth and Development Iowa State University Extension and Outreach, Ames, Iowa.

Adamczyk Jr., J. J., J. W. Holloway, B. R. Leonard, and J. B. Graves. 1997. Susceptibility of fall armyworm collected from different plant hosts to selected insecticides and transgenic Bt cotton. J. Cotton Sci. 1: 21-28.

Barros, E. M., J. B. Torres, and A. F. Bueno. 2010. Oviposition, development, and reproduction of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) fed on different hosts of economic importance. Neotrop. Entomol. 39: 996-1001.

Binning, R. R., J. Coats, X. Kong, and R. L. Helmich. 2014. Susceptibility and aversion of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Cry1F Bt maize and considerations for insect resistance management. J. Econ. Entomol. 107: 368-374.

Bush, G. L. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). Evolution 23: 237–251.

Butt, B. A. and E. Cantu. 1962. Sex determination of lepidopterous pupae. United States Department of Agriculture, Agricultural Research Service. Publication USDA-ARS 33-75. 7 pp.

Cañas-Hoyos, N., E. J. Márquez, and C. I. Saldamando-Benjumea. 2014. Differentiation of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) corn and rice strains from central Colombia: a wing morphometric approach. Ann. Entomol. Soc. Am. 107: 575-581

Cano-Calle, D., R. E. Arango-Isaza, and C. I. Saldamando-Benjumea. 2015. Molecular identification of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) corn and rice strains in Colombia by using a PCR-RDLP of the mitochondrial gene cytochrome oxidase I (COI) and a PCR of the gene FR (for rice). Ann. Entomol. Soc. Am.: 1-9

Cruz, L. and F. T. Turpin. 1983. Yield impact of larval infestations of the fall armyworm (Lepidoptera: Noctuidae) to midwhorl growth stages of corn. J. Econ. Entomol. 76: 1052-1054.

Csiszár, I., and G. Tusnády. 1984. Information geometry and alternating mimization procedures. Stat. Decis. 1: 205-237.

Diehl, S. R., and G. L. Bush. 1984. An evolutionary and applied perspective of insect biotypes. Ann. Rev. Entomol. 29: 471-504.

Drès, M., and J. Mallet. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. Phil. Trans. R. Soc. Lond. B. 357: 471-492.

Farias, J. R., D. A. Andow, R. J. Horikoshi, R. J. Sorgatto, P. Fresia, A. C. Santos, and C. Omoto. 2014. Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: Nocutidae) in Brazil. Crop Prot. 64: 150-158.

Fleischer, S. J., C. L. Harding, P. E. Blom, J. White, and J. Grehan. 2005. *Spodoptera frugiperda* pheromone lures to avoid nontarget captures of *Leucania phragmatidicola*. J. Econ. Entomol. 98: 66-71.

Glaser, J. A. and S. R. Matten. 2003. Sustainability of insect resistance management strategies for transgenic Bt corn. Biotechnol. Adv. 22: 45-69.

Georgen, G., P. L. Kumar, S. B. Sankungh, A. Togola, and M. Tamò. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae), a New Alien Invasive Pestnew alien invasive pest in west and central Africa. PLoS ONE 11(10).

Groot, A. T., M. Marr, D. G. Heckel, and G. Schöfl. 2010. The roles and interactions of reproductive isolation mechanisms in fall armyworm (Lepidoptera: Noctuidae) host strains. Ecol. Entomol. 55: 105-118.

Hardke, J. T., B. R. Leonard, F. Huang, and R. E. Jackson. 2011. Damage and survivorship of fall armyworm (Lepidoptera: Noctuidae) on transgenic field corn expressing *Bacillus thuringiensis* Cry proteins. Crop Prot. 30: 168-172.

Harrington, S. 2010. Greenhouse procedures manual: growing rice under controlled conditions. Cornell University. http://ricelab.plbr.cornell.edu/greenhouse_procedures_manual [Accessed 2 February 2017].

Hogg, D. B., H. N. Pitre, and R. A. Anderson. 1982. Assessment of early-season phenology of the fall armyworm (Lepidoptera: Noctuidae) in Mississippi. Environ. Entomol. 11: 705-710.

Jaenike, J. 1981. Criteria for ascertaining the existence of host races. Am. Nat. 117: 830–834.

Jakka, S. R. K., V. R. Knight, and J. L. Jurat-Fuentes. 2014. Fitness costs associated with field-evolved resistance to bt maize in *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Econ. Entomol. 107: 342-351.

Johnson, S. J. 1987. Migration and the life history strategy of the fall armyworm, *Spodoptera frugiperda* in the western hemisphere. Insect Sci. Its Appl. 8: 543-549.

Luginbill, P. 1928. The fall armyworm. USDA Tech. Bull. 34.

Luttrell, R. G., and J. S. Mink. 1999. Damage to cotton fruiting structures by the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Cotton Sci. 3: 35-44.

McDonald, J. H., A. M. Grasso, and L. K. Rejto. 1999. Patterns of temperature adaptation in proteins from *Methanococcus* and *Bacillus*. Mol. Biol. Evol. 16: 1787-1790.

McMichael, M., and D. P. Prowell. 1999. Differences in amplified fragment-length polymorphisms in fall armyworm (Lepidoptera: Noctuidae) host strains. Ann. Entomol. Soc. Am. 92: 175-181.

Meagher, R. L. 2001. Trapping fall armyworm (Lepidoptera: Noctuidae) adults in traps baited with pheromone and a synthetic floral volatile compound. Fla. Entomol. 84: 288-292.

Meagher, R. L., R. N. Nagoshi, C. Stuhl, and E. R. Mitchell. 2004. Larval development of fall armyworm (Lepidoptera Noctuidae) on different cover crop plants. Fla. Entomol. 87: 454-460.

Meagher, R. L., R. N. Nagoshi, and C. J. Stuhl. 2011. Oviposition choice of two fall armyworm (Lepidoptera: Noctuidae) host strains. J. Insect Behav. 24: 337-347.

Mitchell, E. R., J. N. McNeil, J. K. Westbrook, J. F. Silvain, B. Lalanne-Cassou, R. B. Chalfant, S. D. Pair, V. H. Waddill, A. Sotomayor-Rios, and F. I. Proshold. 1991. Seasonal periodicity of fall armyworm (Lepidoptera: Noctuidae) in the Caribbean basin and northward to Canada. J. Entomol. Sci. 26: 39-50.

Murúa, M. G., R. N. Nagoshi, D. A. Dos Santos, M. M. Hay-Roe, R. K. Meagher, and J. C. Vilardi. 2015. Demonstration using field collections that Argentina fall armyworm populations exhibit strain-specific host plant preferences. J. Econ. Entomol. 108: 2305-2315.

Nagoshi, R. N., and R. Meagher. 2008. Review of fall armyworm (Lepidoptera: Noctuidae) genetic complexity and migration. Fla. Entomol. 91: 546-554.

Nagoshi, R. N., R. L. Meagher, J. J. Adamczyk, S. K. Braman, R. L. Brandenburg, and G. Nuessly. 2006a. New restriction fragment length polymorphisms in the cytrochrome oxidase I gene facilitate host strain identification of fall armyworm (Lepidoptera: Noctuidae) populations in the southeastern United States. J. Econ. Entomol. 99: 671-677.

Nagoshi, R. N., R. L. Meagher, G. Nuessly, and D. G. Hall. 2006b. Effects of fall armyworm (Lepidoptera: Noctuidae) interstrain mating in wild populations. Environ. Entomol. 35: 561-568.

Nagoshi, R. N., P. Silvie, and R. L. Meagher. 2007. Comparison of haplotype frequencies differentiate fall armyworm (Lepidoptera: Noctuidae) corn-strain populations from Florida and Brazil. J. Econ. Entomol. 100: 954-96.

Nagoshi, R. N., S. Fleischer, and R. L. Meagher. 2009. Texas is the overwintering source of fall armyworm in central Pennsylvania: implications for migration into the northeastern United States. Environ. Entomol. 38: 1546-1554.

Nagoshi, R. N., R. L. Meagher, and M. Hay-Roe. 2012. Inferring the annual migration patterns of fall armyworm (Lepidoptera: Noctuidae) in United States from mitochondrial haplotypes. Ecol. Evol. 2: 1458-1467.

Nagoshi, R. N., N. Rosas-García, R. L. Meagher, S. J. Fleischer, J. K. Westbrook, T. W. Sappington, M. Hay-Roe, J. M. G. Thomas, and G. M. Murua. 2015. Haplotype profile comparisons between *Spodoptera frugiperda* (Lepidoptera: Noctuidae) populations from Mexico with those form Puerto Rico, South America, and the United States and their implications to migratory behavior. J. Econ. Entomol. 108: 135-144.

Niu, Y., R. L. Meagher, F. Yang, and F. Huang. 2013. Susceptibility of field populations of the fall armyworm (Lepidoptera: Noctuidae) from Florida and Puerto Rico to purified Cry1F protein and corn leaf tissue containing single and pyramided Bt genes. Fla. Entomol. 69: 701-713.

Niu, Y., F. Yang, V. Dangal, and F. Huang. 2014. Larval survival and plant injury of Cry1F-susceptible, -resistant, and –heterozygous fall armyworm (Lepidoptera: Noctuidae) on non-Bt and Bt corn containing single or pyramided genes. Crop Prot. 59: 22-28.

Pair, S. D., J. R. Raulston, A. N. Sparks, J. K. Westbrook, and G. K. Douce. 1986. Fall armyworm distribution and population dynamics in the southeastern States. Fla. Entomol. 69: 468-487.

Pashley, D. 1986. Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): a sibling species complex? Ann. Entomol. Soc. Am. 79: 898-904.

Pashley, D. 1988. Current status of fall armyworm host strains. Fla. Entomol. 71: 227-234.

Pashley, D., and J. A. Martin. 1987. Reproductive incompatibility between host strains of the fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 80: 731-733.

Pashley, D., S. J. Johnson, and A. N. Sparks. 1985. Genetic population structure of migratory moths: the fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 78: 756-762.

Pashley, D., T. C. Sparks, S. W. Quisenberry, T. Jamjanya, and P. F. Dowd. 1987. Two fall armyworm strains feed on corn, rice and Bermuda grass. Louisiana Agric. 30: 8-9.

Pashley, D., A. M. Hammond, and T. N. Hardy. 1992. Reproductive isolating mechanisms in fall army worm host strains (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 84: 400-405.

Perkins, W. D. 1979. Laboratory rearing of the fall armyworm. Fla. Entomol. 62: 87-91.

Pitre, H. N., J. E. Mulrooney, and D. B. Hogg. 1983. Fall armyworm (Lepidoptera: Noctuidae) oviposition: crop preferences and egg distribution on plants. J. Econ. Entomol. 76: 463-466.

Prowell, D., P. M. McMichael, and J. –F. Silvain. 2004. Multilocus genetic analysis of host use, introgression, and speciation in host strains of fall armyworm (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 97: 1034-1044.

Raulston, J. R., S. D. Pair, A. N. Sparks, J. Loera G., F. A. Pedraza M., A. Palamon T., A. Ortega, J. Ruiz Sanchez M., P. Marquez C., H Rueles A., J. Perez M., R. Rodriguez R., H. Carrillo R., R. Archundia R., and F. Herrera R. 1986. Fall armyworm distribution and population dynamics in the Texas-Mexico gulf coast area. Fla. Entomol. 69: 455-467.

Ríos-Díez, J. D., B. Siegfried, and C. I. Saldamando-Benjumea. 2012. Susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains from central Colombia to Cry1Ab and Cry1Ac entotoxins of *Bacillus thuringiensis*. Southwest. Entomol. 37: 281-293.

Saldamando, C. I., and A. M. Vélez-Arango. 2010. Host plant association and genetic differentiation of corn and rice strains of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) in Colombia. Neotrop. Entomol. 39: 921-929.

Santos, L. M., L. R. Redaelli, L. M. G. Diefenbach, and C. F. S. Efrom. 2002. Larval and pupal stage of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) in sweet and field corn genotypes. Braz. J. Biol. 63: 627-633.

Schöfl, G., D. C. Heckel, and A. T. Groot. 2009. Time-shifted reproductive behaviors among fall armyworm (Noctuidae: *Spodoptera frugiperda*) host strains: evidence for differing modes of inheritance. J. Evol. Biol. 22: 1447-1459.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry, 3rd ed. W. H. Freeman and Company, New York, NY.

Sparks, A. N. 1979. A review of the biology of the fall armyworm. Fla. Entomol. 62: 82-87.

Sparks, A. N. 1986. Fall armyworm (Lepidoptera: Noctuidae) potential for area-wide management. Fla. Entomol. 69: 603-314.

Stuhl, C., R. L. Meagher, and R. N. Nagoshi. 2008. Genetic variation in neonate behavior of fall armyworm (Lepidoptera: Noctuidae). Fla. Entomol. 91: 151-158.

Storer, N. P., J. M. Babcock, M. Schlenz, T. Meade, G. T. Thompson, J. W. Bing, and R. M. Huckaba. 2010. Discovery and characterization of field resistance to Bt maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico. J. Econ. Entomol. 103: 1031-1038.

Stuhl, C. J., R. L. Meagher, and R. N. Nagoshi. 2008. Genetic variation in neonate behavior of fall armyworm (Lepidoptera: Noctuidae). Fla. Entomol. 91: 151-158.

Téllez-Rodriguez, P., B. Raymond, I. Morán-Bertot, L. Rodríguez-Cabreara, D. J. Wright, C. G. Borroto, and C. Ayra-Pardo. 2014. Strong oviposition preference for Bt over non-Bt maize in *Spodoptera frugiperda* and its implications for the evolution of resistance. BMC Biol. 12: 48.

Thrash, B., J. J. Adamczyk, G. Lorenz, A. W. Scott, J. S. Armstrong, R. Pfannelstein, and N. Taillon. 2013. Laboratory evaluations of Lepidopteran-active soybean seed treatments on survivorship of fall armyworm (Lepidoptera: Noctuidae) larvae. Fla. Entomol. 96: 724-728.

Veenstra, K. H., D. P. Pashley, and J. A. Ottea. 1995. Host-plant adaptation in fall armyworm host strains: comparisons of food consumption, utilization, and detoxification enzyme activities. Ann. Entomol. Soc. Am. 88: 80-91.

Vélez, A. M., T. A. Spencer, A. P. Alves, D. Moellenbeck, R. L. Meagher, H. Chirakkal, and B. D. Siegfried. 2013. Inheritance of Cry1F resistance, cross-resistance and frequency of resistant alleles in *Spodoptera frugiperda* (Lepidoptera: Noctuidae). B. Entomol. Res. 103: 700-713.

Vélez, A. M., T. A. Spencer, A. P. Alves, L. B. Crespo, and B. D. Siegfried. 2014. Fitness costs of Cry1F resistance in fall armyworm *Spodoptera frugiperda*. J. Appl. Entomol. 138: 315-325.

Virla, E. G., A. Álvares, F. Loto, L. M. Pera, and M. Baigorí. 2008. Fall armyworm strains (Lepidoptera: Noctuidae) in Argentina, their associate host plants and response to different mortality factors in laboratory. Fla. Entomol. 9: 63-69.

Whitford, F., S. S. Quisenberry, T. J. Riley, and J. W. Lee. 1988. Oviposition preference, mating compatibility, and development of two fall armyworm strains. Fla. Entomol. 71: 234-243.

Young, J. R. 1979. Fall armyworm: control with insecticides. Fla. Entomol. 62: 130-133.