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Integrated pest management of the Mexican rice borer in Louisiana and Texas sugarcane and rice

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INTEGRATED PEST MANAGEMENT OF THE MEXICAN RICE BORER IN LOUISIANA AND TEXAS SUGARCANE AND RICE

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Interactions between sugarcane, *Saccharum* spp., and the invasive species Mexican rice borer, *Eoreuma loftini* (Dyar), were evaluated in field experiments and in the greenhouse with rice, *Oryza sativa* L., as an additional host. By determining adult emergence holes together with percent bored internodes, a novel method for evaluating sugarcane cultivar resistance was developed. In 2001, LCP 85-384 had the greatest moth production per hectare, significantly higher ($P \leq 0.05$) than HoCP 85-845. High levels of sodium and magnesium salt stress in the soil were associated with higher *E. loftini* injury in all cultivars except HoCP 91-555 and CP 70-321.

Irrigation reduced injury in both susceptible (LCP 85-384) and resistant (HoCP 85-845) cultivars by 2.5-fold. The combination of irrigation, plant resistance, and insecticide applications of tebufenozide decreased injury from 70% bored internodes to less than 10%. Several free amino acids essential for insect development increased in sugarcane leaves under drought stressed conditions, which exacerbated *E. loftini* infestations.

Drought stressed sugarcane was 1.8-fold more attractive based on egg masses/plant than non stressed sugarcane. Based on egg masses/plant and eggs/egg mass, cultivar LCP 85-384 was more attractive than the resistant HoCP 85-845. Egg masses were 9.2-fold more abundant on sugarcane than on rice. Oviposition on sugarcane occurred exclusively on dry leaf material, and the number of dry leaves was positively correlated with egg masses per plant. Several free amino acids essential for insect development increased in sugarcane leaves under drought stressed conditions, and were

highly correlated with egg masses per plant. Rice leaves, despite being less attractive for oviposition, had higher levels of free amino acids than sugarcane.

Based on boundary movement monitoring with pheromone traps, the average rate of spread from 1980 (Weslaco, TX) to 2004 (Chambers County, TX) was 23.2 km/yr. From 2000 to 2004, annual mean centroids of moth trap counts moved 29.3 km, however 95% C.I. overlapped across years. Minimizing sugarcane stress will play a major role in managing this invasive pest when it becomes established in Louisiana.

INTRODUCTION

The Mexican rice borer, *Eoreuma loftini* (Dyar), is the major insect pest of sugarcane in the Lower Rio Grande Valley of Texas, representing more than 95% of stalk borer populations on sugarcane (Legaspi et al. 1999c). Introduced from Mexico, it was first detected in the Lower Rio Grande Valley of Texas in 1980 (Johnson and van Leerdam 1981). Sugarcane damage has averaged between 20 and 30% bored internodes (Meagher et al. 1993, Legaspi et al. 1997a). Estimates of the effects of borer damage, based on a 20% level of bored internodes, on revenue have varied between $575/ha, when considering only the producer's loss (Meagher et al. 1994), and $1,181.04/ha, when considering the effect on all involved parties (producer and mill) (Legaspi et al. 1999a).

The detection of the insect in Texas in 1980 created concern among Louisiana and Florida sugarcane growers (Fors 1981). By 1989, its range had expanded into the rice production area of Texas (Browning et al. 1989) where it is responsible for increasing yield loss in rice. With *E. loftini* moths discovered in the sugarcane production area near Beaumont, Texas, invasion of Louisiana sugarcane fields is expected (Reagan et al. 2005). Pheromone traps can be valuable in monitoring the movement of invasive insect pests (Robacker and Landholt 2002), and were used in the Texas Rice Belt to follow moth movement and to estimate the rate of spread (Chapter 5).

Prior to the establishment of *E. loftini* in Louisiana sugarcane and rice, effective management programs are needed. Insecticide studies (Johnson 1985, Meagher et al. 1994, Legaspi et al. 1999c) and extensive attempts at classical biological control involving several millions of dollars (Meagher et al. 1998) have not resulted in effective

E. loftini control programs. These efforts led to our investigation of alternative management approaches. The evaluation of Louisiana and Texas sugarcane cultivars for resistance to *E. loftini* was a primary goal in this work. The development of a novel method of resistance assessment to *E. loftini* was based not only on plant injury, but also on moth production per hectare (Chapter 2). In addition to reducing pest injury on an individual field basis, areawide pest management aims to reduce population levels of the target organism over a large geographical area. Under heavy infestations, the use of plant resistance may not be sufficient to maintain injury levels below economic thresholds. A field study was therefore conducted to evaluate a combination of several management tactics, namely irrigation management, plant resistance, and insecticide applications (Chapter 3). To assist in understanding the insect-crop interactions, plant physiology measurements were made on sugarcane in field management experiments at Ganado, TX, as well as on plants in greenhouse studies, where the oviposition preference of *E. loftini* was determined on both sugarcane and rice (Chapter 4). Because of the limited mobility of first instar larvae for feeding and survival, oviposition of Lepidoptera insects is a critical step in their life cycle (Feeny et al. 1983). Determining causal factors underlying oviposition patterns and quantifying oviposition preference of insect pests for host crops can help understand insect-plant relationships (Renwick and Chew 1994) and therefore assist in developing pest management strategies (Showler 2004a).

This work has provided critical information on a devastating insect pest of sugarcane and rice. The use of several control tactics in combination creates a more effective and environmentally friendly pest management strategy with greater permanency. Furthermore, by providing underlying biochemical mechanisms, this work

has enhanced our understanding of the population dynamics of *E. loftini* on both sugarcane and rice. The study of both these crops has allowed a holistic approach to managing this pest in the Louisiana and Texas agroecosystem, a necessity when conceptualizing areawide cross-regional management strategies.

CHAPTER 1: LITERATURE REVIEW

1.1. Mexican Rice Borer Taxonomy

The Mexican rice borer *Eoreuma loftini* (Dyar) is a stalk borer belonging to the family Crambidae. Dyar (1917) first described this species while studying single specimens reared from different host plants in Arizona and described two nominal species: *Chilo loftini* (from sugarcane) and *Chilo opinionellus* (from wheat). Bleszynski (1967) classified *C. loftini* in the genus *Acigona* Hübner, while Klots (1970) showed that the two nominal species described by Dyar were conspecific, and should be moved to *Eoreuma* Ely, belonging to the tribe *Chiloini* (Klots 1970) or *Chilonini* (Gaskin 1973).

1.2. Mexican Rice Borer Distribution and Host Plants

Following Dyar's description, *E. loftini* was found on commercial sugarcane on the west coast of Mexico (Morill 1925, Van Zwalunwenburg, 1926) in the states of Baja California, Sonora, Sinaloa, Nayarit, Jalisco, Colima, Michoacán and Huastecas (Van Zwalunwenburg 1950, Riess 1981, Johnson 1984). The range later expanded to eastern Mexico, with recoveries made in Nuevo Leon, Tamaulipas, San Luis Potosi and Veracruz (Rodriguez-del-Bosque et al. 1989), and to southeastern Mexico in Oaxaca (Rodriguez-del-Bosque and Smith 1991) and Yucatan (Klots 1970).

After Dyar's initial recovery of the insect in Arizona, more specimens were found in southern Arizona (Van Zwalunwenburg, 1926) and in the Imperial Valley in California, close to the Mexican border (Osborn and Phillips 1946). *Eoreuma loftini* was first detected in the Lower Rio Grande Valley of Texas in 1980 (Johnson and van

Leerdam 1981), and by the end of the 1980s, its range had expanded well into the rice production area of Texas (Browning et al. 1989).

The Mexican rice borer has a wide range of host plants, like many Crambidae stalk borers. Recoveries have been made on sugarcane, *Saccharum officinarum* L., rice, *Oryza sativa* L., milo maize, *Sorghum* sp., wheat, *Triticum aestivum* L., Johnsongrass, *Sorghum halpense* L., corn, *Zea mays* L., Panicum grass, sorghum, *Sorghum bicolor* (L.) Moench, barley, *Hordeum vulgare* L., yellow bristlegrass, *Setaria lutescens* (Weigel) Hubbard, bulrush, *Scirpus validus* Vahl, lemon grass, *Cymbopogon citrates* (DC) Stapf, millet, *Pennisetum glaucum* L., pampasgrass, *Cortaderia selloana* (Schultes) Ascherson & Graebner, and sudan grass, *Sorghum vulgarae* var. sudanense [synonym of *S. sudanense* (Piper) Stapf] (Dyar 1917, Morill 1925, Osborn and Phillips 1946, Van Zwalunwenburg, 1926, Johnson 1984).

1.3. Biology of the Mexican Rice Borer

Eoreuma loftini is the major economic pest in Texas sugarcane, surpassing the sugarcane borer *Diatraea saccharalis* F. in economic importance almost as soon as it was discovered in 1980. The decline of the impact of the sugarcane borer appears to be due to the successful introduction of the parasite *Cotesia flavipes* Cameron (Fuchs et al. 1979b). The inability to control *E. loftini* can be explained by biological characteristics that render the insect less accessible to control agents. Female *E. loftini* moths oviposit in cryptic sites on dried sugarcane leaves located on the lower portion of the plant, i.e. between the soil surface and 80 cm height (van Leerdam et al. 1984, 1986). The globular cream-colored eggs are usually laid in groups of 5 to 100. Newly hatched Mexican rice borer larvae disperse from dry leaves where the eggs are deposited to green parts of the plant

(van Leerdam et al. 1986). By rapidly mining into leaf sheath and stalk, *E. loftini* larvae become protected from parasitoids and predators. In contrast, *D. saccharalis* lays its eggs in flat clusters of about 25 on green leaf blades and larvae do not enter the stalk as quickly *E. loftini*. As a result, the eggs and the newly emerged larvae of *D. saccharalis* are more exposed to parasitism and predation than *E. loftini*.

The Mexican rice borer completes the egg stage in 14 days at 20ºC and 5 days at 32ºC when reared at constant temperatures. In appearance, the larvae have an orange-brown head capsule and four parallel purple-red lines that run along the cream-colored body. The young larvae migrate from the oviposition site to green and moist parts of the plant suitable for feeding. Laboratory tests show developmental polymorphism, with five, six, or even seven instars (van Leerdam 1986). The number of stadia is affected by sex, with a higher number for females (six) than for males (five), not unlike the sugarcane borer (Roe 1981). Temperature inversely affects this number, with a six stadia larval development at 23ºC, and five stadia at 29ºC (van Leerdam 1986). The mean duration of each *E. loftini* stadium decreases with increasing temperature, with an average of 78 days at 20ºC and 21days at 32ºC for completion of all larval stages.

Towards the end of larval development, the 19 to 25 mm larvae have tunneled in the stalk both vertically and horizontally (Legaspi et al. 1997a). Pupation takes place in frass packed tunnels after mature larvae have constructed an emergence window covered by one or two layers of plant tissue. This is a relatively protected environment compared to the sugarcane borer, which produces a hollow cavity (with less frass), and is therefore more accessible than *E. loftini* to parasitism (Legaspi et al. 1997b). The *E. loftini* pupal stage lasts 21 days at 20º and 7 days at 32ºC (van Leerdam 1986). The developmental

times were measured using stalk sections of the sugarcane cultivar NCo 310. The results showed extended durations compared with those obtained with an artificial diet, suggesting that sugarcane may be a less favorable food (van Leerdam 1986). Different cultivars may also affect developmental times (Kennedy and Kishabi 1976).

Temperature affects total fecundity, with an average of 259 eggs per female at 20ºC and 406 eggs at 26ºC. The mean oviposition rate varies from 29 eggs/day at 20ºC to 64 eggs/day at 32ºC (van Leerdam 1986). Most females begin to oviposit 2 days after emergence, and have an oviposition peak the same day. Increasing temperature appears to decrease the time between emergence and oviposition peak (van Leerdam 1986). Studies have shown that *E. loftini* is active throughout the growing season in the Lower Rio Grande Valley of Texas, with a proportion of larvae continuing to feed and to develop in the winter, and some adults have been observed to oviposit throughout the winter (Johnson 1985, van Leerdam et al. 1986). *Eoreuma loftini* has a facultative larval diapause which is regulated by the interaction of photoperiod and temperature (van Leerdam 1986), similar to the sugarcane borer (Fuchs et al. 1979a).

1.4. Introduction of Parasitoids

Since the early 1980s, research has focused almost exclusively on the introduction of parasitic insects to control *E. loftini* in Texas. The Braconid, *Allorhogas pyralophagus* Marsch was collected on Johnsongrass in Monterrey, N.L., Mexico in 1984 (Browning and Melton 1984, Marsch 1984). Evaluations of this parasite prior to field liberation were very promising because of the insect's ability to tolerate extreme climatic conditions and to attack a wide age range of *E. loftini* instars (Smith et al. 1987). However, the parasite was unable to effectively suppress infestations of *E. loftini* in the field, probably

due to lack of host accessibility when the larvae are deep in the stalk. The parasite may still be useful as a component of an overall integrated pest management program (Hawkins et al. 1987, Meagher et al. 1998).

The Commonwealth Institute imported the parasite *Rhacanotus roslinensis* Lal. from Pakistan with releases beginning in the Lower Rio Grande Valley of Texas in 1983. No recoveries were made (Browning and Melton 1984). Laboratory as well as greenhouse studies evaluating several *Trichogramma* species showed promising results (Browning and Melton 1987, Greenberg et al. 1998). Field evaluations have yet to confirm these results but assessing the success by recoveries of the parasites in feral eggs is difficult because of their extreme concealment in the field by *E. loftini* moths (Lagaspi et al. 1997b). This behavior may reduce the efficiency of *Trichogramma* wasps, although several species are successfully used against various stemborers in several sugarcane pest management programs around the world (Jaipal, 1996; Mohyuddin 1991).

The gregarious pupal parasite *Pediobius furvus* Gahan was imported from Kenya, Africa, and introduced in the Lower Rio Grande Valley of Texas in 1983 and 1984 (Browning et al. 1985). Laboratory tests have shown that *E. loftini* is an acceptable host for oviposition and development of *P. furvus* (Pfannenstiel et al. 1996). However, the parasite appears not to have access to the host under field conditions due to frass-filled tunnels and sealed emergence windows (Pfannenstiel et al. 1992).

A previously unrecorded tachinid was collected in 1988 in Jalisco, Mexico, from *E. loftini* (Rodriguez-del-Bosque and Smith 1989), although further surveys in other sugarcane growing areas of Mexico were unsuccessful in finding the fly (Rodriguez-del-Bosque and Smith 1996). The fly was described in 1994 as a new species, *Lydella jalisco*

Woodley (Woodley 1994). Promising laboratory evaluations, as well as an apparent specificity for *E. loftini*, led to some optimism concerning the potential of this species as an efficient biocontrol agent. Beginning in 1989, field releases were made of the "Jalisco fly" with disappointing results. Recent recoveries after massive releases (61,369 from 1987-2003) on sugarcane in Texas have been very low (Meagher et al. 1998). The lack of effectiveness is likely caused by the higher temperatures of the Lower Rio Grande Valley of Texas compared to the native region of the fly, which may affect the efficiency of the parasite (Legaspi et al. 2000a). A recent laboratory study of the biology of *L. jalisco* affected by temperature confirmed this hypothesis (Lauzière et al. 2002).

A new *Mallochia* species, *M. pyralidis* Wharton, was recovered from *E. loftini* on sugarcane in the state of Sinaloa in 1983 (Wharton 1985). Since field releases began in 1984, recoveries in the Lower Rio Grande Valley of Texas have shown a very low parasitization rate, also suggesting that *M. pyralidis* will not be a prime mortality factor for *E. loftini* (Smith et al. 1990, Legaspi et al. 1997a). Further releases were made in sugarcane fields with *Agathis stigmateris* Cresson, *Goniozus natalensis* Gordh, *Macrocentrus prolificus* Wharton, and *Xanthopimpla stemmator* (Pfannenstiel et al. 1989). Of the four parasites, only *M. profilicus* was recovered, and only at low parasitism rates.

Some parasitoids have also been evaluated to control *E. loftini* on rice. These parasitoids include *A. pyralophagus*, *Alabagrus stigma* Brulle, *G. natalensis*, *Apanteles minator* Muesebeck, and *M. prolificus*. In field tests, only *A. pyralophagus* and *G. natalensis* parasitized more than 5% of the available *E. loftini* eggs (Pfannenstiel and Browning 1995).

The two most effective parasites of *E. loftini* in Texas are both indigenous braconids, *Chelonus sonorensis* Cameron and *Digonogastra solitaria* Wharton & Quicke, which represent together more than 75% of field parasite recoveries (Legaspi et al. 1997a). *Chelonus sonorensis*, a solitary egg and larval endoparasite, appears to have the same distribution as *E. loftini* in Mexico and Texas, following the range expansion of the host pest. *Digonogastra solitaria* is an endoparasite with a distribution ranging from northeastern Mexico to southern Texas.

Various entomophagous nematodes have also been assessed for their use as biocontrol agents against *E. loftini*. While laboratory results have had very promising results (Ring and Browning 1990), field tests have been disappointing, possibly due to the ineffectiveness of field application methods (Legaspi et al. 2000b). Despite entomophagous fungi being effective against various stemborers (Riba 1984, Maniania 1993, Chiuo and Hou 1993), tests on sugarcane against *E. loftini* using *Beauveria bassiana* have not been successful, possibly for the same reasons as for entomophagous nematodes (Legaspi et al. 2000c).

Although some of these biocontrol agents introduced in the Lower Rio Grande Valley of Texas are now established as parasites of *E. loftini*, none provide economic control (Meagher et al. 1998). Biological control of *E. loftini*, from both naturally occurring and introduced arthropod parasitoids and predators, should be a component of any integrated pest management program.

1.5. The Use of Pheromones

Sex pheromones have been used in many crops to disrupt mating of insect pests. Brown et al. (1988) were the first to provide evidence of the female sex pheromone for

E. loftini. The synthetic pheromone was subsequently described (Shaver et al. 1988), and field experiments showed the efficiency of pheromone-baited traps as a survey and monitoring tool (Shaver et al. 1991). Mating disruption using the pheromone has been evaluated with mating reductions up to 95.5 % in small fields (Shaver and Brown 1993). Tests in larger fields were not effective in disrupting mating and reducing crop injury levels (Spurgeon et al. 1997, Legaspi et al. 1999b). Both studies concluded that *E. loftini* mating disruption was not an efficient control method.

1.6. Chemical Control

The life stage of *E. loftini* targeted for chemical control is the neonate larva, which migrates from the ovipositional site on dry leaves at the base of the plant to green parts of the plant (Meagher et al. 1994). Weekly applications of insecticides can reduce the percentage of bored internodes, but the effect on sugarcane yield is rarely significant (Johnson 1985, Meagher et al. 1994, Legaspi et al. 1999c). Insecticides have had such limited success in controlling *E. loftini* that most sugarcane growers in the Lower Rio Grande Valley of Texas have abandoned this control method (Legaspi et al 1997a). The narrow window during which *E. loftini* larvae are potentially exposed to insecticides reduces the impact of a chemical. The difficulty of applying pesticides to foliage in the lower parts of sugarcane further reduces the efficiency of applying insecticides.

The growth regulator tebufenozide has shown excellent efficacy against the sugarcane borer in both laboratory (Rogriguez et al. 2001) and field tests (Rodriguez et al. 1995). Furthermore, the compound's ovicidal activity may assist in controlling populations of this pest on an areawide basis (Rodriguez et al. 2001). Laboratory studies revealed that tebufenozide is toxic to *E. loftini*, even though it is less toxic than

cyfluthrin-based insecticides (Legaspi et al. 1999c). Both these insecticides showed some potential when applications were carefully timed in small plot and aerial tests (Reagan et al. 2000). Tebufenozide also appeared to be less toxic to the parasitoids *A. pyralophagus* (Legaspi et al. 1999c) and *Cotesia chilonis* (Matsumura) (Reagan et al. 1997), suggesting the importance of using a biorational chemical. Tebufenozide also has a reduced impact on other non-target and predatory arthropods for the control of *D. saccharalis* in Louisiana (Reagan and Posey 2001).

1.7. Cultivar Resistance

Studies on various sugarcane cultivars in Texas showed variability in damage due to *E. loftini* (Pfannenstiel and Meagher 1991, Legaspi et al. 1999a). Cultivar CP 70-321 suffered less injury than NCo 310 in breeding evaluations (Pfannenstiel and Meagher 1991). Field studies on farms have confirmed these results (Meagher et al. 1993, Legaspi et al. 1999a), however results from five growing seasons in another study indicated that CP 70-321 had less bored internodes in only 28% of the comparisons (Meagher et al. 1996). An increase in bored internodes does correlate with decreased yield; however the yield of cultivar CP 70-321 is more severely affected by *E. loftini* injury than cultivar NCo 310 (Legaspi et al. 1999a). In greenhouse and laboratory studies, ovipositional preference did not appear to be as important as a resistance mechanism as antibiosis (Meagher et al. 1996), which is the category of plant resistance that represents the negative effects of a resistant cultivar on the biology of an insect feeding on the plant (Smith 1989). Diet incorporation bioassays as well as larval establishment tests detected significant differences among sugarcane cultivars, suggesting the importance of larval antibiosis (Meagher et al. 1996).

The snowdrop lectin (*Galanthus nivalis* agglutinin, GNA) was expressed in transgenic sugarcane, which was evaluated as a potential pest management strategy for control of *E. loftini* and *D. saccharalis* (Sétamou et al. 2002b). Transgenic leaf material incorporated into artificial diet had no effects on life history parameters of *D. saccharalis*, but did reduce larval survival, adult emergence, and fecundity of *E. loftini*. *Diatraea saccharalis* fed with this transgenic sugarcane in the laboratory did however have negative effects on the parasitoid *C. flavipes*, which is responsible for maintaining sugarcane borer populations below economic threshold levels in Texas (Sétamou et al. 2002a).

1.8. Components of Plant Physiology under Stress

Stress can be defined at the whole plant level as environmental conditions that limit the rate of dry-matter biomass of at least one component of the vegetation below its genetic potential (Grime 1979). However, genetic potential may be difficult to quantify, which is problematic when studying the effects of stress on plants (Jones and Jones 1989). Measurements of yield under stress conditions may be more appropriate than dry-matter biomass in agriculture. The definition of stress becomes less clear when one considers that the environment is rarely optimal for plant growth, and stress can even be benefical in some cases (Grierson 1999). Factors responsible for stress can be abiotic (e.g. temperature, water, soil nutrients, air pollution) or biotic (e.g. competition, infections by bacteria, nematodes, fungi, viruses, and insect feeding). Stress is considered here as any external factor that results in reduced growth rate relative to more optimal conditions, which has been observed on plants under many types of stress (Munns 2002, Oksanen and Saleem 1999).

The reduction in growth can sometimes cause a decrease in demand of photosynthetic assimilates, which leads to a loss of photosynthetic activity and a disruption of the photosynthesis apparatus (Godde 1999), of which chlorosis is an indicator (Ottander et al. 1995). Carbohydrates are known to accumulate under stress conditions (Lacerda et al. 2002), as well as other compounds such as potassium, organic acids, chloride and free amino acids (Cutler and Rains 1978, Jones et al. 1980). Such accumulation contributes to maintaining an adequate turgor necessary for growth by osmoregulation (Wyn Jones and Pritchard 1989).

Free amino acids (FAA) are involved in numerous cell processes, and have a prominent role in osmotic adjustment (Rabe 1994). Studies on plants such as alfalfa, *Medicago sativa* L., wheat, sesame, *Sesamum indicum* L., and rice have shown that their accumulation can result from reduced rates of protein synthesis (Crocomo and Basso 1974, Dell'Aquila 1992, Dubey and Rani 1990, Pessarakli and Huber 1991). Other studies on pearl millet, *Pennisetum typhoides* (Burm) Stapf and Hubbard, tobacco, *Nicotiana tabacum* L., and cotton, *Gossypium hirsutum* L., have shown that the increase in available nitrogen results from increased levels of FAAs (Albassum 2001, Rufty et al. 1990, Hanower and Brzozowska, 1975).

Levels of FAAs vary with plant species (Showler 2001), plant stage (Weibull 1987) and numerous stresses (Rabe 1994) including soil and water salinity (Perez-Alfocea et al. 1993), water deficit (Gzik 1996, Bussis and Heineke 1999), temperature (Wilding et al. 1960, Walgenbach et al. 1981), shade (Showler 2002), soil nutrients (Hoff et al. 1974), weed competition (Showler 2002, Showler and Reagan 1991), pesticides (Gilliam et al. 1981, Starratt and Lazarovits 1996), and infections by bacteria (Meon et al.

1978), fungi (Reddy and Rao 1976), viruses (Blua et al. 1994) and nematodes (Showler et al. 1990). Drought stress has been shown to induce accumulations of FAAs in numerous plants including Bermuda grass, *Cynodon dactylon* (L.) (Barnett and Naylor 1966), chick pea, *Cicer arietinum* (Singh et al. 1985), cotton (Showler 2002), flatpea, *Lathyrus sylvestris* L. (Shen et al. 1989), maize (Ranieri et al. 1989), peanut, *Arachis hypogaea* L. (Ali-Ahmad and Basha 1998), sugar beet, *Beta vulgaris* L. (Gzik 1996), sugarcane (Muqing and Ru-Kai 1998), tomato, *Solanum esculentum* L. (Franco et al. 1999), wheat (Sarker et al. 1999), and wild watermelon, *Citrullus lanatus* sp. (Kawasaki et al. 2000).

Free proline appears to be the most widespread and consistent amino acid that responds to water deficit stress conditions (Aspinall and Paleg 1981). Proline biosynthesis is thought to be regulated by Δ'-pyrroline-5-carboxylate synthetase, which is inhibited by proline (feedback inhibition) (Delauney and Verma 1993). Under water stress, this feedback is lost (Boggess et al. 1976a).

The rapid accumulation of free proline when plants are subject to dehydration lowers the water potential of the cells, and thus reduces water loss by osmoregulation (Heuer 1994). Free proline can also interact with various proteins in the cytoplasm in order to increase their stability (Schobert and Tschesche 1978). Free proline also provides a readily available source of energy and nitrogen by being transported throughout the plant during stress conditions, which can help the plant recover following the stress (Singh et al. 1973). Free proline, among other nitrogen-containing compounds, is also thought to act as a sink for the accumulation of ammonia resulting from a general reduction in growth rate under stress conditions. High levels of ammonia are known to occur during drought stress, and the build up in stressed plants is due to the accumulation

of nitrate and the degradation of amino acids that would normally be incorporated into protein. No feedback inhibition for nitrogen uptake and nitrate reduction exists, so such concentrations of ammonia can become toxic if not rapidly eliminated. The detoxification of high levels of ammonia therefore can result from sequestering ammonia into nitrogen-containing compounds such as free proline (Rabe 1994).

1.8. Response of Herbivores to Drought Stressed Host Plants

The resistance of plants to insect herbivores has a genetic basis, which can be modified by abiotic and biotic factors of the environment (Smith 1989). Biotic factors affecting the susceptibility of plants to insects include disease infection (Hardy et al. 1985, Ahmad et al. 1987), weeds (Showler and Reagan 1991, Levine 1993), and host plant attributes such as density (Miller et al. 1993), height, and age (Gurr and McGrath 2001). Abiotic factors affecting plant resistance include temperature (Thindwa and Teetes 1994, Hilbeck and Kennedy 1998), soil fertility (Barbour et al. 1991, Rao 2002), light (Elden and Kenworthy 1995, Kennedy et al. 1981), pesticides (Wu et al. 2001) and water (Kumar 1994).

Water is a limiting factor in many agroecosystems in the world, and 70% of the water taken from rivers and groundwater is used for irrigation (United Nations 2003). Though difficult to estimate, average annual crop losses caused by drought have increased in recent years, from an estimated $700 million in the Great Plains region of the United Staes in 1975, to a national total of $6-8 billion in 1995 (Wilhite 2000). Drought-related problems in agriculture will increase in some areas in the 21st century with global warming (Rind 2000), which will likely affect the dynamics of insect herbivores (Masters et al. 1998).

Studies on the effects of drought stress on the biology and infestations of insect pests have shown a diversity of responses. The plant stress hypothesis postulates that outbreaks of insect herbivores occur under plant stress conditions because of the increased nutritional value of the host plant, mainly due to an increase in available nitrogen (White 1984). The plant vigor hypothesis predicts enhanced insect performance on healthy and rapidly growing plants (Price 1991). Examples supporting both theories can be found in the literature, representing two extremes of plant-insect interactions with intermediate relationships also existing.

Aphids have shown diverse responses to drought stressed host plants. Water deficit stress can either increase (Kennedy et al. 1950) or decrease (Kennedy et al. 1958) infestations of *Aphis fabae* Scop. on sugar beet. Drought stress has been shown to decrease abundance, survival and fecundity of *A. fabae* on spindle tree, *Euonymus europaeus* L., in laboratory experiments (Kennedy et al. 1958). Fecundity and survival of *Rhopalosiphum padi* L. and *Sitobion avenae* F. decreased under drought-stressed winter wheat. (Pons and Tatchell 1995). Field experiments have shown smaller *Acyrthosiphon pisum* (Harris) population sizes on drought-stressed pea plants, *Pisum sativum* L. (McVean and Dixon 2001). Factors responsible for such a diverse response to droughtstress include aphid species (Wearing and van Emden 1967) and plant and leaf age (Wearing 1967, 1972). Despite increases in FAA levels in the phloem sap, water stress has a detrimental effect on phloem feeders by causing a loss of turgor and decreased sap pressure (Risebrow and Dixon 1987). The intensity of drought stress can also impact the response of herbivores (McMillin and Wagner 1995). A non-linear relationship was established between the level of drought stress on bush beans, *Phaseolus*

vulgaris L. and abundance of the two spotted spider mite, *Tetranychus urticae* (Koch) in a field experiment, which may also help explain the diversity of responses of herbivores to drought stressed plants described in the literature (English-Loeb 1990).

Among other herbivores, drought stress has been shown to decrease longevity and fecundity of the greenbug, *Schizaphis graminum* (Rondani) on wheat (Sumner et al. 1983). Drought stress on cotton has also been shown to increase bollworm [*Helicoverpa zea* (Boddie)] larval survival (Slosser 1980). Resistance of corn to *Chilo partellus* (Swinhoe) decreased under drought stress conditions (Kumar 1994). Increased plant susceptibility associated with the whitefly *Bemisia argentifolii* Bellows & Perring was observed on water-stressed cotton (Paris et al. 1993, Flint et al. 1994). Periods of drought have also caused outbreaks of the gypsy moth in birch forests (Koltunov and Andreeva 1999). Population growth of *T. urticae* was greater on drought-stressed greenhouse peppermint plants, *Mentha piperita* L. (Hollingsworth and Berry 1982). Developmental rates of the pacific spider mite, *Tetranychus pacificus* McGregor, were faster on drought-stressed almond (Oi et al. 1989). Plant water stress has also increased populations of spider mites on corn (Chandler et al. 1979) and on sorghum (Kattes and Teetes 1978).

Numerous modifications of host plant physiology under drought stress can impact herbivores (Holtzer et al. 1988). Drought stress can sometimes increase levels of carbohydrates in plants, which may benefit insect herbivores (Mattson and Haack 1991). However, these compounds have received limited attention in insect-plant interaction studies, and may not impact herbivore populations as profoundly as other factors (Holtzer et al. 1988). An increase in water potential under drought stress is an indicator of a reduction in the availability of water for insects, which can affect the digestibility of

ingested food (Schmidt and Reese 1987). Drought stress can also increase or decrease levels of plant secondary metabolites, which have roles of chemical defense against herbivores (Gershenzon 1984). The availability of amino acids in host plants is a critical factor in population growth of many insect herbivores (McNeil and Southwood 1978). Insects can respond to changes in the nutritional quality of a plant (Rhoades 1983) and modified nitrogen metabolism under drought stress is thought to have a significant impact on insect populations (White 1984). The majority of nitrogen is acquired by insects through absorption in the gut (Brodbeck and Strong 1987). Three potential physiological mechanisms may explain the enhanced nutritional quality of plants under stress: (1) FAAs are nutritionally superior to proteins, (2) FAAs are more readily available than proteins because of the absence of any proteinase inhibitors, and (3) FAAs are physically more accessible to herbivores because of increased solubility (Cockfield 1988). Certain amino acids are known to be essential for insect development (Vanderzant 1958, Nation 2002). Artificial diets with amino acid distributions simulating anthers were adequate for survival and development of the tobacco budworm, *Heliothis virescens* (F.) (Hedin et al. 1991). Understanding the relationships between plant physiological changes under dought stress to herbivore biology and ecology can assist in developing more long term IPM programs based on insect-crop interactions.

CHAPTER 2: RESISTANCE TO THE MEXICAN RICE BORER AMONG LOUISIANA AND TEXAS SUGARCANE CULTIVARS[1]

2.1. Introduction

Mexican rice borer, *Eoreuma loftini* (Dyar), was first reported as a major pest of commercial sugarcane, *Saccharum* spp., in 1924 on the west coast of Mexico in the state of Sinaloa (Van Zwalunwenburg 1926). In the United States, after an initial discovery of the insect in Arizona (Dyar 1917), specimens were again found in southern Arizona in 1945 and in the Imperial Valley of California, near the Mexican border (Osborn and Phillips 1946). *Eoreuma loftini* was first detected in the Lower Rio Grande Valley of Texas in 1980 and became the dominant pest of sugarcane (Johnson and van Leerdam 1981). By 1990, its range had expanded into the rice production area of Texas (Browning et al. 1989). *Eoreuma loftini,* now the major insect pest of sugarcane in the Lower Rio Grande Valley of Texas, represents >95% of the sugarcane stalk borer population there (Legaspi et al. 1999a). With *E. loftini* established only 80~100 km from new sugarcane production near Beaumont, TX, the invasion of Louisiana sugarcane fields in the near future is expected (Way and Reagan 2001). Efforts are underway to develop more adequate management strategies in both Louisiana and Texas.

Sugarcane damage in the Lower Rio Grande Valley of Texas has averaged 20-30% bored internodes (Meagher et al. 1993, Legaspi et al. 1997a). Estimates of the effects of borer damage on revenue, based on a 20% level of bored internodes, have varied between $575/ha, when considering only the producer's loss (Meagher et al. 1994), and $1,181/ha, when considering the effect on all involved parties (producer and mill) (Legaspi et al. 1999a). Insecticides have had such little success in controlling *E.*

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loftini that most sugarcane growers in the Lower Rio Grande Valley of Texas have abandoned their use (Legaspi et al. 1997a).

Cultivar resistance allows for a more permanent control of pest populations (Smith 1989) and is compatible with other tactics (Kogan 1994). Resistant sugarcane cultivars have been a major component of the integrated pest management (IPM) system to control the key insect pest, the sugarcane borer, *Diatraea saccharalis* (F.), in Louisiana (Bessin et al. 1990a). Until excessive variation in sugarcane cultivar injury resistance rankings encouraged the use of other criteria (White and Hensley 1987), the most commonly used method for assessing borer susceptibility among cultivars for many years was percentage of bored internodes (Reagan and Martin 1988). The evaluation of sugarcane cultivars with the Louisiana State University Agricultural Center currently involves estimating resistance by using percentage of bored internodes as well as counting adult emergence holes. Percentage of bored internodes is a useful criterion for estimating resistance to young larvae before penetration into the stalk (Reagan 2001). The presence of adult emergence holes represents a seasonal record of moth production for each sugarcane cultivar (Bessin et al. 1990b) and can be used to determine the relative survival of larvae entering the stalk.

The impact of cultivars can be estimated from the buildup of borer populations on an areawide basis (Bessin et al. 1991). In addition to reducing pest injury on an individual field basis, areawide pest management aims to reduce population levels of the target organism over a large geographical area. This method offers long-term solutions, as opposed to field -to- field solutions, which may not allow sustainable pest management. Areawide pest management frequently uses environmentally friendly

techniques and offers minimal costs to the growers (Chandler and Faust 1998). The objectives of this study were to determine the relative resistance to *E. loftini* among selected Louisiana and Texas sugarcane cultivars based on plant injury as well as their contribution to the enhancement or the suppression of adult borer populations on an areawide basis and to assess selected candidate cultivars for possible future releases for commercial production in Louisiana or Texas.

2.2. Materials and Methods

Four cultivars that are commercially recommended in Louisiana, CP 70-321, LCP 85-384, HoCP 85-845, and HoCP 91-555, were planted on 2 October 2000. An additional cultivar, NCo 310, commercially produced in Texas (formerly a major cultivar in Louisiana), was planted as an *E. loftini*-susceptible check. All cultivars were planted in 6.1-m-long and 1.8-m-wide one-row plots in a randomized block design with five replications at the Texas A&M University Agricultural Research and Extension Center annex in Hidalgo County, Weslaco, TX. A second test was planted on 12 December 2001 at the same site with the same cultivars, in addition to TCP 87-3388 and CP 72-1210, in 44.6-m^2 plots (four 6.1-m-long and 1.8-m-wide rows) in a randomized block design, also with five replications. Plots were irrigated every 2 wk as recommended for commercial production. A third experiment was planted on 3 October 2001, also with five replications in a randomized block design, at the Texas A&M University Agricultural Experiment Station site near Ganado in Jackson County, Texas. Cultivars in this test were NCo 310, CP 70-321, LCP 85-384, HoCP 85-845, and HoCP 91-555, plus the candidate Louisiana cultivar HoCP 96-540, in 6.1-m-long and 1.8-m-wide one row plots. The sugarcane at this site was not irrigated. With the exception of the dryland

regime at Ganado, studies at all three test locations were conducted under normal management practices, with a single application of ethoprop at 2.69 kg (AI)/ha at planting to control wireworms (Coleoptera: Elateridae) at each location.

Twenty stalks were randomly removed from each plot for *E. loftini* damage assessment on 1 November 2001 at the first Weslaco test, on 14 November 2002 at both Weslaco tests (including the first ratoon study), and on 24 November 2002 at the Ganado test. Numbers of bored internodes and emergence holes were recorded, and the positions of injury on the stalks were verified by mechanically splitting the stalks.

In accordance with the method of Bessin et al. (1990b), a relative index was used to estimate the survival of larvae to adulthood (relative survival=no. of exit holes/no. of bored internodes). Moth production per hectare was calculated as the number of adult exit holes per stalk times the stalk density. Average Louisiana sugarcane stand counts based on outfield tests from the variety development program 1996-2001 (Orgeron et al. 2001) were used to project potential commercial stalk density per hectare. For cultivar NCo 310, combined plant cane density means across outfield locations from 1978 (Report of the Contact Committee of the American Sugar Cane League 1979) were adjusted for current Louisiana stand counts with CP 70-321 used as a comparative reference. For cultivars TCP 87-3388 and CP 72-1210, stand counts were used from the experiment in Weslaco and were adjusted for current Louisiana stand counts with CP 70-321 as reference. Although damage by *E. loftini* might differ from Texas in Louisiana field conditions, by using Louisiana stand counts gives a potential estimate of moth production per hectare for Louisiana. On 12 December 2002, one soil sample from each plot (five probes per plot) was collected for analysis from depths of 0-15 cm and 15-30

cm across each replication of the sugarcane cultivar experiment at Ganado. Means for each plot were analyzed using a one-way analysis of variance (ANOVA) (PROC MIXED, SAS Institute 1999), and Tukey's honestly significant difference (HSD) test (Tukey 1953) was used for mean separation. Damage in replication three (20 stalks) was also compared with that in the four other replications (80 stalks) at Ganado (PROC MIXED).

2.3. Results

The percentage of bored internodes in the first Weslaco test varied from 5.3% (HoCP 85-845) to 13.8% (HoCP 91-555) (Table 2.1). Cultivar HoCP 91-555 was significantly more susceptible to infestation than cultivar HoCP 85-845. Moth production per hectare varied from 3,038 ± 2,353 for HoCP 85-845 to 17,052 ± 3,956 for LCP 85-384. Cultivar LCP 85-384 produced significantly more moths than cultivar HoCP 85-845 in 2001. In 2002, the percentage of bored internodes varied from 4.2% (CP 70-321) to 9.8% (HoCP 91-555). Moth production per hectare ranged from 4,197 ± 2,023 (HoCP 85-845) to 13,994 ± 8,918 (LCP 85-384) (Table 2.1).

Results from the second Weslaco experiment showed similar trends for the previously tested cultivars (data not shown). Percentage of bored internodes ranged from 5.6% (TCP 87-3388) to 14.6% (HoCP 91-555). However, differences were not detected among cultivars in percentage of bored internodes ($F = 1.79$; df = 6, 24; $P = 0.1447$) and moth production ($F = 1.12$; df = 6, 24; $P = 0.3804$). CP 72-1210 was among the more susceptible cultivars (14.1% bored internodes).

Table 2.1. Injury (± SEM) by *E. loftini* to five commercial sugarcane cultivars, resultant survival (± SEM) of older larvae inside the stalks, and moth production (± SEM) at Weslaco, Hidalgo County, TX, 2001-2002.

Cultivar	% Bored internodes		Relative survival[a]		Moth emergence/ha[b]	
	2001	2002	2001	2002	2001	2002
HoCP 91-555	13.84 ± 2.40a	9.84 ± 1.17a	0.089 ± 0.023a	0.050 ± 0.019a	15,071 ± 4,671ab	7,868 ± 4,126a
LCP 85-384	12.06 ± 1.97ab	6.48 ± 1.31ab	0.111 ± 0.028a	0.112 ± 0.051a	17,052 ± 3,956a	13,994 ± 8,918a
NCo 310	9.03 ± 1.95ab	6.40 ± 0.99ab	0.047 ± 0.016a	0.091 ± 0.044a	4,926 ± 1,847ab	5,483 ± 2,875a
CP 70-321	7.63 ± 1.81ab	4.15 ± 1.58b	0.040 ± 0.029a	0.167 ± 0.044a	3,805 ± 2,370ab	6,225 ± 3,021a
HoCP 85-845	5.29 ± 0.80b	4.94 ± 0.70ab	0.034 ± 0.022a	0.083 ± 0.040a	3,038 ± 2,353b	4,197 ± 2,023a
F[c]	3.37	3.58	2.00	1.10	4.34	0.62
$P > F$	0.035	0.029	0.144	0.388	0.0145	0.652

Means within the same column followed by the same letter are not significantly different ($P<0.05$; Tukey's [1953] HSD).
[a] Based on a ratio of *E. loftini* exit holes to bored internodes.
[b] Estimated as the product of the mean number of exit holes and the number of stalks per hectare.
[c] df = 4, 16.

Results from the Ganado test in 2002 (Table 2.2) indicated that the number of bored internodes ranged from 28.32% (CP 70-321) to 67.46% (LCP 85-384). LCP 85-384, HoCP 91-555, and HoCP 96-540 were more susceptible based on percent bored internodes than NCo 310 and CP 70-321. Moth production per hectare varied from 39,140 ± 5,477 (CP 70-321) to 165,097 ± 65,424 (HoCP 91-555). The proportion of bored internodes at different stalk positions was consistently affected by the cultivars (Fig. 2.1) and significant differences were detected among cultivars for the bottom (F = 9.54; df = 5, 20; P = 0.0001), middle (F = 17.85; df = 5, 20; P = 0.0001), and top (F = 27.40; df = 5, 20; P = 0.0001) portions of the stalk. Analysis of the subsoil showed high levels of combined salts (sodium and magnesium) in replication three (1,554 ppm) compared with the other four replications (627 ppm). The comparison of replication three to the other four showed a significant interaction between cultivar and replication effect for the percentage of bored internodes (F = 3.52; df = 5, 490; P = 0.05) (Fig. 2). Replication three showed either a strong trend or significance (P = 0.05) for an increase in percentage of bored internodes in all cultivars except HoCP 91-555 (susceptible) or CP 70-321 (resistant). Differences in larval survival were not detected between any of the cultivars; however, general trends were consistent in both 2001 and 2002, with HoCP 85-845 showing the lowest relative survival.

2.4. Discussion

This study provides a new method for evaluating sugarcane cultivar resistance to *E. loftini* by using adult exit holes and percentage of bored internodes. Previous studies have focused on bored internodes as the only criterion for evaluating resistance (Pfannenstiel and Meagher 1991, Meagher et al. 1993, Legaspi et al. 1999a). As the

Table 2.2. Injury (± SEM) by *E. loftini* to six sugarcane cultivars, resultant survival (± SEM) of older larvae inside the stalks, and moth production (± SEM) at Ganado, Jackson County, TX, 2002.

Cultivar	% Bored internodes	Relative survival[a]	Moth Emergence/ha[b]
LCP 85-384	67.46 ± 5.70a	0.225 ± 0.065a	112,255 ± 3,7504a
HoCP 96-540	62.45 ± 6.80ab	0.200 ± 0.030a	105,590 ± 7,886a
HoCP 91-555	57.53 ± 3.43abc	0.363 ± 0.144a	165,097 ± 65,424a
HoCP 85-845	47.23 ± 4.90cd	0.150 ± 0.039a	62,669 ± 16,966a
NCo 310	36.15 ± 3.10de	0.166 ± 0.035a	53,057 ± 13,429a
CP 70-321	28.32 ± 1.86e	0.171 ± 0.023a	39,140 ± 54,77a
F^c	34.01	1.27	2.12
$P > F$	< 0.0001	0.316	0.106

Means within the same column followed by the same letter are not significantly different (P<0.05; Tukey's [1953] HSD).
[a] Based on a ratio of *E. loftini* exit holes to bored internodes. [b] Estimated as the product of the mean number of exit holes and the number of stalks per hectare. [c] df = 5, 20.

sugarcane stalk grows, it provides a seasonal record of adult emergence as well as stalk borer damage. Therefore, the contribution of each cultivar to enhancement or suppression of pest populations can be estimated on an areawide basis. The relative survival index does not estimate actual survival of the larvae entering the stalk, because one larva can make several entrance holes, but only one emergence hole. Relative survival can be used to determine the relationship of damage to life cycle completion. If moth production per hectare were to be estimated using only percentage of bored internodes, it would be expected that cultivar LCP 85-384 would produce the most adults

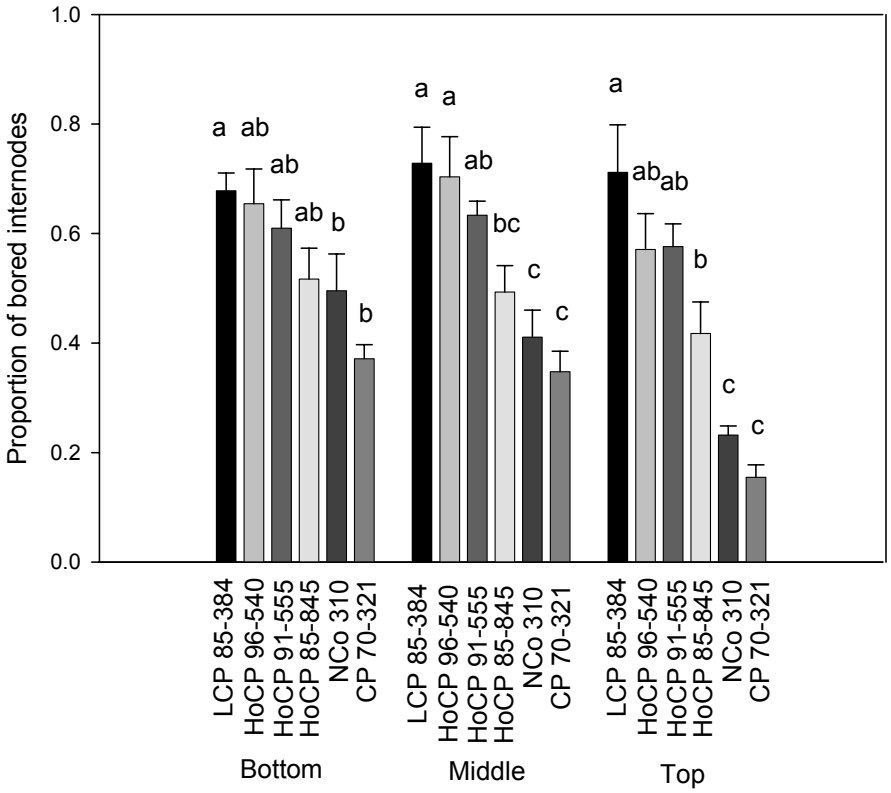

Fig. 2.1. Proportion of bored internodes (± SEM) by stalk position on different sugarcane cultivars on 26 November 2002 at Ganado, TX. Within each position of damage (bottom, middle, top), bars followed by the same letter are not significantly different according to Tukey's [1953] HSD.

when one considers the results from the Ganado experiment (Table 2.2). However, because of a trend for greater relative survival of *E. loftini* larvae in cultivar HoCP 91-555, more moths might be expected to emerge from this cultivar compared with LCP 85-384. Future *E. loftini* management programs should assess plant damage and the potential impact of each cultivar on the buildup or the reduction of pest populations. Field conditions have a major impact on damage to sugarcane by *E. loftini*. Using stand counts from the Louisiana sugarcane industry to determine moth production does provide some insight on the impact of cultivars on moth production per hectare, even if damage

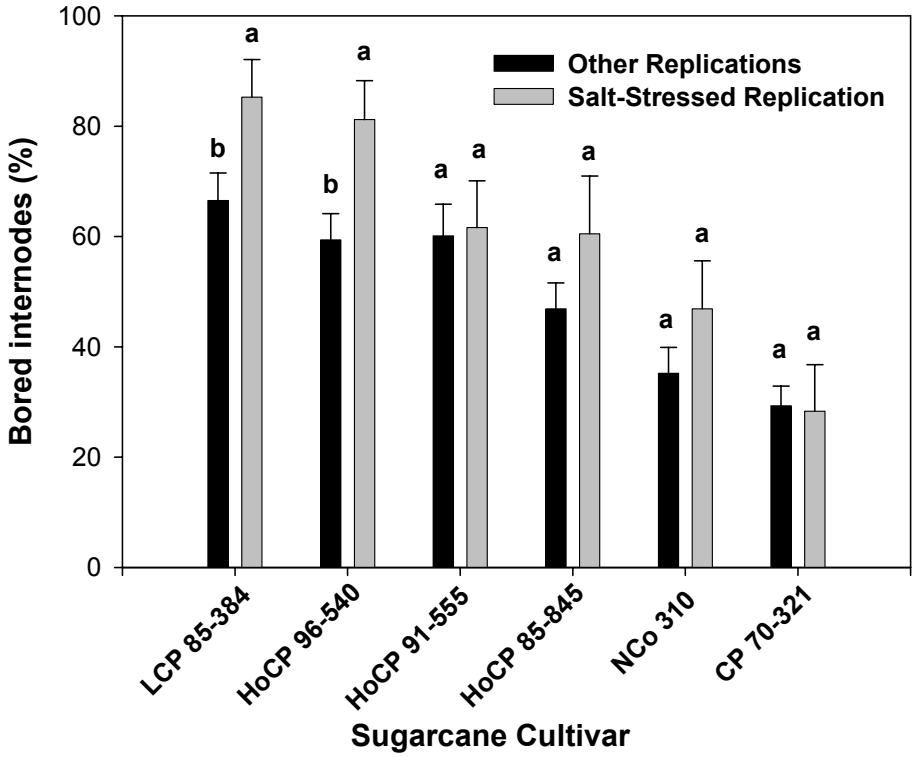

Fig. 2.2. Percent bored internodes (± 95% confidence interval) of the salt-stressed replication (rep. 3) compared to percent bored internodes (± 95% confidence interval) of replications 1, 2, 4 and 5 for different sugarcane cultivars on 26 November 2002 at Ganado, TX.

is likely to differ from our experiments in Texas when the insect becomes established in Louisiana. We would expect the relationships among the cultivars to be similar in both conditions, because they were generally in the two Texas test locations.

Cultivars HoCP 91-555, LCP 85-384, and HoCP 96-540 were among the more susceptible to *E. loftini,* even more so than NCo 310, previously regarded as being the most susceptible commercial cultivar (Pfannenstiel and Meagher 1991, Legaspi et al. 1999a). As in previous studies (Pfannenstiel and Meagher 1991, Legaspi et al. 1999a),

CP 70-321 was the most resistant in both locations. Under the heavy infestation pressure at the Ganado location, LCP 85-384 was the most susceptible. This cultivar now comprises >85%[2] of the production area in Louisiana (Legendre and Gravois 2003).

All cultivars included in the Ganado test and the first Weslaco test have now been released for commercial use in Louisiana (Table 2.3). Of the materials evaluated in test 2, NCo 310 and CP 70-321 were also released in Texas along with CP 72-1210 and TCP 87-3388. HoCP 96-540 was released for commercial use in Louisiana for planting in fall 2003. Based on yield trials reported annually in the sugarcane research progress report of the LSU AgCenter, cultivars NCo 310, CP 70-321, and HoCP 85-845 are classified as resistant to the sugarcane borer, *D. saccharalis,* although CP 70-321 is the least tolerant of the three. The remaining three cultivars, LCP 85-384, HoCP 91-555, and HoCP 96-540, are classified as susceptible and intolerant to the sugarcane borer. It is interesting that HoCP 96-540 is a progeny of LCP 85-384. The current studies should be useful in determining whether any of these cultivars have cross-resistance (or susceptibility) to the two major stalk borers affecting sugarcane in Texas and possibly in Louisiana in the future. The more resistant cultivars to *D. saccharalis* show a general trend to yield less than the more susceptible cultivars in the absence of moderate-to-high sugarcane borer damage (Waguespack et al. 2002).

Under heavier *E. loftini* infestation at Ganado, the level of resistance in HoCP 85-845 was reduced, suggesting that the best use of this cultivar is in moderate to low infestation conditions. Similar changes in resistance within cultivars with varying insect densities have been reported previously (Smith 1989). The differences in injury to LCP 85-384 at Ganado and Weslaco suggest that major differences in both plant damage and

[2] The current estimate is over 90% (Legendre and Gravois 2005).

Table 2.3. Name of cultivar, year released for commercial use, parents and sugarcane borer *D. saccharalis* resistance for cultivars grown commercially in Louisiana.

Cultivar	Year released for commercial use in Louisiana	Parents		Sugarcane borer resistance[1]
		Male	Female	
LCP 85-384	1993	CP 77-310	CP 77-409	S
HoCP 91-555	1999	CP 83-644	CP 82-94	S
HoCP 85-845	1993	CP 72-370	CP 77-403	R
CP 70-321	1978	CP 61-39	CP 57-614	R
NCo 310	1954	Co 421	Co 312	R
HoCP 96-540	2003	LCP 86-454	LCP 85-384	S

[1] R = resistant, S = susceptible

adult moth production occur at the two locations. By using *E. loftini* pheromone traps (Shaver et al. 1991) and counting adults once a week throughout the season, a maximum of 250 moths was caught in the trap near the sugarcane field at Ganado, whereas a maximum of only 27 moths was caught in the Weslaco area. Although no statistical comparisons are possible, the observed trends imply differences in areawide population abundance (which also occurred in plant injury; Table 2.1 versus 2.2).

Plant resistance to insect pests can be induced by changes in environmental conditions, such as altered levels of fertilization and irrigation (Smith 1989). The cultivar experiment conducted in Ganado had a significant replication effect, with more damage in the third replication compared with the other four (Fig. 2). The stressed appearance of

the sugarcane across this replication was confirmed by soil analysis, which showed higher levels of sodium and magnesium in the subsoil (15-30 cm) compared with all other replications. Our interpretation of the greater *E. loftini* damage in this replication is that the sugarcane across the entire replication suffered from salt stress and thus became more prone to *E. loftini* damage. The differential response to the stress among sugarcane cultivars is a first step toward determining management strategies in sugarcane-stressed areas.

Research on the effects of cultural practices on the control of *E. loftini* in sugarcane has been sparse, and no replicated field experiments have been conducted to quantitatively assess the relationship of *E. loftini* to stressed sugarcane. Field observations conducted in the early 1980s on *E. loftini* damage in Texas sugarcane fields indicated that irrigation had the potential to reduce the damage caused by *E. loftini* (K.J.R. Johnson, S.D. Hensley, and T.E. Reagan, unpublished data), suggesting that *E. loftini* infestation is favored by drought stress. This might explain the differences in damage between our Weslaco and Ganado experiments, the sugarcane in Weslaco was irrigated, and in Ganado was dryland. A survey of *E. loftini* in Texas in 1989 showed an average of 20% bored internodes, with important variability among fields (from 3.7 to 39.1% bored internodes for NCo 310) (Meagher et al. 1993). It was suggested that irrigation was responsible. Greenhouse studies have indicated that a moderate drought stress enhances larval weight, internode damage, and larval density (M. Sétamou, A.T. Showler, and F.P.F. Reay-Jones. unpublished data). Sugarcane growers in the Lower Rio Grande Valley of Texas are able to irrigate their crop when sufficient water is available, thus reducing damage due to this pest. In Louisiana, a less arid climate precludes the

common use of irrigation. Therefore, damage in Louisiana is likely to be generally less drastic due to different moisture availability. However, extended periods of drought in recent years have created ideal conditions for enhancing *E. loftini* populations. Cultivar resistance based on drought resistance is an important consideration when constructing areawide and cross-regional control strategies. Cultivar resistance to *E. loftini* is anticipated to serve a major role in keeping infestations below economic thresholds, as well as decreasing populations on an areawide basis.

CHAPTER 3: INTEGRATED TACTICS FOR MANAGING THE MEXICAN RICE BORER IN SUGARCANE[3]

3.1. Introduction

The Mexican rice borer, *Eoreuma loftini* (Dyar), has been the dominant insect pest of sugarcane, *Saccharum* spp., in the Lower Rio Grande Valley of Texas since it was introduced from Mexico in 1980 (Johnson and van Leerdam 1981). *Eoreuma loftini* now represents >95% of the sugarcane stalk borer population in the Lower Rio Grande Valley (Legaspi et al. 1999a). By 1989, its range had expanded into the rice production area of Texas (Browning et al. 1989). With *E. loftini* moths discovered in the sugarcane production area near Beaumont, Texas, invasion of Louisiana sugarcane fields is expected (Reagan et al. 2005).

Although applications of insecticides have been shown to reduce the percentage of *E. loftini* bored internodes, the effect on sugarcane yield has rarely been significant (Johnson 1985, Meagher et al. 1994, Legaspi et al. 1999c) and producers in Texas have discontinued using insecticides (Legaspi et al. 1997a). Extensive attempts at classical biological control have not resulted in the establishment of effective *E. loftini* parasitoids (Meagher et al. 1998). In contrast, the use of cultivar resistance has shown potential to reduce both injury and the production of *E. loftini* in sugarcane (Reay-Jones et al. 2003).

High salt concentrations in soil can enhance *E. loftini* infestations in some sugarcane cultivars (Reay-Jones et al. 2003), but few studies have focused on cultural practices to reduce *E. loftini* infestations and population pressure. A survey of *E. loftini* in Texas in 1989 showed an average of 20% bored internodes, with substantial variability among fields (3.7-39.1% bored internodes for the cultivar NCo 310), and it was

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suggested that irrigation management practices might explain the variability (Meagher et al. 1993). Statements regarding unpublished greenhouse studies by Sétamou and Showler have since indicated that a degree of drought stress enhances larval weight, internode injury, and survival to adulthood. Combined with other control tactics, managing host plant stress might assist in reducing *E. loftini* populations and damage in sugarcane.

Accumulations of host plant free amino acids (FAAs) have been associated in many plants with numerous stresses (Rabe 1994, Showler 2004b), including drought (Gzik 1996, Showler 2002). Accumulated FAAs lower the water potential of cells, and may reduce water loss through osmoregulation (Heuer 1994). In sugarcane, free amino acids are known to increase with nematode infestation (Showler et al. 1990), pathogen infection (Singh et al. 1993), drought stress (Muqing and Ru-Kai 1998), and salt stress (Joshi and Naik 1980). The availability of amino acids in host plants is a critical factor in population growth of many insect herbivores (McNeil and Southwood 1978). When unbound to molecules such as proteins, free amino acids do not require proteolysis, thus saving energy for the insect (Helms et al. 1971, Brodbeck and Strong 1987). Insects can respond to changes in host plant nutritional quality (Rhoades 1983), and modified nitrogen metabolism under plant stressed conditions can increase insect herbivore populations (White 1984).

The objectives of this study were to determine the effects of irrigation management, selected cultivars, and insecticide applications on *E. loftini* injury to sugarcane and ramifications for adult borer populations. Levels of free amino acids were

measured to identify potential biochemical mechanisms that might be important in these relationships.

3.2. Material and Methods

A 2-year study was conducted using a split-split plot design at the Texas A&M University Agricultural Research and Extension Station site near Ganado, in Jackson County, TX. Irrigation was assigned to main plots (2 replications). Irrigation occurred on 1 May (13.7 cm), 29 May (7.6 cm) and 22 August (13.7 cm) 2003, and on 30 July (13.7 cm) and 26 September (13.7 cm) 2004. Sub-plot treatments (n = 2 per main plot) consisted of two commercial sugarcane cultivars, LCP 85-384 and HoCP 85-845, planted on 22 January 2003 in three 6.1-m-long by 1.8-m-wide rows in each sub-plot. Sub-sub plots, 3 m of the center row of each sub-plot, were either treated or not with seven applications of tebufenozide at 0.56 kg [AI]/ha with surfactant Latron CS-7 (Dow AgroSciences, Indianapolis, IN) from June to August in 2003 and 2004 at two-week intervals with a Knapsack Solo sprayer (Solo, Newport News, VA) with a pressure of 11.3-15.9 kg PSI at 187 liters/ha. A single soil-incorporated application of ethoprop, covered by hand-raking, at 2.69 kg [AI]/ha was used at planting to control wireworms (Coleoptera: Elateridae). Plots were fertilized with 89.7 kg/ha nitrogen on 20 June 2003 and on 7 April 2004. Rainfall during the main crop production period was recorded from April to June in 2003 and 2004 at the Jackson County weather station, located 16 km from this study (Crop Weather Program for South Texas, Texas A&M University Agricultural Research and Extension Station, Corpus Christi).

Twenty stalks were randomly removed from the center row of each experimental plot for *E. loftini* injury/damage assessment on 1 November 2003 and on 19 October

2004. Number of internodes per plant, bored internodes per plant, and moth emergence holes were recorded. Plant height was measured for each stalk and plant weight was determined for each sampled stalk bundle from each plot. A relative index was used to estimate the survival of larvae to adulthood (relative survival = no. exit holes/no. bored internodes) (Reay-Jones et al. 2003). Means for each plot were analyzed using a generalized mixed linear model with year, cultivar, insecticide and irrigation regime as factors (PROC GLIMMIX, SAS Institute 2004) with a binomial distribution for percentage of bored internodes and relative survival, and a Poisson distribution for number of exit holes per plot. A logarithm transformation of the number of stalks per plot was used as an offset for the total number of exit holes. The Kenward-Roger method (Kenward and Roger 1997) was used to compute denominator degrees of freedom for the test of fixed effects for all variables. The generalized mixed linear model used for binomial data predicted the probability of a success (i.e. the internode is bored), which is reported for all treatment combinations rather than percentage values. Least square means are presented to account for the variance-covariance structure of the design. Confidence intervals were more appropriate as estimates of variability about a mean using the generalized mixed linear model because transformed intervals from a logarithm scale to a linear scale were asymmetric, which could not be accounted for with a simple standard error value.

The brix of juice was obtained with a hand-held refractometer from the bottom, middle and top portions of four stalks from each sub-subplot in the 2004 experiment. Stand counts were also taken in each sub-subplot, and tons of sugarcane/ha were calculated using the average stalk weight. Sugar per ton of sugarcane was calculated by

multiplying the average Brix reading for each stalk by a constant of 5.6 kg/unit of Brix. The method assumes a juice purity of 85% to obtain juice sucrose and a factor of 0.85 to convert juice sucrose to normal juice sucrose. For every unit of normal juice sucrose, a mill should recover approximately 7.8 kg of sugar. The constant of 5.6 kg/unit of Brix is thereby derived by multiplying 0.85 × 0.85 × 7.8 kg. Sugar yield per hectare was determined by multiplying sugar per ton of cane by tons of sugarcane per hectare. Effects of cultivar, insecticide, and irrigation regime were tested using a three-way ANOVA (PROC MIXED, SAS Institute 1999) and Kenward-Roger's method to compute denominator degrees of freedom for the test of fixed effects for all yield variables (Kenward and Roger 1997).

On 7 June 2003, the third leaf from the apex of sugarcane plants from both irrigation regimes and both cultivars (n = 8 per treatment) was excised and water potential was measured with a Model 610^{TM} pressure bomb (PMS Instrument Co., Corvalis, Oregon). The second sugarcane leaf from the top of the plant was selected at the same time as water potential measurements were taken. Each 1-g leaf tissue sample was homogenized with 10 ml 0.1 N HCl using a Virtishear homogenizer (Virtis, Gardiner, New York). A 5 g homogenate from each sample was placed in separate 10-ml tubes and centrifuged at 10,000 rpm for 30 min. Samples were stored at -80°C. One milliliter of supernatant from each sample was filtered through a 0.5-µl filter fitted to a 5-ml plastic syringe. Samples were placed in the autosampler of an Agilent 1100 Series (Agilent Technologies, Atlanta, Georgia) reversed-phase high-performance liquid chromatograph (HPLC) with a binary pump delivering solvent A [1.36 g sodium acetate trihydrate + 500 ml purified HPLC grade water + 90 µl triethylamine (TEA) + sufficient

acetic acid to bring the pH to 7.2 ± 0.05 (95% C.I.)] and solvent B [1.36 g sodium acetate trihydrate + 100 ml purified HPLC grade water (acetic acid added to this mixture to bring the pH to 7.2 ± 0.05 [95% C.I.]) + 200 ml acetonitrile + 200 ml methanol] at 100 and 1.0 ml/min on a Zorbax Eclipse AAA 4.6 × 150 mm 3.5 µ column (Agilent Technologies). Absorbances at 262 and 338 nm were monitored on a variable wavelength detector for 48 min per sample. The autosampler measured and mixed 6 µl sodium borate buffer (0.4 N, pH 10.2 in water), 1 µl 9-fluorenylmethylchloroformate (FMOC), and 1 µl ophthalaldehyde (OPA) derivitizing agents, and 2 µl of sample, then injected 2 µl for chromatographic separation of FAAs. Identification and quantification of 17 derivitized FAAs, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine, were achieved by calibrating with a standard mixture of amino acids. Peak integration accuracy was enhanced by manual establishment of peak baselines using Agilent software. Water potential and free amino acid concentrations were analyzed using a two-way analysis of variance with factors cultivar and irrigation regime (PROC MIXED, SAS Institute 1999).

3.3. Results

Irrigation significantly reduced the probability of occurrence of a bored internode by 2.5-fold and moth exit holes/stalk by 2.5-fold for the average of both cultivars over both years (Table 3.1 and Fig. 3.1). Cultivar LCP 85-384 had 1.8 times greater probability of occurrence of a bored internode than HoCP 85-845 and 1.9 times more moth exit holes/stalk. Applications of insecticide reduced probability of occurrence of a

Table 3.1. Statistical comparisons of *E. loftini* injury and adult emergence from irrigation/cultivar/insecticide experiment on sugarcane at Ganado, TX, 2003-2004.

Management factor combination	% Bored internodes			Moth exit holes		
	df	*F*	*P* > *F*	df	*F*	*P* > *F*
Irrigation	1, 2.074	34.72	0.0254	48	21.32	<0.0001
Cultivar	1, 10.35	32.53	0.0271	48	10.77	0.0019
Irrigation × Cultivar	1, 10.35	0.33	0.5758	48	0.32	0.5730
Insecticide	1, 12.99	271.81	<0.0001	48	55.10	<0.0001
Irrigation × Insecticide	1, 12.99	0.95	0.3478	48	3.44	0.0696
Cultivar × Insecticide	1, 12.99	0.00	0.9717	48	0.78	0.3816
Irrigation × Cultivar × Insecticide	1, 12.99	1.06	0.3231	48	0.00	0.9913
Year	1, 48	8.19	0.0062	48	3.08	0.0856
Irrigation × Year	1, 48	90.72	<0.0001	48	4.06	0.0495
Cultivar × Year	1, 48	17.58	0.0001	48	0.06	0.8033
Irrigation × Cultivar × Year	1, 48	1.54	0.2214	48	0.00	0.9591
Insecticide × Year	1, 48	121.80	<0.0001	48	4.35	0.0423
Irrigation × Insecticide × Year	1, 48	76.22	<0.0001	48	5.29	0.0259
Cultivar × Insecticide × Year	1, 48	2.31	0.1350	48	0.07	0.7962
Irrigation × Cultivar × Insecticide × Year	1, 48	1.27	0.2652	48	0.03	0.8604

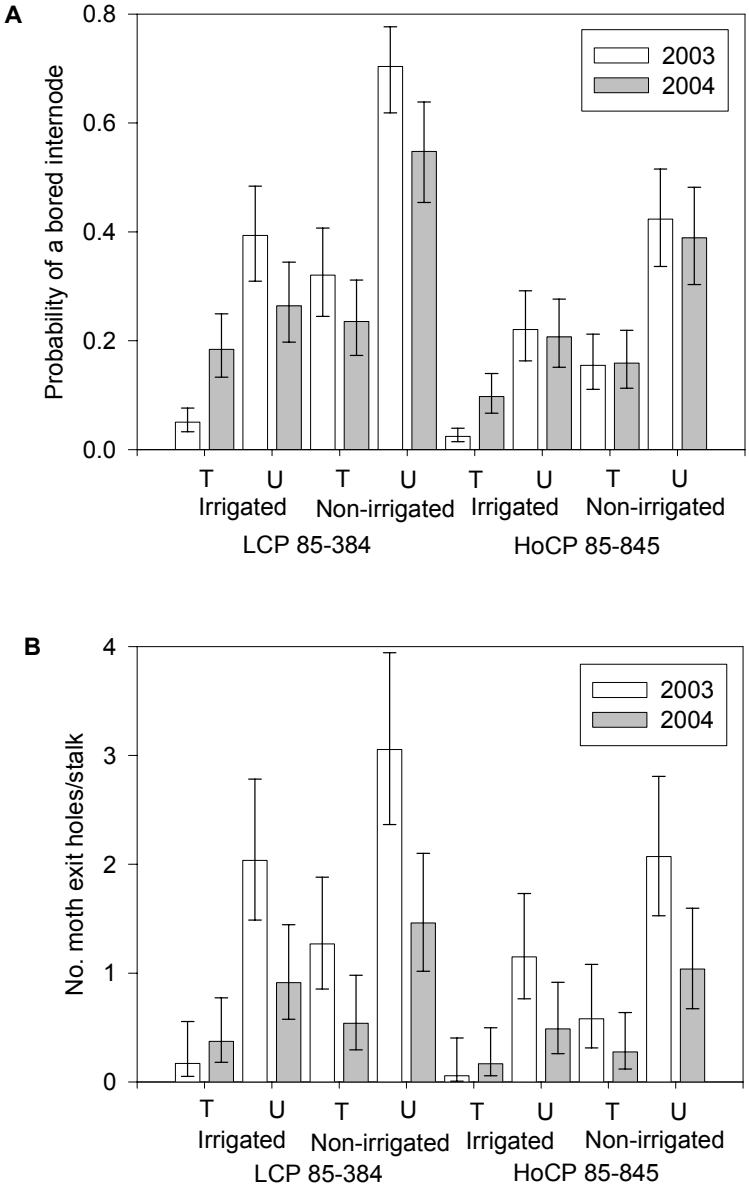

Fig. 3.1. A. Mean (± 95 C.I.) probability of an *E. loftini* bored internode (equivalent to proportion of bored internodes), and B. number of moth emergence holes per stalk (± 95 C.I.) in sugarcane irrigation, cultivar, and insecticide (T = insecticide treated; U = untreated) experiment at Ganado, TX, 2003-2004.

bored internode 3.1-fold and moth exit holes/stalk 4.4-fold. The probability of occurrence of a bored internode was 1.1-fold higher in 2003 than in 2004. A significant interaction was detected between year and irrigation for probability of occurrence of a bored internode and moth exit holes/stalk (Table 3.1). In 2003, irrigation reduced probability of occurrence of a bored internode 3.5-fold, and in 2004 1.8-fold. Similar trends were observed for moth exit holes/stalk, which were reduced by applying irrigation water 3.8-fold in 2003 and 1.7-fold in 2004. A significant interaction was detected between year and cultivar for probability of occurrence of a bored internode (Table 3.1). From 2003 to 2004, probability of occurrence of a bored internode increased from 0.149 to 0.194 for cultivar HoCP 85-845, but decreased from 0.307 to 0.294 for cultivar LCP 85-384. A significant interaction was detected between year and insecticide for probability of occurrence of a bored internode and moth exit holes/stalk (Table 3.1). In 2003, probability of occurrence of a bored internode was reduced from 0.429 in untreated plots to 0.093 in insecticide treated plots, and in 2004 from 0.341 to 0.162. The application of insecticides also reduced moth exit holes/stalk from 2.0 to 0.3 in 2003 and 0.9 to 0.3 in 2004. Climatic data showed that rainfall from April to June totaled 17 cm in 2003 and 62 cm in 2004. From June 1 to July 31, 290 *E. loftini* moths were caught in the pheromone trap in 2003 and 439 in 2004.

Probability of relative survival of *E. loftini* larvae was significantly reduced in insecticide treated plots ($\bar{x}$ = 0.184; 95% C.I. = [0.143, 0.234]) compared to untreated plots ($\bar{x}$ = 0.277; 95% C.I. = [0.231, 0.328]) (F = 10.71; df = 1, 39.98; P = 0.0022). Probability of relative survival decreased significantly from 0.260 (95% C.I. = [0.200, 0.332]) in 2003 to 0.197 (95% C.I. = [0.145, 0.261]) in 2004 (F = 10.60; df = 1, 48; P =

0.0021). Probability of relative survival in cultivar LCP 85-384 ($\bar{x}$ = 0.253; 95% C.I. = [0.193, 0.325]) was 25% greater than HoCP 85-845 ($\bar{x}$ = 0.203; 95% C.I. = [0.152, 0.265])], however the difference was not statistically significant (F = 3.17; df = 1, 40.03; P = 0.0825).

Water potential was significantly elevated in the irrigated treatment (10.31 ± 0.43 [SE] barr) compared to non-irrigated treatment (9.0 ± 0.40 [SE] barr) (F = 4.31; df = 1, 28, P = 0.0472). Cultivar did not affect water potential (F = 0.39; df = 1,28; P = 0.5386). Free alanine, aspartic acid, glutamic acid, glycine, histidine, leucine, methionine, phenylalanine, serine, threonine, tyrosine and valine were detected in both sugarcane cultivars under both irrigation regimes (Table 3.2). Isoloeucine was found in both cultivars, but only under non-irrigated conditions. Glutamic acid was 1.34-fold more abundant in cultivar LCP 85-384 than in HoCP 85-845. Free proline levels were 1.2-fold greater in cultivar HoCP 85-845, which also contained 1.4 times more tyrosine than LCP 85-384. Higher levels of aspartic acid (1.4-fold), histidine (2-fold), isoleucine (from 0 in non-irrigated to 12.8 in irrigated sugarcane), proline (1.2-fold), and serine (1.8-fold) were contained in non-irrigated sugarcane leaves ($P < 0.05$). Lower amounts of leucine (1.6-fold), methionine (14-fold) and tyrosine (1.5-fold) were found in non-irrigated sugarcane leaves ($P < 0.05$).

Applying irrigation water significantly increased the height (cm) of sugarcane stalks from 74.7 ± 4.0 [SE] to 101.6 ± 4.0 [SE] (F = 20.06; df = 1, 2; P = 0.046) over both years. In 2004, reduced tonnage of sugarcane per hectare was observed in the non-irrigated (26.0 ± 6.1 [SE]) compared to the irrigated treatment (51.0 ± 6.1 [SE]), however

Table 3.2. Free amino acid accumulations (nanomoles per 10 µL sugarcane juice) in sugarcane leaves from cultivars LCP 85-384 and HoCP 85-845 in irrigated and non-irrigated plots, Ganado, TX, 2003.

Free amino acid	LCP 85-384		HoCP 85-845		Cultivar		Stress		Cultivar × stress	
	Irrigated	Non-irrigated	Irrigated	Non-irrigated	F[a]	$P > F$	F[a]	$P > F$	F[a]	$P > F$
Alanine	256.8	351.9	385.9	231.9	0.01	0.9385	0.25	0.6177	4.54	0.0400
Arginine	0	0	0	0	-	-	-	-	-	-
Aspartic acid	195.7	400.0	241.2	211.2	3.08	0.0875	4.57	0.0395	8.26	0.0068
Glutamic acid	308.6	439.4	283.6	276.3	5.83	0.0210	2.51	0.1216	3.14	0.0849
Glycine	130.8	227.2	237.2	180.0	1.12	0.2971	0.49	0.4892	7.52	0.0095
Histidine	101.5	343.9	191.9	234.5	0.14	0.7075	32.04	< 0.0001	15.73	0.0003
Isoleucine	0	13.0	0	12.6	0.00	0.9671	7.61	0.0090	0.00	0.9671
Leucine	123.9	141.5	255.0	101.3	2.42	0.1287	5.43	0.0256	8.59	0.0058
Lysine	40.7	141.5	75.0	46.7	1.16	0.2891	0.10	0.7492	10.70	0.0024
Methionine	53.8	7.77	76.9	1.59	1.28	0.2645	66.13	< 0.0001	3.85	0.0576
Phenylalanine	47.0	53.7	57.5	57.8	0.48	0.4931	0.11	0.7412	0.09	0.7626
Proline	145.6	206.3	195.3	211.2	10.99	0.0021	21.68	< 0.0001	7.41	0.0099
Serine	85.1	336.6	215.2	205.6	0.00	0.9919	6.95	0.0123	8.11	0.0072
Threonine	52.7	90.2	78.9	62.9	0.00	0.9577	1.01	0.3204	6.28	0.0169
Tyrosine	123.2	125.6	230.6	116.6	5.87	0.0205	7.57	0.0092	8.24	0.0068
Valine	24.1	58.6	66.9	27.0	0.09	0.7667	0.02	0.8874	3.89	0.0563
Total	1689.5	2859.7	2591.2	1977.2	0.00	0.9724	1.02	0.3184	10.53	0.0025
Sum[1]	443.7	772.7	802.1	544.4	0.60	0.4435	0.18	0.6737	12.20	0.0013

[a] df = 1, 36.
[1] Sum of concentrations of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine.

the difference was not significant (F = 8.33; df = 1, 2; P = 0.102). Similar observations were made for theoretical tons of recoverable sugar per hectare, 2448.3 ± 537.7 [SE] in non-irrigated plots and 5122.4 ± 537.7 [SE] in irrigated plots (F = 12.37; df = 1, 2; P = 0.072). Cultivar LCP 85-384 (34.1 ± 4.5 [SE]) produced a lower tonnage than HoCP 85-845 (42.9 ± 4.5 [SE]) (F = 14.40; df = 1, 22; P = 0.001). Estimated tons of recoverable sugar per hectare were also lower in cultivar LCP 85-384 (3406.5 ± 406.7 [SE]) than in HoCP 85-845 (4164.2 ± 406.7 [SE]) (F = 6.89; df = 1, 22; P = 0.015). Increased theoretical tons of recoverable sugar per hectare was observed in insecticide treated (4036.5 ± 406.7 [SE]) compared to untreated treatments (3534.2 ± 406.7 [SE]), however the difference was not significant (F = 3.03; df = 1, 22; P = 0.096).

3.4. Discussion

Irrigation reduced the percentage of bored internodes (equivalent to probability of a bored internode × 100) by 2.5-fold, and in combination with fortnightly insecticide applications achieved the best *E. loftini* control for both susceptible and resistant cultivars. Together with trends for reduced tonnage of sugarcane and estimated tons of recoverable sugar per hectare under non-irrigated conditions, our results indicate the value of using irrigation to better manage *E. loftini* in sugarcane. Non-irrigated sugarcane plants were significantly shorter than irrigated plants in both years and had a significantly higher water potential in June 2003. Observations of plants showed increased numbers of dry leaves on non-irrigated sugarcane. A water deficit stress occurs when insufficient water is present in the plant's environment. An appropriate term to qualify the state of non-irrigated sugarcane was therefore water-stressed. Stress can be defined at the whole plant level as environmental conditions that limit the rate of dry

matter biomass of at least one component of the vegetation below its genetic potential (Grime 1979). Irrigated plots in our study received sufficient water to preclude being qualified as water-stressed. The decision to apply irrigation water was based on a combination of observed stress symptoms as previously verified with the pressure bomb, and rainfall patterns.

Rainfall patterns were different in 2003 and 2004, which may partially explain the significant interaction between year and irrigation for the percentage of bored internodes. Rainfall from April to June was 3.6-fold greater in 2004 than in 2003. The greater injury in 2003 likely was caused by an increase in drought stress and adult pest populations, as indicated by increased *E. loftini* moths caught in the pheromone trap in 2003. Growers in Texas can irrigate sugarcane, which seems to reduce their *E. loftini* injury. In contrast, the majority of Louisiana growers are not able to irrigate, and extended periods of drought in recent years have produced conditions that could aggravate *E. loftini* infestations. Partial cultivar resistance, combined with cultural practices to minimize plant stress, are expected to become major components of the *E. loftini* pest management system when the Mexican rice borer becomes established in Louisiana. Insecticides reduced infestations in this study, but required numerous applications that might not be economically feasible in commercial sugarcane production.

The irrigation regime was assigned to the main plots in our experiment to minimize movement of water between plots. Because of more degrees of freedom associated with estimates of experimental error for sub-plot and sub-sub-plot effects, main plot effects are not as precisely estimated. The irrigation regime had such a strong influence on *E. loftini* injury and the number of moth exit holes per stalk that significant

differences were detected despite the low number of degrees of freedom. However, yield components in 2004 (sugarcane per hectare and tons of recoverable sugar per hectare) were not significantly affected by irrigation, even though strong trends were observed. Our decision to use irrigation treatments in the main plot decreased the statistical power for detecting mean differences of this factor in the design.

As shown in previous work (Reay-Jones et al. 2003), cultivar LCP 85-384 was more susceptible to *E. loftini* than HoCP 85-845 based both on percentage of bored internodes and moth emergence holes per stalk. The differences in moth emergence holes would be further exacerbated when considering stand counts from the Louisiana sugarcane industry to determine moth production per hectare, as LCP 85-384 has a substantially higher stalk density than HoCP 85-845 (Orgeron et al. 2003). Adult emergence holes represent a seasonal record of moth production from sugarcane and might assist in choosing a cultivar that would suppress *E. loftini* populations on an areawide basis. Reduced pest injury in individual fields is a desired goal when choosing a resistant cultivar. An equally valuable attribute is reducing pest populations over large geographical areas, which provides more of a long term management solution. Implementation of cultural practices can be valuable in achieving this goal.

Applications of tebufenozide reduced injury and moth emergence holes per stalk in both cultivars. However, our study did not demonstrate an increase in yield during the second year when four subsamples were assessed with the refractometer. In a previous study, tebufenozide showed potential for control of *E. loftini* with applications carefully timed in a small plot test (Reagan et al. 2001), but a separate field experiment did not detect effects on yield or juice quality (Legaspi et al. 1999c). Weekly applications of

other insecticides (i.e. monocrotophos, azinphosmethyl, carbofuran) also reduced the percentage of *E. loftini* bored internodes, but significant effects on yield were not detected (Johnson 1985, Meagher et al. 1994, Legaspi et al. 1999c), further indicating the exceptionally high variability involving sugarcane yield studies. Tebufenozide is less toxic than the pyrethroid cyfluthrin to the *E. loftini* parasitoid *Allorhogas pyralophagus* Marsh (Legaspi et al. 1999c), and it has a substantially reduced impact on non-target arthropods in comparison to the pyrethroids lambda-cyhalothrin, esfenvalerate and cyfluthrin used to control the sugarcane borer, *Diatraea saccharalis* (F.), in Louisiana (Reagan and Posey 2001).

The life stage of *E. loftini* targeted for chemical control is the neonate larva, which disperses from dry leaves where the eggs are deposited to green parts of the plant (van Leerdam et al. 1986). The difficulty of applying pesticides to foliage in the lower parts of sugarcane, combined with the narrow window during which larvae are exposed, diminishes the effectiveness of insecticides. Our use of a back pack sprayer enhanced coverage and enabled insecticides to be applied low on the plant. Unfortunately, aerial application normally is not similarly effective at reaching this area of the plant. Sugarcane growers in Louisiana currently make 1-3 insecticide applications annually against *D. saccharalis*. Because a greater frequency of applications is needed to reduce *E. loftini* infestations (Johnson 1985, Meagher et al. 1994, Legaspi et al. 1999c), tebufenozide was applied seven times on a fortnightly basis in our study in an attempt to achieve adequate control. The need for alternate control tactics for *E. loftini* will arise once the pest becomes established in Louisiana sugarcane.

Because water potential recorded on 25 June 2003 was higher in non-irrigated plots only by a small margin (1.25 barr), total free amino acid levels were not significantly increased with this treatment; however drought stress effects were detected for several free amino acids in sugarcane, including free proline, which has previously been shown to be an indicator of water deficit stress (Showler 2002). Discontinuance of daily watering of sugarcane in greenhouse pots for 12 days increased levels of proline by 2.5-fold (Muqing and Ru-Kai 1998). Other types of stress have also increased levels of free proline in sugarcane leaves 1.6-fold (salt stress) (Joshi and Naik 1980), 6.2-fold (*Colletotrichum falcatum* Went infection) (Singh et al. 1993), and 1.2-fold (iron cholorosis) (Jain and Shrivastava 1998). When plants are subject to dehydration, which lowers the water potential of the cells and thus reduces water loss by osmoregulation, free proline accumulates (Heuer 1994). Free proline levels also increase under drought stress in sugar beets, *Beta vulgaris* L., by 12-fold (Gzik 1996) and in cotton, *Gossypium hirsutum* L., by 58-fold (Showler and Moran 2003). Free proline appears to be the most widespread and consistent amino acid that responds to drought stress (Aspinall and Paleg 1981). Proline biosynthesis is thought to be regulated by Δ'-pyrroline-5-carboxylate synthetase, which undergoes feedback inhibition by accumulation of proline (Delauney and Verma 1993), but under water stress, this feedback is lost (Boggess et al. 1976a). A major pathway of proline biosynthesis is thought to emanate from glutamic acid derived from carbohydrates (Boggess et al. 1976b). Glutamic acid levels were lower in the resistant cultivar HoCP 85-845, which also had higher levels of proline. This cultivar may have more efficient proline metabolism involving glutamic acid which may render the plant more tolerant to drought stress.

Certain amino acids are known to be essential for insect development (Vanderzant 1958, Nation 2002). Artificial diets with amino acid concentrations simulating that found in anthers were adequate for survival and development of the tobacco budworm, *Heliothis virescens* (F.) (Hedin et al. 1991). Free amino acids can elicit electrophysiological responses of the sensilla of the adult tobacco budworm, the corn earworm, *Heliothis armigera* (Hübner), and *Spodoptera littoralis* (Boisdval) (Blaney and Simmonds 1988). Positive correlations have been established between free amino acid levels in phloem sap of wheat and barley and the rate of population increase of the bird cherry oat aphid, *Rhopalosiphum padi* L. (Weibull 1987). Similar observations were made with the cabbage aphid, *Brevicoryne brassica* (L.), and *Brassica* species (Cole 1997). Fecundity of *R. padi* was also correlated to free amino acid levels in wheat phloem (Kazemi and van Emden 1992). Environmental conditions that cause accumulations of free amino acids in plants can sometimes increase the suitability of these plants to insect herbivores. The plant stress hypothesis postulates that outbreaks of insect herbivores occur under plant stress conditions because of the increased nutritional value of the host plant, mainly due to an increase in available nitrogen (White 1984). Drought stress did not affect the sum of free amino acids in our study, but some free amino acids essential for insect growth and development increased under drought stress in both cultivars, which might have affected *E. loftini* infestations and adult emergence. Reducing plant stress with irrigation may assist in managing *E. loftini* in sugarcane by decreasing the nutritional value of the crop for this insect.

A balance of control tactics is nearly always a recommended approach to achieve a greater permanency in integrated pest management programs (Luckmann and Metcalf

1994). Several control tactics (plant resistance, insecticides, and arthropod predation) have been shown to be effective in reducing infestations and areawide populations of *D. saccharalis* in Louisiana sugarcane (Bessin et al. 1990, 1991). Growers currently achieve adequate control using insecticides without a widespread use of resistant cultivars (Rodriguez et al. 2001). However, there is concern regarding the potential for insecticide resistance (Reay-Jones et al. 2005). Our study regarding anticipated management of *E. loftini* has demonstrated the need to employ multiple practices to achieve sufficient levels of control and population reduction on sugarcane. No single management tactic was effective alone in controlling *E. loftini* infestations. Therefore, a combination of control strategies will be necessary for farmers to achieve adequate control when the insect becomes established in the Louisiana sugarcane agroecosystem.

CHAPTER 4: OVIPOSITION OF THE MEXICAN RICE BORER ON SUGARCANE AND RICE

4.1. Introduction

Oviposition of Lepidoptera insects is a critical step in their life cycle because of the limited mobility of first instar larvae to feed and survive (Feeny et al. 1983). Visual, olfactory, gustatory and mechanical senses are used by ovipositing females in host plant selection (Ramaswamy 1988). Plant phenotypic characters that influence acceptability for insect oviposition include leaf pubescence (Sosa 1988), color (Levinson et al. 2003), phenological stage (Moré et al. 2003), and leaf shape (Mackay and Jones 1989). In addition, stress (Showler and Moran 2003), nutritional status (Myers 1985), and secondary metabolites (Feeny et al. 1983) also influence host selection by insect herbivores. Determining causal factors underlying oviposition patterns and quantifying oviposition preference of insect pests for host crops can assist in the development of pest management strategies (Renwick and Chew 1994, Showler 2004a).

The availability of host plant free amino acids (FAA) is a critical factor in population growth of many insect herbivores (McNeil and Southwood 1978) and insects can respond to changes in the nutritional quality of a plant (Rhoades 1983). Accumulations of host plant FAAs have been associated in many plants with numerous stresses (Rabe 1994, Showler 2004b), including drought (Gzik 1996, Showler 2002). Accumulated FAAs lower the water potential of cells, and may reduce water loss through osmoregulation (Heuer 1994).

The Mexican rice borer, *Eoreuma loftini* (Dyar), is known to feed on over 15 plant species in North America (see chapter 1) where it is known to be a pest of corn, *Zea*

mays L., sorghum, *Sorghum bicolor* (L.) Moench (Youm et al. 1988), rice, *Oryza sativa* L. (Way et al. 1999) and sugarcane, *Saccharum* spp. (Meagher et al. 1993). *Eoreuma loftini* has been the dominant insect pest of sugarcane in the Lower Rio Grande Valley of Texas since it was introduced from Mexico in 1980 (Johnson and van Leerdam 1981). *Eoreuma loftini* now represents >95% of the sugarcane stalk borer population in this area (Legaspi et al. 1999a). By 1989, its range had expanded into the rice production area of Texas (Browning et al. 1989) where it is responsible for major yield loss in rice. With *E. loftini* moths discovered in the sugarcane production area near Beaumont, Texas, invasion of Western Louisiana, where sugarcane and rice are grown in close proximity, is imminent (Reagan et al. 2005). Determining oviposition preference on both sugarcane and rice will assist in understanding insect-crop dynamics in Texas and Louisiana. The objectives of this study were to quantify *E. loftini* oviposition on sugarcane and rice cultivars at different phenological stages, and to identify the potential biochemical mechanisms behind these relationships.

4.2. Materials and Methods

This study was conducted at the Texas A&M Agricultural Experiment Station in Weslaco, Texas, during the summers of 2003 and 2004. Sugarcane plants (cultivars LCP 85-384, HoCP 85-845) were grown in a greenhouse in 3.8 L pots containing nursery potting soil (Metromix 300, Scotts, Maryville, OH). Sugarcane nodes collected in fields in the Lower Rio Grande Valley of Texas were planted individually in pots and fertilized when neccessary (N: 33 kg/ha; P: 6 kg/ha; K: 12 kg/ha). Plants were watered (1.5 L) three times weekly. The two phenological stages of sugarcane used in this study were plants with 5-6 internodes (~89 cm from soil to apex), representative of plants in early

July in Louisiana, and 10-11 internodes (~158 cm from soil to apex), representative of plants in mid-August. Drought-stressed sugarcane plants were watered once a week for two weeks prior to the start of the experiments. Non-drought stressed sugarcane continued to receive the standard (1.5 L × 3/week) watering regime. Rice cultivars Cocodrie and XL8 were grown in the greenhouse in 1.1 L soil containing pots (3 plants/pot), and received two amounts of urea at 207 kg/ha of nitrogen one week and five weeks after emergence. Rice was flooded 6 weeks after emergence. The four phenological stages used in this study were the 4-5 leaf tillering stage, 8-10 leaf tillering stage (3 weeks after emergence), boot stage (6 weeks after emergence), and full panicle stage (10-11 weeks after emergence).

4.2.1. Plant Phenology Measurements

At the end of each experiment, measurements of number of internodes, green and dry leaves were taken on each sugarcane plant. Measurements on rice plants were number of tillers, green and dry leaves. Dry weight was determined for 3 plants of each treatment after 5 days in an oven at 75ºC. In experiments 1 and 3 (Table 4.1), the third leaf from the apex of each sugarcane plant was excised and water potential measured with a Model 610^{TM} (PMS Instrument Co., Corvalis, Oregon) pressure bomb.

4.2.2. Plant Physiochemical Measurements

The second leaf from the apex of the plant was excised and placed on dry ice for all sugarcane treatments in experiments 1 and 7 and for rice treatments in experiments 2 and 6 (n = 8 per treatment). Each 1-g leaf tissue sample was homogenized with 10 ml of 0.1 N HCl using a Virtishear homogenizer (Virtis, Gardiner, New York). At least 5 g

homogenate from each sample was placed in separate 10-ml tubes and centrifuged at 10,000 rpm for 30 min. Samples were stored at –80°C.

One milliliter of supernatant from each sample was filtered through a 0.5-µl filter fitted to a 5-ml plastic syringe. Samples were placed in the autosampler of an Agilent 1100 Series (Agilent Technologies, Atlanta, Georgia) reversed-phase high-performance liquid chromatograph (HPLC) with a binary pump delivering solvent A [1.36 g sodium acetate trihydrate + 500 ml purified HPLC grad water + 90 µl triethylamine (TEA) + sufficient acetic acid to bring the pH to 7.2 ± 0.05 (95% C.I.)] and solvent B [1.36 g sodium acetate trihydrate + 100 ml purified HPLC grade water (acetic acid added to this mixture to bring the pH to 7.2 ± 0.05 [95% C]) + 200 ml acetonitrile + 200 ml methanol] at 100 and 1.0 ml/min on a Zorbax Eclipse AAA 4.6 × 150 mm 3.5 µ column (Agilent Technologies). Absorbances at 262 and 338 nm were monitored on a variable wavelength detector for 48 min per sample. The autosampler measured and mixed 6 µl sodium borate buffer (0.4 N, pH 10.2 in water), 1 µl 9-fluorenylmethylchloroformate (FMOC), and 1 µl ophthalaldehyde (OPA) derivitizing agents, and 2 µl of sample, then injected 2 µl for chromatographic separation of FAAs. Identification and quantification of 17 derivitized FAAs, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine, were achieved by calibrating with a standard mixture of amino acids. Peak integration accuracy was enhanced by manual establishment of peak baselines using Agilent software.

4.2.3. Oviposition Choice Test

Eoreuma loftini adults were obtained from a laboratory colony at the Texas A&M Agricultural Experiment Station in Weslaco. The insects were reared on artificial diet (Martinez et al. 1988) at 25ºC, 65% RH, and a photoperiod of 14:10 (L:D). Pupae were separated by sex and placed in 3.8 L plastic containers for emergence in the same conditions. Adult *E. loftini* had emerged less than 48 h prior to the beginning of the experiment. Each test consisted of placing four treatments per cage replicated in four greenhouse cages (2 × 2 × 2 m). Two plants of each treatment were randomly placed on a 1.5 m circle in the center of each cage. Seven experiments (j = 7) were conducted to cover all treatments (i = 16) (Table 4.1). Oviposition tests were initiated with the release of 30 male and 30 female moths in each cage and ended 6 days later. Number of eggs, number of egg masses and location on plant were recorded.

4.2.4. Data Analyses

Oviposition choice based on egg masses, eggs per egg mass and eggs per plant were analyzed for non-randomness by performing chi-square tests (Zar 1999) in each experiment and over all seven experiments. Expected frequencies of egg laying for each of the four treatments per experiment were 1/4, 1/4, 1/4, and 1/4.

The study of host preference has used several types of models predicting patterns of oviposition or predation on different hosts (Manly et al. 1972, Chesson et al. 1978, Wilson and Gutierrez 1980, Murphy et al. 1991). Our preference coefficients and those of Chesson (1978), Manly et al. (1972) and Wilson and Gutierrez (1980) can be obtained with varying host availability, which is useful when comparing observations in the field at different host densities. Measurements of preference can be viewed as departures from

the probability of accepting a host based solely on availability, with choice at random. Preference coefficients (based on egg masses, eggs per egg mass, and eggs per plant) derived using our model ranged from 0.0 (no eggs laid) to 1.0 (maximum number laid). Predicted numbers of egg masses, eggs per egg mass, or eggs per plant were derived using the following equation:

$$P_{ij} = \frac{\hat{\alpha}_{ij} A_i}{\sum_{i=1}^{i} \hat{\alpha}_{ij} A_i} \qquad [1]$$

where:

$\hat{\alpha}_{ij}$ = the estimated preference shown for the ith host of the jth dataset.

A_i = relative density of the ith host, with a value of 1 for sugarcane categories (one plant per pot) and 3 for rice categories (three plants per pot).

Models were fit using iterative nonlinear least squares regression with the modified Gauss-Newton method (JMP, SAS Institute 2002) combining average mean preference estimates (a_{ij} values from equation 2) from all seven experiments. Because two treatments overlapped between each experiment, this allowed an overall adjustment of preference estimates. The parameter with the greatest value (i.e. the parameter estimating preference for the most attractive host) was locked to a value of 1. Treatments where oviposition did not occur were assigned a locked preference estimate of 0. The model was refit with parameters now scaled from 0 to 1.

Prior to conducting regression and correlation analyses, preference coefficients were established for each host category and each experiment, therefore accounted for between experiment variability. The estimated oviposition preference coefficient estimate a_{ij} for the ith category and jth experiment was obtained using the

Table 4.1. Design of *E. loftini* oviposition studies, Weslaco, TX, 2003-2004.

Species	Cultivar	Stage	Stress (sugarcane only)	Host type	Experiment						
					1	2	3	4	5	6	7
Sugarcane	LCP 85-384	5 internodes	Non drought stressed	1	X						
			Drought stressed	2	X	X					
		11 internodes	Non drought stressed	3						X	X
			Drought stressed	4							X
	HoCP 85-845	5 internodes	Non drought stressed	5	X	X					
			Drought stressed	6	X						
		11 internodes	Non drought stressed	7							X
			Drought stressed	8						X	X
Rice	Cocodrie	Tillering 3-4 leaves		9				X			
		Tillering 6-7 leaves		10			X	X	X		
		Boot		11		X	X				
		Heading		12					X	X	
	XL8	Tillering 3-4 leaves		13				X			
		Tillering 6-7 leaves		14			X	X	X		
		Boot		15		X	X				
		Heading		16					X	X	

following equation:

$$a_{ij} = \frac{n_{ij}}{\max n_j} \quad [2]$$

where n_{ij} = number of eggs laid on treatment i in experiment j; max n_j = maximum number of eggs laid in experiment j. These coefficients represent unadjusted estimates that need to be modified to account for between experiment variability. Deviations were calculated using the following equation:

$$D_j = \sum_{i=1}^{j} (a_{ij}\beta_j - \overline{\alpha}_i)^2 \quad [3]$$

Parameter β_j was determined for each dataset as the value that minimized D. The adjusted preference coefficient estimate for the i^{th} category for the j^{th} experiment was obtained from the following equation:

$$\alpha_{ij} = a_{ij}\beta_j \quad [4]$$

Regressions were performed using preference coefficients α_{ij} as responses and plant phenology and physiochemical measurements as variables (PROC REG, SAS Institute, 1999). Preference coefficients α_{ij} were used to establish Pearson correlations with plant phenology and physiochemical measurements (PROC CORR, SAS Institute, 1999). Plant phenology and physiochemical measurements were pooled across experiments and analyzed with a one-way analysis of variance (PROC MIXED, SAS Institute 1999). Means were compared using contrasts ($\alpha = 0.05$).

4.3. Results

Over all seven experiments, eggs ($\chi^2 = 886.1$; d.f. = 21; $P < 0.0001$) and eggs per egg mass ($\chi^2 = 83.0$; d.f. = 21; $P < 0.0001$) per plant did not result from random choices

by *E. loftini*. The chi-square test for goodness of fit was not significant for egg masses per plant across all seven experiments (χ^2 = 24.2; d.f. = 21; P = 0.284); however, non-randomness was detected in experiment two (χ^2 = 8.9; d.f. = 3; P = 0.0304).

A total of 1,130 egg masses ($\bar{x}$ = 5.0 ± 0.51 [SE] egg masses per plant) and 29,337 eggs ($\bar{x}$ = 131 ± 12.57 [SE] eggs/plant) were laid in this study, corresponding to an average egg mass size of 29.1 ± 1.6 [SE]. Regression models showed a good fit between observed and predicted values for number of egg masses per plant (R^2 = 0.983), eggs per egg mass per plant (R^2 = 0.965), and eggs per plant (R^2 = 0.967). The preference coefficients (Fig. 4.1) derived using equation 1 show values ranging from 1.0 (drought stressed sugarcane cultivar LCP 85-384 at the 5 internode stage) to 0.0 (both rice cultivars at the 3-4 leaf tillering stage). Sugarcane was more attractive for oviposition than rice by 9.2-fold based on egg masses per plant, 7.0-fold based on eggs per egg mass per plant, and 12.9-fold based on eggs per plant. Drought stress increased attractiveness by 1.8-fold based on egg masses per plant, 1.03-fold based on eggs per egg mass per plant, and 1.6-fold based on eggs per plant. Egg masses per plant (1.2-fold), eggs per egg mass per plant (1.6-fold), and eggs per plant (1.5-fold) were greater on cultivar LCP 85-384 than on cultivar HoCP 85-845. The young sugarcane (5 internodes) was more attractive than the old sugarcane (11 internodes) based on egg masses per plant (1.1-fold), eggs per egg mass per plant (1.2-fold), and eggs per plant (1.3-fold). On rice, cultivar XL8 was more attractive than cultivar Cocodrie by 1.4-fold based on egg masses per plant, 1.7-fold based on eggs per egg mass per plant, and 1.9-fold based on eggs per plant. Preference estimates increased with rice phenology for all preferences estimates on cultivar Cocodrie, and for egg masses per plant on XL8. The boot stage was the most

attractive for cultivar XL8 based on eggs per egg mass per plant and eggs per plant. On sugarcane, 100% of the egg masses were laid on dry leaves or dry tips of leaves. On rice, 46% of the eggs were laid on dry leaves and 54% on green leaves, leaf sheaths and on the stem.

Rice plants had a lighter weight (0.027-fold), fewer leaves (0.52-fold), fewer dry leaves (0.52-fold) (Tables 4.2 and 4.3), and higher levels of arginine (4.2-fold), aspartic acid (2.5-fold), glutamic acid (8.0-fold), glycine (1.7-fold), histidine (3.9-fold), isoleucine (4.0-fold), leucine (8.4-fold), lysine (3.6-fold), phenylalanine (6.2-fold), serine (5.5-fold), threonine (3.4-fold), tyrosine (1.8-fold), valine (3.2-fold), total FAAs (3.0-fold) and the sum of essential FAAs (4.1-fold) than sugarcane ($P < 0.05$) (Tables 4.4 and 4.5). Cultivar HoCP 85-845 had higher levels of glycine (1.5-fold) and tyrosine (2.0-fold), but fewer leaves (0.93-fold) and fewer dry leaves (0.75-fold) than LCP 85-384 ($P < 0.05$). Stressed sugarcane had a lighter weight (0.82-fold), more dry leaves (1.5-fold), a greater water potential (2.2-fold), and higher levels of arginine (4.1-fold), aspartic acid (2.3-fold), glycine (2.9-fold), isoleucine (3.6-fold), leucine (6.8-fold), phenylalanine (5.1-fold), and the sum of essential FAAs (1.9-fold) than non-stressed sugarcane ($P < 0.05$). Sugarcane at the 5-6 internode stage had higher levels of arginine (2.6-fold), aspartic acid (1.7-fold), proline (5.0-fold), valine (3.0-fold) and less glycine (0.50-fold), fewer leaves (0.8-fold), fewer dry leaves (0.8-fold), and had a lower weight (0.33-fold) than the 10-11 internode stage ($P < 0.05$). Rice cultivar Cocodrie had fewer leaves (0.76-fold), fewer tillers (0.69-fold), and higher levels of aspartic acid (1.7-fold), glycine (1.9-fold), isoleucine (3.1-fold), leucine (4.3-fold), lysine (4.8-fold), phenylalanine (4.3-fold), threonine (1.4-fold), tyrosine (4.5-fold), sum of essential FAAs (1.5-fold), but lower

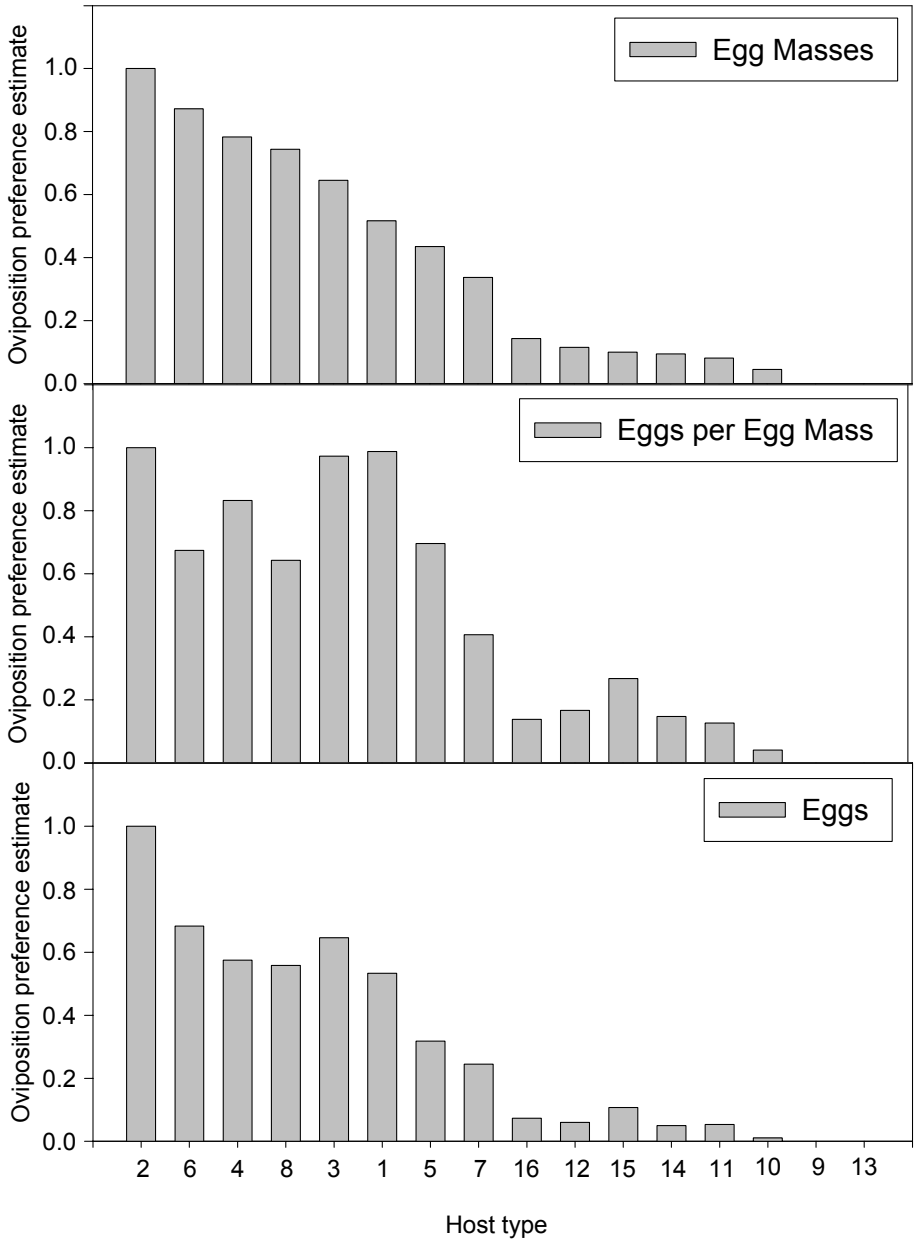

Fig. 4.1. Oviposition preference estimates from non-linear regression models ranging from 0 (no oviposition) to 1 (maximum preference). Host types are reported in Table 4.1.

Table 4.2. Sugarcane and rice phenology and physiochemical measurements from greenhouse oviposition test, Weslaco, TX, 2003-2004.

Species	Cultivar	Stage	Stress (sugarcane only)	Leaves	Dry leaves	Water potential (sugarcane) [barr]	Tillers (rice)
Sugarcane	LCP 85-384	5 internodes	No	17.2	7.2	8.7	-
			Yes	16.9	10.6	14.7	-
		11 internodes	No	19.2	9.2	5.4	-
			Yes	21.0	14.4	26.4	-
	HoCP 85-845	5 internodes	No	15.0	5.2	4.1	-
			Yes	14.6	8.9	23.0	-
		11 internodes	No	19.6	7.2	13.5	-
			Yes	19.7	9.7	5.7	-
Rice	Cocodrie	Tillering 3-4 leaves	-	4.0	0.0	-	1.0
		Tillering 6-7 leaves	-	7.7	0.7	-	1.6
		Boot	-	9.9	1.2	-	1.7
		Heading	-	10.6	1.9	-	2.0
	XL8	Tillering 3-4 leaves	-	4.6	0.04	-	1.5
		Tillering 6-7 leaves	-	10.6	0.5	-	2.6
		Boot	-	14.0	1.5	-	3.1
		Heading	-	13.3	1.7	-	2.5

Table 4.3. Multiple contrasts of plant phenology measurements on rice and sugarcane from greenhouse oviposition test, Weslaco, TX, 2003-2004.

	Dry weight (g)	Leaves	Dry leaves	Water potential (sugarcane only) [barr]	Tillers (rice only)
F	320.29	55.34[b]	66.95[b]	9.67[c]	14.73[e]
P > *F*	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
			Contrasts (F-values)[e]		
Rice vs. sugarcane[b]	2840.8***	564.1***	783.4***	-	-
LCP 85-384 vs. HoCP 85-845[b]	7.71**	6.33*	35.53***	6.09*	-
Stressed sugarcane vs. non-stressed[b]	59.73***	0.32NS	71.82***	40.69***	-
5 vs. 11 internode stage (sugarcane)	1318.21***	56.06***	24.56***	0.83NS	-
Cocodrie vs. XL8[b]	0.50NS	30.70***	0.00NS	-	43.82***
Boot vs. heading[b]	1.92NS	0.00NS	0.87NS	-	18.63***
Tillering vs. boot and heading	3.00NS	124.95***	10.94**	-	26.23***

[a] df = 15, 68
[b] df = 15, 96
[c] df = 7, 24
[d] df = 7, 40
[e] df = 7, 56
[e] * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$, NS $P > 0.05$

levels of arginine (0.68-fold), and histidine (0.69-fold), compared to XL8 ($P < 0.05$). Rice heading stage had higher levels of aspartic acid (1.3-fold), glycine (72.3-fold), isoleucine (3.9-fold), leucine (24.4-fold), lysine (0 for boot and 125.6 nanomoles per 10 μL juice for heading stage), phenylalanine (18.1-fold), tyrosine (117.8-fold), valine (1.3-fold), sum of essential FAAs (1.7-fold), but lower levels of histidine (0.62-fold), proline (0.024-fold), and serine (0.64-fold) compared to the boot stage ($P < 0.05$). The boot and heading rice stages had more leaves (1.8-fold), dry leaves (3.4-fold), and tillers per plant (3.8-fold) than the tillering stage ($P < 0.05$).

On rice, subsets of variables that best predict egg masses per plant using regression models yielded R^2 values ranging from 1.0 to 0.9996 (Table 4.6). In the best model ($R^2 = 1.0$), the variable total leaves had a negative parameter, indicating an increase in egg masses per plant as total leaves decrease, holding valine constant. Levels of valine increased with egg masses, holding total leaves constant. Positive associations were established between eggs masses per rice plant and variables valine, aspartic acid and threonine, as indicated by positive parameter estimates in regression models and positive correlation coefficients (Table 4.7). For eggs per egg mass, the ten best regression models had R^2 values ranging from 1.0 to 0.9984 (Table 4.6). Positive associations were established between eggs per egg mass per rice plant and levels of alanine, as indicated by the positive parameter estimate in regression models and positive correlation coefficients (Table 4.7). Subsets of variables that best predict eggs per plant using regression models yielded R^2 values ranging from 0.9673 to 0.9113 (Table 4.8). Positive associations were established between eggs per rice plant and dry leaves, arginine, alanine, glutamic acid and the sum of essential FAAs, as indicated by the

Table 4.4. Free amino acid accumulations (nanomoles per 10 µL juice) in rice and sugarcane leaves from greenhouse oviposition test, Weslaco, TX, 2003-2004.

	Sugarcane								Rice			
	5 internode				11 internode				Boot		Heading	
	LCP 85-384		HoCP 85-845		LCP 85-384		HoCP 85-845					
	Non-stressed	Stressed	Non-stressed	Stressed	Non-stressed	Stressed	Non-stressed	Stressed	Cocodrie	XL8	Cocodrie	XL8
Alanine	1441.6	1278.4	447.0	1238.2	172.7	526.7	177.9	801.8	888.8	1458.4	1454.4	489.2
Arginine	31.0	59.4	14.1	41.6	3.1	48.7	10.3	90.9	41.5	286.9	212.8	86.1
Aspartic acid	378.9	618.1	252.3	756.9	174.9	255.4	161.3	576.6	843.5	894.2	1617.4	591.2
Glutamic acid	96.2	329.1	237.7	634.8	203.2	386.7	249.3	696.2	2117.9	3432.4	3584.9	2150.4
Glycine	0	148.0	42.9	169.5	104.3	187.2	131.6	310.0	12.64	0	599.8	322.1
Histidine	250.7	145.6	91.8	167.1	87.4	112.2	85.7	126.3	351.2	924.7	492.5	298.8
Isoleucine	9.4	49.5	12.3	77.9	0	29.2	35.2	48.6	43.8	62.3	350.4	66.9
Leucine	12.6	37.9	23.5	43.4	0	84.5	5.2	113.1	16.0	36.7	1069.2	218.1
Lysine	0	12.1	0	12.4	0	10.2	5.0	29.5	0	0	104.1	21.5
Methionine	3.8	37.1	0	2.7	0	3.0	0	12.0	1.4	0	19.0	0
Phenylalanine	4.1	42.4	32.8	62.5	3.1	38.7	0	58.9	0	39.3	610.2	102.0
Proline	1679.5	570.4	458.9	421.1	125.8	168.1	153.4	172.2	1211.6	1150.4	0	55.6
Serine	416.2	281.7	176.8	218.4	89.7	182.8	134.6	253.7	1025.0	1931.9	1366.3	513.1
Threonine	176.1	170.1	42.1	163.3	30.6	88.6	28.9	142.6	287.5	393.1	537.3	209.3
Tyrosine	18.0	168.1	144.3	273.1	96.8	94.9	199.5	141.6	0	8.66	839.7	180.1
Valine	124.9	86.0	56.4	84.9	35.6	18.4	62.4	0	89.3	232.8	339.5	93.0
Total	4642.8	4033.9	2032.9	4675.1	1127.2	2196.7	1440.4	3960.1	6930.0	10852.3	13197.4	5397.4
Sum[1]	612.4	640.2	273.0	709.9	232.8	622.0	159.8	433.7	830.8	1975.7	3735.0	1095.7

[1] Sum of concentrations of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine.

Table 4.5. Multiple contrasts of free amino acid accumulations (nanomoles per 10 µL juice) in rice and sugarcane leaves from greenhouse oviposition test, Weslaco, TX, 2003-2004.

			Contrasts (F-values)					
	F[a]	$P > F$	Rice vs. sugarcane[b]	LCP 85-384 vs. HoCP 85-845[b]	Stressed sugarcane vs. non-stressed[b]	5 vs. 11 internode stage (sugarcane)	Cocodrie vs. XL8[b]	Boot vs. heading[b]
Alanine	2.37	0.0251	2.90NS	1.02NS	2.61NS	8.19**	0.39NS	0.40NS
Arginine	13.58	<0.0001	68.95***	0.05NS	7.52**	0.01NS	6.39*	0.40NS
Aspartic acid	15.61	<0.0001	85.30***	1.18NS	17.65*	8.07**	21.83***	5.08*
Glutamic acid	18.19	<0.0001	171.5***	1.94NS	3.72NS	0.53NS	0.04NS	0.09NS
Glycine	22.45	<0.0001	18.60***	4.27*	26.65***	12.9**	15.63**	153.4***
Histidine	12.87	<0.0001	84.70***	0.42NS	0.03NS	1.61NS	7.79**	12.68**
Isoleucine	21.09	<0.0001	64.42***	1.78NS	5.89*	0.21NS	43.02***	59.28***
Leucine	102.9	<0.0001	267.1***	0.29NS	7.73**	1.18NS	197.6***	436.8***
Lysine	6.63	<0.0001	10.83**	0.51NS	3.21NS	0.46NS	13.19**	30.43***
Methionine	1.15	0.3526	0.11NS	0.99NS	2.92NS	0.90NS	0.95NS	0.70NS
Phenylalanine	66.60	<0.0001	156.3***	0.95NS	6.99*	0.32NS	128.3***	264.1***
Proline	2.04	0.0528	0.37NS	1.66NS	1.11NS	5.19*	0.00NS	9.14**
Serine	35.73	<0.0001	274.4**	0.29NS	0.08NS	2.88NS	0.07NS	30.19***
Threonine	8.26	<0.0001	59.19***	0.34NS	3.61NS	2.99NS	4.34*	0.38NS
Tyrosine	18.90	<0.0001	14.51**	5.74*	1.63NS	0.06NS	40.48***	97.64***
Valine	12.43	<0.0001	62.52***	0.88NS	1.72NS	8.60**	3.60NS	4.13*
Total	13.84	<0.0001	101.7***	2.28NS	2.73NS	4.07NS	3.73NS	0.16NS
Sum[1]	33.90	<0.0001	190.4***	0.04NS	4.51*	2.04NS	18.70**	34.31***

[a] Df = 1, 36

[b] Df = 36; * $P < 0.05$, ** $P < 0.001$, ***$P < 0.0001$, NS $P > 0.05$

positive parameter estimate in regression models and positive correlation coefficients (Table 4.7).

On sugarcane, subsets of three variables that best predict egg masses per plant using regression models yielded R^2 values ranging from 0.9845 to 0.9562 (Table 4.9). Positive associations were established between egg masses per sugarcane plant and variables dry leaves, methionine, and aspartic acid, as indicated by positive parameter estimates in regression models and positive correlation coefficients (Table 4.7). For eggs per egg mass, the ten best regression models had R^2 values ranging from 0.9041 to 0.7918 (Table 4.9). Positive associations were established between eggs per egg masses per sugarcane plant and variables dry leaves, methionine, and aspartic acid, as indicated by positive parameter estimates in regression models and positive correlation coefficients (Table 4.7). Subsets of variables that best predict eggs per plant using regression models yielded R^2 values ranging from 0.9673 to 0.9113 (Table 4.10). Positive associations were established between eggs per sugarcane plant and variables dry leaves, methionine, threonine, alanine and aspartic acid, as indicated by positive parameter estimates in regression models and positive correlation coefficients (Table 4.7).

4.4. Discussion

4.4.1. Methodology

This study provided a method that allows oviposition preference estimates to be determined from multiple experiments, each with a reduced number of host types. For practical reasons, all 16 hosts in our study could not be used in a single experiment. Allowing 2 of the 4 hosts for each experiment to occur in several tests enabled preference coefficients to be estimated using non-linear regression. By locking the coefficient of the

Table 4.6. Regression analyses (n = 4) of *E. loftini* oviposition estimates (egg mass and eggs per egg mass per plant) using plant phenology and physiochemical measurements on rice.

Dependant variable	Number of parameters	R^2	Intercept	Independent variables			
				Name	Estimate	Name	Estimate
Egg mass	2	1.0000	0.23685	Total leaves	-0.0120	Valine	0.000921
	2	0.9999	-0.04077	Tillers	0.0248	Aspartic acid	0.000266
	2	0.9999	0.04273	Threonine	0.000589	Isoleucine	0.000223
	2	0.9998	0.22643	Dry leaves	-0.0864	Threonine	0.000589
	2	0.9998	0.00263	Threonine	0.00104	Alanine	-0.0000872
	2	0.9997	0.03476	Threonine	0.000646	Lysine	0.000528
	2	0.9997	0.02584	Aspartic acid	0.000226	Arginine	0.000208
	2	0.9996	0.03604	Threonine	0.000638	Leucine	0.0000542
	2	0.9996	-0.02445	Aspartic acid	0.000197	Glutamic acid	0.0000398
	2	0.9996	0.03440	Threonine	0.000646	Tyrosine	0.0000658
Eggs per egg mass	2	1.0000	0.34342	Glutamic acid	-0.000142	Alanine	0.000451
	2	1.0000	0.24346	Tyrosine	-0.00161	Isoleucine	0.00456
	2	1.0000	0.10077	Threonine	0.000724	Proline	0.000111
	2	1.0000	0.23049	Isoleucine	0.00543	Leucine	-0.00154
	2	0.9998	0.39935	Total leaves	-0.0208	Serine	0.000208
	2	0.9998	0.33439	Dry weight	-0.0382	Sum FAAs	0.0000222
	2	0.9995	-0.11435	Valine	-0.00271	Sum FAAs	0.000118
	2	0.9994	0.44098	Dry weight	-0.0473	Valine	0.000644
	2	0.9990	0.31087	Tyrosine	-0.00993	Leucine	0.00797
	2	0.9984	0.45684	Tillers	-0.162	Histidine	0.000694

Table 4.7. Correlation coefficients ($P < 0.1$) of *E. loftini* oviposition estimates with plant phenology and physiochemical measurements.

Preference estimates	Sugarcane				Rice			
	Plant variable	n	r	*P*	Plant variable	n	r	*P*
Egg mass	Dry leaves	12	0.740	0.0059	Dry leaves	16	0.809	0.0001
	Arginine	8	0.823	0.0122	Threonine	4	0.988	0.0125
	Phenylalanine	8	0.821	0.0125	Aspartic acid	4	0.982	0.0176
	Aspartic acid	8	0.796	0.0181	Essential FAAs	4	0.971	0.0287
	Essential FAAs	8	0.776	0.0236	Valine	4	0.963	0.0372
	Water potential	8	0.750	0.0322	Total FAAs	4	0.954	0.0461
	Threonine	8	0.730	0.0399	Dry weight	16	0.470	0.0662
	Methionine	8	0.706	0.0503	Methionine	4	0.915	0.0852
	Isoleucine	8	0.690	0.0585				
	Lysine	8	0.689	0.0587				
	Leucine	8	0.647	0.0827				
Eggs/egg mass	-				Dry leaves	16	0.732	0.0013
					Tillers	16	0.506	0.0456
					Alanine	4	0.933	0.0674
					Total leaves	16	0.456	0.0740
					Serine	4	0.903	0.0967
Eggs	Methionine	8	0.850	0.0076	Dry leaves	16	0.758	0.0007
	Threonine	8	0.763	0.0277	Alanine	4	0.985	0.0149
	Dry leaves	12	0.626	0.0294	Glutamic acid	4	0.963	0.0374
	Essential FAAs	8	0.749	0.0325	Tillers	16	0.515	0.0414
	Aspartic acid	8	0.739	0.0403	Serine	4	0.936	0.0639
	Arginine	8	0.709	0.0492	Total FAAs	4	0.936	0.0640
	Alanine	8	0.689	0.0587	Arginine	4	0.919	0.0815
	Water potential	8	0.662	0.0737	Total leaves	16	0.442	0.0862

Table 4.8. Regression analyses (n = 4) of *E. loftini* eggs per plant oviposition estimate using plant phenology and physiochemical measurements on rice.

Number of parameter	R^2	Intercept	Independent variables			
			Name	Estimate	Name	Estimate
2	1.0000	-0.09023	Methionine	-0.00539	Sum FAAs	0.0000351
2	1.0000	-0.02238	Serine	0.000162	Glycine	0.000119
2	1.0000	0.00674	Arginine	0.000260	Alanine	0.000143
2	1.0000	-0.02331	Dry weight	-0.0594	Dry leaves	0.222
2	0.9999	-0.04380	Aspartic acid	0.000112	Histidine	0.000246
2	0.9997	-0.03943	Histidine	0.000182	Threonine	0.000410
2	0.9996	0.01289	Serine	0.0000998	Valine	0.000359
2	0.9989	0.19389	Phenylalanine	0.00504	Leucine	-0.00280
2	0.9988	0.00467	Serine	0.000118	Essential FAAs	0.0000283
2	0.9988	-0.15178	Glutamic acid	0.000117	Proline	0.0000380

Table 4.9. Regression analyses (n = 8) of *E. loftini* oviposition estimates (egg mass and eggs per egg mass per plant) using plant phenology and physiochemical measurements on sugarcane.

Dependant variable	R^2	Intercept	Independent variables					
			Name	Estimate	Name	Estimate	Name	Estimate
Egg mass	0.9845	0.589	Alanine	0.000763	Tyrosine	-0.00136	Proline	-0.000675
	0.9755	0.477	Alanine	0.000982	Isoleucine	-0.00586	Proline	-0.000792
	0.9709	1.1221	Dry leaves	0.0817	Total leaves	-0.0707	Methionine	0.00519
	0.9702	0.591	Serine	-0.00288	Arginine	0.00491	Alanine	0.000671
	0.9635	0.359	Dry weight	-0.00414	Dry leaves	0.0527	Glycine	0.000621
	0.9610	0.318	Dry weight	-0.00392	Dry leaves	0.0586	Glutamic acid	0.000140
	0.9610	0.193	Dry weight	-0.00257	Dry leaves	0.0557	Aspartic acid	0.000290
	0.9575	0.301	Height	-0.00256	Dry leaves	0.0589	Aspartic acid	0.000308
	0.9564	0.510	Dry weight	-0.00489	Dry leaves	0.0598	Valine	-0.00135
	0.9562	0.483	Alanine	0.00116	Aspartic acid	-0.000805	Proline	-0.000794
Eggs per egg mass	0.9041	0.475	Water pot.	0.0203	Aspartic acid	0.00121	Isoleucine	-0.0161
	0.8870	0.775	Alanine	0.00104	Isoleucine	-0.0142	Proline	-0.000696
	0.8662	1.265	Serine	-0.00356	Alanine	0.000883	Tyrosine	-0.00271
	0.8649	0.938	Serine	-0.00272	Threonine	0.00753	Isoleucine	-0.0118
	0.8498	0.434	Dry leaves	0.0659	Alanine	0.00112	Essential FAAs	-0.00245
	0.8330	0.533	Water pot.	0.0156	Threonine	0.00276	Isoleucine	-0.00952
	0.7971	0.988	Serine	-0.00332	Alanine	0.00103	Isoleucine	-0.00833
	0.7934	0.741	Threonine	0.00580	Isoleucine	-0.0128	Proline	-0.000370
	0.7926	1.401	Threonine	0.0213	Leucine	0.00516	Essential FAAs	-0.00681
	0.7918	-0.178	Dry weight	-0.0236	Height	0.0217	Isoleucine	-0.00419

Table 4.10. Regression analyses (n = 8) of *E. loftini* eggs per plant oviposition estimate using plant phenology and physiochemical measurements on sugarcane.

Dependant variable	R^2	Intercept	Independent variables					
			Name	Estimate	Name	Estimate	Name	Estimate
Eggs	0.9673	-0.671	Dry weight	-0.0327	Height	0.0288	Proline	-0.000270
	0.9624	1.024	Histidine	-0.00617	Threonine	0.0164	Essential FAAs	-0.00303
	0.9436	0.400	Serine	-0.00118	Threonine	0.00313	Methionine	0.0109
	0.9316	-0.299	Dry weight	-0.0292	Height	0.0245	Valine	-0.00344
	0.9296	0.383	Alanine	0.00110	Isoleucine	-0.00849	Proline	-0.000808
	0.9212	0.139	Dry leaves	0.0199	Aspartic acid	0.000388	Methionine	0.0105
	0.9189	0.120	Histidine	0.00353	Methionine	0.0149	Proline	-0.000315
	0.9137	0.159	Dry leaves	0.0184	Threonine	0.00144	Methionine	0.0103
	0.9122	0.365	Aspartic aci	0.000561	Tyrosine	-0.000822	Methionine	0.0115
	0.9113	0.307	Threonine	0.00216	Methionine	0.0105	Proline	-0.000121

most preferred treatment to 1, all preference estimates were scaled to this value. Across experiment variability is effectively corrected by the least squares estimation of the preference coefficients. Rather than comparing independent subsets of treatments separately in simultaneous choice experiments, our method allows preference comparisons to be made among a multitude of host plant types across different experiments.

4.4.2. Oviposition on Sugarcane

Eggs on sugarcane were laid exclusively on dry leaves, dry tips of leaves or dry leaf sheaths. Eggs have been observed on sugarcane in the field between the leaf sheath and the stalk (van Zwaluwenberg 1926, Flanders 1930, Fors 1981) and on dead leaves (van Leerdam 1984). A greenhouse bioassay revealed that 99% of *E. loftini* oviposition occurred in cryptic sites on dried sugarcane leaves located on the lower part of the plant, i.e. between ground level and 80 cm height (van Leerdam et al. 1986). These results concur with the significant correlation between oviposition and dry leaves on sugarcane in our study, where all eggs were laid on dry leaves or dry tips of leaves. The numbers of eggs laid per sugarcane plant increased under drought stressed conditions with the number of dry leaves per plant. A field study has shown that both *E. loftini* injury and production of moths on sugarcane can be reduced by irrigation (see chapter 3). Preference for drought stressed sugarcane provides a mechanism which partially explains the breakdown of resistance observed in the field.

Oviposition of *E. loftini* in cryptic sites on dried sugarcane leaves has been suggested as a mechanism to protect eggs from predation or parasitism (van Leerdam et al. 1986). The African pyralid, *Eldana saccharina* Walker, also oviposits on dry

sugarcane leaves (Leslie 1990), and the very mobile newly hatched larvae are vulnerable to predation (Girling 1978). Predation, mainly by ants from the genera *Paratrachina* and *Solenopsis*, have been shown to destroy up to 60% of artificially placed *E. saccharina* eggs on sugarcane in South Africa (Leslie 1982). Ant predation was also observed to be high on artificially positioned *E. loftini* eggs in sugarcane (Meagher and Pfannenstiel, unpublished data). Despite efforts to conceal artificially positioned eggs, naturally laid eggs may escape detection by predators and parasitoids more efficiently. Insecticide studies (Meagher et al. 1994, Legaspi et al. 1999c) and extensive attempts at classical biological control (Meagher et al. 1998) have not resulted in effective *E. loftini* control programs. Oviposition in cryptic sites on the lower portion of the plant may assist in explaining this lack of success.

Sugarcane cultivar LCP 85-384 was more attractive for oviposition than HoCP 85-845. Greenhouse and laboratory studies have previously shown only slight differences in *E. loftini* oviposition among several sugarcane cultivars, whereas differences in larval establishment indicated antibiosis as a more important resistance mechanism (Meagher et al. 1996). A field study has shown that sugarcane cultivar LCP 85-384 was more susceptible to *E. loftini* than HoCP 85-845 based on both percentage of bored internodes and moth emergence per hectare (Reay-Jones et al. 2003). Cultivar LCP 85-384 had more dry leaves than HoCP 85-845 in our study, which likely affected oviposition preference. The decreased oviposition on HoCP 85-845 therefore is a mechanism conferring resistance to *E. loftini*.

4.4.3. Oviposition on Rice

Eoreuma loftini eggs were distributed on rice on green leaves, leaf sheaths, stems, and dry leaves. Oviposition did not occur in sites as cryptic as on sugarcane, indicating potential increased exposure in the field to mortality factors such as parasitoids, predators and insecticides. The relative concealment of eggs on sugarcane may explain the preference over rice. The tillering stages were not as attractive as both the boot and heading stage, possibly due to reduced number of oviposition sites (i.e. green and dry leaves) on young rice plants. The pest status of *E. loftini* on rice in the Texas Rice Belt has not yet achieved the same degree of severity as on sugarcane in the Lower Rio Grande Valley of Texas. However, field insecticide trials on rice have shown yield losses as much as 50% or greater (treated versus untreated) attributed to stem borers (*E. loftini* and *Diatraea saccharalis* (F.)) (Way 1999). Insecticidal control is more effective on rice than on sugarcane, apparently due to increased egg and larval exposure.

4.4.4. Drought Stress Effects on Sugarcane Physiology

Drought stress significantly increased water potential and levels of several FAAs (arginine, aspartic acid, glycine, isoleucine, leucine, phenylalanine) in sugarcane; however effects were not detected for free proline, which has previously been shown to be an indicator of water deficit stress (see chapter 3, Showler 2002). Discontinuance of daily watering of sugarcane in greenhouse pots for 12 days increased levels of proline by 2.5-fold (Muqing and Ru-Kai 1998). Other types of stress have also increased levels of free proline in sugarcane leaves 1.6-fold (salt stress) (Joshi and Naik 1980), 6.2-fold (*Colletotrichum falcatum* Went infection) (Singh et al. 1993), and 1.2-fold (iron cholorosis) (Jain and Shrivastava 1998). When plants are subject to dehydration, which

lowers the water potential of the cells and thus reduces water loss by osmoregulation, free proline accumulates (Heuer 1994). Free proline levels also have been shown to increase under drought stress in sugar beets, *Beta vulgaris* L., by 12-fold (Gzik 1996) and in cotton, *Gossypium hirsutum* L., by 58-fold (Showler and Moran 2003). Free proline appears to be the most widespread and consistent amino acid related to drought stress (Aspinall and Paleg 1981). In our study, reducing irrigation two weeks prior to the beginning of the experiment may not have been sufficient to elicit an accumulation of proline, even though stress symptoms, such as increased dry leaves, were visible.

4.4.5. Relationships between Oviposition Preference and Larval Performance

Insects often oviposit on plants that maximize their survival and development (Showler 2001, Singer 1972, 1983). Greenhouse studies have shown that survival and development of *E. loftini* on sugarcane is enhanced within a certain range of drought stress (Sétamou and Showler, unpublished data). Our study demonstrated increased attractiveness of drought stressed sugarcane for oviposition. A positive correlation may exist between preference and performance on sugarcane. Performance of *E. loftini* on rice has not been studied, however a field study has shown that cultivar XL8, despite being more attractive for oviposition in our study, showed a trend to being more resistant to stem borers than Cocodrie (Way 2002). Poor relationships between ovipositional preference and performance can be explained by several hypotheses (Thompson 1988). The time hypothesis states that when a novel plant is introduced into an area, females may oviposit on this plant even if it is unsuitable or fatal for the insect's offspring (Legg et al. 1986). Selection may take several generations to affect this trend. *Eoreuma loftini* was first described on sugarcane in experimental plots in Arizona in 1917 (Dyar 1917).

The insect was then found on commercial sugarcane on the west coast of Mexico (Morill 1925, Van Zwalunwenburg, 1926) and was observed on rice plants in California (Osborn and Philips 1946). The laboratory colony used in this study was initiated from larvae collected in sugarcane fields in the early 1980s, with sporadic field collections of *E. loftini* added at various other times. The insect may have had a sufficient number of generations to preclude the importance of this hypothesis on sugarcane, but not on rice. The enemy-free space hypothesis suggests that natural enemies may affect performance as much as plant characteristics (Jeffries and Lawton 1984, Thompson 1988). Natural enemies of *E. loftini* have not yet been studied on rice. Preference for drought stressed sugarcane may be correlated with both enhanced performance and relative concealment on dry leaves from natural enemies. The patch dynamic hypothesis considers changes in space and time of the composition of plant communities that can affect the quality of host plants for insects (Thompson 1988). Because of such variation, natural selection may not favor preference for a particular plant species, therefore preventing a positive correlation with performance. In the parasite/grazer hypothesis (Thompson 1988), an insect able to develop on a single plant (parasite) may have a stronger association between preference and performance than an insect that has to move among several plants during the course of their development (grazer).

4.4.6. Mechanisms of Oviposition Preference

The majority of nitrogen is acquired by insects through absorption in the gut (Brodbeck and Strong 1987). Three potential physiological mechanisms may explain the enhanced nutritional quality of plants under stress: (1) FAAs are nutritionally superior to proteins, (2) FAAs are more readily available than proteins because of the absence of any

proteinase inhibitors, and (3) FAAs are physically more accessible because of increased solubility (Cockfield 1988). Certain amino acids are known to be essential for insect development (Vanderzant 1958, Nation 2002). Artificial diets with amino acid distributions simulating anthers were adequate for survival and development of the tobacco budworm, *Heliothis virescens* (F.) (Hedin et al. 1991). Moths possess contact chemoreceptors on antennae, proboscis, tarsi and ovipositors, which assist in accepting or rejecting a host plant based on presence or absence of secondary or primary compounds (Städler 1984). FAAs can elicit electrophysiological responses of the sensilla of the adult tobacco budworm, the corn earworm, *Heliothis armigera* (Hübner), and *Spodoptera littoralis* (Boisdval) (Blaney and Simmonds 1988). Oviposition of the beet armyworm was increased on cotton under drought stress, which was correlated with greater levels of essential FAAs (Showler and Moran 2003). Assuming that *E. loftini* can detect host plant FAA levels, and that such levels influence oviposition preference, increased levels of essential FAAs may help explain the observed variability in egg laying.

Host plant selection by moths and butterflies can be viewed as a sequence of behavioral events consisting of (1) searching, orientation, and encounter, (2) landing and contact evaluation, and (3) acceptance or rejection (Renwick and Chew 1994). Alighting on a potential host plant is the result of integrating information perceived by the moth, which includes visual, olfactory, gustatory and mechanical cues (Ramaswamy 1988). Contact chemoreception is the most predominant sensory modality involved in host acceptance (Ramaswamy 1988). Host location and acceptance in oviposition preference studies are reflected by number of egg masses per plant. The size of each egg mass may reflect the moth's perception of host plant suitability. Smaller egg masses may occur on

plants that are perceived as having low suitability. Moths may assess host acceptability and host suitability using different mechanisms, which likely involve different host cues. In our study, multiple regression and correlation analyses yielded associations between several plant characteristics and the different oviposition parameter estimates (see Tables 4.6, 4.7, 4.8 4.9 and 4.10), which may reflect such behavioral steps.

Drought stressed sugarcane cultivar LCP 85-384 (5-6 internodes) was the most attractive plant based both on egg masses and eggs per egg mass, indicating that this plant is not only preferred for host location and acceptance, but is also perceived as the most suitable plant by *E. loftini*. In our study, oviposition on sugarcane was associated with arginine (egg masses per plant) and aspartic acid (eggs laid per plant), which both increased under stress. Modified nitrogen metabolism under plant stressed conditions can increase insect herbivore populations (White 1984). Reducing plant stress with irrigation may assist in decreasing *E. loftini* oviposition in sugarcane by decreasing both the nutritional value of the crop for this insect and the number of ovipositional sites (i.e. dry leaves). Young sugarcane (5-6 internodes), despite having fewer dry leaves than old sugarcane (10-11 internodes), was more attractive for egg-laying, likely due to the higher levels of several FAAs essential for insect development (alanine and valine). On rice, associations were established between egg masses per plant and essential FAAs (threonine and valine) and dry leaves. Rice cultivar XL8, which was more attractive for oviposition compared to Cocodrie, had higher levels of several essential FAAs (arginine and histidine), with positive associations for both variables with eggs per plant. Cultivar XL8 did however have more tillers which were positively associated with egg mass per plant. *Eoreuma loftini* laid more eggs on rice plants of large biomass, a common

response in oviposition behavior among other insects (Asman 2002, Vasconcellosneto and Monteiro 1993).

Early instar *E. loftini* larvae have limited mobility and must feed on, or very near, the plant on which the eggs are laid. Levels of antixenosis can help control pests of crops in some IPM systems (Smith 1989), and may assist in developing a defense strategy against *E. loftini*. Sugarcane cultivar HoCP 85-845, with a reduced number of dry leaves, is less attractive than LCP 85-384. Leaf FAA levels varied with host species, cultivar, stress, and phenology and were correlated with oviposition preference estimates. Reducing drought stress decreases both host plant suitability and attractiveness for oviposition. Because sugarcane is more attractive than rice, populations from rice fields will be expected to contribute to enhancing infestations on proximate sugarcane in some areas in Louisiana. Despite being more attractive for oviposition, rice cultivar XL8 was more resistant to stem borers in a field study (Way 1999). The use of this resistant rice cultivar may be effective in reducing infestations on proximate host crops if the resistance mechanisms are antibiotic. Using our models, preference estimates can be determined at varying levels of host availability, which is useful when comparing observations in the field at different host densities. Understanding the population dynamics on both sugarcane and rice is necessary to conceptualize areawide cross-regional management strategies. Once *E. loftini* becomes established in Louisiana, cultivar resistance will likely serve a major role in keeping infestations below economic injury levels, as well as decreasing populations on an areawide basis.

CHAPTER 5: MONITORING THE MOVEMENT OF THE MEXICAN RICE BORER IN THE TEXAS RICE BELT

5.1. Introduction

An estimated 50,000 non-indigenous species have been introduced in the United States, causing $138 billion in economic damage and control measures (Pimentel et al. 2002). Arthropods account for 11% of these non-indigenous species (Pimentel et al. 2002), which include the Mexican rice borer, *Eoreuma loftini* (Dyar), an invasive species introduced into Texas from Mexico in 1980 (Johnson and van Leerdam 1981). Described from different host plants in Arizona (Dyar 1917), *E. loftini* was first reported as a pest of commercial sugarcane, *Saccharum* spp., in the state of Sinaloa on the west coast of Mexico (Morill 1925, Van Zwalunwenburg 1926). The range later expanded to eastern and southeastern Mexico in Oaxaca (Rodriguez-del-Bosque et al. 1989, Rodriguez-del-Bosque and Smith 1991). After Dyar's initial recovery of the insect in Arizona, more specimens were found in southern Arizona (Van Zwalunwenburg 1926) and in the Imperial Valley in California, close to the Mexican border (Osborn and Phillips 1946). In Texas, the range of *E. loftini* has continued to expand well into the rice (*Oryza sativa* L.) production area along the Gulf coast (Browning et al. 1989) and represents a growing risk for the Louisiana sugarcane and rice industries (Reagan et al. 2005).

Eoreuma loftini has been the dominant insect pest of sugarcane in the Lower Rio Grande Valley of Texas since it was introduced from Mexico in 1980 (Johnson and van Leerdam 1981), surpassing the sugarcane borer *Diatraea saccharalis* (F.) in economic importance the same year (Johnson 1981). *Eoreuma loftini* now represents >95% of the sugarcane stalk borer population in the Lower Rio Grande Valley (Legaspi et al. 1999c).

Rice was reported as a host in 1926 (Van Zwalunwenburg 1926) and can suffer from substantial yield loss in the Texas Rice Belt (Way et al. 1999).

Brown et al. (1988) first demonstrated the existence of the *E. loftini* female sex pheromone by studying the response of adult males to ovipositor extracts. The synthetic pheromone was subsequently described (Shaver et al. 1988), and field experiments demonstrated the efficiency of pheromone-baited traps as a survey and monitoring tool (Shaver et al. 1991). Synthetic pheromones are commonly used to monitor populations of numerous insects including the gypsy moth, *Lymantria dispar* (L.) (Sharov et al. 1997), the European corn borer, *Ostrinia nubilalis* Hübner (Ngollo et al. 2000), the millet stem borer, *Coniesta ignefusalis* (Hampson) (Youm and Beevor 1995), and the spotted stem borer, *Chilo partellus* (Partellus) (Unnithan and Saxena 1990). Using the female sex pheromone to monitor the movement of the invasive species *E. loftini* will allow researchers and farmers to anticipate the establishment of this devastating pest in major crops along the Texas Gulf Coast region. The objectives of this work were to monitor the movement of *E. loftini* throughout the Texas Rice Belt and to estimate the rate of movement since the insect was first introduced into Texas in 1980.

5.2. Material and Methods

Counties of the Texas Rice Belt where *E. loftini* monitoring occurred from 2000 to 2004 are reported in Table 5.1. Additional monitoring occurred in 1999 in Waller County from 15 May to 15 October. Two bucket-type pheromone traps separated ~100 meters were set up in each county adjacent to the same field and baited with a synthetic female *E. loftini* sex pheromone lure (Luresept, Hercon Environmental, Emigsville, PA) replaced every 3 weeks. An insecticidal strip (Vaportape II, Hercon Environmental,

Emigsville, PA) was placed in each bucket to kill all trapped insects and prevent them from damaging each other. Insecticidal strips were replaced every 6 weeks. The traps were attached to a metal pole at a height of ~1 m above the soil surface. Trap collections were placed in plastic bags and frozen for later identification and enumeration. Frequency of trap monitoring, reported in Table 5.1, sometimes varied slightly with county and year.

For each trap count, average numbers of moths across both traps were determined for each county. Between each average trap count k, a daily rate was calculated:

$$\alpha_{ijk} = \frac{n_{ijk}}{d_{ijk} - d_{ijk-1}} \qquad [1]$$

where α = interpolated number of moths caught per day, n = average number of moths caught from both traps in county i, year j, and trap count k. From date d_{ijk} to d_{ijk-1}, α_{ijk} was the daily interpolated rate of moth catches. Because trap monitoring did not begin on the same date across counties and across years (Table 5.1), extrapolated rates were determined to establish daily trap counts over identical time periods for each year for each county when interpolated rates were not available. For each year of trapping, extrapolated daily rates began when the first day of actual moth trapping occurred in at least one of the counties and ended when the last day of actual moth trapping occurred:

$$\hat{\alpha}_{ijl} = \frac{\alpha_{i..}\alpha_{..l}}{\alpha_{...}} \qquad [2]$$

where $\hat{\alpha}_{ijl}$ = extrapolated number of moths caught per day l, $\alpha_{i..}$ = average number of moths caught per day for county i across all trap dates for all years, $\alpha_{..l}$ = average

Table 5.1. Dates and frequency of *E. loftini* trapping in the Texas Rice Belt 2000-2004.

County	Code	2000			2001			2002			2003			2004		
		Start	End	N[a]	Start	End	N[a]	Start	End	N[a]	Start	End	N[a]	Start	End	N[a]
Austin	1				5/31	8/11	8	6/25	12/10	11	6/27	12/29	5			
Brazoria	2	5/19	11/15	13				5/25	11/30	23	5/10	11/22	29	5/1	11/30	30
Calhoun	3	7/19	11/7	17	6/12	8/24	11	5/28	12/3	26						
Chambers	4	5/20	11/10	25	5/20	11/10	2	5/2	11/10	2	4/11	11/28	34	4/4	11/5	30
Colorado	5	5/22	11/14	26	5/11	11/30	29	5/2	11/26	30	5/23	12/2	27	5/9	11/29	30
Fort Bend	6	5/22	9/7	13	6/25	8/22	7	6/10	8/22	8						
Galveston	7							5/9	10/24	24	5/27	12/17	28	5/3	12/6	31
Harris	8				5/26	11/30	26	5/22	11/26	26						
Jackson	9	5/15	11/13	43	5/23	11/13	25	4/23	10/4	23	5/23	11/13	22	6/13	8/31	7
Jefferson	10	5/24	11/8	25	5/18	11/8	2	5/3	11/8	2	4/11	11/28	34	4/9	11/5	30
Liberty	11	6/10	11/10	23	6/10	11/10	2	6/10	11/10	2	6/10	11/10	2	7/12	11/15	18
Matagorda	12	5/16	10/6	32	6/15	10/26	20	5/24	11/9	26				5/15	10/11	13
Orange	13	8/2	11/15	16	8/2	11/15	2	8/2	11/15	2	8/2	11/15	2	6/1	10/5	18
Waller[b]	14	5/24	11/8	23				6/9	12/29	30				5/10	12/06	30
Wharton	15	5/30	11/13	24	5/29	11/26	18	5-29	12/5	33	5/16	11/14	27	5/3	11/9	28

[a] Number of trap counts.

[b] Monitoring also occurred from 15 may to 15 October 1999.

number of moths caught per day for trap date l across all counties, and $\alpha_{...}$ = average number of moths caught per day across all counties and all years.

For each trapping date, the latitude and longitude centroid of moth counts across all counties was determined by weighting the latitude and longitude of the traps in each county with the number of moths caught per day. Because of the proximity (~ 100 m) of the two traps, a single set of coordinates was used for each county:

$$x_{.jl} = \frac{\sum_{i=1}^{i=15} x_i \alpha_{ijl}}{\sum_{i=1}^{i=15} \alpha_{ijl}} \qquad [3]$$

where $x_{.jk}$ = longitude of the centroid for trap date l and year j across all counties; x_i = longitude of trap i.

$$y_{.jk} = \frac{\sum_{i=1}^{i=15} y_i \alpha_{ijk}}{\sum_{i=1}^{i=15} \alpha_{ijk}} \qquad [4]$$

where $y_{.jk}$ = latitude of the centroid for trap date l and year j across all counties; y_i = latitude of trap i. Sample variances and covariance were determined for each latitude and longitude value for every date where interpolated or extrapolated values were available:

$$s_{x}{}^{2}{}_{.jk} = \frac{\sum_{i=1}^{i=15} f_{.jk} x_{.jk} - \frac{\left(\sum_{i=1}^{i=15} f_{.jk} x_{.jk}\right)^2}{\sum_{i=1}^{i=15} \alpha_{ijk}}}{\left(\sum_{i=1}^{i=15} \alpha_{ijk}\right) - 1} \qquad [5]$$

where $s_{x}^{2}{}_{.jk}$ = variance of longitude for trap date k and year j; $f_{.jk}$ = frequency of observations with longitude $x_{.jk}$.

$$s_{y}^{2}{}_{.jk} = \frac{\sum_{i=1}^{i=15} f_{.jk} y_{.jk} - \frac{\left(\sum_{i=1}^{i=15} f_{.jk} y_{.jk}\right)^{2}}{\sum_{i=1}^{i=15} \alpha_{ijk}}}{\left(\sum_{i=1}^{i=15} \alpha_{ijk}\right) - 1} \qquad [6]$$

where $s_{y}^{2}{}_{.jk}$ = variance of latitude for trap date k and year j. $f_{.jk}$ = frequency of observations with latitude $y_{.jk}$.

$$s_{xy}{}_{.jk} = \frac{\sum_{i=1}^{i=15} \left(f_{.jk} x_{.jk}\right)\left(f_{.jk} y_{.jk}\right) - \frac{\left(\sum_{i=1}^{i=15} f_{.jk} x_{.jk}\right)\left(\sum_{i=1}^{i=15} f_{.jk} y_{.jk}\right)}{\sum_{i=1}^{i=15} \alpha_{ijk}}}{\left(\sum_{i=1}^{i=15} \alpha_{ijk}\right) - 1} \qquad [7]$$

Principle axes and 95% confidence intervals of the bivariate scattergram of mean annual centroids were calculated using the method of Sokal and Rohlf (1969).

5.3. Results

A total of 78,666 *E. loftini* moths were caught in pheromone traps in this study, corresponding to an average number of 5.2 ± 0.22 [SE] moths per trap in each county for each day when trapping occurred. Seven of the fifteen counties were monitored for *E. loftini* populations during every year of the study (Table 5.1). The date of initial trap deployment in each county varied from 4 April to 2 August. The last date of trapping ranged from 11 August to 29 December (Table 5.1). Pheromone traps in adjacent counties were distant on average by 58.3 km.

Eoreuma loftini moths were found in counties previously unknown to be infested in 2000 (Colorado, Wharton, Fort Bend, Brazoria), 2001 (Austin and Harris), and 2002 (Galveston) (Fig. 5.1). However, traps were not deployed in these counties in years prior to *E. loftini* discovery; therefore it is unknown if these new infestations represent immediate moth movement. For our analysis, we conservatively assume that *E. loftini* moths were present in these counties prior to initial discovery. The counties of Chambers and Liberty were however uninfested prior to 2004, and Waller uninfested prior to 2000, when *E. loftini* moths were first caught in pheromone traps (Fig. 5.1). The traps in Waller, Liberty and Chambers counties were located from the initial discovery in Weslaco, TX, at a distance of 473.9, 538.9, and 556.5 km, respectively. From Weslaco to the trap in Waller County, the average rate of *E. loftini* spread was 23.7 km/yr from 1980 to 2000. Using the Chambers county trap, the average rate of spread from 1980 to 2004 was 23.2 km/yr.

The surface area of the 95% C.I. of the centroids and the length of the minor axis showed a trend to increase from 2000 to 2004 (Table 5.2). The slopes of the principal and minor axes decreased from 2000 to 2004, indicating a spatial shift in the ellipse in a northeastern direction (Table 5.2 and Fig. 5.2). Annual mean centroids moved 10.4 km in 2001, 8.2 km in 2002, 6.2 km in 2003, and 3.6 km in 2004 (Fig. 5.2). From 2000 to 2004, annual mean centroids moved 17.6 km, however 95% C.I. overlapped across years. The average rate of spread of the centroids was 4.4 km/yr.

5.4. Discussion

The monitoring of *E. loftini* in our study was not conducted with standardized sampling procedures across counties and years. Sites sampled, dates and frequencies of

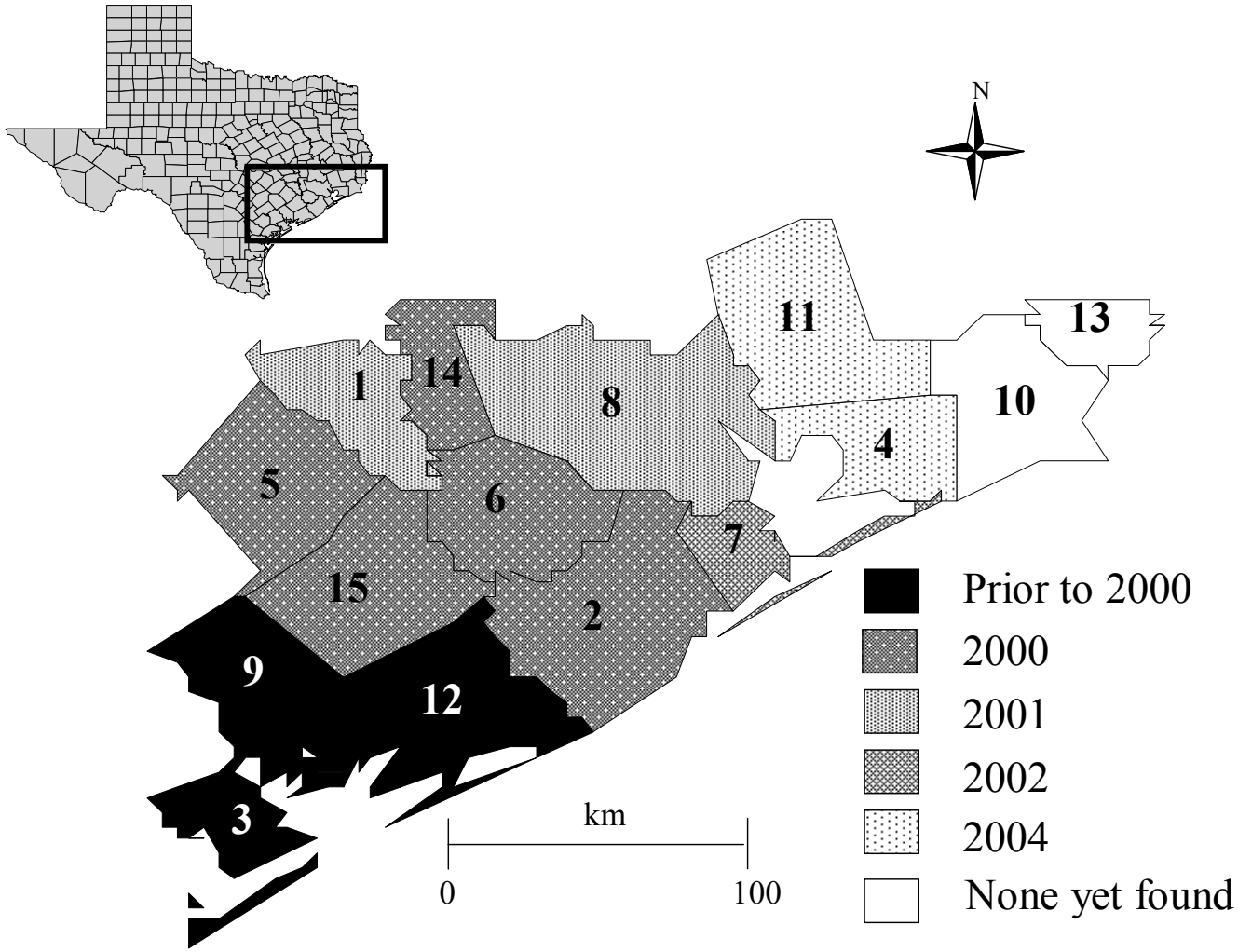

Fig. 5.1. Movement of *Eoreuma loftini* through the Texas Rice Belt, 2000-2004, TX. County names are reported in Table 5.1.

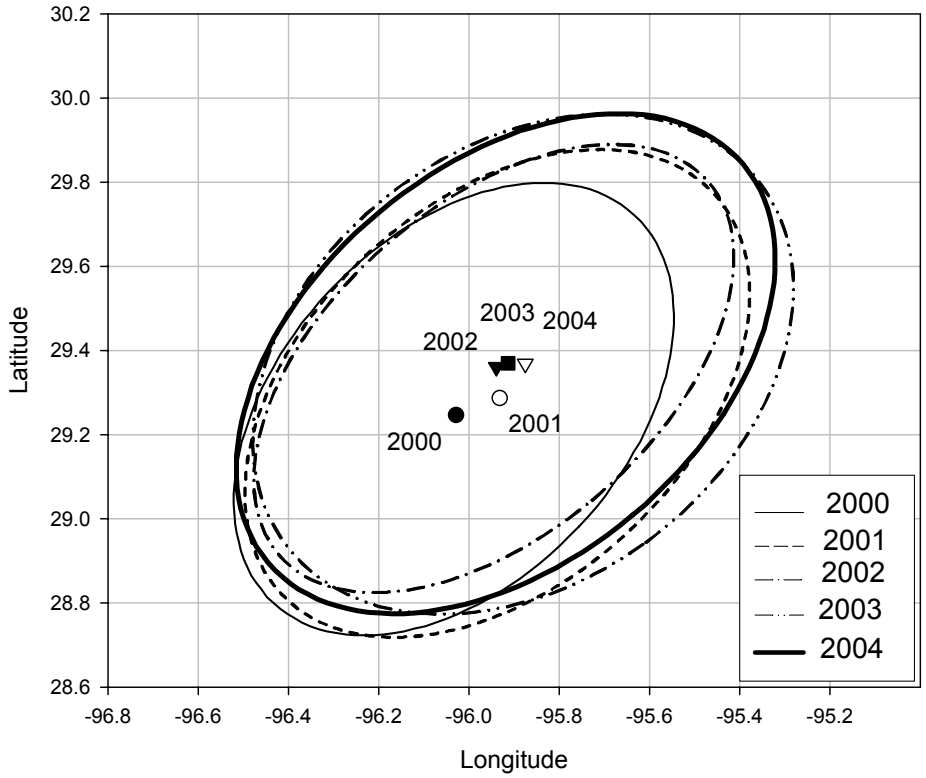

Fig. 5.2. Average annual centroid of *E. loftini* moths and 95% confidence region.

Table 5.2. Characteristics of 95% confidence interval of annual centroids.

	Principal axis		Minor axis		Surface area
	Slope	Length (km)	Slope	Length (km)	(km^2)
2000	1.052	128.6	-0.951	81.8	8,268.7
2001	0.931	140.9	-1.074	93.5	10,349.2
2002	0.859	133.5	-1.163	82.2	8,618.6
2003	0.763	138.6	-1.310	102.2	11,124.3
2004	0.812	145.9	-1.223	97.2	11,137.8

sampling varied with county and year. The analysis of spatial and temporal distributions of insects using traps typically requires the use of fixed permanent sites where consistent measurement techniques are used (Yang et al. 1998). Our study provides a method of interpolating and extrapolating trap counts when traps are not consistently monitored. The development of a homogeneous dataset across counties and years allowed estimated population centroids and their 95% C.I. ellipse to be determined.

Spread rates based on the first recorded sighting have been determined for several other invasive species (i.e. Augustin et al. 2004, Leibhold et al. 1992). An average rate of spread of 23.2 km/yr was established in our study, which was faster than the annual rate of centroid movement (7.3 km/yr). The movement of annual centroids reflects shifts in the areawide abundance of *E. loftini* populations, which can not be estimated with simple boundary spread rates. The main finding of our analysis is the 1.7-fold increase from 2000 to 2004 in ellipse surface area of the 95% C.I. around the centroids, indicating an expansion of the distribution of *E. loftini* populations. The spread of the ellipse is slower than the spread based on boundary movement, partially due to the invasion of only three new counties during this study, where infestations were initially low (Waller in 2000, and Chambers and Liberty counties in 2004).

Pheromone traps can be invaluable in monitoring the movement of invasive insect pests (Robacker and Landholt 2002). However the ability to detect movement of insects relies on appropriate deployment of traps. Prior to setting traps up in the field, a monitoring program must establish the scale of insect movement that needs to be detected, which will depend on the objectives of the study. If detection of *E. loftini* moths is to be determined during the year of invasion, the distance between traps should

not exceed the expected rate of spread. The average rate of spread in our study was 23.2 km/yr. Assuming spread rates to be constant, the intertrap distance must therefore not exceed 23.2 km for annual movement to be detected. The accuracy of spread rates estimate decreases as intertrap distances increases (Sharov et al. 1997). The average distance between *E. loftini* pheromone traps in our study was 58.3 km. Reducing intertrap distance is expected to assist in developing a more accurate monitoring program.

Spread rates of invasive insects vary with species and can be strongly influenced by human activities. Analysis of historical records of the gypsy moth, *Lymantria dispar* (L.), in North America showed varying rates of spread from 9.45 km/yr to 20.78 km/yr, with varying rates linked to human-caused movement (Leibhold et al. 1992). Spread of the leafminer, *Cameraria ohridella* Deschka and Dimič, was monitored in Eastern France using pheromone traps from 2001-2003, with annual spread rates varied from 17.0 to 37.9 km/yr (Augustin et al. 2004). Establishing estimates of variability for spread rate of insects can assist in improving monitoring programs.

The first interception of *E. loftini* in a Lower Rio Grande Valley of Texas port of entry from Mexico occurred in 1959 in Brownsville when a single larva was found in sugarcane (Johnson 1984). Records of interception in Texas, despite being incomplete, reported four more interceptions at this port between 1980 and 1982, eight interceptions at the Roma port between 1971 and 1980, and nine interceptions at the Hidalgo port between 1966 and 1980 (Johnson 1984). A vast majority (94%) of the interceptions were from sugarcane, with additional interceptions from corn, lemon grass and tomatoes (Johnson 1984). The movement of infested crops is likely to have accelerated the spread of *E. loftini* from Mexico into Texas. The Louisiana sugarcane and rice industries face

similar issues, with regulatory programs playing a vital role in preventing the movement of insect pests into areas not currently infested (Reagan et al. 2005). Invasive pests of agricultural crops represent a growing threat because of the increased volume, speed of travel, and types of commodities that are being transported throughout the world (Schwalbe and Hallman 2002). An estimated 500 species of insect and mite crop pests have been introduced into the United States, causing approximately $13.5 billion per year in crop losses (Pimentel et al. 2002). Estimates of the effects of borer damage on revenue, based on a 20% level of bored internodes, have varied between $575/ha, when considering only the producer's loss (Meagher et al. 1994), and $1,181/ha, when considering the effects on all involved parties (producer and mill) (Legaspi et al. 1999c). In rice, yield losses attributed to *E. loftini* and to *D. saccharalis*, have exceeded 50% in certain replicated experiments conducted in the Texas Rice Belt (Way 1999). This invasive species has the potential to cause major yield losses in the Louisiana sugarcane and rice industries. In September 2004, *E. loftini* was detected for the first time (via pheromone trapping) in the sugarcane-producing region of South East Texas, prompting a previously agreed to quarantine by the Louisiana Department of Agriculture and Forestry and the Texas Department of Agriculture, immediately costing producers over $300,000 (Reagan et al. 2005). A 1.6-km radius quarantine was initiated around the *E. loftini* trap capture, prohibiting the transport of sugarcane into Louisiana for processing. Effective monitoring programs are a necessity to detect this devastating insect pest especially during the first year of presence in a newly infested area.

SUMMARY

Field and greenhouse studies were conducted to identify management practices that effectively control *E. loftini* in sugarcane and also to assist in understanding the population dynamics of this invasive pest insect in the sugarcane and rice agroecosystems. An overview of the data indicates that management tactics are available to reduce *E. loftini* injury and reduce production of moths in sugarcane by selecting less susceptible cultivars and by minimizing plant stress. By counting moth exit holes, production of moths was determined from sugarcane stalks at the end of the season, estimating the contribution of each cultivar to the enhancement or suppression of areawide pest populations. Cultivars HoCP 85-845 and CP 70-321 were among the more resistant cultivars; however, HoCP 85-845 exhibited less resistance under heavy *E. loftini* infestation pressure, suggesting its value only in moderate to low infestation conditions. Cultivar LCP 85-384, which now represents more than 90% of the sugarcane production area in Louisiana, was highly susceptible to *E. loftini* injury. In a portion of the test at a high infestation location, high levels of sodium and magnesium salt (15-30 cm soil depth) causing shorter more stressed plants were associated with higher *E. loftini* damage in all cultivars except HoCP 91-555 and CP 70-321. The differential response to the stress among sugarcane cultivars is a first step toward determining management strategies in sugarcane-stressed areas.

Applying irrigation water 3 times in 2003 and twice in 2004 reduced injury in both susceptible (LCP 85-384) and resistant (HoCP 85-845) sugarcane cultivars grown in replicated field plots by an average of 2.5-fold. Together with trends for reduced tonnage

of sugarcane and estimated tons of recoverable sugar per hectare under non-irrigated conditions, our results indicate the value of using irrigation to better manage *E. loftini* in sugarcane. Growers in Texas can irrigate sugarcane, which seems to reduce their *E. loftini* injury. In contrast, the majority of Louisiana growers are unable to irrigate, and extended periods of drought in recent years have produced conditions expected to aggravate *E. loftini* infestations. Partial cultivar resistance, combined with cultural practices to minimize plant stress, are expected to become major components of the pest management system when *E. loftini* becomes established in Louisiana. Irrigation, host plant resistance, and insecticide applications of tebufenozide decreased injury from 70% bored internodes to less than 10% during both years. The use of multiple control tactics was substantially better at suppressing *E. loftini* in sugarcane than solely relying on insecticide applications. A combination of control strategies will be necessary for farmers to effectively manage this insect when it becomes established in the Louisiana sugarcane agroecosystem.

Drought stress effects were detected for several free amino acids in sugarcane, including free proline, which has previously been shown to be an indicator of water deficit stress. Several free amino acids (histidine and isoleucine) essential for insect growth and development were increased in sugarcane leaves by drought-stressed conditions, which in turn exacerbated *E. loftini* infestations. Reducing plant stress with irrigation may assist in managing *E. loftini* in sugarcane by decreasing the nutritional value of the crop for this insect.

A method was developed that allows oviposition preference estimates to be determined from multiple experiments, each with a reduced number of host types, using

non-linear regression models. Drought-stressed sugarcane (1.5 L water/week) was 1.8-fold more attractive based on egg masses per plant than non drought-stressed sugarcane (4.5 L water/week). Preference for drought stressed sugarcane provides a mechanism which partially explains the breakdown of resistance observed in the field. Oviposition in concealed sites on the lower portion of the plant may assist in explaining the lack of success of both insecticide and biological control attempts. The *E. loftini* susceptible sugarcane cultivar LCP 85-384 was more attractive than HoCP 85-845 based on egg masses and eggs per egg mass per plant.

Egg masses were 9.2-fold more abundant and 7.0-fold larger on sugarcane than on rice. *Eoreuma loftini* eggs were distributed on rice on green leaves, leaf sheaths, stems, and dry leaves. Oviposition did not occur in sites as concealed as on sugarcane, indicating potential increased exposure in the field to mortality factors such as parasitoids, predators and insecticides. The relative concealment of eggs on sugarcane may therefore help explain the preference over rice, by providing increased protection from predation or parasitism. Oviposition on sugarcane occurred exclusively on dry leaf material. The number of dry leaves, which increased under drought stress, was positively correlated with egg masses ($r = 0.740$) and eggs ($r = 0.626$) per plant. Several free amino acids essential for insect growth and development increased in sugarcane leaves under drought-stressed conditions, and were highly correlated with egg masses per plant. Rice leaves, despite being less attractive for oviposition, had higher levels of certain free amino acids than sugarcane. The more resistant but more attractive rice cultivar XL8 had higher levels of several free amino acids than the susceptible Cocodrie. Because sugarcane is more attractive than rice, populations from rice fields will be expected to

contribute to enhancing infestations on proximate sugarcane in some areas in Louisiana. Levels of antixenosis can help control pests of crops in some IPM systems, and may assist in developing a defense strategy against *E. loftini*.

Introduced in Texas from Mexico in 1980, the range of *E. loftini* has continued to expand well into the rice production area along the Gulf Coast and represents a growing risk to the Louisiana sugarcane and rice industries. Pheromone baited traps were used to monitor the movement of *E. loftini* through the Texas Rice Belt towards Louisiana from 2000 to 2004. Based on boundary movement, the average rate of spread from 1980 (Weslaco) to 2004 (Chambers County) was 23.2 km/yr. Daily trap counts were determined by interpolation and extrapolation because frequency of trap monitoring varied among counties and years. The 1.7-fold increase from 2000 to 2004 in ellipse surface area of the 95% C.I. around the centroids indicates an increase in the variability of the covariance of latitude and longitude due to the expansion of the distribution of *E. loftini* populations. The annual mean centroid of moths moved 15.3 km in 2001, 5.9 km in 2002, 9.2 km in 2003, and 6.1 km in 2004. From 2000 to 2004, annual mean centroids moved 29.3 km, however 95% CI overlapped across years. The average rate of spread of annual centroids was 4.4 km/yr. If moth movement continues to occur at similar rates, *E. loftini* will reach Louisiana in the next two years, assuming no human-caused movement accelerates the spread. This proactive work has provided a basis for developing an integrated pest management program for this devastating insect pest of several important crops along the Texas and Louisiana Gulf Coasts.

It is anticipated that future work will investigate the role that alternate host plant species have on the dynamics of *E. loftini* in the Louisiana and Texas sugarcane and rice

agroecosystems. Because *E. loftini* is able to develop on so many host species, an areawide approach to reduce populations will require an understanding of the interactions among these different plants and the herbivore. Late season cultural practice differences may substantially impact *E. loftini* population dynamics, in addition to the role of ratoon rice. Studies may investigate the effects of varying heights of harvesting and phenological differences among sugarcane fields on overwintering insects. Studies may also include the oviposition preference, survivorship and production of adults from additional key hosts in the Gulf Coast region. The tri-trophic interactions between host plants, *E. loftini*, and arthropod predators and parasitoids may also be investigated. Pest management programs need to integrate the biological control component, which will assist in developing more permenent and sustainable control strategies.

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APPENDIX A: SAS CODE FOR CHAPTER 2

Program 1: Injury, moth production/ha and relative survival, Ganado, TX, 2002.

```
dm'log;clear;output;clear';
Title'Francis Reay-Jones – Chapter 1, Ganado 2002';
options nodate nonumber ps=55 ls=78;
data Ganado2002;
input cultivar$ replication boredinternodes mothproduction survival;
cards;
A       1       77.8846         238413.8614   0.413580247
A       2       66.5116         131661.3861   0.258741259
A       3       81.5287         64051.48515   0.140625
A       4       61.3636         14233.66337   0.02962963
A       5       50      113869.3069   0.283185841
B       1       56.6225         70462.48047   0.128654971
B       2       49.6324         48683.16832   0.118518519
B       3       57.384 121707.9208    0.294117647
B       4       40.8784         54768.56436   0.148760331
B       5       31.6456         18256.18812   0.06
C       1       61.1111         85776.60891   0.162337662
C       2       62.8959         130380.4455   0.273381295
C       3       57.8947         418589.8515   0.924242424
C       4       61.5741         144104.703    0.315789474
C       5       44.1964         48034.90099   0.141414141
D       1       32.3529         49007.67327   0.181818182
D       2       30.9353         40839.72772   0.174418605
D       3       27.5081         19058.5396    0.082352941
D       4       29.1667         49007.67327   0.214285714
D       5       21.6301         38117.07921   0.202898551
E       1       41.8251         75445.54455   0.218181818
E       2       32      9927.045337   0.0375
E       3       45.0549         88019.80198   0.227642276
E       4       33.0986         44009.90099   0.14893617
E       5       28.7625         53440.59406   0.197674419
F       1       66.4093         74514.59094   0.151162791
F       2       60.7407         98015.34653   0.219512195
F       3       81.106 100737.995     0.210227273
F       4       65.0641         68066.21287   0.123152709
F       5       38.9474         89847.40099   0.297297297
;
run;
proc mixed data=Ganado2002;
class cultivar replication ;
model mothproduction  = cultivar ;
random replication;
```

```
lsmeans cultivar / adjust=tukey ;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'C:\Documents and Settings\FReayJones.AGCENTER\Desktop\Francis
summer 2004\Stats PhD\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
quit;
```

Program 2: Injury, moth production/ha and relative survival, Weslaco, TX, 2001a.

```
dm'log;clear;output;clear';
Title'Francis Reay-Jones – Chapter 1, Weslaco 2001';
options nodate nonumber ps=55 ls=78;
data Weslaco2001;
input cultivar$ replication boredinternodes mothproduction survival;
cards;
A       1       19.61325967   17762  0.070422535
A       2       10.04672897   17762  0.11627907
A       3       8.446866485   3552.4 0.032258065
A       4       12.02346041   28419.2       0.195121951
A       5       10.16949153   17762  0.138888889
B       1       5.263157895   0      0
B       2       4.439252336   0      0
B       3       4.106280193   3037.55       0.058823529
B       4       8.391608392   12150.2       0.111111111
B       5       4.265402844   0      0
C       1       6.707317073   10275.75      0.136363636
C       2       10.6017192    0      0
C       3       18.32298137   20551.5       0.101694915
C       4       13.96011396   17126.25      0.102040816
C       5       19.62365591   27402  0.108108108
D       1       9.285714286   0      0
D       2       13.73493976   8154.15       0.052631579
D       3       6.887755102   10872.2       0.148148148
D       4       4.556354916   0      0
D       5       3.67816092    0      0
E       1       5.555555556   0      0
E       2       8.75 3079.0   5      0.028571429
E       3       10.09463722   9237.15       0.09375
E       4       15.83577713   9237.15       0.055555556
E       5       4.927536232   3079.05       0.058823529
;
proc mixed data=Weslaco2001;
class cultivar replication ;
```

```
model mothproduction  = cultivar ;
random replication;
lsmeans cultivar / adjust=tukey ;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'C:\Documents and Settings\FReayJones.AGCENTER\Desktop\Francis
summer 2004\Stats PhD\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
quit;
```

Program 3: Injury, moth production/ha and relative survival, Weslaco, TX, 2001b.

```
dm'log;clear;output;clear';
Title'Francis Reay-Jones – Chapter 1, Weslaco 2002';
options nodate nonumber ps=55 ls=78;
data Weslaco2002;
input cultivar$ replication boredinternodes mothproduction survival;
cards;
A       1       6.162465        12621.6         0.136363636
A       2       11.1782477      48714.94737     0.297297297
A       3       3.9755352       4207.2 0.076923077
A       4       6.993007        4428.631579     0.05
A       5       4.0697674       0       0
B       1       4.0431267       0       0
B       2       5.6701031       0       0
B       3       3.8461538       3372.6 0.090909091
B       4       3.7634409       10117.8         0.214285714
B       5       7.3770492       7494.666667     0.111111111
C       1       10.1226994      3933.9 0.03030303
C       2       14.004914       23603.4         0.105263158
C       3       9.8412698       3933.9 0.032258065
C       4       7.8947368       0       0
C       5       7.3170732       7867.8 0.083333333
D       1       1.0498688       2829.35         0.25
D       2       3.9702233       0       0
D       3       10.0628931      16976.1         0.1875
D       4       3.880597        8488.05         0.230769231
D       5       1.8018018       2829.35         0.166666667
E       1       3.4810127       0       0
E       2       5.5214724       2741.25         0.055555556
E       3       9.4076655       2741.25         0.037037037
E       4       7.4766355       16447.5         0.25
E       5       6.122449        5482.5 0.111111111
;
```

```
proc mixed data=Weslaco2002;
class cultivar replication ;
model mothproduction  = cultivar ;
random replication;
lsmeans cultivar / adjust=tukey ;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'C:\Documents and Settings\FReayJones.AGCENTER\Desktop\Francis
summer 2004\Stats PhD\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
quit;
```

Program 4: Injury, moth production/ha and relative survival, Weslaco, TX, 2001b.

```
dm'log;clear;output;clear';
Title'Francis Reay-Jones – Chapter 1, Weslaco 2002, second test';
options nodate nonumber ps=55 ls=78;
data Weslaco2002b;
input cultivar$ replication boredinternodes mothproduction  survival;
cards;
A       1       6.10687         3833.6 0.125
A       2       10.699588       9584    0.192307692
A       3       9.163347        7667.2 0.173913043
A       4       25.925926       30668.8         0.253968254
A       5       4.471545        0       0
B       1       6.228374        1639    0.055555556
B       2       4.651163        1639    0.083333333
B       3       3.690037        4917    0.3
B       4       13.309353       8195    0.135135135
B       5       3.6     0       0
C       1       17.90393        9241    0.12195122
C       2       12.946429       11089.2         0.206896552
C       3       12.236287       1848.2 0.034482759
C       4       9.342561        2772.3 0.074074074
C       5       20.37037        11089.2         0.136363636
D       1       6.550218        1466.6 0.066666667
D       2       2.439024        1466.6 0.166666667
D       3       16.143498       16132.6         0.305555556
D       4       2.727273        0       0
D       5       12.272727       1466.6 0.037037037
E       1       12.173913       10160  0.214285714
E       2       16.964286       10885.71429   0.157894737
E       3       7.657658        5442.857143   0.176470588
E       4       4.333333        6350    0.384615385
```

```
E       5       9.895833        0       0
F       1       5.283019        2000    0.071428571
F       2       3.030303        0       0
F       3       2.790698        2000    0.166666667
F       4       2.857143        0       0
F       5       14.07767        4285.714286   0.068965517
G       1       2.150538        0       0
G       2       12.429379       4000    0.090909091
G       3       16.292135       4000    0.068965517
G       4       22.049689       28500   0.267605634
G       5       17.5    18000   0.257142857

;
run;
proc mixed data= Weslaco2002b;
class cultivar replication ;
model survival  = cultivar ;
random replication;
lsmeans cultivar / adjust=tukey ;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'C:\Documents and Settings\FReayJones.AGCENTER\Desktop\Francis
summer 2004\Stats PhD\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
quit;
```

Program 5: Injury by stalk position, Ganado, TX, 2002.

```
dm'log;clear;output;clear';
Title'Francis Reay-Jones, – Chapter 1, Ganado 2002, Injury Positon';
options nodate nonumber ps=55 ls=78;
data Ganado2002b;
input cultivar$ replication bottom middle top;
cards;
A 1 0.7384      0.8370 0.8176
A 2 0.69466     0.75257         0.63313
A 3 0.74343     0.89689         0.93876
A 4 0.6425      0.57961         0.74201
A 5 0.57123     0.57566         0.42651
B 1 0.45372     0.39754         0.42471
B 2 0.59484     0.60658         0.44313
B 3 0.6645      0.61351         0.61164
B 4 0.53161     0.44058         0.33503
B 5 0.33943     0.40726         0.2735
```

```
C 1 0.65643    0.69595        0.62053
C 2 0.74653    0.63011        0.61631
C 3 0.64718    0.68694        0.52199
C 4 0.55668    0.57943        0.67792
C 5 0.44165    0.57347        0.44357
D 1 0.42359    0.38469        0.19855
D 2 0.43654    0.3777 0.14491
D 3 0.3378     0.32304        0.18941
D 4 0.30169    0.43639        0.1695
D 5 0.35701    0.21579        0.07131
E 1 0.66674    0.51227        0.18276
E 2 0.36264    0.33437        0.28741
E 3 0.64702    0.54662        0.22347
E 4 0.43607    0.35714        0.22976
E 5 0.36529    0.30256        0.23641
F 1 0.66216    0.77373        0.64208
F 2 0.67791    0.64668        0.51131
F 3 0.80508    0.88357        0.77282
F 4 0.70612    0.76086        0.54856
F 5 0.42027    0.4537 0.37829
run;
proc mixed data=Ganado2002b cl covtest;
classes cultivar replication;
model Ganado2002b = cultivar / htype=3;
random replication;
lsmeans cultivar / adjust=tukey ;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'C:\Documents and Settings\FReayJones.AGCENTER\Desktop\Francis
summer 2004\Stats PhD\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
quit;
```

Program 6: Injury in Salt Stressed Replication, Ganado, TX, 2002.

```
dm'log;clear;output;clear';
Title'Francis Reay-Jones – Chapter 1, Injury Replication 3, Ganado';
options nodate nonumber ps=55 ls=78;
data Ganado2002;
input cultivar$ stalk boredinternodes;
cards;
LCP85-384    1        100.0000
LCP85-384    2        100.0000
LCP85-384    3        100.0000
```

LCP85-384	4	71.4286
LCP85-384	5	100.0000
LCP85-384	6	88.8889
LCP85-384	7	100.0000
LCP85-384	8	62.5000
LCP85-384	9	100.0000
LCP85-384	10	85.7143
LCP85-384	11	77.7778
LCP85-384	12	90.0000
LCP85-384	13	70.5882
LCP85-384	14	66.6667
LCP85-384	15	66.6667
LCP85-384	16	83.3333
LCP85-384	17	100.0000
LCP85-384	18	61.5385
LCP85-384	19	80.0000
LCP85-384	20	100.0000
HoCP85-845	1	43.7500
HoCP85-845	2	40.0000
HoCP85-845	3	77.7778
HoCP85-845	4	40.0000
HoCP85-845	5	50.0000
HoCP85-845	6	57.1429
HoCP85-845	7	81.8182
HoCP85-845	8	36.3636
HoCP85-845	9	50.0000
HoCP85-845	10	91.6667
HoCP85-845	11	78.5714
HoCP85-845	12	100.0000
HoCP85-845	13	75.0000
HoCP85-845	14	50.0000
HoCP85-845	15	35.2941
HoCP85-845	16	50.0000
HoCP85-845	17	20.0000
HoCP85-845	18	77.7778
HoCP85-845	19	91.6667
HoCP85-845	20	62.5000
HoCP91-555	1	66.6667
HoCP91-555	2	50.0000
HoCP91-555	3	46.6667
HoCP91-555	4	77.7778
HoCP91-555	5	63.6364
HoCP91-555	6	100.0000
HoCP91-555	7	43.7500
HoCP91-555	8	41.6667
HoCP91-555	9	100.0000

HoCP91-555	10	63.6364
HoCP91-555	11	42.8571
HoCP91-555	12	60.0000
HoCP91-555	13	58.3333
HoCP91-555	14	66.6667
HoCP91-555	15	31.2500
HoCP91-555	16	66.6667
HoCP91-555	17	80.0000
HoCP91-555	18	46.6667
HoCP91-555	19	64.2857
HoCP91-555	20	61.5385
CP70-321	1	15.7895
CP70-321	2	28.5714
CP70-321	3	26.3158
CP70-321	4	41.6667
CP70-321	5	47.3684
CP70-321	6	35.2941
CP70-321	7	42.8571
CP70-321	8	15.0000
CP70-321	9	8.3333
CP70-321	10	5.8824
CP70-321	11	37.5000
CP70-321	12	12.5000
CP70-321	13	43.7500
CP70-321	14	33.3333
CP70-321	15	50.0000
CP70-321	16	66.6667
CP70-321	17	0.0000
CP70-321	18	12.5000
CP70-321	19	36.3636
CP70-321	20	7.1429
NCo310	1	35.7143
NCo310	2	56.2500
NCo310	3	66.6667
NCo310	4	30.0000
NCo310	5	64.7059
NCo310	6	50.0000
NCo310	7	18.7500
NCo310	8	37.5000
NCo310	9	27.2727
NCo310	10	41.6667
NCo310	11	20.0000
NCo310	12	70.0000
NCo310	13	47.0588
NCo310	14	36.3636
NCo310	15	60.0000

```
NCo310          16      81.8182
NCo310          17      35.7143
NCo310          18      54.5455
NCo310          19      75.0000
NCo310          20      28.5714
HoCP96-540      1       100.0000
HoCP96-540      2       91.6667
HoCP96-540      3       87.5000
HoCP96-540      4       90.9091
HoCP96-540      5       45.4545
HoCP96-540      6       76.9231
HoCP96-540      7       75.0000
HoCP96-540      8       87.5000
HoCP96-540      9       80.0000
HoCP96-540      10      92.3077
HoCP96-540      11      91.6667
HoCP96-540      12      92.3077
HoCP96-540      13      90.0000
HoCP96-540      14      58.3333
HoCP96-540      15      77.7778
HoCP96-540      16      90.0000
HoCP96-540      17      66.6667
HoCP96-540      18      54.5455
HoCP96-540      19      100.0000
HoCP96-540      20      75.0000
;
run;
Proc sort;
by cultivar;
run;
Proc means mean n stderr clm;
var boredinternodes;
by cultivar;
run;
```

Program 8: Injury in Other Replications, Ganado, TX, 2002

```
dm'log;clear;output;clear';
Title'Francis Reay-Jones – Chapter 1, Injury Other Replications, Ganado';
options nodate nonumber ps=55 ls=78;
data ganado2002;
input trt$ stalk bored;
cards;
LCP85-384       1       63.6364
LCP85-384       2       44.4444
LCP85-384       3       100.0000
```

LCP85-384	4	66.6667
LCP85-384	5	90.0000
LCP85-384	6	77.7778
LCP85-384	7	100.0000
LCP85-384	8	100.0000
LCP85-384	9	86.6667
LCP85-384	10	75.0000
LCP85-384	11	71.4286
LCP85-384	12	80.0000
LCP85-384	13	52.9412
LCP85-384	14	63.6364
LCP85-384	15	71.4286
LCP85-384	16	64.2857
LCP85-384	17	100.0000
LCP85-384	18	100.0000
LCP85-384	19	87.5000
LCP85-384	20	100.0000
LCP85-384	21	84.6154
LCP85-384	22	57.1429
LCP85-384	23	63.6364
LCP85-384	24	23.5294
LCP85-384	25	58.3333
LCP85-384	26	50.0000
LCP85-384	27	33.3333
LCP85-384	28	71.4286
LCP85-384	29	100.0000
LCP85-384	30	90.9091
LCP85-384	31	30.7692
LCP85-384	32	90.0000
LCP85-384	33	75.0000
LCP85-384	34	66.6667
LCP85-384	35	50.0000
LCP85-384	36	100.0000
LCP85-384	37	100.0000
LCP85-384	38	92.3077
LCP85-384	39	90.9091
LCP85-384	40	58.3333
LCP85-384	41	60.0000
LCP85-384	42	100.0000
LCP85-384	43	50.0000
LCP85-384	44	41.6667
LCP85-384	45	23.0769
LCP85-384	46	87.5000
LCP85-384	47	58.3333
LCP85-384	48	41.6667
LCP85-384	49	46.1538

LCP85-384	50	100.0000
LCP85-384	51	55.5556
LCP85-384	52	40.0000
LCP85-384	53	71.4286
LCP85-384	54	100.0000
LCP85-384	55	80.0000
LCP85-384	56	61.1111
LCP85-384	57	62.5000
LCP85-384	58	75.0000
LCP85-384	59	68.7500
LCP85-384	60	66.6667
LCP85-384	61	33.3333
LCP85-384	62	66.6667
LCP85-384	63	33.3333
LCP85-384	64	71.4286
LCP85-384	65	33.3333
LCP85-384	66	55.5556
LCP85-384	67	66.6667
LCP85-384	68	85.7143
LCP85-384	69	56.2500
LCP85-384	70	75.0000
LCP85-384	71	31.5789
LCP85-384	72	66.6667
LCP85-384	73	41.6667
LCP85-384	74	22.2222
LCP85-384	75	70.0000
LCP85-384	76	58.3333
LCP85-384	77	37.5000
LCP85-384	78	42.8571
LCP85-384	79	62.5000
LCP85-384	80	40.0000
HoCP85-845	1	43.7500
HoCP85-845	2	47.0588
HoCP85-845	3	36.8421
HoCP85-845	4	64.7059
HoCP85-845	5	58.8235
HoCP85-845	6	68.4211
HoCP85-845	7	42.1053
HoCP85-845	8	71.4286
HoCP85-845	9	66.6667
HoCP85-845	10	50.0000
HoCP85-845	11	37.5000
HoCP85-845	12	53.3333
HoCP85-845	13	77.7778
HoCP85-845	14	72.7273
HoCP85-845	15	47.3684

HoCP85-845	16	50.0000
HoCP85-845	17	72.7273
HoCP85-845	18	63.1579
HoCP85-845	19	83.3333
HoCP85-845	20	81.8182
HoCP85-845	21	15.7895
HoCP85-845	22	60.0000
HoCP85-845	23	15.3846
HoCP85-845	24	78.5714
HoCP85-845	25	33.3333
HoCP85-845	26	36.8421
HoCP85-845	27	87.5000
HoCP85-845	28	38.4615
HoCP85-845	29	66.6667
HoCP85-845	30	80.0000
HoCP85-845	31	11.7647
HoCP85-845	32	100.0000
HoCP85-845	33	71.4286
HoCP85-845	34	53.3333
HoCP85-845	35	53.3333
HoCP85-845	36	88.8889
HoCP85-845	37	71.4286
HoCP85-845	38	15.7895
HoCP85-845	39	30.7692
HoCP85-845	40	50.0000
HoCP85-845	41	20.0000
HoCP85-845	42	41.1765
HoCP85-845	43	75.0000
HoCP85-845	44	50.0000
HoCP85-845	45	41.6667
HoCP85-845	46	46.6667
HoCP85-845	47	50.0000
HoCP85-845	48	25.0000
HoCP85-845	49	25.0000
HoCP85-845	50	57.1429
HoCP85-845	51	53.3333
HoCP85-845	52	26.6667
HoCP85-845	53	19.0476
HoCP85-845	54	27.7778
HoCP85-845	55	56.2500
HoCP85-845	56	38.8889
HoCP85-845	57	41.6667
HoCP85-845	58	40.0000
HoCP85-845	59	71.4286
HoCP85-845	60	38.8889
HoCP85-845	61	33.3333

HoCP85-845	62	13.3333
HoCP85-845	63	26.6667
HoCP85-845	64	50.0000
HoCP85-845	65	20.0000
HoCP85-845	66	54.5455
HoCP85-845	67	33.3333
HoCP85-845	68	50.0000
HoCP85-845	69	33.3333
HoCP85-845	70	28.5714
HoCP85-845	71	37.5000
HoCP85-845	72	18.7500
HoCP85-845	73	20.0000
HoCP85-845	74	38.8889
HoCP85-845	75	33.3333
HoCP85-845	76	21.0526
HoCP85-845	77	52.9412
HoCP85-845	78	21.0526
HoCP85-845	79	22.2222
HoCP91-555	1	35.2941
HoCP91-555	2	64.2857
HoCP91-555	3	57.1429
HoCP91-555	4	63.6364
HoCP91-555	5	23.0769
HoCP91-555	6	93.7500
HoCP91-555	7	88.8889
HoCP91-555	8	50.0000
HoCP91-555	9	40.0000
HoCP91-555	10	81.8182
HoCP91-555	11	90.9091
HoCP91-555	12	100.0000
HoCP91-555	13	58.3333
HoCP91-555	14	33.3333
HoCP91-555	15	40.0000
HoCP91-555	16	50.0000
HoCP91-555	17	40.0000
HoCP91-555	18	84.6154
HoCP91-555	19	100.0000
HoCP91-555	20	100.0000
HoCP91-555	21	60.0000
HoCP91-555	22	71.4286
HoCP91-555	23	100.0000
HoCP91-555	24	66.6667
HoCP91-555	25	64.7059
HoCP91-555	26	72.7273
HoCP91-555	27	57.1429
HoCP91-555	28	80.0000

HoCP91-555	29	85.7143
HoCP91-555	30	42.8571
HoCP91-555	31	50.0000
HoCP91-555	32	92.8571
HoCP91-555	33	37.5000
HoCP91-555	34	72.7273
HoCP91-555	35	53.3333
HoCP91-555	36	5.8824
HoCP91-555	37	84.6154
HoCP91-555	38	90.0000
HoCP91-555	39	42.8571
HoCP91-555	40	91.6667
HoCP91-555	41	66.6667
HoCP91-555	42	90.0000
HoCP91-555	43	30.0000
HoCP91-555	44	58.3333
HoCP91-555	45	58.3333
HoCP91-555	46	81.8182
HoCP91-555	47	87.5000
HoCP91-555	48	40.0000
HoCP91-555	49	20.0000
HoCP91-555	50	72.7273
HoCP91-555	51	90.9091
HoCP91-555	52	81.8182
HoCP91-555	53	85.7143
HoCP91-555	54	77.7778
HoCP91-555	55	30.0000
HoCP91-555	56	37.5000
HoCP91-555	57	66.6667
HoCP91-555	58	33.3333
HoCP91-555	59	15.3846
HoCP91-555	60	100.0000
HoCP91-555	61	87.5000
HoCP91-555	62	38.8889
HoCP91-555	63	22.2222
HoCP91-555	64	35.2941
HoCP91-555	65	50.0000
HoCP91-555	66	85.7143
HoCP91-555	67	54.5455
HoCP91-555	68	28.5714
HoCP91-555	69	25.0000
HoCP91-555	70	90.9091
HoCP91-555	71	33.3333
HoCP91-555	72	66.6667
HoCP91-555	73	28.5714
HoCP91-555	74	21.4286

HoCP91-555	75	53.3333
HoCP91-555	76	50.0000
HoCP91-555	77	50.0000
HoCP91-555	78	16.6667
HoCP91-555	79	26.6667
HoCP91-555	80	100.0000
CP70-321	1	27.2727
CP70-321	2	27.7778
CP70-321	3	7.1429
CP70-321	4	54.5455
CP70-321	5	40.0000
CP70-321	6	6.6667
CP70-321	7	12.5000
CP70-321	8	58.3333
CP70-321	9	33.3333
CP70-321	10	56.2500
CP70-321	11	10.5263
CP70-321	12	60.0000
CP70-321	13	50.0000
CP70-321	14	28.5714
CP70-321	15	35.2941
CP70-321	16	31.2500
CP70-321	17	31.5789
CP70-321	18	26.6667
CP70-321	19	31.2500
CP70-321	20	31.5789
CP70-321	21	47.0588
CP70-321	22	50.0000
CP70-321	23	37.5000
CP70-321	24	11.7647
CP70-321	25	21.4286
CP70-321	26	7.1429
CP70-321	27	50.0000
CP70-321	28	66.6667
CP70-321	29	14.2857
CP70-321	30	23.5294
CP70-321	31	18.7500
CP70-321	32	20.0000
CP70-321	33	54.5455
CP70-321	34	38.8889
CP70-321	35	46.6667
CP70-321	36	12.5000
CP70-321	37	46.6667
CP70-321	38	33.3333
CP70-321	39	17.6471
CP70-321	40	28.5714

CP70-321	41	21.4286
CP70-321	42	33.3333
CP70-321	43	28.5714
CP70-321	44	70.0000
CP70-321	45	54.5455
CP70-321	46	5.8824
CP70-321	47	28.5714
CP70-321	48	37.5000
CP70-321	49	30.7692
CP70-321	50	44.4444
CP70-321	51	43.7500
CP70-321	52	25.0000
CP70-321	53	6.2500
CP70-321	54	11.1111
CP70-321	55	25.0000
CP70-321	56	23.5294
CP70-321	57	33.3333
CP70-321	58	33.3333
CP70-321	59	27.2727
CP70-321	60	21.4286
CP70-321	61	33.3333
CP70-321	62	20.0000
CP70-321	63	26.3158
CP70-321	64	29.4118
CP70-321	65	31.5789
CP70-321	66	21.4286
CP70-321	67	13.3333
CP70-321	68	25.0000
CP70-321	69	21.0526
CP70-321	70	16.6667
CP70-321	71	13.3333
CP70-321	72	0.0000
CP70-321	73	10.5263
CP70-321	74	0.0000
CP70-321	75	41.6667
CP70-321	76	33.3333
CP70-321	77	7.1429
CP70-321	78	7.6923
CP70-321	79	26.3158
CP70-321	80	52.9412
NCo310	1	14.2857
NCo310	2	50.0000
NCo310	3	40.0000
NCo310	4	31.2500
NCo310	5	90.9091
NCo310	6	5.5556

NCo310	7	50.0000
NCo310	8	28.5714
NCo310	9	80.0000
NCo310	10	25.0000
NCo310	11	18.1818
NCo310	12	31.2500
NCo310	13	46.1538
NCo310	14	54.5455
NCo310	15	60.0000
NCo310	16	37.5000
NCo310	17	100.0000
NCo310	18	37.5000
NCo310	19	42.8571
NCo310	20	57.1429
NCo310	21	69.2308
NCo310	22	25.0000
NCo310	23	8.3333
NCo310	24	0.0000
NCo310	25	63.6364
NCo310	26	31.2500
NCo310	27	50.0000
NCo310	28	36.3636
NCo310	29	68.7500
NCo310	30	50.0000
NCo310	31	16.6667
NCo310	32	7.6923
NCo310	33	17.6471
NCo310	34	40.0000
NCo310	35	62.5000
NCo310	36	0.0000
NCo310	37	28.5714
NCo310	38	25.0000
NCo310	39	9.0909
NCo310	40	26.6667
NCo310	41	40.0000
NCo310	42	25.0000
NCo310	43	33.3333
NCo310	44	7.6923
NCo310	45	47.0588
NCo310	46	75.0000
NCo310	47	29.4118
NCo310	48	45.4545
NCo310	49	11.7647
NCo310	50	43.7500
NCo310	51	41.6667
NCo310	52	40.0000

NCo310	53	21.4286
NCo310	54	18.7500
NCo310	55	16.6667
NCo310	56	28.5714
NCo310	57	46.1538
NCo310	58	43.7500
NCo310	59	30.7692
NCo310	60	11.1111
NCo310	61	10.5263
NCo310	62	31.2500
NCo310	63	5.8824
NCo310	64	33.3333
NCo310	65	25.0000
NCo310	66	15.3846
NCo310	67	22.2222
NCo310	68	33.3333
NCo310	69	64.2857
NCo310	70	42.8571
NCo310	71	29.4118
NCo310	72	68.7500
NCo310	73	5.2632
NCo310	74	61.5385
NCo310	75	16.6667
NCo310	76	20.0000
NCo310	77	28.5714
NCo310	78	33.3333
NCo310	79	37.5000
HoCP96-540	1	86.6667
HoCP96-540	2	75.0000
HoCP96-540	3	81.8182
HoCP96-540	4	50.0000
HoCP96-540	5	70.0000
HoCP96-540	6	62.5000
HoCP96-540	7	81.8182
HoCP96-540	8	58.8235
HoCP96-540	9	90.9091
HoCP96-540	10	43.7500
HoCP96-540	11	64.7059
HoCP96-540	12	85.7143
HoCP96-540	13	33.3333
HoCP96-540	14	88.8889
HoCP96-540	15	75.0000
HoCP96-540	16	40.0000
HoCP96-540	17	71.4286
HoCP96-540	18	81.8182
HoCP96-540	19	68.7500

HoCP96-540	20	35.2941
HoCP96-540	21	50.0000
HoCP96-540	22	56.2500
HoCP96-540	23	68.4211
HoCP96-540	24	43.7500
HoCP96-540	25	60.0000
HoCP96-540	26	76.4706
HoCP96-540	27	72.7273
HoCP96-540	28	33.3333
HoCP96-540	29	66.6667
HoCP96-540	30	40.0000
HoCP96-540	31	75.0000
HoCP96-540	32	71.4286
HoCP96-540	33	61.5385
HoCP96-540	34	35.0000
HoCP96-540	35	75.0000
HoCP96-540	36	76.9231
HoCP96-540	37	62.5000
HoCP96-540	38	75.0000
HoCP96-540	39	80.0000
HoCP96-540	40	94.1176
HoCP96-540	41	66.6667
HoCP96-540	42	64.7059
HoCP96-540	43	43.7500
HoCP96-540	44	72.7273
HoCP96-540	45	38.8889
HoCP96-540	46	61.1111
HoCP96-540	47	47.6190
HoCP96-540	48	47.3684
HoCP96-540	49	76.4706
HoCP96-540	50	37.5000
HoCP96-540	51	81.2500
HoCP96-540	52	76.9231
HoCP96-540	53	100.0000
HoCP96-540	54	66.6667
HoCP96-540	55	92.3077
HoCP96-540	56	57.1429
HoCP96-540	57	100.0000
HoCP96-540	58	60.0000
HoCP96-540	59	50.0000
HoCP96-540	60	57.1429
HoCP96-540	61	50.0000
HoCP96-540	62	31.2500
HoCP96-540	63	50.0000
HoCP96-540	64	58.3333
HoCP96-540	65	66.6667

```
HoCP96-540   66      37.5000
HoCP96-540   67      56.2500
HoCP96-540   68      71.4286
HoCP96-540   69      50.0000
HoCP96-540   70      6.2500
HoCP96-540   71      25.0000
HoCP96-540   72      28.5714
HoCP96-540   73      28.5714
HoCP96-540   74      16.6667
HoCP96-540   75      83.3333
HoCP96-540   76      40.0000
HoCP96-540   77      20.0000
HoCP96-540   78      41.1765
HoCP96-540   79      11.7647
run;
Proc sort;
by trt;
run;
Proc print;
run;
Proc means mean n stderr clm;
var bored;
by trt;
run;
```

APPENDIX B: SAS CODE FOR CHAPTER 3

Program 1: Injury, Moth Exit Holes, and Relative Survival, Ganado, TX, 2003.

```
dm'output;clear;log;clear';
Title'Francis Reay-Jones - Intregration of Control Tactics';
data Ganado20032004;
 input year$     Row$  Column$        Irrigation$      Cultivar$        Insecticide$     Stalk
          TotalI  BoredMRB     EMRB Height;
 logstalk=log(stalk); /* offset variable -- analysis is on log scale so take log of offset */
 EMRB=EMRB+1; /* you have 1 cell in the fixed effects table for which all counts are
zero */
 logEMRB=Log(EMRB); /* for plotting */
 LogEMRBStalk=Log(EMRB/Stalk);
 r=BoredMRB/TotalI;
 LogitBoredMRB=Log(r/(1-r));
 sur=EMRB/BoredMRB;
cards;
Year2003 3 4 0        HoCP85845   0       20      271     131     38      39.9
Year2003 1 4 0        HoCP85845   0       20      323     136     27      51.15
Year2003 4 1 0        HoCP85845   0       20      261     121     38      36.1
Year2003 2 1 0        HoCP85845   0       20      381     123     59      54.95
Year2003 2 1 0        HoCP85845   1       17      278     23      2       58.35294118
Year2003 1 4 0        HoCP85845   1       17      278     38      11      47.11764706
Year2003 3 4 0        HoCP85845   1       17      317     68      9       51.41176471
Year2003 4 1 0        HoCP85845   1       18      316     59      14      55.34722222
Year2003 1 2 1        HoCP85845   0       20      367     96      18      57.75
Year2003 4 3 1        HoCP85845   0       20      328     92      28      57.3
Year2003 3 2 1        HoCP85845   0       20      361     80      17      63.1
Year2003 2 3 1        HoCP85845   0       20      352     46      25      54.05
Year2003 3 2 1        HoCP85845   1       19      312     12      0       58.15789474
Year2003 4 3 1        HoCP85845   1       18      306     10      0       54.61111111
Year2003 2 3 1        HoCP85845   1       17      310     3       0       58.76470588
Year2003 1 2 1        HoCP85845   1       17      326     5       0       54.41176471
Year2003 2 4 0        LCP85384    0       20      217     150     64      32.1
Year2003 4 4 0        LCP85384    0       20      215     181     63      31.45
Year2003 3 1 0        LCP85384    0       17      179     136     80      29.11764706
Year2003 1 1 0        LCP85384    0       20      280     153     25      45
Year2003 4 4 0        LCP85384    1       20      291     57      9       39.8
Year2003 3 1 0        LCP85384    1       19      265     111     38      38.21052632
Year2003 1 1 0        LCP85384    1       19      267     73      38      48.05263158
Year2003 2 4 0        LCP85384    1       20      229     97      10      32.05
Year2003 4 2 1        LCP85384    0       20      358     91      24      56.55
Year2003 2 2 1        LCP85384    0       20      356     167     59      54.85
Year2003 3 3 1        LCP85384    0       20      345     120     26      48.4
Year2003 1 3 1        LCP85384    0       17      270     145     44      40.64705882
```

```
Year2003 4 2 1      LCP85384    1    19    245    21    0    46.73684211
Year2003 3 3 1      LCP85384    1    14    288    22    5    53.92857143
Year2003 1 3 1      LCP85384    1    18    316    8     2    54.88888889
Year2003 2 2 1      LCP85384    1    14    268    5     0    61.92857143
Year2004 3 4 0      HoCP85845   0    20    210    127   32   83.25
Year2004 1 4 0      HoCP85845   0    20    191    43    11   98.8
Year2004 4 1 0      HoCP85845   0    20    187    85    18   95.7
Year2004 2 1 0      HoCP85845   0    20    235    65    18   117.1
Year2004 2 1 0      HoCP85845   1    20    249    34    5    142.7
Year2004 1 4 0      HoCP85845   1    20    197    42    5    103.35
Year2004 3 4 0      HoCP85845   1    20    241    46    4    113.4
Year2004 4 1 0      HoCP85845   1    20    192    19    4    97.65
Year2004 1 2 1      HoCP85845   0    20    300    46    4    176.75
Year2004 4 3 1      HoCP85845   0    20    255    48    8    145.5
Year2004 3 2 1      HoCP85845   0    20    266    90    17   154.05
Year2004 2 3 1      HoCP85845   0    20    284    46    6    154.25
Year2004 3 2 1      HoCP85845   1    20    277    23    3    153.35
Year2004 4 3 1      HoCP85845   1    19    252    23    3    149.6842105
Year2004 2 3 1      HoCP85845   1    20    241    22    1    152
Year2004 1 2 1      HoCP85845   1    19    265    33    2    142.8947368
Year2004 2 4 0      LCP85384    0    20    236    133   29   95.4
Year2004 4 4 0      LCP85384    0    20    209    129   32   88.6
Year2004 3 1 0      LCP85384    0    20    206    93    14   95.5
Year2004 1 1 0      LCP85384    0    20    234    127   38   129.55
Year2004 4 4 0      LCP85384    1    20    183    90    22   81.55
Year2004 3 1 0      LCP85384    1    20    207    29    3    106.2
Year2004 1 1 0      LCP85384    1    20    245    26    6    132.65
Year2004 2 4 0      LCP85384    1    20    228    58    8    117.55
Year2004 4 2 1      LCP85384    0    20    261    53    19   135.9
Year2004 2 2 1      LCP85384    0    20    245    86    23   144.25
Year2004 3 3 1      LCP85384    0    20    254    88    23   126.55
Year2004 1 3 1      LCP85384    0    20    261    45    4    145.65
Year2004 4 2 1      LCP85384    1    18    227    36    6    140.5555556
Year2004 3 3 1      LCP85384    1    20    290    59    6    158.85
Year2004 1 3 1      LCP85384    1    20    267    51    6    145.55
Year2004 2 2 1      LCP85384    1    20    231    42    7    149.65;
Proc Sort Data=Ganado20032004;
 By Irrigation Cultivar Insecticide Year Column Row;
Run;
proc glimmix data=Ganado20032004 plots=all;
class Irrigation Cultivar Insecticide column year row;
model EMRB/BoredMRB = Irrigation|Cultivar|Insecticide|year  / htype=3
DDFM=KENWARDROGER dist=binomial link=logit;
random  column(irrigation)  cultivar*row*column(irrigation)
insecticide*cultivar*row*column(irrigation);
lsmeans Irrigation|Cultivar|Insecticide|year / diff cl adjust=tukey;
```

```
run;
proc glimmix data=Ganado20032004 plots=all;
class Irrigation Cultivar Insecticide column year row;
model BoredMRB/TotalI = Irrigation|Cultivar|Insecticide|year  / htype=3
DDFM=KENWARDROGER dist=binomial link=logit;
random  column(irrigation)  cultivar*row*column(irrigation)
insecticide*cultivar*row*column(irrigation);
lsmeans  Irrigation|Cultivar|Insecticide|year   / diff cl adjust=tukey;
run;
proc glimmix data=Ganado20032004 plots=all;
class Irrigation Cultivar Insecticide column year row;
model EMRB = Irrigation|Cultivar|Insecticide|year
        / htype=3 DDFM=KENWARDROGER dist=Poisson link=log offset=logstalk;
*random  column(irrigation)  cultivar*row*column(irrigation)
insecticide*cultivar*row*column(irrigation);
random _residual_; /* fit as overdispersed model */
*lsmeans irrigation*insecticide*year / slice=(year irrigation insecticide
irrigation*insecticide);
lsmeans  Irrigation|Cultivar|Insecticide|year   /  ILINK diff cl adjust=tukey;
run;
proc mixed data=Ganado20032004;
class Irrigation Cultivar Insecticide column year row;
model height = Irrigation|Cultivar|Insecticide|year  / htype=3
DDFM=KENWARDROGER;
random  column(irrigation)  cultivar*row*column(irrigation)
insecticide*cultivar*row*column(irrigation);
lsmeans Irrigation|Cultivar|Insecticide|year / diff cl adjust=tukey;
run;
/*
 * The following proc mixed code was used as a check to see what was
 * causing some of the difficulties above. Appears that there is really
 * little to no variability that can be attributed to the higher level
 * random effects. Apparently, the emergence counts depend upon the
 * fixed effects, but there is very little variation that can be ascribed
 * to the design and spatial arrangements of plots.
 */
proc mixed data=Ganado20032004 update;
class Irrigation Cultivar Insecticide column year row;
model LogEMRBStalk = Irrigation|Cultivar|Insecticide|year
        / htype=3 DDFM=KENWARDROGER residual;
random  column(irrigation)  cultivar*row*column(irrigation)
insecticide*cultivar*row*column(irrigation);
run;
quit;
```

Program 9: Sugarcane Yield Components, Ganado, TX, 2003

```
dm'log;clear;output;clear';
Title'Francis Reay-Jones';
options nodate nonumber ps=55 ls=78;
data ganado2003;
input Trt$ Row Column Irrigation$ Cultivar$ Insecticide weight
      grams TRS cane TRSbyha;
cards;
384AIT 4 2 1 LCP85384 1 1.261111111 572.0273889 98.37333333 48.797722
4800.394573
384AIU 4 2 1 LCP85384 0 1.33 603.2747 98.56 51.46332444 5072.225257
384AT 4 4 0 LCP85384 1 0.5325 241.536675 95.85333333 10.77895908 1033.199158
384AU 4 4 0 LCP85384 0 0.5925 268.752075 90.16 11.99348969 1081.33303
384BIT 3 3 1 LCP85384 1 1.36 616.8824 109.8533333 54.4968969 5986.665781
384BIU 3 3 1 LCP85384 0 0.9325 422.972675 88.34 37.3664385 3300.951177
384BT 3 1 0 LCP85384 1 0.785 356.06815 95.57333333 23.02444321 2200.522786
384BU 3 1 0 LCP85384 0 0.74 335.6566 93.98666667 21.70457067 2039.940249
384CIT 2 2 1 LCP85384 1 1.0675 484.207325 106.7733333 52.3308463
5587.538895
384CIU 2 2 1 LCP85384 0 1.11739 506.8369301 89.88 54.77654739 4923.31608
384CT 2 4 0 LCP85384 1 0.8725 395.757275 104.5333333 19.34332765 2022.022517
384CU 2 4 0 LCP85384 0 0.6225 282.359775 100.893333313.80082689 1392.411428
384DIT 1 3 1 LCP85384 1 1.035 469.46565 107.52 40.76113415 4382.637144
384DIU 1 3 1 LCP85384 0 1.08 489.8772 100.1466667 42.53335738 4259.573963
384DT 1 1 0 LCP85384 1 0.9525 432.044475 111.906666731.34748923 3507.993028
384DU 1 1 0 LCP85384 0 0.94 426.3746 94.15 30.93610486 2912.634272
845AIT 4 3 1 HoCP85845 1 1.394736842 632.6386842 99.07333333 49.16681942
4871.120689
845AIU 4 3 1 HoCP85845 0 1.385 628.22215 97.62666667 48.82357936 4766.483307
845AT 4 1 0 HoCP85845 1 0.845 383.28355 97.48666667 32.23119106 3142.111379
845AU 4 1 0 HoCP85845 0 0.78 353.8002 57.77333333 29.75186867 1718.864626
845BIT 3 2 1 HoCP85845 1 1.335 605.54265 103.7866667 59.56157213 6181.697033
845BIU 3 2 1 HoCP85845 0 1.2095 548.617105 87.73333333 53.9623382
4734.295804
845BT 3 4 0 HoCP85845 1 0.9625 436.580375 98.09333333 28.62822131
2808.237656
845BU 3 4 0 HoCP85845 0 0.5275 239.268725 86.84666667 15.68975246
1362.602702
845CIT 2 3 1 HoCP85845 1 1.285 582.86315 108.4533333 47.24482728 5123.859001
845CIU 2 3 1 HoCP85845 0 1.2225 554.513775 107.4266667 44.94692712
4828.498557
845CT 2 1 0 HoCP85845 1 1.1925 540.906075 90.72 56.15964713 5094.803188
845CU 2 1 0 HoCP85845 0 0.795 360.60405 106.12 37.43976475 3973.107836
845DIT 1 2 1 HoCP85845 1 1.197368421 543.1143421 105.84 55.56452134
      5880.948938
```

```
845DIU 1 2 1 HoCP85845 0 1.59 721.2081 98.37333333 73.78479954 7258.456681
845DT 1 4 0 HoCP85845 1 0.775 351.53225 73.45333333 26.67973968 1959.715812
845DU 1 4 0 HoCP85845       0 0.7625 345.862375 111.3466667    26.2494213
2922.785563
;
proc mixed data=Ganado2003;
class Irrigation Cultivar Insecticide column;
model TRSbyha =     Irrigation|Cultivar|Insecticide  / htype= 1 3
DDFM=KENWARDROGER ;
random  column(irrigation)  cultivar*column(irrigation)
insecticide*cultivar*column(irrigation) insecticide*cultivar*column(irrigation);
lsmeans  Irrigation|Cultivar|Insecticide   / diff cl adjust=tukey;
run;
quit;
```

APPENDIX C: FIELD MAPS AND SUPPLEMENTARY DATA FOR CHAPTER 2

REP	HoCP 91-555 (10ft)	**E** (10ft)	**E** (10ft)	**E** (10ft)	**E** (10ft)	**E** (10ft)	**E** (10ft)	HoCP 91-555 (10ft)
	Alley	5ft	5ft	5ft	5ft	5ft	5ft	
I	HoCP 91-555	**F**	**E**	**D**	**C**	**B**	**A**	HoCP 91-555
	Alley	5ft	5ft	5ft	5ft	5ft	5ft	
II	HoCP 91-555	**A**	**F**	**B**	**D**	**C**	**E**	HoCP 91-555
	Alley	5ft	5ft	5ft	5ft	5ft	5ft	
III	HoCP 91-555	**C**	**B**	**D**	**E**	**F**	**A**	HoCP 91-555
	Alley	5ft	5ft	5ft	5ft	5ft	5ft	
IV	HoCP 91-555	**D**	**E**	**B**	**A**	**C**	**F**	HoCP 91-555
	Alley	5ft	5ft	5ft	5ft	5ft	5ft	
V	HoCP 91-555	**D**	**B**	**A**	**F**	**C**	**E**	HoCP 91-555
	Alley	5ft	5ft	5ft	5ft	5ft	5ft	
	HoCP 91-555 (10ft)	HoCP 91-555 (10ft)	HoCP 91-555 (10ft)	HoCP 91-555 (10ft)	HoCP 91-555 (10ft)	HoCP 91-555 (10ft)	HoCP 91-555 (10ft)	HoCP 91-555 (10ft)

Viewing from highway, front-end border is triangular (10 ft on left down to 3 ft on right), at least 10 ft border at back end of test (from rep 5), 1-row continuous border of HoCP 91-555on each side of test. One row plots 20 ft long, alleys 5 ft at beginning and end of each plot (not planted).

A. NCo 310
B. CP 70-321
C. LCP 85-384
D. HoCP 85-845
E. HoCP 91-555
F. HoCP 96-540

**not drawn to scale

Table C.1. Soil analyses from sugarcane irrigation/cultivar/insecticide test, Ganado, TX, 12 December 2002.

Replication - position[1]	Copper (ppm)	Iron (ppm)	Manganese (ppm)	Zinc (ppm)	Calcium (ppm)	Magnesium (ppm)	pH	Phosphorus (ppm)	Potassium (ppm)	Sodium (ppm)	% Organic matter
1 - U	1.250	217.54	19.62	0.733	1527.8	349.8	5.94	33.1	141.9	80.4	2.285
1 - L	1.86	57.39	3.02	0.312	2284.7	560.6	6.65	12.8	137.4	277.1	1.102
2 - U	1.16	141.09	8.07	0.341	1395.1	350.2	6.62	21.0	129.8	220.6	1.429
2 - L	1.30	52.17	1.84	0.264	1587.1	445.5	6.97	11.6	141.7	392.0	0.912
3 - U	1.13	204.56	26.75	0.469	1444.7	363.6	6.37	34.6	168.1	209.7	1.809
3 - L	1.71	39.96	2.75	0.371	2459.3	761.8	7.20	14.4	277.8	792.6	1.089
4 - U	1.19	99.34	13.78	0.282	1354.0	287.1	5.89	20.2	119.2	55.1	1.116
4 - L	1.92	39.19	7.71	0.180	1792.2	399.4	6.40	11.9	138.7	49.3	0.545
5 - U	1.45	147.35	59.44	0.491	1348.3	257.8	6.19	21.7	131.2	31.0	1.089
5 - L	1.59	34.86	10.38	0.192	1771.7	351.5	6.38	12.9	121.2	33.7	0.518

[1] U = Upper depth (0-15 cm), L = Lower depth (15-30 cm)

APPENDIX D: FIELD MAPS AND SUPPLEMENTARY DATA FOR CHAPTER 3

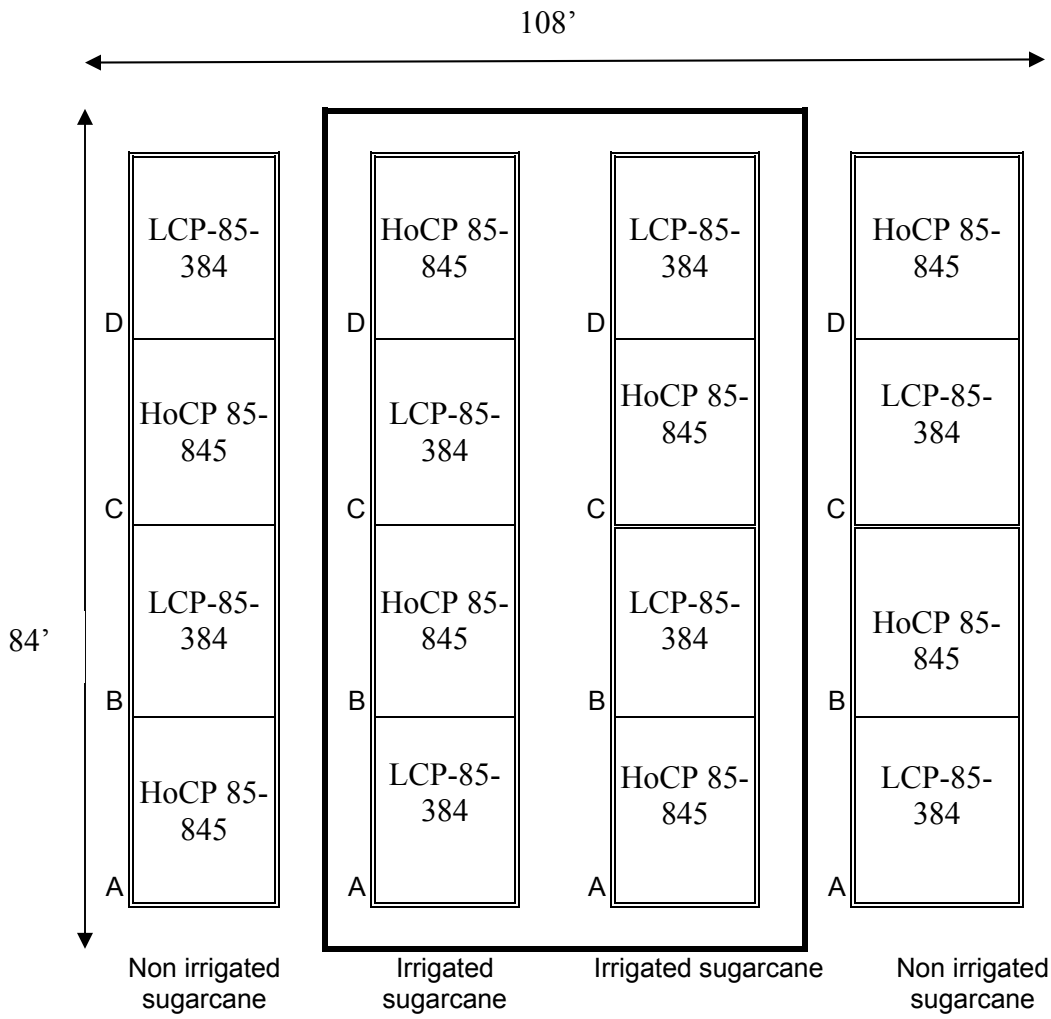


Table D.1. Soil analyses (part 1) from sugarcane irrigation/cultivar/insecticide test, Ganado, TX, 2003.

Cultivar[1]	Irrigation	Row	Position[2]	Calcium (ppm)	Magnesium (ppm)	pH	Phosphorus (ppm)
A	No	A	U	756.7	167.6	5.77	12.90
A	No	A	L	1239.4	290.1	6.48	4.76
A	No	D	U	688.2	139.3	5.68	22.18
A	No	D	L	915.4	187.2	5.87	1.62
A	No	C	U	997.0	256.3	6.16	9.08
A	No	C	L	1759.8	486.9	6.77	15.30
A	No	B	U	813.1	221.7	6.23	22.40
A	No	B	L	1550.5	435.4	6.80	2.43
B	No	D	U	868.0	207.5	5.67	10.68
B	No	D	L	1052.0	257.1	6.31	5.65
B	No	B	U	1022.2	216.1	6.21	7.10
B	No	B	L	1977.2	462.3	6.73	3.26
B	No	C	U	812.7	183.4	5.67	8.74
B	No	C	L	835.8	203.0	6.02	4.66
B	No	A	U	838.4	195.6	5.98	28.47
B	No	A	L	1323.2	346.6	6.76	4.46
A	Yes	C	U	683.2	139.4	5.79	11.96
A	Yes	C	L	759.2	153.4	5.91	1.02
A	Yes	B	U	708.0	168.4	5.88	17.54
A	Yes	B	L	886.4	234.1	6.02	2.04
A	Yes	A	U	700.0	157.8	5.56	23.78
A	Yes	A	L	837.5	182.8	6.03	7.89
A	Yes	D	U	766.2	168.5	5.82	9.96
A	Yes	D	L	942.7	217.4	6.27	6.74
B	Yes	B	U	721.7	161.5	5.75	11.58
B	Yes	B	L	825.2	188.0	6.13	3.00
B	Yes	A	U	711.8	161.4	5.82	12.42
B	Yes	A	L	874.9	195.3	6.21	5.05
B	Yes	C	U	829.2	171.3	5.89	5.46
B	Yes	C	L	694.2	148.3	5.80	13.27
B	Yes	D	U	802.0	172.0	6.00	13.05
B	Yes	D	L	857.1	186.8	6.28	3.88

[1] A = LCP 85-384, B = HoCP 85-845

[2] U = Upper depth (0-15 cm), L = Lower depth (15-30 cm)

Table D.2. Soil analyses (part 2) from sugarcane irrigation/cultivar/insecticide test, Ganado, TX.

Cultivar[1]	Irrigation	Row	Position[2]	Potassium (ppm)	Sodium (ppm)	% Organic matter
A	No	A	U	88.47	41.60	1.469
A	No	A	L	75.54	122.49	0.871
A	No	D	U	183.18	25.49	1.456
A	No	D	L	120.10	36.87	0.667
A	No	C	U	146.83	116.73	1.660
A	No	C	L	159.07	415.36	0.994
A	No	B	U	212.42	109.19	1.605
A	No	B	L	248.42	392.87	1.184
B	No	D	U	92.80	43.87	1.564
B	No	D	L	60.51	76.43	0.749
B	No	B	U	97.27	87.58	1.374
B	No	B	L	135.23	364.35	1.116
B	No	C	U	161.57	60.29	1.143
B	No	C	L	121.19	62.39	0.667
B	No	A	U	133.64	68.59	1.891
B	No	A	L	156.55	263.25	1.116
A	Yes	C	U	156.95	22.21	2.067
A	Yes	C	L	97.61	32.93	1.007
A	Yes	B	U	83.74	38.46	1.646
A	Yes	B	L	79.90	56.38	1.184
A	Yes	A	U	125.60	35.88	1.714
A	Yes	A	L	70.00	38.61	1.075
A	Yes	D	U	99.20	39.75	1.660
A	Yes	D	L	59.69	54.38	0.749
B	Yes	B	U	119.89	40.58	1.605
B	Yes	B	L	77.48	48.41	0.844
B	Yes	A	U	58.77	30.05	1.388
B	Yes	A	L	75.62	41.21	0.762
B	Yes	C	U	73.68	39.77	0.762
B	Yes	C	L	108.64	27.02	1.646
B	Yes	D	U	84.29	40.50	1.605
B	Yes	D	L	75.18	37.67	0.790

[1] A = LCP 85-384, B = HoCP 85-845

[2] U = Upper depth (0-15 cm), L = Lower depth (15-30 cm)

APPENDIX E: DATA FOR CHAPTER 4

Table E.1. Host type codes for data oviposition tables.

Species	Cultivar	Stage	Stress (sugarcane only)	Host type
Sugarcane	LCP 85-384	5 internodes	Non drought stressed	1
			Drought stressed	2
		11 internodes	Non drought stressed	3
			Drought stressed	4
	HoCP 85-845	5 internodes	Non drought stressed	5
			Drought stressed	6
		11 internodes	Non drought stressed	7
			Drought stressed	8
Rice	Cocodrie	Tillering 3-4 leaves	-	9
		Tillering 6-7 leaves	-	10
		Boot	-	11
		Heading	-	12
	XL8	Tillering 3-4 leaves	-	13
		Tillering 6-7 leaves	-	14
		Boot	-	15
		Heading	-	16

Table E.2. Data for oviposition experiment, date 1. A.

Date	Cage	Host code	Leaves	Dry leaves	Plant height (cm)	Water potential (barr)	Dry weight (g)
6/19/2003	1	5	14	3	92	6	43
6/19/2003	1	5	14	8	100	6	-
6/19/2003	2	5	14	4	99	6.5	-
6/19/2003	2	5	14	5	66	9	-
6/19/2003	3	5	16	5	95	10.5	38.8
6/19/2003	3	5	14	5	82	8.5	47.4
6/19/2003	4	5	14	2	82	8.5	-
6/19/2003	4	5	14	6	77	10	-
6/19/2003	1	6	17	11	67	14	18.1
6/19/2003	1	6	14	8	86	12	14.9
6/19/2003	2	6	17	11	71	10	-
6/19/2003	2	6	14	14	43	40	-
6/19/2003	3	6	13	8	74	40	17.4
6/19/2003	3	6	13	4	76	10.5	-
6/19/2003	4	6	13	5	69	17.5	-
6/19/2003	4	6	16	10	70	40	-
6/19/2003	1	1	18	6	126	9	-
6/19/2003	1	1	16	6	117	7	51.2
6/19/2003	2	1	18	8	109	10	49.6
6/19/2003	2	1	20	11	114	6	51.6
6/19/2003	3	1	18	8	113	12	-
6/19/2003	3	1	14	4	114	8	-
6/19/2003	4	1	16	6	115	9	-
6/19/2003	4	1	18	9	114	8.5	-
6/19/2003	1	2	19	13	90	40	-
6/19/2003	1	2	19	13	79	10.5	-
6/19/2003	2	2	16	9	85	40	21.4
6/19/2003	2	2	15	10	85	11	19.2
6/19/2003	3	2	18	12	90	40	-
6/19/2003	3	2	18	11	90	13	17.2
6/19/2003	4	2	17	10	88	40	-
6/19/2003	4	2	17	12	85	40	-

Table E.3. Data for oviposition experiment, date 1. B.

Date	Cage	Host code	Eggs laid	Egg masses	Aspartic[1]	Glutamic[1]	Valine[1]	Serine[1]
6/19/2003	1	5	84	13	132.8	76.6	0.0	46.3
6/19/2003	1	5	301	22	283.9	434.4	36.2	108.9
6/19/2003	2	5	140	4	505.4	492.6	73.7	288.5
6/19/2003	2	5	225	14	229.5	0.0	64.8	176.4
6/19/2003	3	5	28	4	322.1	227.6	137.9	316.9
6/19/2003	3	5	237	9	80.5	226.3	34.3	110.3
6/19/2003	4	5	267	13	351.7	443.8	69.1	178.2
6/19/2003	4	5	0	0	112.6	0.0	34.9	188.8
6/19/2003	1	6	555	25	468.1	964.4	40.6	131.9
6/19/2003	1	6	365	18	1752.0	1496.4	91.8	280.5
6/19/2003	2	6	1095	51	778.2	329.1	39.2	130.6
6/19/2003	2	6	79	8	-	-	-	-
6/19/2003	3	6	163	13	466.5	732.5	82.8	230.4
6/19/2003	3	6	135	12	366.6	332.1	68.5	166.9
6/19/2003	4	6	65	3	444.1	864.7	62.6	180.3
6/19/2003	4	6	385	22	1779.9	359.2	209.0	626.5
6/19/2003	1	1	120	5	590.0	0.0	62.2	171.0
6/19/2003	1	1	44	4	275.9	0.0	627.9	2197.9
6/19/2003	2	1	225	6	358.1	127.6	47.9	183.3
6/19/2003	2	1	958	37	239.7	0.0	48.6	136.5
6/19/2003	3	1	415	22	302.4	60.0	44.2	107.4
6/19/2003	3	1	169	6	439.0	0.0	65.4	312.0
6/19/2003	4	1	74	4	321.1	88.1	48.1	165.1
6/19/2003	4	1	216	6	504.8	494.1	54.7	56.5
6/19/2003	1	2	550	12	59.1	222.4	52.5	146.4
6/19/2003	1	2	579	29	377.1	163.4	42.8	134.2
6/19/2003	2	2	149	6	398.8	265.5	54.1	273.7
6/19/2003	2	2	230	12	446.1	350.4	53.0	193.8
6/19/2003	3	2	804	47	555.5	452.0	45.7	220.4
6/19/2003	3	2	690	18	746.1	597.8	60.1	227.8
6/19/2003	4	2	350	14	1682.4	0.0	308.6	868.6
6/19/2003	4	2	818	35	679.5	580.9	71.2	188.4

[1] nanomoles per 10 μL of leaf juice.

Table E.4. Data for oviposition experiment, date 1. C.

Date	Cage	Host code	Histidine[1]	Glycine[1]	Threonine[1]	Arginine[1]	Alanine[1]
6/19/2003	1	5	39.0	47.7	0.0	0.0	130.4
6/19/2003	1	5	68.2	0.0	42.2	0.0	431.9
6/19/2003	2	5	158.4	113.0	117.4	33.9	894.3
6/19/2003	2	5	84.8	65.2	53.2	0.0	340.3
6/19/2003	3	5	214.2	117.0	0.0	51.9	692.3
6/19/2003	3	5	48.2	0.0	33.3	0.0	204.2
6/19/2003	4	5	65.8	0.0	58.1	27.3	685.1
6/19/2003	4	5	55.9	0.0	32.6	0.0	197.4
6/19/2003	1	6	145.7	165.6	125.0	44.5	936.0
6/19/2003	1	6	224.9	368.6	252.9	55.0	1164.5
6/19/2003	2	6	97.9	118.6	113.1	0.0	671.0
6/19/2003	2	6	-	-	-	-	-
6/19/2003	3	6	163.7	119.0	121.7	57.8	1087.2
6/19/2003	3	6	140.4	108.6	88.7	0.0	941.4
6/19/2003	4	6	127.4	105.7	134.5	46.0	974.5
6/19/2003	4	6	436.6	370.2	470.2	129.6	2892.7
6/19/2003	1	1	68.5	0.0	65.7	18.4	800.1
6/19/2003	1	1	1394.6	0.0	889.2	0.0	7329.3
6/19/2003	2	1	75.2	0.0	82.6	31.8	605.7
6/19/2003	2	1	96.4	0.0	45.1	0.0	229.8
6/19/2003	3	1	56.9	0.0	72.4	159.0	353.7
6/19/2003	3	1	152.2	0.0	134.5	39.0	1165.2
6/19/2003	4	1	76.9	0.0	59.4	0.0	405.4
6/19/2003	4	1	84.8	0.0	59.8	0.0	643.6
6/19/2003	1	2	108.2	126.9	109.2	26.8	832.9
6/19/2003	1	2	96.8	152.4	112.8	18.6	987.4
6/19/2003	2	2	127.2	204.7	94.4	27.5	377.6
6/19/2003	2	2	114.0	107.5	104.0	23.1	690.8
6/19/2003	3	2	120.1	127.6	114.3	55.1	854.0
6/19/2003	3	2	129.9	106.7	122.5	57.5	1304.9
6/19/2003	4	2	361.3	237.0	550.0	243.6	3836.1
6/19/2003	4	2	107.3	121.2	154.0	22.9	1343.1

[1] nanomoles per 10 μL of leaf juice.

Table E.5. Data for oviposition experiment, date 1. D.

Date	Cage	Host code	Tyrosine[1]	Methionine[1]	Phenylalanine[1]	Isoleucine[1]
6/19/2003	1	5	49.8	0.0	0.0	0.0
6/19/2003	1	5	99.0	0.0	0.0	0.0
6/19/2003	2	5	140.0	0.0	34.2	0.0
6/19/2003	2	5	176.2	0.0	65.5	0.0
6/19/2003	3	5	311.5	0.0	114.8	98.3
6/19/2003	3	5	79.5	0.0	0.0	0.0
6/19/2003	4	5	182.1	0.0	47.7	0.0
6/19/2003	4	5	116.8	0.0	0.0	0.0
6/19/2003	1	6	130.6	0.0	0.0	33.4
6/19/2003	1	6	217.6	0.0	50.7	65.2
6/19/2003	2	6	141.2	0.0	0.0	0.0
6/19/2003	2	6	-	-	-	-
6/19/2003	3	6	449.0	18.8	88.2	77.0
6/19/2003	3	6	272.2	0.0	77.7	76.6
6/19/2003	4	6	104.8	0.0	19.5	29.4
6/19/2003	4	6	596.4	0.0	201.2	263.8
6/19/2003	1	1	0.0	0.0	0.0	0.0
6/19/2003	1	1	0.0	0.0	0.0	0.0
6/19/2003	2	1	0.0	0.0	0.0	0.0
6/19/2003	2	1	0.0	0.0	0.0	0.0
6/19/2003	3	1	0.0	30.2	32.4	33.5
6/19/2003	3	1	0.0	0.0	0.0	41.3
6/19/2003	4	1	0.0	0.0	0.0	0.0
6/19/2003	4	1	143.6	0.0	0.0	0.0
6/19/2003	1	2	90.0	22.5	48.4	26.0
6/19/2003	1	2	46.9	0.0	0.0	0.0
6/19/2003	2	2	137.4	0.0	27.7	22.3
6/19/2003	2	2	55.5	0.0	0.0	25.5
6/19/2003	3	2	110.3	0.0	53.4	43.9
6/19/2003	3	2	140.3	24.4	49.2	25.8
6/19/2003	4	2	676.4	227.6	160.9	219.4
6/19/2003	4	2	88.3	22.1	0.0	33.1

[1] nanomoles per 10 μL of leaf juice.

Table E.6. Data for oviposition experiment, date 1. E.

Date	Cage	Host code	Leucine[1]	Lysine[1]	Proline[1]	Total FAA[1]	Essential FAA[1]
6/19/2003	1	5	0.0	0.0	166.0	1981.3	39.0
6/19/2003	1	5	0.0	0.0	179.1	22762.3	146.6
6/19/2003	2	5	30.4	0.0	216.4	1733.9	447.9
6/19/2003	2	5	29.8	0.0	795.5	980.6	298.0
6/19/2003	3	5	85.4	0.0	180.0	3238.8	702.6
6/19/2003	3	5	0.0	0.0	903.4	2760.3	115.9
6/19/2003	4	5	42.4	0.0	392.9	1442.7	310.3
6/19/2003	4	5	0.0	0.0	838.3	2242.6	123.4
6/19/2003	1	6	0.0	0.0	481.0	2981.7	389.1
6/19/2003	1	6	65.0	0.0	530.6	2543.9	805.6
6/19/2003	2	6	0.0	0.0	368.6	2352.1	250.2
6/19/2003	2	6	-	-	-	-	-
6/19/2003	3	6	61.4	0.0	387.7	3091.5	671.4
6/19/2003	3	6	0.0	0.0	329.7	4186.8	451.9
6/19/2003	4	6	34.7	0.0	292.6	10312.5	454.1
6/19/2003	4	6	142.9	86.7	557.3	4021.1	1939.9
6/19/2003	1	1	0.0	0.0	205.4	688.6	214.8
6/19/2003	1	1	0.0	0.0	10047.5	1683.8	2911.7
6/19/2003	2	1	0.0	0.0	221.6	3098.1	237.6
6/19/2003	2	1	0.0	0.0	184.6	2081.0	190.1
6/19/2003	3	1	36.9	0.0	1949.6	2869.9	465.6
6/19/2003	3	1	40.3	0.0	371.6	1719.9	472.6
6/19/2003	4	1	0.0	0.0	278.5	2544.1	184.5
6/19/2003	4	1	23.4	0.0	177.2	1577.3	222.7
6/19/2003	1	2	30.0	0.0	1080.4	3666.7	423.5
6/19/2003	1	2	0.0	0.0	411.4	6615.9	271.0
6/19/2003	2	2	0.0	0.0	341.2	2787.5	353.3
6/19/2003	2	2	0.0	0.0	618.0	0.0	319.4
6/19/2003	3	2	46.4	0.0	292.9	4143.6	478.8
6/19/2003	3	2	35.3	0.0	558.4	2969.5	504.8
6/19/2003	4	2	151.3	97.2	692.1	3420.6	2319.9
6/19/2003	4	2	40.4	0.0	568.7	9122.1	451.0

[1] nanomoles per 10 μL of leaf juice.

Table E.7. Data for oviposition experiment, date 2. A.

Date	Cage	Host code	Dry leaves	Plant height	Dry weight	No. tillers	Eggs laid
6/26/2003	1	11	3	-	-	5	108
6/26/2003	1	11	3	-	-	5	17
6/26/2003	2	11	2	-	-	4	0
6/26/2003	2	11	1	-	-	5	97
6/26/2003	3	11	4	-	-	6	7
6/26/2003	3	11	5	-	4.6	4	0
6/26/2003	4	11	5	-	4.3	6	39
6/26/2003	4	11	3	-	2.1	4	8
6/26/2003	1	5	11	75	-	-	0
6/26/2003	1	5	3	80	-	-	30
6/26/2003	2	5	7	87	-	-	161
6/26/2003	2	5	8	109	54.5	-	43
6/26/2003	3	5	4	85	-	-	0
6/26/2003	3	5	4	91	42.3	-	175
6/26/2003	4	5	7	94	-	-	224
6/26/2003	4	5	1	75	33.4	-	24
6/26/2003	1	2	2	104	-	-	515
6/26/2003	1	2	10	82	-	-	106
6/26/2003	2	2	10	64	-	-	282
6/26/2003	2	2	11	82	32.4	-	17
6/26/2003	3	2	13	98	-	-	284
6/26/2003	3	2	10	98	32.3	-	236
6/26/2003	4	2	12	92	-	-	10
6/26/2003	4	2	12	100	38.8	-	296
6/26/2003	1	15	4	-	-	9	129
6/26/2003	1	15	6	-	4.9	11	60
6/26/2003	2	15	3	-	5.5	11	77
6/26/2003	2	15	7	-	-	11	0
6/26/2003	3	15	5	-	-	8	0
6/26/2003	3	15	8	-	-	18	0
6/26/2003	4	15	7	-	-	12	265
6/26/2003	4	15	4	-	3.7	7	22

Table E.8. Data for oviposition experiment, date 2. B.

Date	Cage	Host code	Egg masses	Aspartic[1]	Glutamic[1]	Serine[1]	Histidine[1]
6/26/2003	1	11	7	944.1	2184.6	951.3	309.3
6/26/2003	1	11	1	1147.0	1866.7	866.0	268.3
6/26/2003	2	11	0	681.5	1875.5	1335.7	398.9
6/26/2003	2	11	9	702.6	1499.7	774.8	423.7
6/26/2003	3	11	1	692.2	1457.1	826.6	335.8
6/26/2003	3	11	0	722.2	2024.3	1194.8	356.4
6/26/2003	4	11	1	900.5	4241.1	1359.9	407.7
6/26/2003	4	11	2	958.1	1794.1	890.8	309.4
6/26/2003	1	5	0	-	-	-	-
6/26/2003	1	5	1	-	-	-	-
6/26/2003	2	5	11	-	-	-	-
6/26/2003	2	5	3	-	-	-	-
6/26/2003	3	5	0	-	-	-	-
6/26/2003	3	5	6	-	-	-	-
6/26/2003	4	5	8	-	-	-	-
6/26/2003	4	5	2	-	-	-	-
6/26/2003	1	2	20	-	-	-	-
6/26/2003	1	2	15	-	-	-	-
6/26/2003	2	2	7	-	-	-	-
6/26/2003	2	2	2	-	-	-	-
6/26/2003	3	2	18	-	-	-	-
6/26/2003	3	2	11	-	-	-	-
6/26/2003	4	2	2	-	-	-	-
6/26/2003	4	2	14	-	-	-	-
6/26/2003	1	15	5	837.3	1950.3	1738.6	622.9
6/26/2003	1	15	3	874.5	2328.9	1562.2	673.7
6/26/2003	2	15	11	1002.9	4236.6	2068.5	934.2
6/26/2003	2	15	0	849.0	2236.6	1792.4	1232.0
6/26/2003	3	15	0	905.1	4466.8	1402.1	763.4
6/26/2003	3	15	0	871.6	4680.9	2486.1	1357.1
6/26/2003	4	15	6	1023.4	4255.5	2685.7	1138.9
6/26/2003	4	15	2	795.6	3303.5	1719.3	674.9

[1] nanomoles per 10 μL of leaf juice.

Table E.9. Data for oviposition experiment, date 2. C.

Date	Cage	Host code	Glycine[1]	Threonine[1]	Arginine[1]	Alanine[1]	Tyrosine[1]
6/26/2003	1	11	0.0	398.2	24.7	869.6	0.0
6/26/2003	1	11	0.0	266.0	46.8	691.9	0.0
6/26/2003	2	11	0.0	223.1	65.2	923.7	0.0
6/26/2003	2	11	0.0	354.0	33.0	1148.2	0.0
6/26/2003	3	11	62.4	217.3	29.5	647.6	0.0
6/26/2003	3	11	38.8	269.2	59.5	1174.0	0.0
6/26/2003	4	11	0.0	359.8	33.0	690.2	0.0
6/26/2003	4	11	0.0	212.6	40.5	963.2	0.0
6/26/2003	1	5	-	-	-	-	-
6/26/2003	1	5	-	-	-	-	-
6/26/2003	2	5	-	-	-	-	-
6/26/2003	2	5	-	-	-	-	-
6/26/2003	3	5	-	-	-	-	-
6/26/2003	3	5	-	-	-	-	-
6/26/2003	4	5	-	-	-	-	-
6/26/2003	4	5	-	-	-	-	-
6/26/2003	1	2	-	-	-	-	-
6/26/2003	1	2	-	-	-	-	-
6/26/2003	2	2	-	-	-	-	-
6/26/2003	2	2	-	-	-	-	-
6/26/2003	3	2	-	-	-	-	-
6/26/2003	3	2	-	-	-	-	-
6/26/2003	4	2	-	-	-	-	-
6/26/2003	4	2	-	-	-	-	-
6/26/2003	1	15	0.0	316.1	263.3	1288.0	0.0
6/26/2003	1	15	0.0	428.8	166.5	1142.9	22.7
6/26/2003	2	15	0.0	459.5	205.5	1351.8	0.0
6/26/2003	2	15	0.0	363.5	289.3	1514.9	0.0
6/26/2003	3	15	0.0	318.2	132.7	1435.2	0.0
6/26/2003	3	15	0.0	518.8	339.4	1808.1	0.0
6/26/2003	4	15	0.0	495.5	571.8	1702.9	46.6
6/26/2003	4	15	0.0	244.7	326.8	1423.4	0.0

[1] nanomoles per 10 μL of leaf juice.

Table E.10. Data for oviposition experiment, date 2. D.

Date	Cage	Host code	Valine[1]	Methionine[1]	Phenylalanine[1]	Isoleucine[1]	Leucine[1]
6/26/2003	1	11	84.2	0.0	0.0	0.0	0.0
6/26/2003	1	11	92.1	11.5	0.0	58.5	40.1
6/26/2003	2	11	86.1	0.0	0.0	58.3	0.0
6/26/2003	2	11	101.1	0.0	0.0	49.0	28.1
6/26/2003	3	11	86.1	0.0	0.0	60.5	25.7
6/26/2003	3	11	84.3	0.0	0.0	58.3	0.0
6/26/2003	4	11	109.4	0.0	0.0	66.0	34.2
6/26/2003	4	11	71.2	0.0	0.0	0.0	0.0
6/26/2003	1	5	-	-	-	-	-
6/26/2003	1	5	-	-	-	-	-
6/26/2003	2	5	-	-	-	-	-
6/26/2003	2	5	-	-	-	-	-
6/26/2003	3	5	-	-	-	-	-
6/26/2003	3	5	-	-	-	-	-
6/26/2003	4	5	-	-	-	-	-
6/26/2003	4	5	-	-	-	-	-
6/26/2003	1	2	-	-	-	-	-
6/26/2003	1	2	-	-	-	-	-
6/26/2003	2	2	-	-	-	-	-
6/26/2003	2	2	-	-	-	-	-
6/26/2003	3	2	-	-	-	-	-
6/26/2003	3	2	-	-	-	-	-
6/26/2003	4	2	-	-	-	-	-
6/26/2003	4	2	-	-	-	-	-
6/26/2003	1	15	277.6	0.0	0.0	80.9	50.4
6/26/2003	1	15	198.4	0.0	42.7	66.2	32.6
6/26/2003	2	15	263.7	0.0	119.3	85.8	44.3
6/26/2003	2	15	192.2	0.0	0.0	60.9	22.3
6/26/2003	3	15	178.4	0.0	80.1	58.7	35.5
6/26/2003	3	15	278.3	0.0	0.0	73.1	41.9
6/26/2003	4	15	358.5	0.0	71.8	72.9	39.4
6/26/2003	4	15	115.2	0.0	0.0	0.0	26.9

[1] nanomoles per 10 μL of leaf juice.

Table E.11. Data for oviposition experiment, date 2. E.

Date	Cage	Host code	Lysine[1]	Proline[1]	Total FAA[1]	Essential FAA[1]	Leaves
6/26/2003	1	11	0.0	411.8	6177.9	816.5	20
6/26/2003	1	11	0.0	2566.8	7921.8	783.4	18
6/26/2003	2	11	0.0	1509.3	7157.3	831.6	25
6/26/2003	2	11	0.0	245.3	5359.5	988.8	24
6/26/2003	3	11	0.0	1131.2	5572.0	755.0	25
6/26/2003	3	11	0.0	794.9	6776.6	827.7	37
6/26/2003	4	11	0.0	2619.0	10820.7	1010.1	23
6/26/2003	4	11	0.0	414.7	5654.5	633.7	22
6/26/2003	1	5	-	-	-	-	18
6/26/2003	1	5	-	-	-	-	13
6/26/2003	2	5	-	-	-	-	18
6/26/2003	2	5	-	-	-	-	17
6/26/2003	3	5	-	-	-	-	15
6/26/2003	3	5	-	-	-	-	14
6/26/2003	4	5	-	-	-	-	18
6/26/2003	4	5	-	-	-	-	13
6/26/2003	1	2	-	-	-	-	13
6/26/2003	1	2	-	-	-	-	17
6/26/2003	2	2	-	-	-	-	16
6/26/2003	2	2	-	-	-	-	17
6/26/2003	3	2	-	-	-	-	18
6/26/2003	3	2	-	-	-	-	16
6/26/2003	4	2	-	-	-	-	16
6/26/2003	4	2	-	-	-	-	18
6/26/2003	1	15	0.0	979.6	8405.2	1611.3	34
6/26/2003	1	15	0.0	1098.5	8638.6	1608.9	39
6/26/2003	2	15	0.0	5003.3	15775.3	2112.3	33
6/26/2003	2	15	0.0	583.2	9136.4	2160.2	48
6/26/2003	3	15	0.0	238.5	10014.5	1566.9	36
6/26/2003	3	15	0.0	483.6	12938.9	2608.6	63
6/26/2003	4	15	0.0	620.4	13083.2	2748.8	49
6/26/2003	4	15	0.0	195.8	8826.1	1388.6	30

[1] nanomoles per 10 μL of leaf juice.

Table E.12. Data for oviposition experiment, date 3. A.

Date	Cage	Host code	Leaves	Dry leaves	Plant height (cm)	Water potential (barr)	Dry weight (g)
8/07/2003	1	7	18	5	150	10	-
8/07/2003	1	7	23	11	150	11	-
8/07/2003	2	7	19	7	179	12	-
8/07/2003	2	7	21	6	170	9	-
8/07/2003	3	7	19	7	155	8	105.2
8/07/2003	3	7	19	7	144	15	-
8/07/2003	4	7	20	10	137	23	108.1
8/07/2003	4	7	18	5	151	20	99.4
8/07/2003	1	8	21	16	165	10	-
8/07/2003	1	8	22	16	146	14	-
8/07/2003	2	8	20	13	171	10	95.2
8/07/2003	2	8	20	14	151	10	84.6
8/07/2003	3	8	18	6	142	15	-
8/07/2003	3	8	19	11	130	11	-
8/07/2003	4	8	21	14	154	15	-
8/07/2003	4	8	18	9	154	7	93.1
8/07/2003	1	3	17	8	150	10	108.4
8/07/2003	1	3	21	10	162	10	116.1
8/07/2003	2	3	20	8	154	7	112.3
8/07/2003	2	3	19	8	180	11	-
8/07/2003	3	3	20	9	180	12	-
8/07/2003	3	3	18	9	160	9	-
8/07/2003	4	3	19	9	180	11	-
8/07/2003	4	3	19	10	175	16	-
8/07/2003	1	4	21	15	152	15	-
8/07/2003	1	4	23	17	142	40	105.5
8/07/2003	2	4	17	10	145	27	101.9
8/07/2003	2	4	19	13	175	15	-
8/07/2003	3	4	23	14	173	18	98.6
8/07/2003	3	4	23	16	160	16	-
8/07/2003	4	4	21	14	165	40	-
8/07/2003	4	4	21	16	160	40	-

Table E.13. Data for oviposition experiment, date 3. B.

Date	Cage	Host code	Eggs laid	Egg masses	Aspartic[1]	Glutamic[1]	Serine[1]
8/07/2003	1	7	266	7	131.7	181.4	111.4
8/07/2003	1	7	270	7	204.9	403.8	183.8
8/07/2003	2	7	12	2	190.3	233.4	207.6
8/07/2003	2	7	59	3	129.8	400.7	108.3
8/07/2003	3	7	17	2	144.8	224.8	126.9
8/07/2003	3	7	24	2	211.6	375.7	127.0
8/07/2003	4	7	0	0	138.1	97.4	114.9
8/07/2003	4	7	0	0	139.0	77.2	97.2
8/07/2003	1	8	861	22	627.1	1402.0	176.1
8/07/2003	1	8	191	6	529.0	1113.3	274.7
8/07/2003	2	8	127	4	909.0	2482.2	627.0
8/07/2003	2	8	147	5	791.7	930.4	309.2
8/07/2003	3	8	15	1	293.6	916.3	177.0
8/07/2003	3	8	537	18	266.9	413.5	143.2
8/07/2003	4	8	22	1	245.4	699.4	104.2
8/07/2003	4	8	15	1	949.9	701.4	217.9
8/07/2003	1	3	214	6	202.0	198.1	97.7
8/07/2003	1	3	288	9	158.1	207.2	85.4
8/07/2003	2	3	100	1	178.3	180.7	105.2
8/07/2003	2	3	339	7	170.5	247.3	92.8
8/07/2003	3	3	9	2	203.9	265.5	96.0
8/07/2003	3	3	382	8	139.6	111.3	83.5
8/07/2003	4	3	32	2	176.0	193.6	63.4
8/07/2003	4	3	0	0	171.0	222.1	93.8
8/07/2003	1	4	173	9	533.4	492.4	251.3
8/07/2003	1	4	423	15	69.5	919.6	160.9
8/07/2003	2	4	137	2	260.7	251.5	123.8
8/07/2003	2	4	379	13	408.1	496.0	171.6
8/07/2003	3	4	15	1	309.3	227.8	179.1
8/07/2003	3	4	109	7	202.8	204.2	76.7
8/07/2003	4	4	22	1	79.4	217.0	96.4
8/07/2003	4	4	280	5	179.8	285.2	92.4

[1] nanomoles per 10 μL of leaf juice.

Table E.14. Data for oviposition experiment, date 3. C.

Date	Cage	Host code	Histidine[1]	Glycine[1]	Threonine[1]	Arginine[1]	Alanine[1]
8/07/2003	1	7	55.0	141.7	25.4	0.0	122.9
8/07/2003	1	7	109.8	131.7	32.7	0.0	199.8
8/07/2003	2	7	96.5	118.7	28.0	62.6	244.6
8/07/2003	2	7	60.7	138.8	26.3	0.0	124.2
8/07/2003	3	7	79.1	124.7	31.7	0.0	207.0
8/07/2003	3	7	143.8	182.2	41.7	19.9	213.5
8/07/2003	4	7	81.5	107.8	26.0	0.0	160.6
8/07/2003	4	7	59.1	107.5	19.7	0.0	150.6
8/07/2003	1	8	116.2	349.7	105.0	73.7	588.5
8/07/2003	1	8	120.5	296.9	140.4	80.4	883.9
8/07/2003	2	8	213.3	343.7	377.7	198.4	1706.2
8/07/2003	2	8	171.3	370.1	203.7	132.6	1416.4
8/07/2003	3	8	77.4	122.2	40.0	71.5	308.4
8/07/2003	3	8	104.4	372.9	86.6	62.8	498.1
8/07/2003	4	8	93.0	143.3	47.2	33.3	359.1
8/07/2003	4	8	114.2	481.0	140.3	74.9	654.2
8/07/2003	1	3	88.0	104.1	27.8	0.0	182.7
8/07/2003	1	3	91.4	99.2	30.6	0.0	139.9
8/07/2003	2	3	102.5	101.8	32.1	0.0	135.5
8/07/2003	2	3	87.8	98.7	26.8	0.0	171.8
8/07/2003	3	3	102.7	108.9	46.1	0.0	275.0
8/07/2003	3	3	67.9	126.8	27.5	0.0	166.2
8/07/2003	4	3	62.6	105.9	21.0	0.0	153.2
8/07/2003	4	3	96.2	88.9	32.7	24.6	157.5
8/07/2003	1	4	191.8	243.4	158.8	87.5	1029.1
8/07/2003	1	4	110.1	205.0	111.2	60.9	708.8
8/07/2003	2	4	124.8	222.4	93.8	74.1	451.7
8/07/2003	2	4	106.8	171.1	94.9	63.1	629.4
8/07/2003	3	4	169.0	148.3	118.0	83.7	739.7
8/07/2003	3	4	45.0	148.3	43.6	20.5	177.3
8/07/2003	4	4	69.6	176.0	56.8	0.0	289.2
8/07/2003	4	4	80.5	183.5	31.6	0.0	188.7

[1] nanomoles per 10 μL of leaf juice.

Table E.15. Data for oviposition experiment, date 3. D.

Date	Cage	Host code	Tyrosine[1]	Valine[1]	Methionine[1]	Phenylalanine[1]	Isoleucine[1]
8/07/2003	1	7	116.3	0.0	0.0	0.0	0.0
8/07/2003	1	7	371.3	162.5	0.0	0.0	101.7
8/07/2003	2	7	300.3	126.6	0.0	0.0	87.3
8/07/2003	2	7	108.0	10.4	0.0	0.0	0.0
8/07/2003	3	7	144.8	84.4	0.0	0.0	0.0
8/07/2003	3	7	230.9	62.4	0.0	0.0	92.5
8/07/2003	4	7	166.6	53.0	0.0	0.0	0.0
8/07/2003	4	7	157.6	0.0	0.0	0.0	0.0
8/07/2003	1	8	118.1	0.0	28.1	82.3	41.7
8/07/2003	1	8	147.5	0.0	0.0	51.7	57.4
8/07/2003	2	8	189.0	0.0	68.0	112.1	125.4
8/07/2003	2	8	133.9	0.0	0.0	62.4	76.9
8/07/2003	3	8	188.9	0.0	0.0	0.0	0.0
8/07/2003	3	8	155.2	0.0	0.0	60.5	39.6
8/07/2003	4	8	101.3	0.0	0.0	22.9	0.0
8/07/2003	4	8	98.6	0.0	0.0	79.4	48.2
8/07/2003	1	3	92.6	41.3	0.0	0.0	0.0
8/07/2003	1	3	67.4	25.1	0.0	0.0	0.0
8/07/2003	2	3	81.6	32.1	0.0	0.0	0.0
8/07/2003	2	3	134.4	29.8	0.0	24.9	0.0
8/07/2003	3	3	113.9	59.2	0.0	0.0	0.0
8/07/2003	3	3	81.4	51.0	0.0	0.0	0.0
8/07/2003	4	3	71.6	0.0	0.0	0.0	0.0
8/07/2003	4	3	131.7	46.5	0.0	0.0	0.0
8/07/2003	1	4	165.8	0.0	0.0	54.9	52.0
8/07/2003	1	4	122.6	0.0	24.4	35.3	41.7
8/07/2003	2	4	79.2	49.2	0.0	87.2	25.6
8/07/2003	2	4	131.3	0.0	0.0	47.7	37.7
8/07/2003	3	4	134.2	12.2	0.0	38.9	32.2
8/07/2003	3	4	60.1	0.0	0.0	0.0	0.0
8/07/2003	4	4	0.0	49.9	0.0	0.0	0.0
8/07/2003	4	4	65.5	36.3	0.0	46.0	44.6

[1] nanomoles per 10 μL of leaf juice.

Table E.16. Data for oviposition experiment, date 3. E.

Date	Cage	Host code	Leucine[1]	Lysine[1]	Proline[1]	Total[1]	Essential FAA[1]
8/07/2003	1	7	0.0	0.0	209.5	1095.4	80.5
8/07/2003	1	7	0.0	20.3	205.2	2127.3	426.9
8/07/2003	2	7	0.0	19.7	128.9	1844.6	420.7
8/07/2003	2	7	42.0	0.0	121.3	1270.5	139.4
8/07/2003	3	7	0.0	0.0	134.7	1302.8	195.2
8/07/2003	3	7	0.0	0.0	150.9	1852.3	360.4
8/07/2003	4	7	0.0	0.0	152.9	1098.8	160.5
8/07/2003	4	7	0.0	0.0	123.8	931.8	78.9
8/07/2003	1	8	70.2	22.2	140.3	3941.0	539.3
8/07/2003	1	8	92.2	47.1	180.8	4015.7	589.6
8/07/2003	2	8	213.9	100.7	184.3	7850.8	1409.4
8/07/2003	2	8	171.3	41.9	195.4	5007.3	860.2
8/07/2003	3	8	145.8	0.0	130.7	2471.8	334.7
8/07/2003	3	8	109.7	24.2	178.3	2515.7	487.8
8/07/2003	4	8	20.8	0.0	195.8	2065.6	217.2
8/07/2003	4	8	81.0	0.0	172.1	3813.0	537.8
8/07/2003	1	3	0.0	0.0	175.7	1210.0	157.1
8/07/2003	1	3	0.0	0.0	88.2	992.6	147.1
8/07/2003	2	3	0.0	0.0	112.0	1061.8	166.7
8/07/2003	2	3	0.0	0.0	43.4	1128.0	169.3
8/07/2003	3	3	0.0	0.0	121.0	1392.3	208.0
8/07/2003	3	3	0.0	0.0	123.4	978.7	146.4
8/07/2003	4	3	0.0	0.0	131.6	978.8	83.6
8/07/2003	4	3	0.0	0.0	210.8	1275.7	200.0
8/07/2003	1	4	172.2	48.0	150.4	3631.0	765.2
8/07/2003	1	4	81.7	0.0	218.5	2870.3	465.3
8/07/2003	2	4	0.0	0.0	204.7	2048.6	454.7
8/07/2003	2	4	118.2	0.0	134.1	2609.7	468.3
8/07/2003	3	4	111.1	33.7	168.9	2506.0	598.7
8/07/2003	3	4	43.3	0.0	152.8	1174.7	152.5
8/07/2003	4	4	56.3	0.0	171.7	1262.2	232.5
8/07/2003	4	4	93.5	0.0	144.0	1471.6	332.4

[1] nanomoles per 10 μL of leaf juice.

Table E.17. Data for oviposition experiment, date 4. A.

Date	Cage	Host code	Leaves	Dry leaves	Plant height	Dry weight	Tillers
8/14/2003	1	12	29	8	-	-	5
8/14/2003	1	12	13	3	-	-	4
8/14/2003	2	12	37	7	-	-	6
8/14/2003	2	12	29	8	-	-	6
8/14/2003	3	12	36	9	-	11.3	9
8/14/2003	3	12	19	4	-	8.6	3
8/14/2003	4	12	24	9	-	-	4
8/14/2003	4	12	39	7	-	12.8	8
8/14/2003	1	8	20	9	148	-	-
8/14/2003	1	8	21	7	140	-	-
8/14/2003	2	8	21	8	143	-	-
8/14/2003	2	8	17	3	137	-	-
8/14/2003	3	8	21	8	171	-	-
8/14/2003	3	8	19	6	160	100.1	-
8/14/2003	4	8	19	7	161	112.7	-
8/14/2003	4	8	19	9	158	110.1	-
8/14/2003	1	3	19	9	169	-	-
8/14/2003	1	3	20	10	172	-	-
8/14/2003	2	3	21	12	162	99.7	-
8/14/2003	2	3	20	10	156	-	-
8/14/2003	3	3	16	7	164	-	-
8/14/2003	3	3	20	10	185	-	-
8/14/2003	4	3	19	8	179	85	-
8/14/2003	4	3	19	10	174	106.9	-
8/14/2003	1	16	48	8	-	14.9	10
8/14/2003	1	16	32	5	-	13.6	7
8/14/2003	2	16	26	6	-	-	6
8/14/2003	2	16	37	8	-	-	8
8/14/2003	3	16	41	5	-	-	8
8/14/2003	3	16	23	5	-	-	5
8/14/2003	4	16	31	4	-	-	7
8/14/2003	4	16	48	5	-	17.4	9

Table E.18. Data for oviposition experiment, date 4. B.

Date	Cage	Host code	Eggs laid	Egg masses	Aspartic[1]	Glutamic[1]	Serine[1]
8/14/2003	1	12	172	7	2119.2	0.0	2265.9
8/14/2003	1	12	128	4	1206.7	2898.3	855.7
8/14/2003	2	12	257	7	2188.0	4793.9	1657.8
8/14/2003	2	12	145	4	1019.9	2794.9	673.6
8/14/2003	3	12	65	6	1811.6	3096.6	1431.1
8/14/2003	3	12	93	3	1966.8	4953.2	1111.9
8/14/2003	4	12	82	2	1035.4	4802.0	1563.8
8/14/2003	4	12	31	1	1591.9	5340.3	1370.8
8/14/2003	1	8	623	8	-	-	-
8/14/2003	1	8	0	0	-	-	-
8/14/2003	2	8	122	3	-	-	-
8/14/2003	2	8	18	1	-	-	-
8/14/2003	3	8	72	7	-	-	-
8/14/2003	3	8	323	10	-	-	-
8/14/2003	4	8	78	2	-	-	-
8/14/2003	4	8	170	5	-	-	-
8/14/2003	1	3	352	12	-	-	-
8/14/2003	1	3	370	6	-	-	-
8/14/2003	2	3	374	6	-	-	-
8/14/2003	2	3	168	4	-	-	-
8/14/2003	3	3	431	7	-	-	-
8/14/2003	3	3	282	6	-	-	-
8/14/2003	4	3	52	1	-	-	-
8/14/2003	4	3	328	6	-	-	-
8/14/2003	1	16	0	0	744.0	1636.1	567.3
8/14/2003	1	16	8	1	799.3	2609.5	679.4
8/14/2003	2	16	50	1	708.9	2437.4	747.1
8/14/2003	2	16	175	6	497.0	2151.5	626.2
8/14/2003	3	16	80	3	375.2	1809.3	298.5
8/14/2003	3	16	44	3	415.7	1798.6	355.2
8/14/2003	4	16	0	0	422.1	2176.1	447.5
8/14/2003	4	16	0	0	767.6	2584.9	382.9

[1] nanomoles per 10 μL of leaf juice.

Table E.19. Data for oviposition experiment, date 4. C.

Date	Cage	Host code	Histidine[1]	Glycine[1]	Threonine[1]	Arginine[1]	Alanine[1]
8/14/2003	1	12	75.1	583.1	0.0	395.5	2467.4
8/14/2003	1	12	250.3	397.7	392.9	116.4	885.0
8/14/2003	2	12	736.6	550.8	688.0	209.6	2277.6
8/14/2003	2	12	597.7	642.9	546.6	196.0	1214.8
8/14/2003	3	12	702.2	1151.6	788.3	264.2	1454.4
8/14/2003	3	12	604.4	662.9	624.9	223.0	674.0
8/14/2003	4	12	534.3	378.4	554.4	147.9	1495.6
8/14/2003	4	12	439.5	430.6	703.0	149.5	1166.0
8/14/2003	1	8	-	-	-	-	-
8/14/2003	1	8	-	-	-	-	-
8/14/2003	2	8	-	-	-	-	-
8/14/2003	2	8	-	-	-	-	-
8/14/2003	3	8	-	-	-	-	-
8/14/2003	3	8	-	-	-	-	-
8/14/2003	4	8	-	-	-	-	-
8/14/2003	4	8	-	-	-	-	-
8/14/2003	1	3	-	-	-	-	-
8/14/2003	1	3	-	-	-	-	-
8/14/2003	2	3	-	-	-	-	-
8/14/2003	2	3	-	-	-	-	-
8/14/2003	3	3	-	-	-	-	-
8/14/2003	3	3	-	-	-	-	-
8/14/2003	4	3	-	-	-	-	-
8/14/2003	4	3	-	-	-	-	-
8/14/2003	1	16	194.1	325.8	165.3	62.6	584.0
8/14/2003	1	16	246.8	340.7	263.0	93.7	434.1
8/14/2003	2	16	271.3	324.1	260.2	131.3	705.2
8/14/2003	2	16	306.9	320.3	181.0	50.1	485.6
8/14/2003	3	16	335.7	311.7	146.0	43.7	249.8
8/14/2003	3	16	295.9	289.0	156.8	23.1	419.3
8/14/2003	4	16	405.7	321.3	215.5	168.3	490.7
8/14/2003	4	16	334.1	344.1	287.0	116.0	545.2

[1] nanomoles per 10 μL of leaf juice.

Table E.20. Data for oviposition experiment, date 4. D.

Date	Cage	Host code	Tyrosine[1]	Valine[1]	Methionine[1]	Phenylalanine[1]	Isoleucine[1]
8/14/2003	1	12	1692.6	642.4	0.0	1052.0	676.9
8/14/2003	1	12	379.5	177.6	0.0	366.1	213.9
8/14/2003	2	12	350.1	247.0	0.0	390.5	165.4
8/14/2003	2	12	1473.9	388.8	0.0	769.7	391.8
8/14/2003	3	12	688.3	379.3	152.0	820.4	458.2
8/14/2003	3	12	1253.2	363.0	0.0	580.0	383.1
8/14/2003	4	12	326.1	186.6	0.0	339.0	171.8
8/14/2003	4	12	553.8	331.0	0.0	564.0	342.2
8/14/2003	1	8	-	-	-	-	-
8/14/2003	1	8	-	-	-	-	-
8/14/2003	2	8	-	-	-	-	-
8/14/2003	2	8	-	-	-	-	-
8/14/2003	3	8	-	-	-	-	-
8/14/2003	3	8	-	-	-	-	-
8/14/2003	4	8	-	-	-	-	-
8/14/2003	4	8	-	-	-	-	-
8/14/2003	1	3	-	-	-	-	-
8/14/2003	1	3	-	-	-	-	-
8/14/2003	2	3	-	-	-	-	-
8/14/2003	2	3	-	-	-	-	-
8/14/2003	3	3	-	-	-	-	-
8/14/2003	3	3	-	-	-	-	-
8/14/2003	4	3	-	-	-	-	-
8/14/2003	4	3	-	-	-	-	-
8/14/2003	1	16	118.3	68.9	0.0	80.9	48.9
8/14/2003	1	16	185.1	117.3	0.0	101.3	80.0
8/14/2003	2	16	218.7	104.8	0.0	131.7	86.3
8/14/2003	2	16	262.6	85.4	0.0	92.5	48.7
8/14/2003	3	16	133.3	67.6	0.0	82.6	45.1
8/14/2003	3	16	116.6	70.7	0.0	82.2	51.7
8/14/2003	4	16	139.7	92.6	0.0	93.2	65.9
8/14/2003	4	16	266.1	136.7	0.0	151.5	108.5

[1] nanomoles per 10 μL of leaf juice.

Table E.21. Data for oviposition experiment, date 4. E.

Date	Cage	Host code	Leucine[1]	Lysine[1]	Proline[1]	Total FAA[1]	Essential FAA[1]
8/14/2003	1	12	1593.0	288.9	0.0	13852.0	4723.7
8/14/2003	1	12	659.0	65.8	0.0	8865.0	2242.1
8/14/2003	2	12	625.0	63.4	0.0	14943.7	3125.6
8/14/2003	2	12	1826.7	0.0	0.0	12537.3	4717.3
8/14/2003	3	12	1386.1	135.0	0.0	14719.3	5085.7
8/14/2003	3	12	844.5	103.1	0.0	14348.2	3726.1
8/14/2003	4	12	593.4	83.6	0.0	12212.1	2610.9
8/14/2003	4	12	1025.7	93.0	0.0	14101.5	3648.1
8/14/2003	1	8	-	-	-	-	-
8/14/2003	1	8	-	-	-	-	-
8/14/2003	2	8	-	-	-	-	-
8/14/2003	2	8	-	-	-	-	-
8/14/2003	3	8	-	-	-	-	-
8/14/2003	3	8	-	-	-	-	-
8/14/2003	4	8	-	-	-	-	-
8/14/2003	4	8	-	-	-	-	-
8/14/2003	1	3	-	-	-	-	-
8/14/2003	1	3	-	-	-	-	-
8/14/2003	2	3	-	-	-	-	-
8/14/2003	2	3	-	-	-	-	-
8/14/2003	3	3	-	-	-	-	-
8/14/2003	3	3	-	-	-	-	-
8/14/2003	4	3	-	-	-	-	-
8/14/2003	4	3	-	-	-	-	-
8/14/2003	1	16	187.2	34.6	0.0	4818.1	842.5
8/14/2003	1	16	224.3	50.9	0.0	6225.2	1177.2
8/14/2003	2	16	279.4	27.1	0.0	6433.4	1292.0
8/14/2003	2	16	230.6	19.7	445.2	5803.2	1014.9
8/14/2003	3	16	168.9	19.4	0.0	4086.8	909.0
8/14/2003	3	16	205.7	20.3	0.0	4300.6	906.3
8/14/2003	4	16	174.6	0.0	0.0	5213.0	1215.7
8/14/2003	4	16	274.0	0.0	0.0	6298.7	1407.9

[1] nanomoles per 10 μL of leaf juice.

Table E.22. Data for oviposition experiment, date 5. A.

Date	Cage	Host code	Leaves	Dry leaves	Plant height (cm)	Dry weight (g)	No. tillers
5/31/2004	1	9	12	0	18	0.3	3
5/31/2004	1	9	14	0	20.5	0.05	3
5/31/2004	2	9	12	0	17.5	0.7	3
5/31/2004	2	9	12	0	17	-	3
5/31/2004	3	9	12	0	18.5	-	3
5/31/2004	3	9	10	0	17	-	3
5/31/2004	4	9	12	0	19	-	3
5/31/2004	4	9	12	0	18	-	3
5/31/2004	1	10	16	2	15	1.7	3
5/31/2004	1	10	26	1	22	0.6	5
5/31/2004	2	10	28	3	27	0.7	6
5/31/2004	2	10	22	2	26	-	5
5/31/2004	3	10	15	2	18	-	3
5/31/2004	3	10	18	3	16.5	-	3
5/31/2004	4	10	15	2	18.5	-	3
5/31/2004	4	10	17	3	18	-	3
5/31/2004	1	13	18	0	17	0.2	6
5/31/2004	1	13	15	0	16	0.05	3
5/31/2004	2	13	15	1	16.5	0.2	4
5/31/2004	2	13	12	0	17.5	-	3
5/31/2004	3	13	9	0	19	-	4
5/31/2004	3	13	15	0	16.5	-	6
5/31/2004	4	13	11	0	14	-	3
5/31/2004	4	13	15	0	18	-	6
5/31/2004	1	14	31	1	20	1.2	9
5/31/2004	1	14	25	0	18.5	0.9	7
5/31/2004	2	14	34	2	22	1.5	8
5/31/2004	2	14	34	0	20.5	-	8
5/31/2004	3	14	29	0	21	-	7
5/31/2004	3	14	23	0	24	-	7
5/31/2004	4	14	30	1	21	-	9
5/31/2004	4	14	27	1	20.5	-	7

Table E.23. Data for oviposition experiment, date 5. B.

Date	Cage	Host code	Eggs laid	Egg masses
5/31/2004	1	9	0	0
5/31/2004	1	9	0	0
5/31/2004	2	9	0	0
5/31/2004	2	9	0	0
5/31/2004	3	9	0	0
5/31/2004	3	9	0	0
5/31/2004	4	9	0	0
5/31/2004	4	9	0	0
5/31/2004	1	10	0	0
5/31/2004	1	10	0	0
5/31/2004	2	10	0	0
5/31/2004	2	10	4	1
5/31/2004	3	10	0	0
5/31/2004	3	10	4	3
5/31/2004	4	10	0	0
5/31/2004	4	10	0	0
5/31/2004	1	13	0	0
5/31/2004	1	13	0	0
5/31/2004	2	13	0	0
5/31/2004	2	13	0	0
5/31/2004	3	13	0	0
5/31/2004	3	13	0	0
5/31/2004	4	13	0	0
5/31/2004	4	13	0	0
5/31/2004	1	14	18	3
5/31/2004	1	14	0	0
5/31/2004	2	14	0	0
5/31/2004	2	14	22	1
5/31/2004	3	14	0	0
5/31/2004	3	14	0	0
5/31/2004	4	14	0	0
5/31/2004	4	14	0	0

Table E.24. Data for oviposition experiment, date 6. A.

Date	Cage	Host code	Leaves	Dry leaves	Plant height (cm)	Dry weight	No. tillers
6/13/2004	1	10	18	1	29	1.5	4
6/13/2004	1	10	21	1	28	2.8	5
6/13/2004	2	10	29	1	27	-	6
6/13/2004	2	10	38	0	22	3.5	9
6/13/2004	3	10	34	4	25	-	9
6/13/2004	3	10	25	0	33	-	5
6/13/2004	4	10	18	3	29	-	3
6/13/2004	4	10	27	1	29	-	6
6/13/2004	1	11	35	5	36	6.9	6
6/13/2004	1	11	50	3	35	-	7
6/13/2004	2	11	49	5	33	7.5	9
6/13/2004	2	11	28	3	30	-	4
6/13/2004	3	11	24	5	27	-	3
6/13/2004	3	11	42	3	39	-	7
6/13/2004	4	11	25	2	32	-	3
6/13/2004	4	11	26	5	31	2.9	3
6/13/2004	1	14	43	0	29	-	11
6/13/2004	1	14	31	0	23	2.4	8
6/13/2004	2	14	49	0	29	-	12
6/13/2004	2	14	47	0	29	3.9	11
6/13/2004	3	14	47	7	40	-	10
6/13/2004	3	14	47	4	25	-	12
6/13/2004	4	14	24	3	28	2.9	4
6/13/2004	4	14	41	0	28	-	10
6/13/2004	1	15	45	4	32	7.4	9
6/13/2004	1	15	31	3	39	-	5
6/13/2004	2	15	48	4	34	-	9
6/13/2004	2	15	45	2	38	-	9
6/13/2004	3	15	39	3	38	-	6
6/13/2004	3	15	45	4	40	-	10
6/13/2004	4	15	39	2	35	4.6	6
6/13/2004	4	15	49	6	39	8.5	8

Table E.25. Data for oviposition experiment, date 6. B.

Date	Cage	Host code	Eggs laid	Egg masses
6/13/2004	1	10	9	9
6/13/2004	1	10	0	0
6/13/2004	2	10	0	0
6/13/2004	2	10	25	1
6/13/2004	3	10	0	0
6/13/2004	3	10	0	0
6/13/2004	4	10	0	0
6/13/2004	4	10	0	0
6/13/2004	1	11	0	0
6/13/2004	1	11	129	3
6/13/2004	2	11	0	0
6/13/2004	2	11	0	0
6/13/2004	3	11	103	4
6/13/2004	3	11	25	1
6/13/2004	4	11	0	0
6/13/2004	4	11	40	2
6/13/2004	1	14	0	0
6/13/2004	1	14	0	0
6/13/2004	2	14	0	0
6/13/2004	2	14	0	0
6/13/2004	3	14	274	7
6/13/2004	3	14	46	2
6/13/2004	4	14	193	2
6/13/2004	4	14	0	0
6/13/2004	1	15	75	1
6/13/2004	1	15	0	0
6/13/2004	2	15	84	1
6/13/2004	2	15	0	0
6/13/2004	3	15	0	0
6/13/2004	3	15	299	6
6/13/2004	4	15	146	2
6/13/2004	4	15	0	0

Table E.26. Data for oviposition experiment, date 7. A.

Date	Cage	Host code	Leaves	Dry leaves	Plant height (cm)	Dry weight (g)	No. tillers
6/13/2004	1	10	24	3	26	1.9	4
6/13/2004	1	10	21	2	26	2	4
6/13/2004	2	10	21	3	28	1.4	4
6/13/2004	2	10	29	4	29	-	5
6/13/2004	3	10	22	3	30	-	4
6/13/2004	3	10	24	2	30	-	4
6/13/2004	4	10	23	1	28	-	4
6/13/2004	4	10	25	3	29	-	5
6/13/2004	1	12	39	2	63	-	6
6/13/2004	1	12	33	5	66	14.4	6
6/13/2004	2	12	35	6	73	14	8
6/13/2004	2	12	32	4	68	10.1	7
6/13/2004	3	12	40	5	68	-	6
6/13/2004	3	12	29	4	72	-	5
6/13/2004	4	12	35	6	70	-	5
6/13/2004	4	12	40	5	68	-	7
6/13/2004	1	14	26	2	28	3.3	6
6/13/2004	1	14	21	2	28	-	6
6/13/2004	2	14	25	1	28	2.1	5
6/13/2004	2	14	25	3	24	-	5
6/13/2004	3	14	26	1	26	-	6
6/13/2004	3	14	23	1	27	-	4
6/13/2004	4	14	23	1	29	2.2	7
6/13/2004	4	14	34	6	29	-	8
6/13/2004	1	16	41	6	69	-	7
6/13/2004	1	16	50	5	75	18.1	9
6/13/2004	2	16	41	5	74	16.6	8
6/13/2004	2	16	50	5	75	-	8
6/13/2004	3	16	32	4	86	-	5
6/13/2004	3	16	47	3	82	-	8
6/13/2004	4	16	44	3	78	19.1	7
6/13/2004	4	16	46	5	81	-	8

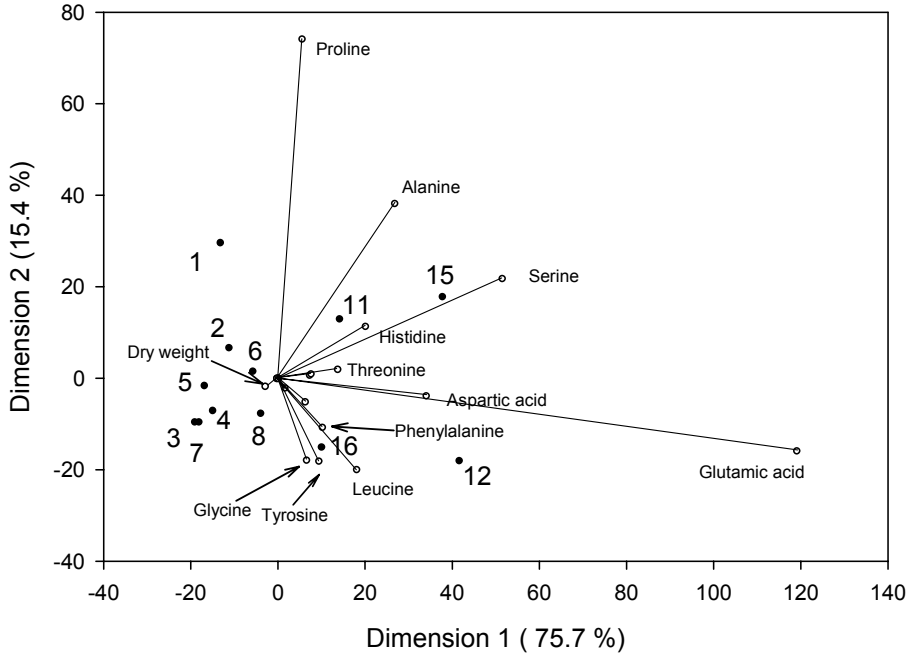


Fig. E.1. Principal component analysis based on covariance matrix of plant phenology and physiochemical measurements. Host types are reported in Table E.1.

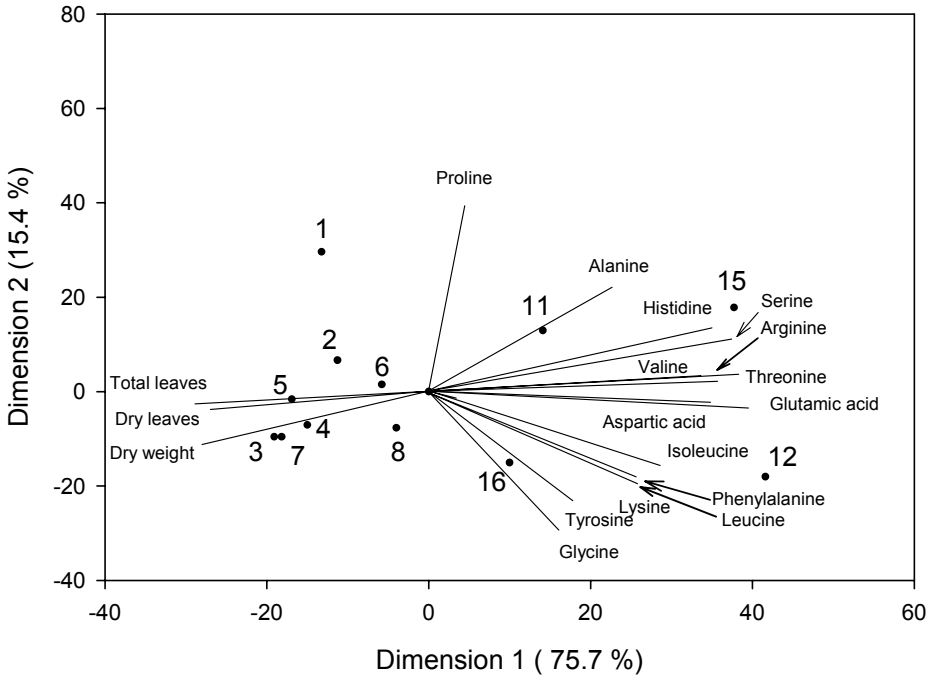
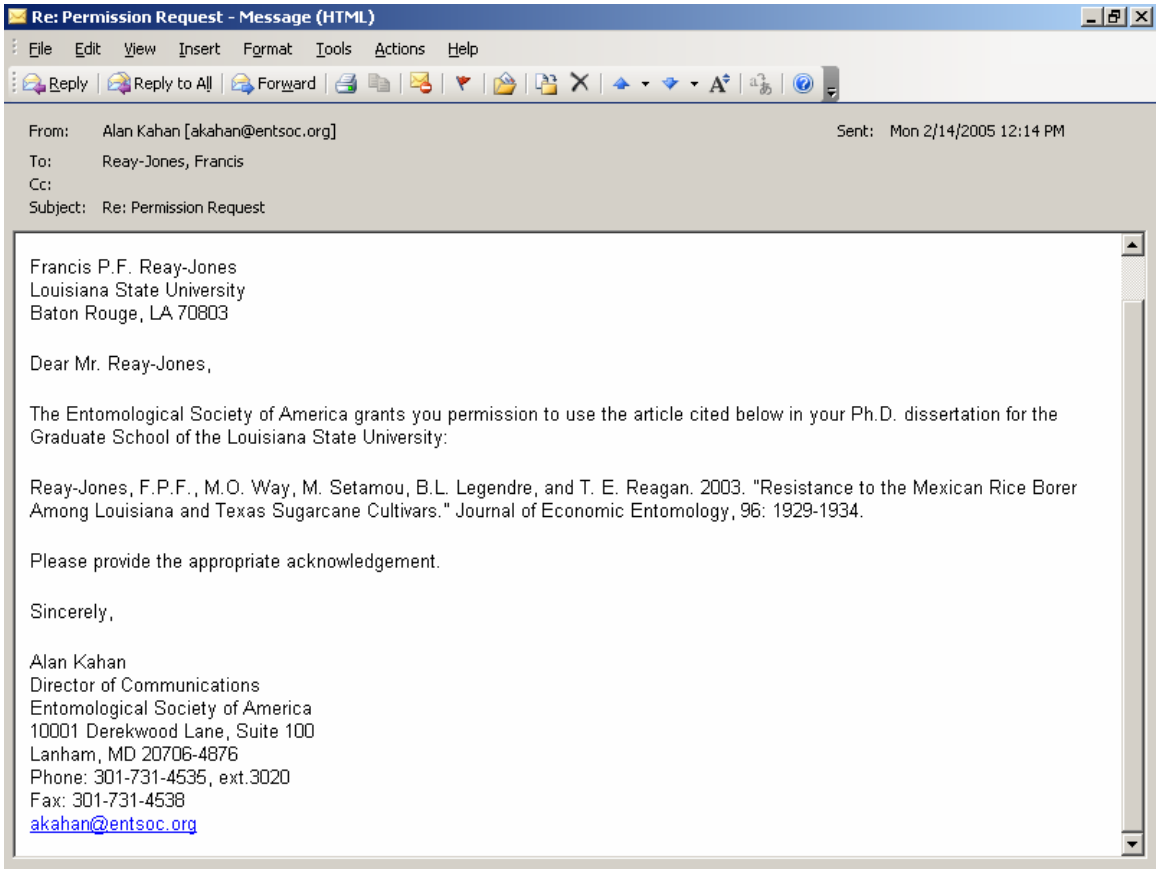


Fig. E.2. Principal component analysis on correlation matrix of plant phenology and physiochemical measurements. Host types are reported in Table E.1.

APPENDIX F: LETTER OF PERMISSION FOR CHAPTER 2



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From: Alan Kahan [akahan@entsoc.org] Sent: Mon 2/14/2005 12:14 PM

To: Reay-Jones, Francis

Cc:

Subject: Re: Permission Request

Francis P.F. Reay-Jones
Louisiana State University
Baton Rouge, LA 70803

Dear Mr. Reay-Jones,

The Entomological Society of America grants you permission to use the article cited below in your Ph.D. dissertation for the Graduate School of the Louisiana State University:

Reay-Jones, F.P.F., M.O. Way, M. Setamou, B.L. Legendre, and T. E. Reagan. 2003. "Resistance to the Mexican Rice Borer Among Louisiana and Texas Sugarcane Cultivars." Journal of Economic Entomology, 96: 1929-1934.

Please provide the appropriate acknowledgement.

Sincerely,

Alan Kahan
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VITA

Francis Peter Fortnum Reay-Jones was born on May 23, 1978, in Oxford, United Kingdom, and moved to live on a vineyard in Sauternes, France, when he was eight years old. He attended the Lycée Jean Moulin in Langon, and after graduating in 1996, he enrolled at the Université Bordeaux 1, where he obtained a bachelor's degree in organism biology in 1999 and a one-year graduate level degree in population and ecosystem biology in 2000. During this time, he conducted an internship with the Entomology Branch of the Forestry Commission in Farnham, United Kingdom. In 2001, he obtained the master's degree in plant technology from the Université d'Angers and the Institut National d'Horticulture in Angers, France. Research for his master's thesis was conducted at the C.I.R.A.D. (Centre de Coopération Internationale en Recherche Agronomique pour le Développement) sugarcane entomology laboratory in Réunion Island in the Indian Ocean on the bionomics and functional response of *Trichogramma chilonis*, a parasitoid of the spotted stalk borer *Chilo saccharisphagus*. In September 2002, Francis began doctoral studies in the Department of Entomology at Louisiana State University with a minor in applied statistics. He is currently completing the requirements for the degree of Doctor of Philosophy and hopes to obtain upon graduation an academic position in research and extension.