

July 2021

## Characterization of Resistance to Two Crambid Stem Borers Among Louisiana Sugarcane Cultivars

Leonardo D. Salgado

*Louisiana State University and Agricultural and Mechanical College*

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**Characterization of Resistance to Two Crambid Stem Borers  
Among Louisiana Sugarcane Cultivars**

A Thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Master of Science

in

The Department of Entomology

by  
Leonardo David Salgado  
B.S., Universidad Nacional de Agricultura, 2019  
August 2021

## **Acknowledgments**

My life has been a result of the sum of influences received by many people and the decisions I have made. However, nothing of this would have been possible without the support and encouragement from my parents, family, and friends. I appreciate all the conversations, lessons, and discussions that have helped me become the person I am now. This thesis is dedicated to my parents Maria Diaz and Elvin Salgado, my brothers Elvin and Eduardo, and all the family and friends who accompanied me on this journey.

I want to highly thank my major advisor, Dr. Blake Wilson, for providing me with the opportunity to pursue a master's degree, being an inspiration and a mentor. Dr. Wilson is always going above and beyond for his graduate students. His passion for agricultural entomological research has helped prepare me to succeed in my career and life because his teachings are valuable for quality research and life. I also want to thank James Villegas for being a mentor, a recruiter, and a friend. James' help in this research range from data collection to analysis and design. I will always remember your support in the process of this research. I also want to thank my committee members, Dr. Kenneth Gravois, Dr. Michael Stout, and Dr. Beibei Guo, for their contribution to improving my research.

Gratitude is extended to Randy Richard and Dr. Hannah Penn from USDA – ARS for providing cultivars, help in data collection, and suggestions for this research. Appreciation is also extended to Dr. Mo Way and his Texas A&M Beaumont Station crew for assistance in field experiments. Additional thanks are due to Katheryn Parraga, Vondel Reyes, and Dr. Jack Losso at the LSU School of Nutrition and Food Sciences for assistance in diet incorporation assays.

Appreciation is also expressed to Megan Mulcahy, Julian Lucero, Ethan Doherty, and Forest Huval. They are the best lab mates someone could wish for. Their help made this research more manageable. I want to thank my roommates, “*Los Perrines del B11.*” Holtman Flores, Adriana Alfaro, and David Galo were always there when I needed any input in this project. I want to thank my friends from UNAs at LSU, Cesar Escalante, Junior Betanco, Teddy Garcia, and others, for all the work invested in founding this association that has helped me strengthen my commitment to researching for a purpose. I also want to show my gratitude to Sarah Bernard, Dr. Hector Zapata, Dr. Lawrence Datnoff, and Dr. William Richardson for helping in the LSU-UNAG internship program that has allowed many UNAG graduates and students to be part of LSU. Finally, I want to thank Kensy Rodriguez. For being a friend and helping me keeping me sane in these crazy times. Kensy, thank you for always listening to me and helping me become a better person.

Sincere gratitude is expressed for financial support from funding agencies. This research was funded by the American Sugarcane League, USDA Hatch Funds, and the LSU College of Agriculture.

Thanks God for your endless blessings.

La vida es bella – Life is beautiful

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## Abstract

Cultivar resistance is a key management strategy for the stem borer complex (Lepidoptera: Crambidae) in Louisiana sugarcane, but mechanisms of resistance are not well understood. This research evaluated the potential mechanisms of cultivar resistance to *Diatraea saccharalis* and *Eoreuma loftini* among commercial sugarcane cultivars and experimental clones through a series of field screenings, greenhouse trials, and diet incorporation assays. The stem borer resistant standard HoCP 85-845 and cultivar L 01-299 were among the cultivars with the lowest borer injury levels in both field and greenhouse trials. Cultivars HoCP 00-950, L 12-201, and HoCP 14-885 were among the most heavily injured in field trials. The variability of results from *E. loftini* field trials suggests that a genotype  $\times$  environment effect might affect the resistance levels of cultivars. Rind hardness from field traits was associated with resistance to *D. saccharalis* in field trials. Differences in oviposition among cultivars in the greenhouse choice study were not detected, suggesting adult preference is not a key factor in resistance, and future studies should compare Louisiana's cultivars to cultivars with known oviposition non-preference. Conversely, results from the no-choice greenhouse experiment revealed up to 9-fold differences were present among cultivars in neonate establishment for both stem borer species. For *D. saccharalis* in the laboratory experiment, lower larval weights and longer days to pupation were observed for resistant germplasm Ho 08-9003, but no significant effects were found among current commercial cultivars. In the *E. loftini* diet experiment, larval weight was reduced by 59.2–86.5% relative to the artificial diet control but did not differ among cultivars. These results suggest a range of resistance levels remains present in sugarcane breeding germplasm and highlight the importance of screening cultivars before commercial release. Future studies of stem borer resistance mechanisms should attempt to devise a measurement of leaf sheath tightness and pubescence.



## Introduction and Justification

Sugarcane (a hybrid of *Saccharum officinarum* L., *S. barberi* Jeswiet, *S. spontaneum* L., and *S. sinense* Roxb) is one of the primary sources of raw sugar in the world. This agricultural crop has been planted in many tropical and subtropical areas worldwide (James 2004). In the continental United States, sugarcane is grown in Florida, Louisiana, and Texas (McConnell 2020). In Louisiana, sugarcane is planted in 24 out of 64 parishes (Gravois 2020, McConnell 2020), being the most valuable row crop in the state (Guidry and Gould 2018, Gravois 2020). Insect pests attack sugarcane from planting to the harvest, increasing the need to develop management strategies that are environmentally friendly and economically profitable (Reagan 2001). The most important insect pests of sugarcane in the United States are the stem borer complex (Reay-Jones et al. 2008, White et al. 2008), which is formed by two species of the family Crambidae (Lepidoptera). The primary pest historically is the sugarcane borer (*Diatraea saccharalis* F.), and the emerging threat is the invasive Mexican rice borer (*Eoreuma loftini* Dyar) (Showler and Reagan 2012, Reagan and Mulcahy 2019, Showler 2019). The management of stem borers in sugarcane relies on integrated pest management (IPM) involving a balance of multiple control tactics (Stern et al. 1959). Advances in IPM practices made over the last several decades have helped Louisiana sugarcane farmers reduce reliance on insecticide applications (Hensley 1971, Reagan 2001, Wilson 2021). Cultivar resistance is a vital part of sugarcane IPM in Louisiana, and a range of resistance is present in current commercial lines (Wilson et al. 2015a, 2020b). Widespread planting of a borer-resistant cultivar, L 01-299, has helped decrease insecticide applications while maintaining a minimal level of crop injury (Gravois 2020, Wilson 2021, Wilson et al. 2021a). Several traits of commercial cultivars and experimental clones of sugarcane have been associated with resistance to stem borers, but these characters have not always been well understood, and mechanisms of resistance in

current cultivars are unknown. A better understanding of the mechanisms that impart resistance in sugarcane to the stem borer complex could help the sugarcane breeding program develop resistant cultivars with desirable agronomic traits. This research attempts to evaluate the mechanisms of resistance of sugarcane to *D. saccharalis*, and *E. loftini* among commercial and experimental cultivars by (1) evaluation of cultivar resistance in the field, (2) examination of oviposition preference among sugarcane cultivars, (3) measuring the effect of sugarcane cultivars on neonate establishment, and (4) assessing nutritional or plant metabolite effects on larval development.

## Chapter 1. Literature Review

### 1.1. Sugarcane borer

The sugarcane borer, *Diatraea saccharalis* (F.), is a stem borer of the family Crambidae. Fabricius first described it in 1794 as *Phalaena saccharalis* (Box 1959), but it was redescribed in 1927 when Dyar and Heinrich described it as *D. saccharalis*. It is difficult to determine *D. saccharalis* historical origin due to the human dispersal of hosts and frequent misidentifications or misnaming of *Diatraea* specimens (Box 1951, 1956). However, although the exact origin of *D. saccharalis* is not known; it is believed it could be located in South America, where several native plant hosts occur (Francischini et al. 2019). *D. saccharalis* might have expanded throughout the western hemisphere with the planting of sugarcane and corn (Box 1951, 1956, Pashley et al. 1990, Fogliata et al. 2019). The first injury effects on sugarcane of *D. saccharalis* were reported in the West Indies between 1786 to 1789 (Box 1959). No precise information is known about how *D. saccharalis* came to the United States, but the species was first reported as a pest of sugarcane in Louisiana in 1854 (Stubbs and Morgan 1902, Holloway et al. 1928). The principal theory of *D. saccharalis* origins suggests that Louisiana and Texas populations slowly diverged from Brazil and Caribbean populations over 400,000 years ago (Pashley et al. 1990, Francischini et al. 2019). Nowadays, the sugarcane borer inhabits a range of areas extending from the warmer parts of the United States' Gulf Coast, including the states of Florida, Louisiana, and Texas throughout the Caribbean (West Indies islands), Mexico, Central, and South America. The sugarcane borer is present with fluctuating population levels from the 30<sup>th</sup> parallel north (30°N) to the 30<sup>th</sup> parallel south (30°S) (Bleszynski 1969, Pemberton and Williams 1969, Capinera 2001, Vargas et al. 2015, Joyce et al. 2016, Fogliata et al. 2019, Roldán et al. 2020). *D. saccharalis* host plants are mainly in the family Poaceae (grasses). It is an economically important pest of sugarcane (*Saccharum*

spp.), corn (*Zea mays* L.), rice (*Oryzae sativa*), and sorghum (*Sorghum bicolor*) (Roe et al. 1981). This insect also feeds on several non-crop grasses, including johnsongrass (*Sorghum halepense*), *Andropogon* spp., crabgrass (*Digitaria* spp.), goosegrass (*Eleusine* spp.), barnyard grass (*Echinochloa* spp.), marsh grasses (*Hymenachne* spp.), sprangletops (*Leptochloa* spp.), vaseygrass (*Paspalum urvillei*) and *Panicum* spp. (Holloway et al. 1928, Bessin and Reagan 1990).

The life cycle of *D. saccharalis* from egg to adult lasts 30 to 40 days with five larval instars and a pupal stage (Holloway et al. 1928). *D. saccharalis* lays its eggs on the upper surface of the green leaves facing the midrib (Sosa 1990, Showler and Reagan 2012). Initially cream-colored, the eggs turn orange through time, then the neonate's head capsule is visible, giving the egg a black color just before eclosion, which will occur 4–6 days after they were laid (Showler and Reagan 2012). The eggs have an oval form and are flattened with about 1.16 mm long by 0.75 mm wide. The eggs are deposited singly or in clusters of 2 to 100 eggs (Holloway et al. 1928). Under laboratory conditions, the egg stage can range from 16.5 days at 15.6°C to 4.6 days at 32°C (King et al. 1975). Larvae can measure from a range of 1.5 to 25.4 mm in length from instars one to five, respectively. Larvae are pale yellow-white with a brown head capsule (Holloway et al. 1928). For larvae raised with an artificial diet, an increase of temperature from 22°C to 30°C can decrease mean larval development time from 32 to 18 days (King et al. 1975). Before pupation, late-instar larvae feeding inside grass stems clean tunnels and chew an enlarged emergence hole, allowing moths to emerge after eclosion (Holloway et al. 1928, White 1993). The pupa is cylindrical, elongate (16–20 mm in length), and yellowish to dark brown in color (Holloway et al. 1928). The pupal duration is 7–8 days under laboratory conditions between 26 and 33°C, and it extends to 13 days at 22°C (King et al. 1975). The pupal weight of *D. saccharalis* females is positively correlated to fecundity (Bessin and Reagan 1990). The adult is a straw-colored moth with wings marked by

black dots on the forewings (Holloway et al. 1928). The wingspan can measure a range between 27–39 mm in females and 18–28 mm in males. The adult stage lasts from 3 to 8 days, and oviposition will on average last four days or less (Holloway et al. 1928). Four to five overlapping generations of *D. saccharalis* occur each year in Louisiana (Hensley 1971). The first generation of *D. saccharalis* occurs when temperatures in the spring start to warm (Hensley et al. 1963, Rodriguez-Del-Bosque et al. 1995). When the temperature starts to cool down, *D. saccharalis* overwinters as a late instar larva (Katiyar and Long 1961). Low temperatures were reported to increase overwintering mortality (Holloway et al. 1928). However, recent studies suggest that only when winter temperatures drop far below average is there a substantial reduction in spring populations (Wilson et al. 2020).

## 1.2. Mexican rice borer

The Mexican rice borer, *Eoreuma loftini* (Dyar), is a stem borer of the family Crambidae, the same family as the economically significant sugarcane borer, *D. saccharalis* (F.). *E. loftini* was initially described as *Chilo loftini* by Dyar (1917). Later on, the species was moved into the genus *Acigona* by Bleszynski (1967), and then into the current know genus *Eoreuma* by Klots (1970). *E. loftini* was first detected in the United States in Texas in 1980 (Johnson and van Leerdam 1981). It was found in Louisiana in 2008 (Hummel et al. 2010), and it has been spreading since then, becoming an important new threat to crop production along the Gulf Coast (Wilson et al. 2015b). *E. loftini* utilizes all the same host plants as *D. saccharalis* with the addition of barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), ryegrass (*Lolium* spp.), brome (*Bromus* spp.), yellow bristlegrass (*Setaria lutescens* (Weigel) Hubbard) bulrush (*Scirpus validus* Vahl), lemongrass (*Cymbopogon citratus* (DC) Stapf), millet (*Pennisetum glaucum* L.), and pampas grass (*Cortaderia selloana* (Schultes) Ascherson & Graebner) (Dyar 1917, Morrill 1925, Van

Zwaluwenburg 1926, Osborn and Phillips 1946, Beuzelin et al. 2011a, 2013; Showler et al. 2011, 2012). Under summer conditions in the Lower Rio Grande Valley, Texas, *E. loftini* life cycle can last 45–50 days with five larval instars and a pupal stage. However, the duration of development depends upon the host plant species and environmental conditions (Browning et al. 1989). Four to six overlapping generations occur in Texas, and all stages of this borer can be found generally throughout the year in the field (Johnson 1985, Meagher et al. 1994, 1996). Eggs of *E. loftini* are rounded with a yellowish cream color (Van Leerdam 1986). Most oviposition occurs on dry leaves of the lower internodes of the sugarcane plant, whereas *D. saccharalis* prefers to lay on green leaves (Showler and Reagan 2012, 2017). However, oviposition is not limited to the lower internodes as it can occur at any other internode in the range between 0–80 cm (Van Leerdam et al. 1986). In comparison with *D. saccharalis*, *E. loftini* moths prefer to lay their eggs in the folds of dead leaves (Van Leerdam et al. 1986, Reay-Jones et al. 2007, Showler and Castro 2010). At constant temperatures, the egg stage can last 14 days at 20°C and five days at 32°C (Van Leerdam et al. 1984, 1986). Once larvae hatch, they migrate to the plant's greener parts and start feeding in leaf sheaths. Larvae of *E. loftini* are described as having a brown to a yellowish-brown head capsule and a white-colored body with two rows of longitudinal, brown-purple markings on either side (Osborn and Phillips 1946, Browning et al. 1989). Being an exception among other borer species of the Crambidae family, *E. loftini* does not have conspicuous hairy plates on the body's dorsal surface (Van Zwaluwenburg 1926, Osborn and Phillips 1946, Capps 1963, Browning et al. 1989). Larval exposure on sugarcane plant surfaces averages less than one week, and some larvae have been documented entering mid-rib tissue one day after eclosion (Wilson et al. 2012). Larvae can undergo four to six molts under laboratory conditions. The number of larval stages varies by sex, with five stages typical in males and six in females (Van Leerdam 1986). The duration of

larval development (21–78 days) is inversely related to temperature (Van Leerdam 1986). *E. loftini* larvae tunnel vertically and horizontally, filling tunnels with frass (Showler and Reagan 2012). This habit makes larvae and pupae less accessible to insecticides and natural enemies in contrast to *D. saccharalis* (Browning et al. 1989, Showler and Reagan 2012). Larvae can reach a length of 19–25 mm before pupation (Van Zwaluwenburg 1926, Browning et al. 1989). The pupal stage duration is between 7 to 21 days, depending on the temperature (Van Leerdam et al. 1986). Also, male pupae are smaller than females for both species (Browning et al. 1989, Legaspi et al. 1997). As shown for *D. saccharalis* by Bessin and Reagan (1990), a positive relationship between fecundity and pupal weight also exists for *E. loftini* (Spurgeon et al. 1995). The adult is a solid light-tan moth of variable size (13–19 mm). This moth has a unique triangular gena and conical frons. However, definitive species-level identification requires examining the male genitalia (Klots 1970, Reiss 1981, Agnew et al. 1988, Vargas et al. 2018). Adult longevity is approximately seven days (Van Zwaluwenburg 1926). Mating probability decreases with time after eclosion; thus, most matings occur the night after eclosion (Shaver et al. 1994). Fecundity ranges from 200–400 eggs/female. However, temperature and host plant species influence fecundity and oviposition rates. Most oviposition occurs two days after adult eclosion (Van Leerdam 1986). Males and females are capable of mating with more than one partner. For both sexes, the highest mating activity occurs between 7–9 hours after sunset; however, females begin emitting pheromone five hours after sunset (Shaver et al. 1994). In adverse temperatures, as seen in the fall and winter, *E. loftini* larvae may enter diapause as needed (Browning et al. 1989). Cold tolerance data from Rodriguez-Del-Bosque et al. (1995), indicate that *E. loftini* can survive winter temperatures, and it has a higher overwintering survival rate than *D. saccharalis*. However, fewer larvae will enter

diapause during mild winters. Larvae have been shown to be freeze tolerant for limited durations (Browning and Smith Jr. 1988, Browning et al. 1989).

### 1.3. Stem borer injury to sugarcane

After hatching, stem borers neonate larvae move in the crop to their preferred entry point into the space between leaf sheaths and stems. Stem borers larvae feed in the inside of sheaths, and after the second or third molt, penetrate the stems (White 1993, Wilson et al. 2012). *D. saccharalis* larvae will move from the green leaves to the nodes around the growing point (Leslie 2004). Stalk penetration will occur in an immature internode. Larval entrance sites and adult emergence holes can be easily identified after leaf sheaths are removed (Bessin et al. 1990, Pfannenstiel and Meagher 1991, White et al. 2001). However, different points of entry may be evaluated by the insect, resulting in multiple entry holes caused by a single larva but leaving only one emergence hole (Bessin et al. 1990a, White 1993). Also, a quantity of frass material may be seen clinging to the stalk, particularly for *D. saccharalis* (Holloway et al. 1928). Tunneling within stalks reduces growth by preventing the flow of nutrients to the vegetative internodes at the top of the plant. Tunneling can also cause stalks to break. Injury to sugarcane from stem borer larval feeding near the plant's apical meristem prior to the formation of internodes can cause a symptom called a "dead top" (Long and Hensley 1972). Injury to sugarcane tillers prior to the formation of internodes results in a "dead heart". *E. loftini* larval feeding occurs in the leaf sheath during early instars; for later instars, they bore into the stalk, feeding transversely through the plant's internodes (Showler and Reagan 2012). Transverse tunneling weakens the stalk's structural strength, increasing the occurrence of breakage relative to *D. saccharalis* (Van Zwaluwenburg 1926). In addition to injury and yield loss from stem borer larvae, tunneled stalks are susceptible to secondary pathogens, principally red rot disease (*Colletotrichum falcatum* Went.) (Ogunwolu et al. 1991). To record the



injury caused by the stem borers, a season-long record called bored internodes has been used to determine borer infestation levels (Hensley and Long 1969, Bessin et al. 1990b, White et al. 2008, Wilson et al. 2015a). This parameter reflects oviposition preference and the establishment of early instar larvae (Wilson et al. 2015a). According to Wilson et al. (2019), every 1% increase in the percentage of bored internodes with a decrease of 0.61% in sugar per hectare (White et al. 2008) will cost an estimated \$19.76 per hectare in lost farm revenue. However, this value could be higher if the molasses price were considered. Sugar per hectare is the product of tonnes of cane per ha, which is the principal yield component that reflects field loss caused by stem borers and theoretical recoverable sugar (TRS), a measure of cane quality that reflects sugar losses in the biomass (White et al. 2008). The percentage of the bored internodes does not reflect the insect survival to adulthood; therefore, Bessin et al. (1990a) created a moth production index using the emergence holes to estimate the insects' adult production. Reay-Jones et al. (2003), created a "Relative Survival Index," which quantifies the proportion of insects that enter the stalk that complete their life cycle. More recently, Wilson et al. 2015a, developed a single index, the relative resistance ratio, that incorporates both measures to evaluate resistance. Ratios approaching 1 indicate a high degree of susceptibility relative to other cultivars evaluated.

#### 1.4. Management of sugarcane stem borers

##### 1.4.1. Chemical Control

Insecticides have been one of the most widely-used controls in managing *D. saccharalis* (Hensley 1971, Bessin et al. 1990c), and include various types of insecticides to target specific life stages. The replacement of inorganic materials and botanical compounds with synthetic organic insecticides was the first factor that improved sugarcane borer control programs in Louisiana. Organochlorines, such as Endrin, were widely-used during the 1960s (Long et al. 1959, Hensley

1971). However, the ban of organochlorines by the United States Environmental Protection Agency and the development of insecticide resistance (Yadav et al. 1965), led to the replacement of organochlorines with organophosphates such as azinphos-methyl (Hensley 1971). The registration of this organophosphate was revoked in 1995 because of a high number of fish kills in the sugarcane planting areas of Louisiana, which lead to environmental concerns (Southwick et al. 1995). These compounds were replaced in the late 1990s with the insect growth regulator, tebufenozide (Rodriguez et al. 1994, Reagan and Posey 2001). Tebufenozide is a selective insecticide that acts as a molting accelerator that affects some Lepidopterous insects' larval growth. This insecticide is an ecdysone agonist that causes larvae to produce a malformed cuticle. Insects affected by this insecticide cannot shed their exuviae and die of dehydration and/or starvation (Dhadialla et al. 1998, Rodriguez et al. 2001). This insecticide was one of the few pesticides available at the time that did not suppress nontarget arthropods allowing for the conservation of naturally occurring biological controls (Woolwine et al. 1997, Beuzelin et al. 2010). As a result of its efficacy and environmental benefits, Louisiana sugarcane farmers used tebufenozide on 91.2% of all the sprayed areas for the 2007 season (Pollet 2008). After field reductions of tebufenozide efficacy (Reay-Jones et al. 2005c), Akbar et al. (2008) selected for resistance to tebufenozide in laboratory-reared *D. saccharalis*, and after 12 generations obtained a 27.1- and 83.3-fold increase in the  $LC_{50}$  and  $LC_{90}$  values, respectively. This resistance in a short period when continuous selection was applied proved the need that environmentally friendly chemistries from different modes of action were needed.

Recent studies demonstrated the effectiveness of new insecticides classes, such as benzoylphenyl ureas and diamides (Beuzelin et al. 2010, Wilson et al. 2017). Novaluron, a chitin inhibitor, disrupts the cuticle formation by exerting its insecticidal activity on egg and larval stages,

and not directly toxic on the adult stage (Barzani 2001, Ishaaya et al. 2003). Novaluron has a different mode of action from other labeled insecticides and is considered a useful component of Louisiana sugarcane IPM (Beuzelin et al. 2010). Chlorantraniliprole is a diamide insecticide that has shown effective control for managing lepidopteran stem borers (Wilson et al. 2017). This insecticide activates ryanodine receptors, which leads to uncontrolled calcium release in the insect muscle that causes not controlled movements in the insect (Lahm et al. 2009). Chlorantraniliprole also has longer residual activity, which increases the number of days of protection (Wilson et al. 2021b). This diamide improved efficacy against the currently used insecticides to control stem borers (Wilson et al. 2017).

Selecting an insecticide to use is essential for stem borer control, but choosing the right application timing is also necessary. Crop consultants scout sugarcane from June through the end of August. The recommended action threshold to initiate insecticide applications for *D. saccharalis* infestation levels is 5% of the stalks with larvae feeding in the leaf sheaths (Hensley 1971, Schexnayder et al. 2001, Posey et al. 2006, Wilson et al. 2018b). Currently, the average number of insecticide applications in Louisiana sugarcane is <0.5 per acre (Wilson et al. 2021). However, the increasing prevalence of *E. loftini* is threatening to destabilize sugarcane IPM.

Insecticidal control of *E. loftini* in Texas sugarcane has, until very recently, been very limited. Previous studies showed that chemical control of *E. loftini* was not enough to improve sugar yield despite numerous applications (Meagher et al. 1994, Reay-Jones et al. 2005b). However, improved application timing, modern insecticide chemistries, and pheromone trap-assisted scouting have improved the management of *E. loftini* in Texas (Wilson et al. 2012, 2017). For *E. loftini*, the same parameter used for *D. saccharalis* (5–10% of the stalks with larvae feeding in the leaf sheaths) has been used to schedule insecticide applications (Meagher et al. 1994).

However, this parameter could be lower because of the rapid entry of *E. loftini* larvae into the sugarcane stalk, which implies yield loss relationships need to be reassessed. (Wilson et al. 2012).

#### 1.4.2. Biological Control

The parasitoid, *Cotesia flavipes* (Hymenoptera: Braconidae), has greatly decreased *D. saccharalis* populations in Brazil, Colombia, Honduras, Florida, and Texas agroecosystems (Fuchs et al. 1979, Hall 1986, Marengo et al. 1988, Machado-Botelho 1992, Meagher et al. 1998, Aya et al. 2017, Roldán et al. 2020). However, *C. flavipes* failed to establish in Louisiana sugarcane after fifteen attempts at St. Mary Parish (Luke and Simon), Lafourche Parish (Clovelly), and Terrebonne Parish (near Houma, LA) due to factors such as annual winter freezes, crop harvest at nine months, and other natural enemies' interference (White et al. 2004). Numerous attempts to import and establish parasitoids for *E. loftini* control in Texas were unsuccessful (Meagher et al. 1998), and the pest is now the most prevalent stem borer in Texas and Mexico (Rodriguez-Del-Bosque et al. 1995).

The first successful attempt to establish biological control for *D. saccharalis* in the Louisiana sugarcane agroecosystem was in 1915 with the Cuban fly, *Lixophaga diatraeae* (Diptera: Tachinidae), which is still present and active (Charpentier et al. 1971). Additionally, some wasps such as *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) and *Alabagrus stigma* Brullé (Hymenoptera: Braconidae) can also be found in the sugarcane fields, but they are not prevalent enough to consistently hold *D. saccharalis* populations levels below the economic threshold (White and Reagan 1999).

Current biological control in Louisiana's sugarcane also benefits from a complex of generalist predators, some of which are spiders (Salticidae and Lycosidae), ants (Formicidae), beetles (Carabidae, Elateridae, and Staphylinidae), and earwigs (Dermaptera) (Negm and Hensley

1967, 1969). Of these, the red imported fire ant, *Solenopsis invicta* Buren, is considered the dominant predator of *D. saccharalis* in Louisiana (Reagan et al. 1972, Bessin et al. 1990a, Showler et al. 1990, Beuzelin et al. 2009) and may contribute to *E. loftini* control (Reay-Jones et al. 2005a). The relative abundance and impact of each group of natural enemies vary through time, season, and location.

#### 1.4.3. Cultural Control

The use of cultural control practices such as burning to facilitate harvest and manually removing infested plant residues for management of *D. saccharalis* has decreased in Louisiana's sugarcane production due to a lack of workforce, costs related inputs, and environmental concerns (Hensley 1971, Meyer et al. 2005). The introduction of the mechanical combine harvester in Louisiana's sugarcane industry in the mid-1990s helped establish "green cane harvesting." The use of the combine harvester has beneficial effects on *D. saccharalis* management because it leaves less overwintering habitat for the insect (Salassi and Champagne 1996, Meyer et al. 2005, Wilson et al. 2020). Current cultural practices used in Louisiana to manage *D. saccharalis* are planting borer-free seed cane, planting corn as far from sugarcane as possible, and maintaining crop residues as overwinter habitat for natural enemies (Gravois et al. 2014). Early planted sugarcane is exposed to increased *D. saccharalis* infestations in the fall, but these did not impact spring infestations (Beuzelin et al. 2011b). Cultural controls for *E. loftini* have been focused on irrigation practices (Reay-Jones et al. 2008), rational nitrogen applications (VanWeelden et al. 2016), and removal of sugarcane trash (Showler 2019). This last one might conflict with the recommendation for *D. saccharalis* of maintaining crop residues, which indicates that it is better to treat both species as a complex than single species (Reagan and Mulcahy 2019).

#### 1.4.4. Cultivar Resistance

Host plant resistance is a key management strategy for stem borer control in Louisiana because it is often inexpensive and easy for sugarcane farmers to implement. Resistance is compatible with almost all other management strategies and minimizes further input costs incurred by the farmer (Smith 2005, Stout and Davis 2009). However, farmers do not always prefer the cultivars with resistance, and frequently plant the cultivars with the highest yield potential, regardless of stem borer susceptibility. Despite that tendency, Louisiana's sugarcane farmers have changed from using a highly susceptible cultivar (LCP 85-384) to a high yielding cultivar with a high level of resistance to stem borers (L 01-299), which is now planted on more than 59% of the state's acreage (Gravois 2020, Wilson et al. 2020). However, overplanting one resistant variety can lead insects to overcome that resistance trait over time (Smith 2005).

Host plant resistance of Louisiana's sugarcane was firstly reported in 1902 by Stubbs and Morgan by showing differences in injury among cultivars. Since then, stem borer cultivar resistance evaluation has focused on borer injury and performance (Reay-Jones et al. 2003, White et al. 2008, Tomaz et al. 2017). To measure the resistance of sugarcane to stem borers, a fixed number of stalks is sampled from the field or plot at the time of harvest of the crop, and injury data is recorded and compared among cultivars (Bessin et al. 1990b, Schexnayder et al. 2001, Reay-Jones et al. 2003). Screening for insect cultivar resistance is a practice performed in breeding programs to avoid releasing a cultivar that might be too susceptible to injury. However, borer resistance is not a key priority of breeding programs because of the availability of effective chemical and cultural practice control measures (Wilson et al. 2017). Despite this, borer resistance remains present in germplasm used in the cultivar development program, and resistant cultivars

with high yield potential are sometimes released. The mechanisms of resistance in recent sugarcane cultivars are unknown.

Knowing the mechanisms and traits associated with resistance can help sugarcane breeding programs increase the level of resistance of future cultivar releases (Meagher et al. 1996a). Some argue that time spent on mechanism studies could be better spent on screening because the ultimate goal is to have a resistant cultivar (Samson 2009). Screening for cultivar resistance is labor-intensive. Identification of resistance mechanisms could lead to the development of a more efficient biological measurement of resistance.

Plant resistance has been divided into three mechanisms: nonpreference (later renamed antixenosis), antibiosis, and tolerance (Painter 1951, 1958, Kogan and Ortman 1978). However, these mechanisms are often interconnected (Kogan and Ortman 1978, Stout 2013). Several traits of sugarcane cultivars have been associated with resistance to stem borers. No clear pattern has been observed for *D. saccharalis* moths' preference for oviposition among sugarcane cultivars in the field (Fuchs and Harding 1978). However, differences in the numbers of eggs and egg clusters have been observed among Brazilian sugarcane cultivars in greenhouse studies (Dinardo-Miranda et al. 2012, Pimentel et al. 2017). External plant characteristics such as leaf sheath pubescence are thought to influence *D. saccharalis* oviposition (Sosa 1988, 1990). Oviposition preference for *E. loftini* has also been studied, though it is similarly not well-understood. Meagher et al. (1996a) asserted that ovipositional non-preference was not a key mechanism of resistance to *E. loftini*. Conversely, Reay-Jones et al. (2007) found that susceptible sugarcane cultivar LCP 85-384 was more attractive than HoCP 85-845 based on the number of eggs per egg mass. Plant physical characteristics, particularly the prevalence of senescent leaves, play a more critical role in host selection for *E. loftini* (VanWeelden et al. 2017). *E. loftini* oviposit exclusively in cryptic sites

within folds of dry leaves (Van Leerdam et al. 1986, Reay-Jones et al. 2007, Showler and Castro 2010). Thus, the absence of senescent leaf tissue may reduce the availability of suitable oviposition sites.

Factors that affect larval or neonate establishment and stalk entry have also been considered important resistance mechanisms for stem borers (Coburn and Hensley 1972). A negative correlation was found between the rind hardness of the first internode accessible to *D. saccharalis* and total bored internodes (Martin et al. 1975). Leaf-sheath tightness around the stalk in resistant cultivar NCo 310 was considered partially responsible for the resistance to *D. saccharalis*, although this parameter was not quantified (Coburn and Hensley 1972). These mechanisms that prolong larval feeding on sugarcane surfaces might increase exposure to natural enemies and insecticide applications and could be a promising tool in IPM programs (Wilson et al. 2012).

Antibiosis traits that influence larval development have been reported to affect both stem borer species (Bessin and Reagan 1990, Meagher et al. 1996a, White et al. 2011). Higher quality host plants, including corn and susceptible sugarcane cultivars, can improve *D. saccharalis* female fecundity compared to resistant cultivars (Bessin and Reagan 1990). Diet incorporation bioassays suggest some genotypes might have an antibiotic effect on *E. loftini* larval development; however, these results are not always consistent with field resistance levels (Meagher et al. 1996a). Germplasm Ho 08-9003 has shown low larval weights in field experiments, which suggest antibiosis as the mechanism of resistance to *D. saccharalis* (White et al. 2011). This germplasm is also resistant to *E. loftini* (Wilson et al. 2015a) and could be used in sugarcane breeding programs as a parent for crossing. Fiber content and pith are also associated with resistance to *D. saccharalis*



(White et al. 2006). However, these traits are negatively associated with sucrose content (Gravois and Milligan 1992).

More tolerance is present in modern sugarcane cultivars that can recover yield losses from borer injury than cultivars grown 50 years ago (White et al. 2008). Susceptible sugarcane cultivars vary significantly in their response to stem borer infestations; they are 5- to 10-fold more injured than resistant cultivars (Wilson et al. 2018a,b). However, susceptible cultivars with a high yield potential may compensate for damage from *D. saccharalis*, but planting susceptible cultivars can have implications by possibly increasing the *D. saccharalis* pressure (Bessin et al. 1990a, White et al. 2008).

## **Chapter 2. Resistance to the sugarcane borer (Lepidoptera: Crambidae) in Louisiana sugarcane cultivars**

### 2.1. Introduction

The sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae), is considered one of the most important sugarcane pests in the Americas (White et al. 2008, Reagan and Mulcahy 2019). In Louisiana (USA), this pest is responsible for more than USD 8 million in losses and control costs annually (Wilson 2021). Cultivar resistance is a vital part of integrated pest management (IPM) in Louisiana sugarcane and has greatly reduced reliance on insecticides (Wilson et al. 2020). A range of resistance is present in current commercial sugarcane, with susceptible cultivars often suffering 5–10-fold greater levels of injury than resistant cultivars (Wilson et al. 2015a, Wilson et al. 2021a). Many resistance mechanisms for *Diatraea saccharalis* have been identified, but the traits responsible for resistance among currently planted cultivars are unknown.

Neonates of *D. saccharalis* feed inside leaf sheaths of immature internodes before boring into stalks, where they feed until pupation (White 1993). Plant traits that affect stem borer adult behavior (e.g. oviposition preference) and larval establishment (e.g. rind hardness and leaf sheath tightness) have been shown to contribute to sugarcane resistance against *D. saccharalis* (Martin et al. 1975, Coburn and Hensley 1972, Sosa 1990).

Fiber content is also associated with resistance to *D. saccharalis* (White et al. 2006), but fiber content is minimized below 13.5% in modern resistant commercial cultivars because it negatively impacts milling throughput and sucrose recovery (Posey et al. 2006, Wilson et al. 2021a). Continuous selection for low borer injury in sugarcane breeding programs may result in the accumulation of traits, including high fiber and low sucrose content, which reduce milling efficiency (Gravois and Milligan 1992, White et al. 2006). However, resistance is present in

recently developed commercial cultivars without detriment to yield, and resistant cultivars are now cultivated on >60% of Louisiana sugarcane acreage (Wilson 2021). Research is needed to identify traits associated with resistance in modern cultivars which are not detrimental to sugar yield or milling efficiency. Therefore, the objectives of this study were to: (1) evaluate resistance to *D. saccharalis* among sugarcane cultivars in the field, (2) examine *D. saccharalis* oviposition preference among sugarcane cultivars, (3) determine the effect of sugarcane cultivars on *D. saccharalis* neonate establishment, and (4) assess the influence nutritional content or plant metabolites on *D. saccharalis* larval development. Resistance was assessed herein through field trials, greenhouse experiments, and laboratory assays.

## 2.2. Materials and methods

**Field trials.** A series of three field trials were conducted during 2018 to 2020 at the LSU AgCenter Sugar Research Station in St. Gabriel, Louisiana, to screen for resistance against *D. saccharalis*. The experimental design for all trials was a randomized complete block design with five replications. Plot size and other production aspects varied among trials (Table 2.1). Seed cane was obtained from the Louisiana State Agricultural Center Sugar Research Station, St. Gabriel, Louisiana, except for cultivars N-21 and Ho 08-9003, which were obtained from the USDA Sugarcane Research Unit located in Houma, Louisiana. In all trials, weed management, fertilization, and other production practices were done according to LSU AgCenter recommendations (Gravois et al. 2014). The 2018 (plant cane) and 2019 (first ratoon) trials included nine advanced experimental clones (L 11-183, Ho 11-573, L 12-201, Ho 12-615, L 13-251, Ho 13-708, Ho 13-739, HoCP 13-740, and HoCP 13-758) in the sugarcane cultivar development program and seven commercial cultivars (L 01-299, L 01-283, HoCP 85-845, HoCP 96-540, HoCP 00-950, HoCP 04-838, and HoCP 09-804). The 2020 plant cane trial included five

experimental sugarcane cultivars (HoCP 14-885, L 14-267, HoL 15-508, Ho 15-971, and L 15-306), eleven commercial cultivars (the seven commercial cultivars from the 2018–2019 trials as well as L 11-183, L 12-201, Ho 12-615, and Ho 13-739, which were released for commercial production from 2017–2019) and N-21 (resistant to *Eldana saccharina* and *E. loftini*) and Ho 08-9003 (*D. saccharalis* resistant germplasm). HoCP 85-845 and HoCP 00-950 were included in all trials as *D. saccharalis*-resistant and susceptible standards, respectively (Reay-Jones et al. 2003, Wilson et al. 2012, 2015a, 2021a).

Table 2.1. Summary of production practices in field evaluations for cultivar resistance to *Diatraea. saccharalis*, at the Sugar Research Station, St. Gabriel, Louisiana, 2018–2020.

<b>Year</b>	<b>Planting Date</b>	<b>Injury data collection</b>	<b>Crop</b>	<b>Cultivars/Clones Evaluated</b>	<b>Plot size</b>
2018	28-Oct-17	30-Oct-18	Plant Cane	16	0.0004-ha
2019	28-Oct-17	23-Oct-19	First Ratoon	16	0.0004-ha
2020	3-Sep-19	27-Oct-20	Plant Cane	18	0.0008-ha

To increase *D. saccharalis* pest pressure in the field trials, corn (*Zea mays*) was intercropped on every third (2018–2019) or fourth row (2020). Individual corn plants were inoculated with approximately 15 first-instar *D. saccharalis* larvae from a laboratory colony (Benzon Research, Carlisle, PA, USA) using a handheld inoculator (Model F9050, Bio Serv, Frenchtown, NJ, USA) in June of each growing season, according to the procedures outlined by White et al. (1996) and Wilson et al. (2021a). Populations of red imported fire ants, *Solenopsis invicta*, were suppressed with insecticidal bait containing hydramethylnon and methoprene (Extinguish Plus<sup>®</sup>, Wellmark International, Schaumburg, Illinois) to further enhance insect pressure.

At the end of the growing season in October of each year, a 12-stalk sample was cut from each plot (five replications = 60 stalks per variety). The number of bored internodes (Hensley and Long 1969, Bessin et al. 1990a, White et al. 2008), total internodes, and moth emergence holes

(Bessin et al. 1990b) from each sample were recorded. Relative survival of larvae was calculated as the ratio of emergence holes divided by the number of bored internodes (Reay-Jones et al. 2003). A relative resistance ratio was calculated based on rankings within replications for percentage bored internodes and relative survival according to the methods of Wilson et al. (2015a). Ratios approaching 1 indicate a high degree of susceptibility relative to other cultivars evaluated. The following resistance categories were used based on the mean relative resistance ratios of all cultivars within each year: highly resistant (0.00–0.19), resistant (0.20–0.39), intermediate (0.40–0.59), susceptible (0.60–0.79), and highly susceptible (0.80–0.99) (Wilson et al. 2015a).

A five-stalk plant cane sample was cut from each plot of selected cultivars (HoCP 85-845, HoCP 00-950, L 01-299, HoCP 04-838, L 12-201, Ho 12-615, and N-21) in the 2020 trial on 21 Aug 2020 (five replications = 25 stalks per variety) to evaluate the force required to penetrate stalk rinds. Measurements were taken using the youngest fully formed internode. These internodes are referred to as target internodes because they are sites of larval entry into the stalk (White 1993). The measurements were done using the procedures outlined by Villegas (2017) using a handheld digital force gauge (Dillon GL, Athens, TX, USA) fitted to a stand with wheel rotation capacity (Dillon CT, Athens, TX, USA). The metal cone point accessory was attached to the force gauge and was aligned to the target internode. The force gauge was lowered until the metal cone point pierced through the internode. Reading at each point was recorded as the force required ( $\text{kg}\cdot\text{m}\cdot\text{s}^{-2}$ ) to penetrate the internode.

Data were analyzed with generalized linear mixed models (PROC GLIMMIX, SAS® Institute 2013). The proportion of bored internode data were analyzed with a binomial distribution and a logit link function (Bessin et al. 1990a, Stroup 2015). Emergence per stalk, relative survival, relative resistance ratio, and rind hardness data were analyzed with Gaussian distributions. Models

for 2018–2019 trials included cultivar, year, and their interaction as fixed effects and replication(year) as a random effect. The slice function was used for comparisons of cultivars within years whenever a significant interaction effect was detected. Models for the 2020 trial included cultivar as a fixed effect and replication as a random effect. In all models, the Kenward–Roger method was used to estimate denominator degrees of freedom (Kenward and Roger 1997), and LS-Means were separated using Tukey’s HSD ( $\alpha=0.05$ ).

**Oviposition preference experiment.** Ten commercial cultivars with varying levels of resistance based on the field trials (HoCP 85-845, HoCP 96-540, HoCP 00-950, L 01-299, L 01-283, HoCP 04-838, HoCP 09-804, L 11-183, L 12-201, and Ho 12-615) and N-21 (resistant to *Eldana saccharina* and *E. loftini*) and Ho 08-9003 (*D. saccharalis* resistant germplasm) were used to determine the host preference of *D. saccharalis* females. Three nodes of each cultivar were planted on 10 Sept 2019 in five 60 L pots in a 1:2:1 mixture of sand silt, soil, and peat moss (Premier Peat Moss Ltd, Riviere-du-Loup, Quebec, Canada). Plants were maintained in a greenhouse under a photoperiod of Light (14 hours): Dark 10 (hours) at  $26 \pm 2$  °C. Sugarcane cultivars for the greenhouse experiments were obtained from the Louisiana State University Agricultural Center Sugar Research Station, St. Gabriel, LA. Pots were fertilized with 16 g of urea when the first internodes were formed. Pots were arranged in a randomized block design with five blocks, and each block consisted of one pot of each of the twelve cultivars arranged around a single release point. Male and female pupae sourced from lab colonies were placed into 3.8 L plastic buckets and were allowed to emerge and mate for 48 hours before release. One hundred thirty adults were released in each block on 17 Jan 2020 and allowed to lay eggs for four days. On 22 Jan 2020, the numbers of eggs, oviposition events (egg clusters separated by a distance of >5 mm), and eggs per oviposition event were recorded for each plant. Forty days later, to allow larvae to

reach maturity, sugarcane stalks were dissected to recover larvae. Larvae were weighed after a three-hour starvation period in an analytical balance.

All data were analyzed with generalized linear mixed models (SAS, PROC GLIMMIX). Oviposition data were analyzed with a negative binomial distribution and a log link function (Sileshi 2006, Stroup 2015). Larval weight data were analyzed with a Gaussian distribution. Models included cultivar as a fixed effect and replication as a random effect. The Kenward–Roger method was used to estimate denominator degrees of freedom. Tukey’s Honest Significant Difference (HSD) ( $\alpha=0.05$ ) test was used for mean separations.

**Neonate establishment experiment.** Nine of the same commercial cultivars (HoCP 85-845, HoCP 96-540, HoCP 00-950, L 01-299, HoCP 04-838, HoCP 09-804, L 11-183, L 12-201, and Ho 12-615) were evaluated for *D. saccharalis* neonate establishment in the greenhouse during fall 2018. Three internodes of each cultivar were planted on 10 Sept 2018, in five 60 L pots in a 1:2:1 mixture of sand, river silt, and peat moss (Premier Peat Moss Ltd, Riviere-du-Loup, Quebec, Canada). Plants were maintained under a photoperiod of Light (14 hours): Dark 10 (hours) at  $26 \pm 2$  °C. Pots were arranged in a completely randomized design with five replications each. Movement of larvae among pots was prevented by spacing pots at least 0.3 m apart. Plants were fertilized with 25 g Miracle-Gro® (24-8-16, N-P-K) and 4 g urea when the first internode was formed. Before the experiment started, pots were thinned to only one stalk per pot. On 1 Dec 2018, each plant was inoculated with *D. saccharalis* egg masses containing a range of 200–213 eggs by attaching wax paper strips containing eggs to the leaves of target internodes using 2.5-cm paper clips in a manner simulating natural oviposition behavior (White 1993, Wilson et al. 2012). The paper strips were removed fourteen days after larvae hatched, and the numbers of hatched and unhatched, presumably nonviable, eggs were counted under a microscope. Frass coming out of the

stem and visible entry holes were considered as confirmation of larval stalk entry. Boring success was calculated as the proportion of larvae entering stalks to the number of hatched inoculated eggs.

Data were analyzed with generalized linear mixed models (SAS, PROC GLIMMIX). Boring success data were analyzed with a binomial distribution and a logit link function. Models included cultivar as a fixed effect and replication as a random effect. The Kenward–Roger method was used to estimate denominator degrees of freedom, and Tukey’s HSD ( $\alpha=0.05$ ) was used for mean separations.

**Diet incorporation assay.** Three resistant cultivars (N-21, L 01-299, and HoCP 85-845), one susceptible cultivar (HoCP 96-540), and one experimental clone from the USDA-ARS recurrent selection program for resistance to the sugarcane borer (Ho 08-9003) were used to determine if there was an effect of plant tissue composition on *D. saccharalis* larval development and survival via a diet incorporation assay based on the methods of Meagher et al. (1996a). The experimental design was a completely randomized design with thirty replications. Sugarcane leaf sheaths attached to the 3<sup>rd</sup> leaf below the apical meristem were collected on 21 Jul 2020 from plants of each cultivar or experimental clones from the 2020 plant cane field trial previously described. Sheaths were stored in a freezer at -80°C until the initiation of the assay. A total of 162 frozen leaf sheaths was freeze-dried with a Virtis SP Scientific lyophilizer (Gardiner, NY, USA) for 72 hours. Lyophilized tissue was ground using a Waring grinder (Model WSG60, Waring Commercial, New Hartford, Conn. USA) for thirty seconds at 20,000 rpm. Ground tissue was passed through a 35-mesh screen (VWR Scientific, Seattle, WA, USA), and the resulting powder was stored in the freezer. During sugarcane borer artificial diet preparation (Southland Products Inc., Lake Village, AR, USA), two concentrations (100 and 250 mg powder/ml diet) of leaf sheath powder of each cultivar were added. An additional 39.2 or 97.9 ml of distilled water, respectively, was added to



the final mix (artificial diet + incorporated powder) to improve blending. A set of 30 larvae were put in an artificial diet with no incorporated tissue used as general control. Each diet cup contained 10 g of the mixed diet. One recently hatched neonate (12-hr old) was placed per plastic cup (Dart Container Corporation, Mason, MI) using a fine camel-hair brush. The cups were placed on trays and maintained under a photoperiod of Light (14 hours): Dark 10 (hours) at  $26^{\circ}\text{C} \pm 1$ ,  $60\% \pm 10\%$  RH in an insect chamber. Data collected included larval weight (after 14 days, with a 3-hour starvation period), days to pupation, and pupal weight. The sex of each pupa was determined following the procedure outlined by Butt and Cantu (1962). To normalize mortality data at 14 days, results were pooled into five replications consisting of six random larvae. A larva was considered dead if it did not respond to prodding. Data were corrected with Abbott's formula to compensate for mortality in the artificial diet control (Abbott 1925).

All data were analyzed with generalized linear mixed models (SAS, PROC GLIMMIX) using Gaussian distributions. Models included cultivar, tissue concentration, and their interaction as fixed effects and replication as a random effect. For the pupae data models, sex was added as a fixed effect along with all two- and three-way interactions. The Kenward–Roger method was used to estimate denominator degrees of freedom, and Tukey's HSD ( $\alpha=0.05$ ) was used for mean separations. Control measurements were not included in the analysis to have a cross-classified factorial.

### 2.3. Results

**Field experiments.** All injury parameters except relative survival differed among cultivars (Table 2.2). Percentage of bored internodes ranged from 3.7–27.7% in the 2018 plant cane and 2019 first ratoon trials and was significantly influenced by cultivar ( $F = 61.04$ ;  $df = 15, 128.0$ ;  $P < 0.001$ ) and the interaction of cultivar and crop year ( $F = 3.39$ ;  $df = 15, 128$ ;  $P < 0.001$ ), but not by

the crop year main effect ( $F = 1.45$ ;  $df = 1, 7.9$ ;  $P = 0.264$ ). Emergence per stalk was influenced by cultivar ( $F = 7.23$ ;  $df = 15, 120.0$ ;  $P < 0.001$ ), year ( $F = 37.37$ ;  $df = 1, 8.0$ ;  $P < 0.001$ ), and the interaction of cultivar and crop year ( $F = 4.0$ ;  $df = 15, 120.0$ ;  $P < 0.001$ ). Adult emergence ranged from 0.02–2.73 emergence holes/stalk. Emergence per stalk across cultivars in 2019 ( $1.4 \pm 0.14$  [SE]) was 7-fold higher relative to 2018 ( $0.2 \pm 0.14$  [SE]). Relative survival was 7-fold higher in 2019 ( $0.58 \pm 0.03$ [SE]) than in 2018 ( $0.08 \pm 0.03$ [SE]) ( $F = 115.41$ ;  $df = 1, 8.0$ ;  $P < 0.001$ ), but did not differ among cultivars ( $F = 1.48$ ;  $df = 15, 120.0$ ;  $P = 0.124$ ) or the interaction of cultivar and crop year ( $F = 1.39$ ;  $df = 15, 120.0$ ;  $P = 0.163$ ). Relative resistance ratio differed among the cultivars ( $F = 6.44$ ;  $df = 15, 128.0$ ;  $P < 0.001$ ), but was not influenced by year ( $F = 0.51$ ;  $df = 1, 128.0$ ;  $P = 0.476$ ) or the interaction [cultivar  $\times$  crop year] ( $F = 0.97$ ;  $df = 1, 128.0$ ;  $P = 0.490$ ).

Differences in the percentage of bored internodes ( $F = 52.39$ ;  $df = 17, 72.0$ ;  $P < 0.001$ ), the number of emergence holes/stalk ( $F = 7.11$ ;  $df = 17, 72.0$ ;  $P < 0.001$ ), and the relative resistance ratio ( $F = 6.42$ ;  $df = 17, 72.0$ ;  $P < 0.001$ ) were detected among the cultivars evaluated in 2020 (Table 2.3). Injury ranged from 3.6–29.4% bored internodes, adult emergence ranged from 0.12–2.07 emergence holes/stalk, and relative resistance ratio ranged from 0.26–0.84. Hardness of the target internode differed statistically among selected cultivars ( $F = 3.97$ ;  $df = 6, 24.0$ ;  $P = 0.007$ ). Internodes of cultivars HoCP 04-838 and HoCP 85-845 were approximately 1.5-fold harder than L 12-201 (Figure 2.1).

Table 2.2. *Diatraea saccharalis* injury, survival, and resistance classification among commercial and experimental sugarcane cultivars, St. Gabriel, Louisiana, 2018–2019.

Cultivar	Percentage of bored internodes (LS means $\pm$ SE) <sup>a,*</sup>		No. emergence holes/stalk (LS means $\pm$ 0.23 [SE]) <sup>*</sup>		Relative survival (LS means $\pm$ 0.042 [SE])	Relative Resistance Ratio (LS means $\pm$ 0.059 [SE]) <sup>*</sup>	Resistance Category
	2018	2019	2018	2019	2018-2019	2018-2019	
HoCP 00-950	21.3 $\pm$ 2.2a	27.7 $\pm$ 2.2a	0.38a	2.73a	0.343	0.719a	Susceptible
L 12-201	20.8 $\pm$ 1.9a	17.3 $\pm$ 1.7bcd	0.18a	1.88ab	0.324	0.622ab	Susceptible
Ho 13-708	18.8 $\pm$ 1.8ab	17.5 $\pm$ 1.7abcd	0.15a	1.78abc	0.299	0.591ab	Intermediate
HoCP 13-740	16.8 $\pm$ 1.6ab	19.2 $\pm$ 1.8abc	0.58a	1.8abc	0.353	0.684a	Susceptible
L 11-183	15.8 $\pm$ 1.6abc	20.1 $\pm$ 1.9ab	0.37a	2.02ab	0.372	0.681a	Susceptible
L 13-251	18.9 $\pm$ 1.8ab	16.5 $\pm$ 1.7bcd	0.20a	1.78abc	0.345	0.647ab	Susceptible
HoCP 13-758	13.1 $\pm$ 1.4abcd	16.2 $\pm$ 1.7bcd	0.12a	1.83ab	0.362	0.600ab	Susceptible
HoCP 09-804	12.2 $\pm$ 1.4abcd	12.8 $\pm$ 1.4b–f	0.27a	1.12bcd	0.314	0.534abc	Intermediate
HoCP 96-540	9.2 $\pm$ 1.2bcd	14.7 $\pm$ 1.6bcde	0.22a	1.40bcd	0.321	0.484abcd	Intermediate
Ho 13-739	12.2 $\pm$ 1.3abcd	10.1 $\pm$ 1.3cdef	0.27a	1.02bcd	0.339	0.556abc	Intermediate
Ho 12-615	8.4 $\pm$ 1.1bcd	8.8 $\pm$ 1.1def	0.12a	1.00bcd	0.356	0.431abcd	Intermediate
Ho 11-573	6.5 $\pm$ 0.9cd	9.5 $\pm$ 1.2cdef	0.10a	1.22bcd	0.398	0.447abcd	Intermediate
L 01-283	7.5 $\pm$ 1.1bcd	8.0 $\pm$ 1.1def	0.13a	0.77bcd	0.356	0.466abcd	Intermediate
L 01-299	6.7 $\pm$ 1.0cd	8.4 $\pm$ 1.1def	0.03a	0.80bcd	0.332	0.369bcd	Resistant
HoCP 04-838	3.7 $\pm$ 0.7d	5.7 $\pm$ 0.9ef	0.03a	0.55cd	0.265	0.281dc	Resistant
HoCP 85-845	4.1 $\pm$ 0.7d	4.5 $\pm$ 0.8f	0.02a	0.32d	0.217	0.194c	Highly Resistant

<sup>a</sup> Standard error for individual means reported for data analyzed with a binomial distribution.

\*Means which share a letter are not significantly different (Tukey's HSD,  $\alpha=0.05$ ).

Table 2.3. *Diatraea saccharalis* injury, survival, and resistance classification among commercial and experimental sugarcane cultivars plant cane, St. Gabriel, Louisiana, 2020.

Cultivar	Percentage of bored internodes (LS means $\pm$ SEM) <sup>a,*</sup>	No. emergence holes/stalk (LS means $\pm$ 0.21 [SE]) <sup>*</sup>	Relative survival (LS means $\pm$ 0.063 [SE])	Relative Resistance Ratio (LS means $\pm$ 0.073 [SE]) <sup>*</sup>	Resistance Category
HoCP 00-950	29.4 $\pm$ 1.5a	2.07a	0.431	0.844a	Highly Susceptible
L 12-201	29.0 $\pm$ 1.6a	1.77ab	0.387	0.806a	Highly Susceptible
Ho 15-971	27.4 $\pm$ 1.5a	1.15abc	0.284	0.694ab	Susceptible
L 11-183	22.6 $\pm$ 1.5ab	1.03abc	0.308	0.689ab	Susceptible
L 15-306	22.3 $\pm$ 1.4ab	1.08abc	0.299	0.678abc	Susceptible
L 14-267	16.5 $\pm$ 1.2bc	0.87bc	0.338	0.644a–d	Susceptible
HoCP 96-540	16.4 $\pm$ 1.2bc	0.90bc	0.287	0.556a–f	Intermediate
HoCP 14-885	15.6 $\pm$ 1.3c	0.45c	0.196	0.517a–f	Intermediate
HoL 15-508	15.1 $\pm$ 1.2cd	0.78bc	0.3	0.578a–e	Intermediate
HoCP 09-804	11.5 $\pm$ 1.1cde	0.42c	0.236	0.489a–f	Intermediate
Ho 12-615	10.0 $\pm$ 0.9de	0.63c	0.339	0.539a–f	Intermediate
Ho 13-739	9.9 $\pm$ 1.0de	0.58c	0.334	0.517a–f	Intermediate
L 01-283	9.7 $\pm$ 1.0de	0.45c	0.294	0.494a–f	Intermediate
N-21	9.4 $\pm$ 1.0e	0.25c	0.17	0.361b–f	Resistant
HoCP 04-838	4.8 $\pm$ 0.7f	0.20c	0.213	0.306cdef	Resistant
L 01-299	4.6 $\pm$ 0.7f	0.12c	0.132	0.194f	Highly Resistant
HoCP 85-845	3.6 $\pm$ 0.6f	0.13c	0.198	0.278def	Resistant
Ho 08-9003	3.6 $\pm$ 0.6f	0.12c	0.187	0.261ef	Resistant

<sup>a</sup> Standard error for individual means reported for data analyzed with a binomial distribution.

\*Means which share a letter are not significantly different (Tukey's HSD,  $\alpha=0.05$ ).

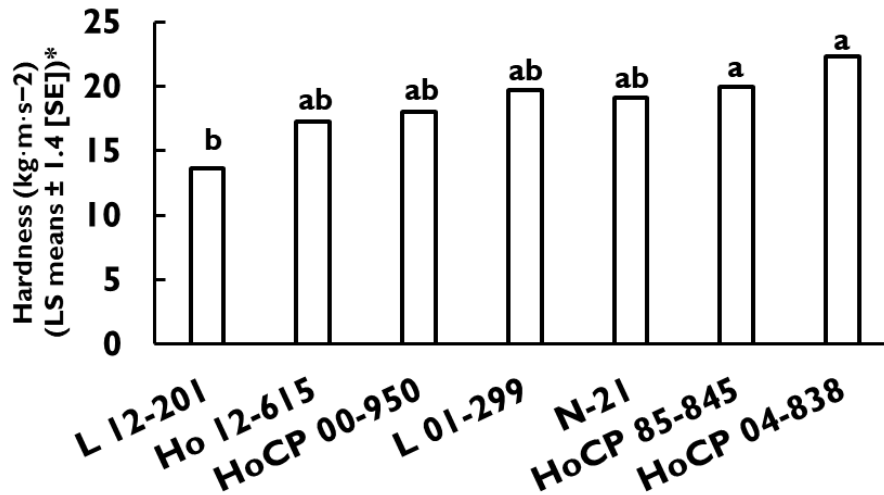


Figure 2.1. Hardness of the target internode from selected cultivars 2020. Columns that share a letter are not significantly different (Tukey's HSD,  $\alpha=0.05$ ).

**Oviposition Preference.** Throughout the experiment, a total of 2,985 eggs (mean:  $60.9 \pm 16.1$  [SE] eggs per plant) and 193 oviposition events (mean:  $3.9 \pm 1.0$  [SE] events per plant) were recorded. No eggs were recorded on cultivar HoCP 85-845 and N-21, and these cultivars were therefore removed from the statistical analyses. Differences were not detected among cultivars in the number of eggs per plant ( $F = 0.84$ ;  $df = 9, 35.0$ ;  $P = 0.581$ ), number of oviposition events per plant ( $F = 1.57$ ;  $df = 9, 35.0$ ;  $P = 0.162$ ), the number of eggs per oviposition event ( $F = 0.42$ ;  $df = 9, 39.0$ ;  $P = 0.918$ ), or recovered larvae weight ( $F = 1.77$ ;  $df = 10, 78.7$ ;  $P = 0.080$ ).

**Neonate Establishment.** Throughout the experiment, a total of 9,000 eggs and 3,446 hatched larvae were monitored for boring success. There was a significant effect of cultivar on boring success ( $F = 7.41$ ;  $df = 8, 36$ ;  $P < 0.001$ ), which ranged from 2.5–23.4%. Boring success was 3-fold greater in cultivars HoCP 96-540 and HoCP 00-950 than cultivars HoCP 04-838 and HoCP 85-845 and 9-fold greater in L 01-299 (Figure 2.2). No differences were detected among the cultivars in the percentage of hatched larvae ( $F = 0.42$ ;  $df = 8, 36.0$ ;  $P = 0.900$ ).

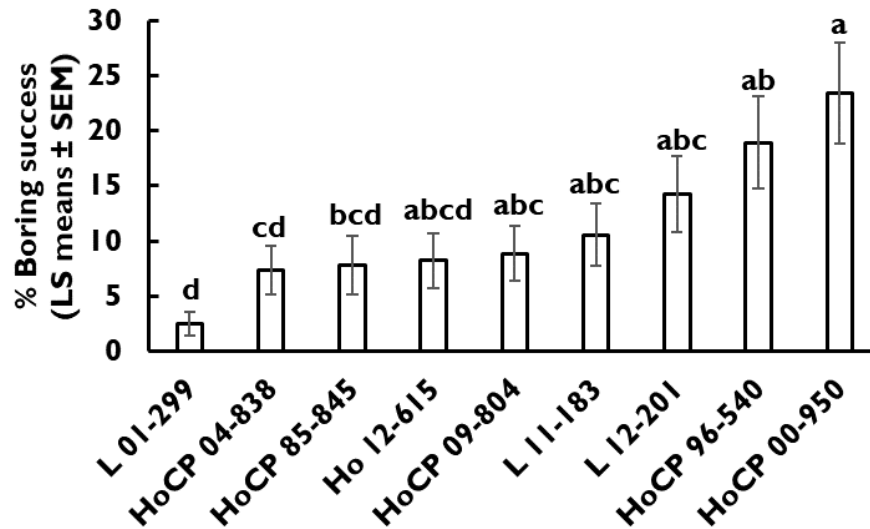


Figure 2.2. *Diatraea saccharalis* neonate establishment among commercial sugarcane cultivars. Columns that share a letter are not significantly different (Tukey's HSD,  $\alpha=0.05$ ).

Diet incorporation assays. Cultivar significantly affected *D. saccharalis* larval weight, pupal weight, and days to pupation. Leaf sheath tissue concentration affected larval weight and days to pupation (Table 2.4). High concentration (250 mg/ml) of leaf sheath tissue decreased larval weight and increased days to pupation. All larvae on the control diet survived to pupation. Larval weight ( $32.8 \pm 3.1$  mg [SEM]), pupal weight ( $80.9 \pm 3.8$  [SEM]), and days to pupation ( $30.8 \pm 1.3$ [SEM]) in the control was relatively similar to that of the other cultivars evaluated. Cultivar Ho 08-9003 had a 2.5-fold decrease in larval weight compared to the artificial diet-only control and HoCP 96-540, and a 3.5-fold decrease compared to HoCP 85-845. Time to pupation on the diet with cultivar Ho 08-9003 was on average ten days longer than in all the other cultivars evaluated. Cultivar had a significant effect on larvae mortality and survival to pupation. Mortality was influenced by cultivar and the interaction. Mortality of larvae was nearly 10-fold higher on cultivar Ho 08-9003 than on HoCP 85-845 and HoCP 96-540, but it did not differ among the other cultivars. Survival to pupation was not affected by leaf sheath concentration but was influenced by cultivar and the interaction, and it was two times higher in cultivar Ho 08-9003 than the other cultivars evaluated.

Table 2.4. *Diatraea saccharalis* development and survival (LS Means  $\pm$  SEM) as influenced by cultivar and leaf tissue dose

		Larval Weight (mg)	Pupal Weight (mg)	Days to pupation	Mortality at 15 days	Survival to pupation	
Dose	100	37.6 $\pm$ 1.2a	66.6 $\pm$ 1.9a	31.8 $\pm$ 0.8a	9.3 $\pm$ 3.4a	68.9 $\pm$ 5.2a	
	250	21.5 $\pm$ 1.4b	64.9 $\pm$ 2.5a	34.5 $\pm$ 1.0b	4.0 $\pm$ 3.4a	75.6 $\pm$ 5.2a	
<i>F</i> =		74.72	0.28	4.83	3.26	1.07	
df =		1, 216	1, 120	1, 120	1, 36.0	1, 36.0	
<i>P</i> =		<0.001	0.597	0.030	0.080	0.307	
Cultivar	HoCP 85-845	41.6 $\pm$ 2.5a	74.1 $\pm$ 3.6a	30.8 $\pm$ 1.4a	1.67 $\pm$ 4.3a	75.3 $\pm$ 7.6a	
	HoCP 96-540	35.8 $\pm$ 2.0a	63.3 $\pm$ 2.4ab	30.7 $\pm$ 0.9a	1.67 $\pm$ 4.3a	91.7 $\pm$ 7.6a	
	L 01-299	35.5 $\pm$ 1.9a	72.1 $\pm$ 2.3a	30.6 $\pm$ 0.9a	5.0 $\pm$ 4.3ab	87.3 $\pm$ 7.6a	
	N-21	23.6 $\pm$ 1.9b	66.0 $\pm$ 4.1ab	30.7 $\pm$ 1.6a	8.3 $\pm$ 4.3ab	67.0 $\pm$ 7.6ab	
	Ho 08-9003	11.1 $\pm$ 2.1c	53.1 $\pm$ 4.6b	43.0 $\pm$ 1.6b	16.67 $\pm$ 4.3b	40.0 $\pm$ 7.6b	
	<i>F</i> =		32.76	5.13	10.6	3.56	8.12
df =		4, 216	4, 120	4, 114.4	4, 36.0	4, 36.0	
<i>P</i> =		<0.001	<0.001	<0.001	0.015	<0.001	
Cultivar $\times$ Dose	N-21	100	33.1 $\pm$ 2.7b	72.1 $\pm$ 3.5a	27.7c	6.7 $\pm$ 5.4b	78.0 $\pm$ 10.5a
		250	14.2 $\pm$ 2.7cd	59.9 $\pm$ 7.5abc	33.7abc	10.0 $\pm$ 5.4ab	56.0 $\pm$ 10.5ab
	L 01-299	100	47.9 $\pm$ 2.6a	79.6 $\pm$ 2.9a	28.6c	6.7 $\pm$ 5.4b	93.3 $\pm$ 10.5a
		250	23.1 $\pm$ 2.7bc	64.7 $\pm$ 3.4bc	32.6bc	3.3 $\pm$ 5.4b	81.3 $\pm$ 10.5a
	HoCP 96-540	100	45.9 $\pm$ 2.6a	65.5 $\pm$ 3.3abc	28.2c	3.3 $\pm$ 5.4b	86.7 $\pm$ 10.5a
		250	25.7 $\pm$ 2.9bc	61.1 $\pm$ 3.4bc	33.3bc	0.0 $\pm$ 5.4b	96.7 $\pm$ 10.5a
	HoCP 85-845	100	54.1 $\pm$ 2.5a	69.2 $\pm$ 4.2abc	28.1c	3.3 $\pm$ 5.4b	57.3 $\pm$ 10.5ab
		250	29.1 $\pm$ 4.2bc	79.1 $\pm$ 5.9a	33.4bc	0.0 $\pm$ 5.4b	93.3 $\pm$ 10.5a
	Ho 08-9003	100	6.8 $\pm$ 3.0d	46.5 $\pm$ 6.6c	46.3a	30.0 $\pm$ 5.4a	29.3 $\pm$ 10.5b
		250	15.4 $\pm$ 2.9cd	59.7 $\pm$ 6.6abc	39.7ab	3.3 $\pm$ 5.4b	50.7 $\pm$ 10.5ab
	<i>F</i> =		11.3	3.45	2.35	3.51	2.72
	df =		4, 216	4, 120	4, 114.3	4, 36.0	4, 36.0
	<i>P</i> =		<0.001	0.010	0.058	0.016	0.044

\*Means which share a letter are not significantly different (Tukey's HSD,  $\alpha=0.05$ ).

## 2.4. Discussion

This work provides further support for the utility of cultivar resistance in *D. saccharalis* IPM and offers new insights into resistance mechanisms in modern Louisiana sugarcane cultivars. This study provides a comprehensive examination of sugarcane resistance to *D. saccharalis*, with the same cultivars evaluated in field trials, choice and no choice greenhouse experiments, and diet incorporation assays. Collectively, our data suggest that multiple resistance mechanisms are likely present in the current Louisiana sugarcane cultivars. Additionally, this research evaluated *D. saccharalis* resistance among experimental clones in advanced stages of the sugarcane breeding program providing the first evaluations for recently released cultivars Ho 12-615, Ho 13-739, and L 12-201 in addition to the South African cultivar, N-21, which has been utilized in Louisiana sugarcane crossing efforts.

Results from field trials are consistent with previous studies demonstrating a wide range of *D. saccharalis* susceptibility among Louisiana sugarcane varieties. The 5–8-fold reduction in *D. saccharalis* injury in resistant relative to susceptible cultivars observed in field studies reported herein is similar to injury reductions in other recent studies (Wilson et al. 2015a, 2018b, 2021a). However, this injury reduction is greater than the 2–3-fold reductions reported in older studies (Martin et al. 1975, White and Hensley 1987, Bessin et al. 1990b, White et al. 2008). This suggests the level of resistance achieved in Louisiana cultivars has increased in recent years, though susceptibility in some cultivars remains high. This increased level of resistance has been one of the factors attributed to aiding in the substantial decline in insecticide use over the past 20 years (Wilson et al. 2020). Our results provide further support for the presence of *D. saccharalis* resistance in HoCP 85-845 (Wilson et al. 2012, Wilson et al. 2015a), HoCP 04-838 and L 01-299 (Gravois et al. 2011, Todd et al. 2018, Wilson et al. 2021a). Similarly, the high level of



susceptibility of HoCP 00-950 in both field and greenhouse experiments agrees with previous studies (Wilson et al. 2012, Wilson et al. 2015a). A range of susceptibility remains in the newest developed sugarcane cultivars, with L 12-201 and L 11-183 ranking as susceptible or highly susceptible, while Ho 12-615 was ranking as intermediate. None of the experimental clones were classified as resistant, however, suggesting future cultivars may not obtain the same reductions in injury and will need to be closely monitored for borer pressure. These results highlight the importance of screening new cultivars before their commercial release.

Our results also provide further support for borer resistance in cultivars N-21 and Ho 08-9003. N-21 has previously shown resistance to the Crambid stem borers, *Eldana saccharina* (Conlong et al. 2004) and *Eoreuma loftini* (Wilson et al. 2015a), but this is the first record of *D. saccharalis* resistance. Ho 08-9003 has previously been shown to be resistant to *D. saccharalis* (White et al. 2011) and *E. loftini* (Wilson et al. 2015a). While these cultivars are not suitable for commercial production in Louisiana, their role in Louisiana's commercial breeding program could be increased. While the presence of *D. saccharalis* resistance among commercial cultivars in Louisiana appears consistent, the mechanisms behind this resistance are more complex. Although no clear oviposition preferences were identified, the complete lack of oviposition on HoCP 85-845 and N-21 suggests resistance demonstrated by these cultivars in field studies may be in part because of non-preference by ovipositing females. The lack of differences among cultivars in the oviposition experiment contrasts with the large differences in *D. saccharalis* injury in the field trial. Other studies have reported differences in *D. saccharalis* oviposition preference among Brazilian cultivars (Dinardo-Miranda et al. 2012, Pimentel et al. 2017). Brazilian cultivars used in these studies were pubescent, which has previously been reported to affect *D. saccharalis* oviposition behavior (Sosa 1988, 1990). However, other studies in corn (*Zea mays* L.) have

reported no clear association between pubescence content and *D. saccharalis* oviposition preference (Greco et al. 1998). Future studies should compare Louisiana's cultivars to Brazilian cultivars with known oviposition non-preference.

Results from the no-choice neonate establishment experiment better align with field observations than oviposition results. The low boring success recorded on cultivars L 01-299, HoCP 04-838, and HoCP 85-845 indicates their resistance likely comes from reduced neonate establishment, which has previously been reported to be effective against stem borers (White 1993, Wilson et al. 2012). This is also supported by the relatively high boring success in cultivars that sustained a greater injury in field trials (HoCP 00-950, HoCP 96-540, and L 12-201). The levels of force required to puncture the stalks of cultivar HoCP 04-838 and HoCP 85-845 in our study suggest resistance to *D. saccharalis* might come in part from rind hardness. High rind hardness of cultivars HoCP 04-838 and HoCP 85-845 and low rind hardness of cultivar L 12-201 agree with field results and boring success. This is consistent with previous studies that showed that higher rind hardness confers resistance to *D. saccharalis* (Martin et al. 1975, White et al. 2006). Direct comparison with rind hardness levels in those studies cannot be made because of different measurement techniques. Not all results from the neonate establishment experiment agree with rind hardness values. L 01-299 (resistant) was not among the cultivars with high rind hardness, and HoCP 00-950 (susceptible) was not among those clones with low rind hardness, which suggests that other factors such as leaf sheath tightness (not measured in this study) may also play a role in neonate establishment. Previous studies have suggested leaf sheath tightness as a resistance mechanism (Coburn and Hensley 1972), but tightness was not quantified. Future studies of stem borer resistance mechanisms should attempt to devise a measurement of leaf sheath tightness.

The lack of differences among commercial cultivars observed in the diet incorporation assay suggests nutritional factors play a minimal role in conferring resistance for commercially grown cultivars. The addition of leaf sheath tissue to artificial diet only slightly reduced survival relative to diet-only controls, suggesting the antibiotic properties of sheath tissue is minimal. Indeed, adding 100 mg/ml of leaf sheath tissue to the normal sugarcane borer rearing diet had a positive effect on *D. saccharalis* growth and development, while adding 250 mg/ml had a negative effect on *D. saccharalis*, which suggests the diet used to rear *D. saccharalis* might gain some missing nutrients or feeding stimulants from the leaf sheath tissue, but that some compounds may become detrimental at higher doses. In contrast to commercial cultivars, Ho 08-9003 negatively affected *D. saccharalis* development in diet incorporation assays suggesting its leaf sheath material might be of poor quality for the insect. This is in agreement with previous studies demonstrating that Ho 08-9003 expresses antibiosis to *D. saccharalis* (White et al. 2011). Future studies should examine the potential antibiotic properties of leaf tissue from this cultivar to determine if these attributes can be bred into commercial germplasm.

A wide range of borer resistance levels remains in Louisiana's sugarcane breeding program. Factors deterring neonate establishment appear to be the most important in conferring *D. saccharalis* resistance in commercial cultivars, but our results suggest oviposition non-preference and antibiosis may contribute in some cultivars. These differences in findings for specific cultivars suggest more research into plant characteristics associated with resistance is needed to improve the utilization of this management strategy. Continuous evaluation of cultivar resistance to *D. saccharalis* is essential in developing improved host plant resistance to this pest in Louisiana. Future studies should also examine the heredity of resistance traits so cultivars expressing resistance can be better utilized as parents in sugarcane breeding programs.

## **Chapter 3. Resistance to the Mexican rice borer (Lepidoptera: Crambidae) in Louisiana sugarcane cultivars**

### 3.1. Introduction

The Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae), is the most significant insect attacking sugarcane in Texas and is increasing in economic importance in Louisiana, where it is predicted to cause annual revenue losses as high as \$220 million by 2035 (Reay-Jones et al. 2008, Showler 2019). *E. loftini* has been expanding eastward and northward into Louisiana sugarcane and rice production regions since 2008, and infestations have been reported with higher frequency in recent years (Wilson et al. 2015b).

One of the most promising management tactics for *E. loftini* is cultivar resistance, but resistance mechanisms are not fully understood (Pfannenstiel and Meagher 1991, Meagher et al. 1996a, Reay-Jones et al. 2003, Wilson et al. 2015a). Resistant cultivars can improve other management strategies by enhancing larval exposure to insecticides and natural enemies (Wilson et al. 2012). However, mechanisms of resistance in sugarcane against *E. loftini* have been less studied than *D. saccharalis* mechanisms. Cultivars with resistance to *D. saccharalis* are not always resistant to *E. loftini*. Cultivar HoCP 04-838, which is resistant to *D. saccharalis*, is susceptible to *E. loftini* (Wilson et al. 2015a, 2021a; Todd et al. 2018). Plant physical characteristics are important to host selection by *E. loftini* (VanWeelden et al. 2017). Oviposition preference for dry leaves is thought to be the key difference between *D. saccharalis* and *E. loftini*. Reay-Jones et al. (2007) showed a 1.8-fold increase in *E. loftini* oviposition events per plant on drought-stressed sugarcane than well-watered sugarcane. *E. loftini* eggs have been reported to be laid in folds of dry leaves, and leaf sheaths (Showler and Castro 2010). Some genotypes may differ in nutritional content or presence of secondary metabolites, which can have an antibiotic effect on *E. loftini* larval development (Meagher et al. 1996). Factors that affect neonate establishment and survival,

such as rind hardness (Martin et al. 1975) and leaf sheath tightness (Coburn and Hensley 1972), can also be present in resistant cultivars to *E. loftini* (Wilson et al. 2012). Continuous evaluations of sugarcane cultivars and their resistance mechanisms are important to incorporating resistant cultivars into stem borer IPM programs. Therefore, the objectives of this study were (1) evaluate sugarcane cultivar resistance to *E. loftini* in the field, (2) examine *E. loftini* oviposition preference among sugarcane cultivars, (3) measure the effect of sugarcane cultivars on *E. loftini* neonate establishment, and (4) determine if a nutritional or plant metabolite effect exists on *E. loftini* larval development.

### 3.2. Materials and methods

**Field screening for resistance.** A field trial was conducted at the Texas A&M AgriLife Beaumont Research Center in Beaumont, Texas, to screen for resistance against *E. loftini*. All cultivars were planted on 11 Sep 2019, at the Texas A&M AgriLife Beaumont Research Center in Beaumont, Texas, in a randomized block design with five replications. Previous studies have demonstrated that high *E. loftini* pest pressure is typically present at this site; thus, plots were exposed to naturally occurring infestations throughout the season (VanWeelden et al. 2015, 2016, Wilson et al. 2015a, Lama et al. 2018). The experiment was conducted in one-row plots measuring 4.9-m (8.9-m<sup>2</sup>), with 1.5-m gaps between. Weed management, fertilization, and other production practices were done according to LSU AgCenter recommendations (Gravois et al. 2014).

The 2020 (plant cane) trial included ten commercial cultivars (HoCP 85-845, HoCP 96-540, HoCP 00-950, L 01-283, L 01-299, HoCP 04-838, HoCP 09-804, L 12-201, Ho 12-615, and Ho 13-739) and two experimental clones (L 14-267 and HoCP 14-885). HoCP 85-845 and HoCP 00-950 were included as *E. loftini*-resistant and susceptible standards, respectively (Reay-Jones et

al. 2003; Wilson et al. 2012, 2015a, 2021a; VanWeelden, et al. 2015). Bored internode data were collected and analyzed as previously described for studies involving *D. saccharalis*.

**Oviposition preference.** Moth preference for oviposition was assessed in greenhouse trials following the procedures previously described for *D. saccharalis*. The experiment was planted on August 18, 2020, in a 1:1:1 of the previous potting soil mixture described for *D. saccharalis* greenhouse experiments. Internodes of resistant cultivars (HoCP 85-845, L 01-299, and N-21), susceptible cultivars (HoCP 00-950 and HoCP 04-838), and commercial cultivars (Ho 12-615 and L 12-201) were planted and maintained as previously described (Chapter 2). Moth release, borer injury data collection, and other procedures were conducted as previously described for the *D. saccharalis* experiment. Data were analyzed as previously described (Chapter 2).

**Neonate establishment.** Resistant cultivars (HoCP 85-845, L 01-299, and N-21), susceptible cultivars (HoCP 00-950 and HoCP 04-838), and commercial cultivars (Ho 12-615 and L 12-201) were used to evaluate *E. loftini* neonate establishment during 2020. Internodes for planting were obtained from the Louisiana State Agricultural Center Sugar Research Station, St. Gabriel, LA. Three internodes of each cultivar were planted on August 18, 2020, in five 60 L pots (each pot was considered as one repetition) in a 1:1:1 potting soil mixture of the previous mix described for *D. saccharalis* greenhouse experiments. On 25 Jan 2021, each plant was inoculated with *E. loftini* egg masses containing a range of 137–208 (average: 173.0) eggs as previously described for *D. saccharalis*, and establishment recorded as previously described. Plant physical characteristics such as the number of green and dry leaves, stalk diameter (third internode from the base of the plant), and plant height (soil to top visible dewlap) were recorded before insect inoculation. Data were analyzed as previously described (Chapter 2).

**Antibiotic effect on cultivars.** Four resistant cultivars (L 01-299, HoCP 85-845, Ho 08-9003, and N-21) and two susceptible cultivars (HoCP 00-950 and HoCP 04-838) were used to determine if antibiotic compounds or nutritional factors affected the biology of *E. loftini*. Methods for diet incorporation assay previously described for *D. saccharalis* were used for *E. loftini*. Data were analyzed as previously described (Chapter 2).

### 3.3. Results

**Field experiments.** A range of borer injury was recorded in the 2020 plant cane trials (Figure 3.1.). Percentage of bored internodes ranged from 6.3–31.9% and were influenced by cultivar ( $F = 49.84$ ;  $df = 11, 48$ ;  $P < 0.001$ ). Cultivar HoCP 85-845 and HoCP 04-838 received approximately 3-fold less injury than cultivars HoCP 00-950, HoCP 09-804, L 12-201 and HoCP 14-885.

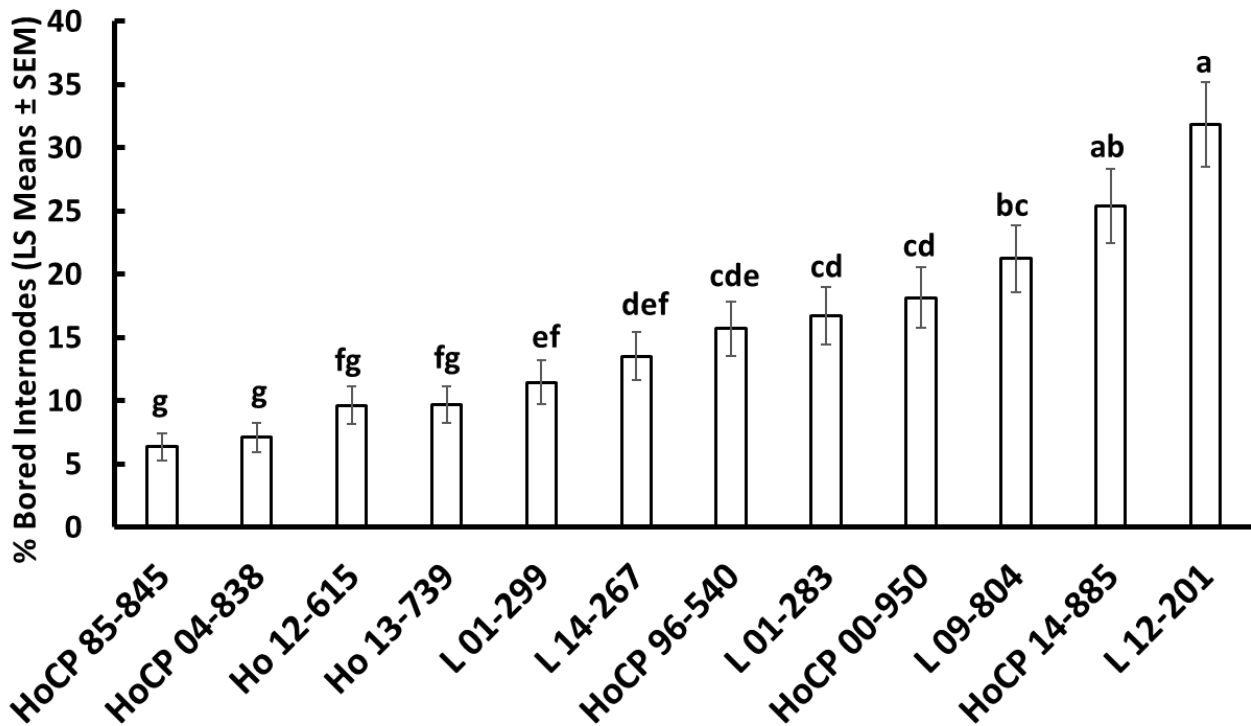


Figure 3.1. *Eoreuma loftini* injury among commercial and experimental sugarcane cultivars, Beaumont, Texas, 2020. Columns that share a letter represent means that do not significantly differ (Tukey’s HSD,  $\alpha=0.05$ ). Standard error for individual means reported for data analyzed with a binomial distribution.

**Oviposition Preference.** Throughout the experiment, a total of 1,329 eggs (mean:  $38.0 \pm 6.5$  [SE] eggs per plant) and 83 oviposition events (mean:  $2.4 \pm 0.5$  [SE] events per plant) were recorded. Differences were not detected among cultivars in the number of eggs per plant ( $F = 0.12$ ;  $df = 6, 24.0$ ;  $P = 0.992$ ), number of oviposition events per plant ( $F = 1.48$ ;  $df = 6, 24.0$ ;  $P = 0.228$ ), and the number of eggs per oviposition event ( $F = 0.52$ ;  $df = 6, 24.0$ ;  $P = 0.786$ ). No significant differences in plant height ( $F = 1.04$ ;  $df = 6, 24.0$ ;  $P = 0.425$ ), internode diameter ( $F = 1.41$ ;  $df = 6, 28.0$ ;  $P = 0.246$ ), number of green leaves ( $F = 0.76$ ;  $df = 6, 24$ ;  $P = 0.605$ ), and dry leaves ( $F = 1.35$ ;  $df = 6, 24.0$ ;  $P = 0.2736$ ) among cultivars were observed.

**Neonate Establishment.** A total of 6,000 eggs and 2,408 hatched neonates were monitored for boring success in this experiment. Percentage of boring success was affected by cultivar ( $F = 4.51$ ;  $df = 6, 28.0$ ;  $P = 0.003$ ), which ranged from 4.5–11.7%. Boring success was 1.5-fold greater in cultivars L 12-201, HoCP 00-950, and HoCP 04-838 relative to Ho 12-615. Other cultivars had intermediate levels of injury. No differences were detected among the cultivars evaluated in the percentage of hatched larvae ( $F = 0.22$ ;  $df = 6, 28.0$ ;  $P = 0.968$ ), plant height ( $F = 1.54$ ;  $df = 6, 24.0$ ;  $P = 0.209$ ), internode diameter ( $F = 1.27$ ;  $df = 6, 28.0$ ;  $P = 0.304$ ), number of green leaves ( $F = 1.20$ ;  $df = 6, 24.0$ ;  $P = 0.338$ ), number of dry leaves ( $F = 1.03$ ;  $df = 6, 24.0$ ;  $P = 0.431$ ), or the number of internodes among cultivars were recorded ( $F = 1.35$ ;  $df = 6, 28.0$ ;  $P = 0.268$ ).



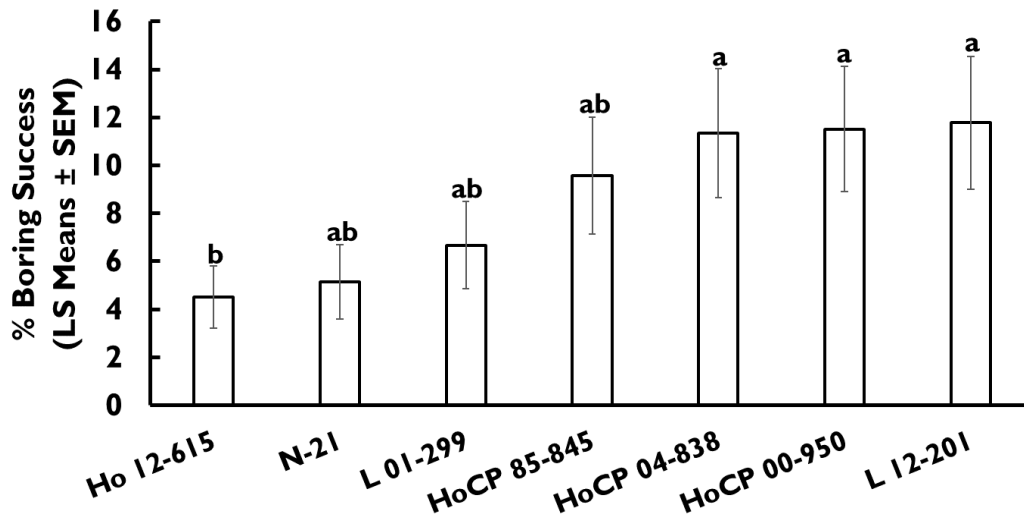


Figure 3.2. *Eoreuma loftini* neonate establishment among commercial sugarcane cultivars. Columns that share a letter are not significantly different (Tukey's HSD,  $\alpha=0.05$ ). Standard error for individual means reported for data analyzed with a binomial distribution.

**Diet incorporation assays.** All the diet cups that had the 250 mg powder/ml diet concentration were contaminated by fungi, which has previously been reported to be a problem while rearing stem borers (Hensley and Hammond 1968). Therefore, all the diet cups contaminated by fungi were not included in the analysis. The cultivars evaluated reduced larval weight by 59.2–86.5% relative to the artificial diet control but were not significantly different from each other ( $F = 12.33$ ;  $df = 6, 146.5$ ;  $P < 0.001$ ; Figure 3.3).

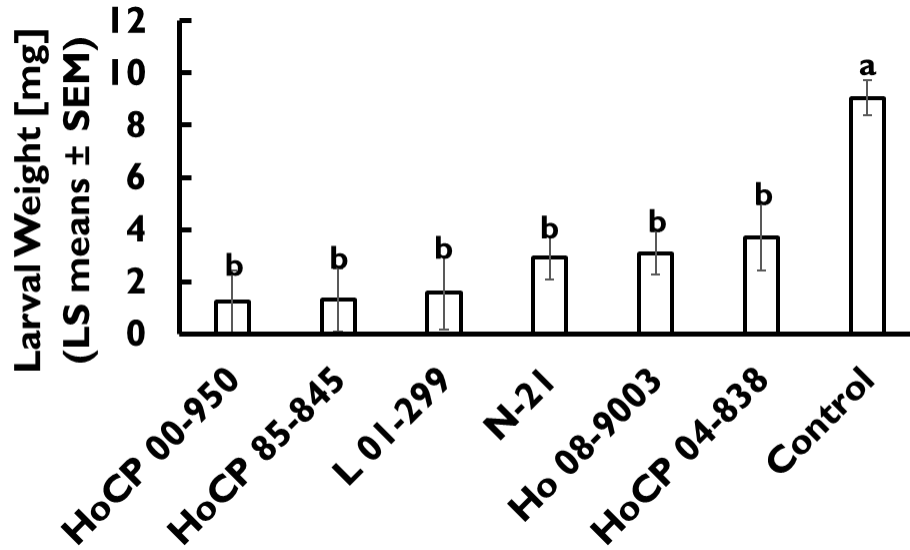


Figure 3.3. *Eoreuma loftini* larval weight as influenced by cultivar. Columns that share a letter are not significantly different (Tukey's HSD,  $\alpha=0.05$ ).

#### 3.4. Discussion

This work provides new insights into cultivar resistance to *E. loftini* in sugarcane and further supports the importance of factors that affect neonate establishment in stem borer resistance. Results from this study are consistent with previous studies suggesting different mechanisms of resistance exist in sugarcane cultivars (Meagher et al. 1996a). This study provides new resistance evaluations for recently released cultivars Ho 12-615, L 12-201, and Ho 13-739 and advanced experimental clones HoCP 14-885 and L 14-267 for the utility of cultivar resistance in *E. loftini* IPM.

Our results indicate a range of resistance to *E. loftini* exists among currently planted Louisiana sugarcane cultivars. Cultivar HoCP 85-845 can still be considered as the standard for resistance in field and greenhouse studies as seen previously (Reay-Jones et al. 2003, Wilson et al. 2012, 2015a, VanWeelden et al. 2015). However, cultivar HoCP 04-838, known as to be susceptible to *E. loftini* (Wilson et al. 2015a), was as resistant as HoCP 85-845 in our field experiment. Also, cultivar HoCP 09-804, which was previously reported as a moderately resistant cultivar (Lama et al. 2018),

was among the most susceptible to *E. loftini*. The variability of field results suggests that a genotype  $\times$  environment interaction might affect the evaluation of resistance (Wilson et al. 2021a). Recently released cultivars L 12-201 and HoCP 14-885 were among the most susceptible to *E. loftini*, while Ho 12-615 and Ho 13-739 were among the most resistant to *E. loftini*. This suggests a range of resistance levels remains present in sugarcane breeding germplasm and highlights the importance of screening cultivars before commercial release.

The absence of differences among cultivars in the oviposition experiment might be related to the lack of differences in plant physical characteristics (e.g. dry leaves) that have been reported to influence *E. loftini* oviposition behavior (Meagher et al. 1996a, Reay-Jones et al. 2007, VanWeelden et al. 2017). However, results from the neonate establishment experiment agree with field observations and previous studies (White 1993, Wilson et al. 2012). Low boring success recorded on cultivars Ho 12-615, N-21, L 01-299, and HoCP 85-845 suggests their resistance comes from reduced neonate establishment. Additionally, cultivars that sustained greater injury in field trials (HoCP 00-950 and L 12-201) also showed high boring success, suggesting that factors such as pubescence and leaf sheath tightness may also play a role in neonate establishment though these factors were not measured in this study. L 12-201 is highly pubescent on the outside of leaf sheaths (Pontif et al. 2019), but is highly susceptible, suggesting pubescence must occur at larval feeding sites to confer resistance. The reduction of larval weight by 59.2–86.5% relative to the artificial diet control observed in the diet incorporation assay suggests nutritional factors might play a role in conferring resistance, as seen in other studies (Meagher et al. 1996a).

Differences in findings for specific cultivars suggest more research into plant characteristics associated with resistance and environmental variation in resistance are needed to improve the utilization of this management strategy.

## Summary and Conclusions

The management of stem borers (*D. saccharalis* and *E. loftini*) in Louisiana sugarcane has relied on IPM programs involving a balance of multiple control tactics (Reagan and Mulcahy 2019, Showler 2019). Cultivar resistance is a vital part of sugarcane IPM that has reduced reliance on insecticide applications (Hensley 1971, Reagan 2001, Wilson et al. 2015a). However, the mechanisms underlying resistance are not always clear, and a better understanding of the mechanisms that impart resistance in sugarcane to the stem borer complex could help the sugarcane breeders develop cultivars with resistance levels with desirable agronomic traits. Therefore, this research evaluated the potential mechanisms of cultivar resistance to *D. saccharalis* and *E. loftini* among sugarcane cultivars and experimental clones through a series of field screenings, greenhouse trials, and diet incorporation assays.

Results suggest the level of resistance present in Louisiana cultivars has increased in recent years, but susceptibility in some newly released cultivars remains high. The resistant standard HoCP 85-845 and L 01-299 were among the cultivars with the lowest borer injury levels in both field and greenhouse trials for both species. HoCP 00-950, L 12-201, and HoCP 14-885 were among the most heavily injured in field trials of both species.

This research indicates that factors that reduce neonate establishment confer resistance in sugarcane to both stem borer species (Lepidoptera: Crambidae) because these results better agree with field observations than oviposition results. Low internode boring success recorded on L 01-299, Ho 12-615, and HoCP 85-845 indicates their resistance likely comes from reduced neonate establishment, which has previously been reported to be effective against stem borers (White 1993, Wilson et al. 2012). This is also supported by the relatively high internode boring success in cultivars that sustained a greater injury in field trials (HoCP 00-950 and L 12-201). Further, the

levels of force required to penetrate the stalk internode rind of cultivar HoCP 04-838 and HoCP 85-845 in our study suggest resistance to *D. saccharalis* in these cultivars might come from rind hardness. Future studies of stem borer resistance mechanisms should attempt to devise a measurement of leaf sheath tightness and pubescence.

Stem borer resistance among commercial cultivars in Louisiana appears to be species-specific in some cultivars (Wilson et al. 2021a, Zhou et al. 2010), because of the complexity of the mechanisms behind this resistance. Continuous evaluation of cultivar resistance to *D. saccharalis* and *E. loftini* is important in developing effective IPM strategies for these pests.

## Appendix A. Sugarcane Borer Varietal Resistance Trial 2018–2019 - Plot Plan

**Cultivars**

Resistant: L 01-299 and HoCP 85-845  
 Susceptible: HoCP 96-540 and HoCP 00-950  
 Others: HoCP 04-838, L 01-283, HoCP 09-804

**2011 Experimental Clones**

L 11-183                      Ho 11-573

**2012 Experimental Clones**

L 12-201                      Ho 12-615

**2013 Experimental Clones**

L 13-251                      Ho 13-708  
 Ho 13-739                      HoCP 13-740  
 HoCP 13-758

	Rep									
Corn	V	L 13-251	L 12-201	HoCP 09-804	HoCP 00-950	← 416 ft →				
		Ho 12-615	HoCP 04-838	Ho 13-739	HoCP 13-758					
		Ho 13-708	HoCP 85-845	L 11-183	Ho 11-573					
		L 01-283	HoCP 13-740	HoCP 96-540	L 01-299					
	IV	HoCP 96-540	L 13-251	Ho 12-615	HoCP 04-838					
		HoCP 13-740	HoCP 85-845	Ho 13-708	HoCP 13-758					
		L 11-183	Ho 13-739	Ho 11-573	L 12-201					
		L 01-299	HoCP 09-804	HoCP 00-950	L 01-283					
	III	HoCP 00-950	L 01-283	HoCP 09-804	L 13-251					
		Ho 13-739	L 12-201	L 01-299	HoCP 13-740					
		Ho 11-573	Ho 13-708	HoCP 13-758	HoCP 85-845					
		HoCP 96-540	HoCP 04-838	Ho 12-615	L 11-183					
	II	L 01-299	L 11-183	HoCP 04-838	L 01-283					
		HoCP 00-950	HoCP 13-758	Ho 13-708	HoCP 96-540					
		HoCP 13-740	L 13-251	Ho 11-573	Ho 13-739					
		HoCP 85-845	HoCP 09-804	L 12-201	Ho 12-615					
	I	Ho 11-573	Ho 13-739	L 01-299	HoCP 85-845					
		HoCP 09-804	Ho 12-615	L 11-183	L 12-201					
		L 13-251	HoCP 00-950	HoCP 13-740	Ho 13-708					
		HoCP 13-758	HoCP 04-838	L 01-283	HoCP 96-540					
13 Rows										

Plot size = 2 rows x 16-ft (0.004 acres) with 4-ft alleys

Randomized Block Design with 5 replications

## Appendix B. Sugarcane Borer Varietal Resistance Trial 2019–2020 - Plot Plan

**Cultivars**

Resistant: L 01-299, HoCP 85-845, N-21, Ho 08-9003

Susceptible: HoCP 96-540, HoCP 00-950, L 12-201

Others: HoCP 04-838, L 01-283, HoCP 09-804, Ho 12-615, L 11-183,

**2013 Experimentals**

Ho 13-739

**2014 Experimentals**

HoCP 14-885            L 14-267

**2015 Experimentals**

HoL 15-508    Ho 15-971    L 15-306

← River Road →

		Approx. 40 ft. Buffer							
Corn	L 12-201	Ho 08-9003	HoCP 00-950	Corn	HoCP 96-540	Ho 15-971	HoCP 04-838	Rep V	
	L 15-306	HoCP 09-804	L 14-267		HoCP 85-845	L 01-299	HoL 15-508		
	L 11-183	Ho 13-739	HoCP 14-885		Ho 12-615	N-21	L 01-283		
	HoCP 96-540	HoCP 04-838	L 01-283		HoL 15-508	L 12-201	Ho 13-739		
	L 14-267	L 01-299	Ho 12-615		HoCP 00-950	L 15-306	HoCP 09-804		Rep IV
	HoCP 14-885	Ho 15-971	HoCP 85-845		N-21	Ho 08-9003	L 11-183		
	L 01-299	L 12-201	Ho 15-971		HoCP 85-845	Ho 12-615	HoL 15-508	Rep III	
	Ho 13-739	N-21	HoCP 00-950		HoCP 14-885	HoCP 09-804	HoCP 96-540		
	L 11-183	L 01-283	L 15-306		Ho 08-9003	HoCP 04-838	L 14-267		
	Ho 12-615	HoCP 09-804	HoL 15-508		L 01-299	Ho 13-739	HoCP 04-838	Rep II	
	HoCP 00-950	L 14-267	HoCP 96-540		L 01-283	L 12-201	Ho 15-971		
	L 15-306	HoCP 14-885	N-21		L 11-183	HoCP 85-845	Ho 08-9003		
	L 15-306	L 12-201	Ho 15-971		L 14-267	HoCP 96-540	Ho 12-615	Rep I	
	L 01-299	Ho 13-739	HoCP 04-838		HoCP 00-950	HoL 15-508	HoCP 09-804		
HoCP 14-885	Ho 08-9003	L 11-183	HoCP 85-845	N-21	L 01-283				
1	2	3	4	5	6	7	8	9	

← Hwy 30 →

Plot size = 1 rows x 23-ft (0.002 acres) with 5-ft alleys  
 Randomized Block Design with 5 replications  
 Planted 9/3/2019 2 stalk lap, 10 stalks per plot.

## Appendix C. Mexican Rice Borer Trial 2019–2020 - Plot Plan

Commercial varieties:

Resistant: L 01-299 HoCP 85-845

Susceptible: HoCP 04-838 HoCP 96-540 HoCP 00-950

Others: HoCP 09-804 Ho 12-615 L 12-201 L 01-283 Ho 13-739

Experimentals:

HoCP 14-885 L 14-267

College Drive/Station Main Building							
↑  210 ft  ↓	L 01-299	HoCP 96-540	L 01-283	Ho 13-739	L 12-201	HoCP 85-845	Rep V
	HoCP 00-950	HoCP 04-838	HoCP 09-804	Ho 12-615	HoCP 14-885	L 14-267	
	HoCP 04-838	HoCP 85-845	HoCP 00-950	HoCP 09-804	Ho 12-615	L 12-201	Rep IV
	Ho 13-739	L 01-299	HoCP 96-540	HoCP 14-885	L 01-283	L 14-267	
	HoCP 14-885	HoCP 85-845	L 12-201	L 01-283	HoCP 96-540	Ho 12-615	Rep III
	HoCP 00-950	Ho 13-739	HoCP 09-804	L 14-267	HoCP 04-838	L 01-299	
	HoCP 00-950	Ho 12-615	L 01-299	Ho 13-739	HoCP 85-845	L 14-267	Rep II
	HoCP 09-804	L 01-283	HoCP 96-540	HoCP 14-885	L 12-201	HoCP 04-838	
	L 01-299	HoCP 14-885	L 12-201	HoCP 09-804	L 14-267	L 01-283	Rep I
	Ho 12-615	HoCP 00-950	HoCP 85-845	Ho 13-739	HoCP 96-540	HoCP 04-838	

Plot size: One-row plots, 16 ft (0.0022 acres), 5-foot gaps

Planted on 9/11/2019



## Appendix D. Cultivars and Experimental Lines Information

	Status	Registration Article	DOI
HoCP 85-845	Cultivar	Crop Sci. 34: 820–820.	10.2135/cropsci1994.0011183X003400030043x
HoCP 96-540	Cultivar	Crop Sci. 45: 785–786.	10.2135/cropsci2005.0785a
HoCP 00-950	Cultivar	J. Plant Regist. 3: 42–50.	10.3198/jpr2008.07.0430crc
L 01-283	Cultivar	J. Plant Regist. 4: 183–188.	10.3198/jpr2009.10.0638crc
L 01-299	Cultivar	J. Plant Regist. 5: 191–195.	10.3198/jpr2010.04.0194crc
HoCP 04-838	Cultivar	J. Plant Regist. 12: 324–332.	10.3198/jpr2017.10.0069crc
Ho 08-9003	Germplasm Clone	J. Plant Regist. 5: 248–253.	10.3198/jpr2010.07.0429crg
HoCP 09-804	Cultivar	J. Plant Regist. 13: 161–169.	10.3198/jpr2017.08.0052crc
Ho 11-573	Cultivar	J. Plant Regist. 13: 187–192.	10.3198/jpr2018.10.0066crc
L 11-183	Cultivar	In Progress	
L 12-201	Cultivar	In Progress	
Ho 12-615	Cultivar	In Progress	
Ho 13-739	Cultivar	In Progress	
N-21	Cultivar	Proc. S. Afr. Sug. Technol. Ass. 68: 28-30	10.1.1.439.9835
L 13-251		Experimental Line	
Ho 13-708		Experimental Line	
HoCP 13-740		Experimental Line	
HoCP 13-758		Experimental Line	
L 14-267		Experimental Line	
HoL 15-508		Experimental Line	
Ho 15-971		Experimental Line	
L 15-306		Experimental Line	

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## Vita

Leonardo David Salgado was born in Honduras, Central America got his bachelor's degree in Agronomy at Universidad Nacional de Agricultura (UNAG), which led him to do an internship in sugarcane and rice integrated pest management under the supervision of Dr. Blake Wilson. Later, he joined the Wilson Lab to start his master's degree in Entomology with a Minor in Applied Statistics at Louisiana State University (LSU) in June 2019. Leo investigated for his master's thesis the mechanisms that confer resistance to sugarcane (*Saccharum* spp. L.) to the stem borers *Diatraea saccharalis* (F.) and *Eoreuma loftini* (Dyar) in a series of field, laboratory, and greenhouse experiments. In addition to his thesis project, he worked on projects related to the timing, volume, and efficacy of insecticide applications in sugarcane and rice (*Oryza sativa*).

Leo plans to receive his Masters this August 2021, and plans to join the Department of Entomology of Cornell University to start his Ph.D. in Fall 2021, working in Applied Insect Ecology under the direction of Dr. Brian Nault.