VanWeelden et al.:
Impact of Nitrogen on *Eoreuma loftini*

For: Crop Protection

M. T. VanWeelden
University of Florida
2976 SR 15
Belle Glade, FL 33430
Phone: 561-996-1656
Fax: 561-996-4831
Email: mvanweel1@ufl.edu

**Impact of Nitrogen Fertilization on Mexican Rice Borer (Lepidoptera: Crambidae) Injury
and Yield in Bioenergy Sorghum**

M. T. VanWeelden¹, B. E. Wilson¹, J. M. Beuzelin², T. E. Reagan¹, and M. O. Way³

¹Department of Entomology, Louisiana State University Agricultural Center, 404 Life Sciences
Building, Baton Rouge, LA 70803

Present address: University of Florida Institute of Food and Agricultural Sciences, Palm Beach
County, 2976 SR 15, Belle Glade, FL 33430

²Dean Lee Research Station, Louisiana State University Agricultural Center, 8105 Tom Bowman
Drive, Alexandria, LA 71302

³Texas A&M AgriLife Research and Extension Center, 1509 Aggie Drive, Beaumont, TX 77713

Abstract

The Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae), is a serious pest of sugarcane (*Saccharum* spp.), sorghum (*Sorghum bicolor* (L.) Moench), corn (*Zea mays* L.), rice (*Oryza sativa* L.), and related graminaceous bioenergy crops. A two-year field study was conducted in Jefferson County, TX to examine the impact of nitrogen (N) fertilization on *E. loftini* infestations and subsequent yields in cultivars of high-biomass and sweet sorghum. In 2013, percentage of bored internodes and number of adult emergence holes per stalk increased with higher N rates; however, only the percentage of bored internodes was impacted by N in 2014. Yields from both years indicated that N rate was positively associated with increases in stalk weight and ethanol productivity, but not sucrose concentration. Because higher N rates were associated with increased yields despite having greater levels of *E. loftini* injury, our data suggest that increases in yield from additional N outweigh decreases from additional *E. loftini* injury. Fertilization rates maintained between the recommended 45 and 90 kg N/ha minimize risks of negative area-wide impacts from increased production of *E. loftini* adults, while still allowing for optimum yields.

Keywords: *Eoreuma loftini*, *Sorghum bicolor*, yield loss, pest management, invasive species

1. Introduction

The Mexican rice borer, *Eoreuma loftini* (Dyar), has become one of the dominant pests of graminaceous crops in Texas and Louisiana since its introduction into the Lower Rio Grande Valley in 1980 (Johnson and van Leerdaam, 1981; Legaspi et al., 1997; Reay-Jones et al., 2007a; Wilson et al., 2015a). Hosts of this invasive species include sugarcane (*Saccharum* spp.), rice (*Oryza sativa* L.), corn (*Zea mays* L.), and grain sorghum (*Sorghum bicolor* (L.) Moench) (Dyar, 1917; Van Zwaluwenburg, 1926; Osborn and Phillips, 1946; Van Zwaluwenburg, 1950; Johnson, 1984; Showler et al., 2012), as well as related bioenergy crops (VanWeelden et al., 2015). Recent demands in the United States for increased production of dedicated bioenergy feedstocks high in sucrose or lignocellulosic biomass (United States Environmental Protection Agency, 2007), including high-biomass sorghum (*Sorghum* spp. hybrids), sweet sorghum (*Sorghum bicolor* (L.) Moench) and energycane (*Saccharum* spp.), are anticipated to increase the host availability for *E. loftini*, which could impact area-wide infestations in conventional and bioenergy crops.

Current integrated pest management tactics to mitigate *E. loftini* infestations in sugarcane include judiciously timed insecticide applications (Wilson et al., 2012), irrigation (Reay-Jones et al., 2005), and host-plant resistance (Reay-Jones et al., 2003; Way et al., 2006; Wilson et al., 2015b; VanWeelden et al., 2015). However, little research has been conducted on management strategies in cultivars of sorghum produced as dedicated bioenergy feedstocks, specifically with regard to the impact of soil fertilization. Current nitrogen (N) fertilization recommendations for sweet sorghum range from 45 to 90 kg/ha in the Gulf Coast region (Viator et al., 2010), but little is known whether this regime is optimal for production of newly emerging high-biomass sorghum cultivars. In addition, the compatibility of recommended N regimes with current

management strategies for *E. loftini* is unknown. Because N is essential for all biological processes and greater availability of N results in greater productivity among organisms, it is considered a limiting factor in ecosystems (Mattson, 1980). Impacts on insect pest biology resulting from enhancements in available N have been documented on numerous crops, including grain sorghum (Wale et al., 2006), corn (Sétamou et al., 1995; Wale et al., 2006), sugarcane (Atkinson and Nuss, 1989; Showler, 2015), and wheat (*Triticum* spp. L.) (Honek, 1991; Gash, 2012). Thus, manipulation of N fertilization can be a useful tool in an integrated pest management strategy. Quantity of available N is linked to many plant characteristics in sugarcane, including concentrations of free amino acids and vigor (Showler, 2015), which in turn can impact host preference for oviposition and larval performance of *E. loftini*. The objective of this study was to assess *E. loftini* injury and yield response across varying N fertilization regimes in high-biomass and sweet sorghum cultivars.

2. Materials and Methods

2.1 Experimental design

A field experiment was conducted in 2013 and 2014 at the Texas A&M AgriLife Research and Extension Center at Beaumont, TX (Jefferson County) to evaluate the impact of N fertilization on *E. loftini* injury and yield in three cultivars of sorghum used in the production of biofuels. Cultivars included two high-biomass sorghum cultivars, ES 5200 and ES 5140 (Blade Energy Crops, Thousand Oaks, CA, USA), and one sweet sorghum cultivar, M81E (MAFES Foundation Seed Stocks, Mississippi State University, MS, USA). High-biomass sorghum cultivars ES 5200 and ES 5140 are *S. bicolor* and *S. bicolor* × *drummondii* (sudangrass) hybrids, respectively, bred to produce thick-stemmed, late maturing crops for use as dedicated bioenergy feedstocks (Blade Energy Crops, 2012). Sweet sorghum cultivar, M81E, is a *S. bicolor* inbred

released by the USDA-ARS U.S. Sugar Crops Field Station for production of fermentable sugar (Broadhead et al., 1981), and has potential for ethanol production (Tew et al., 2008; Erickson et al., 2012).

The experiment was arranged using a randomized block, split-plot design with four blocks (one replication per block). Each block was 22.7 m long and 25.4 m wide (24 rows). Rates of N fertilization were randomized to six-row plots (22.7 m long and 6.4 m wide) and sorghum cultivars were randomized to two-row subplots (22.7 m long and 2.1 m wide). Prior to planting, soil samples consisting of single 2.5 cm wide by 30 cm deep cores at 15 locations within the field were collected and analyzed for available N at the Louisiana State University Agricultural Center Soil Testing and Plant Laboratory, Baton Rouge, LA. Soil analysis showed that available N ranged from 0.08 to 0.13% (mean: 0.10%, standard deviation: 0.02%), and available N in field plots prior to fertilization was not expected to interfere with the experimental design. High-biomass sorghum and sweet sorghum cultivars were planted using a four-row precision cone planter (Kinkaid Equipment Manufacturing, Haven, KS, USA) on 10 May in 2013 and 9 May in 2014 at a rate of 210,000 seeds per hectare. Two weeks after planting, urea granules were applied by hand to plots at one of four fertilization rates: 0, 45, 90, 135 kg N/ha. Nitrogen granules were applied to plots to reduce any confounding effects resulting from N mobility in the soil if applied to smaller subplots. The study was conducted on adjacent field sites in 2013 and 2014, and N fertilization rates and sorghum cultivars were re-randomized each year.

2.2 *Eoreuma loftini* within-season injury

Plants were sampled throughout the growing season during both years to determine within-season *E. loftini* injury. Subplots were sampled on 17 July, 7 August, 4 September, and 26 September in 2013 and 17 July, 4 August, 21 August, 5 September, and 3 October in 2014. At

each date, 10 plants were randomly selected in each subplot and inspected for leaf sheath feeding and bored internodes from *E. loftini*. In addition, the height and total number of internodes were recorded for each plant. Injury was expressed as the percentage of injured internodes [(internodes with leaf sheath feeding or bored internodes / total internodes) $\times$ 100].

2.3 *Eoreuma loftini* end-of-season injury and yield

Experiments were harvested on 2 October in 2013 and 24 October in 2014 to collect *E. loftini* end-of-season injury and yields. Twelve stalks were sampled from each row of each subplot (24 stalks per subplot), stripped of leaf material, and the numbers of internodes, bored internodes, and *E. loftini* adult emergence holes were recorded for each stalk. Each 12-stalk sample was then weighed to determine fresh stalk weight (kg), and crushed using a sugarcane stalk crusher (Skyfood Equipment, Miami, FL, USA) to separate juice from bagasse. A 1-mL aliquot of juice from each sample was analyzed using a handheld refractometer (Reichert Technologies, Depew, NY, USA) to determine Brix (% w/w of soluble solids). From the Brix reading, sucrose concentration in stalks (% w/w) from each sample was calculated using the model (VanWeelden et al., 2015):

$$(1) \text{ Sucrose concentration} = \text{Brix} / 1 \times 0.85 / 1.72$$

where 1 is the factor converting *Brix* to soluble solid concentration in juice (% w/v) assuming a juice relative density of 1; 0.85 is the purity factor for converting juice sucrose to normal juice sucrose (Reay-Jones et al., 2005); and 1.72 is a constant estimated from calculating the relationship between fresh stalk weight and juice volume from data collected in the experiment. Bagasse from each sample was weighed, and a sub-sample of bagasse was removed, weighed,

and dried for two weeks to remove moisture content. Dry weights of samples were used to estimate percent moisture remaining in the bagasse.

Estimation of ethanol productivity (liter/ha) was determined to be the sum of ethanol outputs from both sucrose and lignocellulosic biomass. Predicted ethanol output from sucrose was calculated for each sample using the model (Vasilakoglou et al., 2011):

$$(2) \text{ Predicted sucrose ethanol output} = \text{sucrose concentration} \times \text{fresh biomass} \times 6.5 \times 0.85 \times 1.27$$

where *sucrose concentration* is calculated using model (1) for each sample; *fresh biomass* is the total fresh stalk weight in Mg/ha; 6.5 is the conversion factor of ethanol from sucrose; 0.85 is the efficiency constant from converting sucrose into ethanol; and 1.27 is the specific gravity of ethanol in g/mL. Because stands were low but spatially homogenous, stalk populations were standardized using published stand counts (Blade Energy Crops, 2012) to estimate fresh biomass per hectare. Dry bagasse weight (following adjustment for moisture remaining after crushing) from each sample was multiplied by a factor of 465.3, the theoretical ethanol yield in liter/metric ton from bagasse (United States Department of Energy, 2013) to determine lignocellulosic ethanol productivity.

2.4 Statistical analyses

Data were analyzed separately by year. Within-season *E. loftini* injury data for each subplot were analyzed using a linear mixed model (PROC GLIMMIX) (SAS Institute, 2008) with N fertilization rate, cultivar, and sampling date, along with all two and three-way interactions, as fixed effects. Random effects included block, block $\times$ N fertilization rate, and block $\times$ N fertilization rate $\times$ cultivar. For end-of-season data, a multivariate analysis was conducted using

PROC GLM (SAS Institute, 2008) with a MANOVA statement including all injury (percentage of bored internodes, no. adult emergence holes per stalk) and yield parameters (fresh stalk weight, sucrose concentration, ethanol productivity) because these response parameters were collected from the same observation units (i.e., stalk samples). Fixed effects included N fertilization rate, cultivar, and the N fertilization rate $\times$ cultivar interaction. Random effects included block, block $\times$ N fertilization rate, and block $\times$ N fertilization rate $\times$ cultivar. Subsequently, univariate linear mixed models with the same fixed and random effects as the multivariate linear mixed model were conducted to independently compare percentage of bored internodes, no. adult emergence holes per stalk, fresh stalk weight, sucrose concentration, and ethanol productivity. For all univariate models, the Kenward-Roger method was used to estimate the error degrees of freedom (PROC GLIMMIX) (SAS Institute, 2008). Least square means $\pm$ standard errors from the LSMEANS statement output (Proc GLIMMIX, SAS Institute, 2008) were reported. Means were separated with Tukey's HSD ($\alpha = 0.05$).

Simple linear regressions using sample estimates were conducted using PROC REG (SAS Institute, 2008) to determine the relationships between N fertilization (x) and the percentage of bored internodes (y), and percentage of bored internodes (x) and yield (fresh stalk weight, sucrose concentration, ethanol productivity) (y). Because the effect the cultivar was not significant ($P > 0.05$) in predicting the relationship between N fertilization and the percentage of bored internodes, data were pooled across all cultivars.

3. Results

3.1 *Eoreuma loftini* within-season injury

In 2013, within-season percentage of *E. loftini* injured internodes remained below 2.0% across all sampling dates, ranging from 0.0% on 17 July to 1.4% on 26 September. However,

differences were detected ($F = 17.64$; $df = 3, 781.5$; $P < 0.0001$) among sampling dates, with injury recorded on 26 September exceeding that on 17 July (0.0% injured internodes), 7 August (0.2% injured internodes), and 4 September (0.3% injured internodes). The percentage of injured internodes generally increased with N rate ($F = 3.67$; $df = 3, 27.6$; $P = 0.0241$), ranging from 0.28% at 0 kg N/ha to 0.92% at 135 kg N/ha. A sampling date by N rate interaction was detected ($F = 3.18$; $df = 9, 783.3$; $P = 0.0009$). The percentage of injured internodes for 17 July remained at 0.0% across all N rates, while injury at later sampling dates increased with added N, reaching percentages of injured internodes as high as 0.6% on 4 September and 2.9% on 26 September. Differences among cultivars were not detected ($P > 0.05$).

In 2014, the within-season percentage of injured internodes remained below 0.4% across all sampling dates. Differences were detected only among sampling dates ($F = 6.78$; $df = 783.1$; $P < 0.0001$), peaking late in the season on 5 September at 0.4% injured internodes.

3.2 *Eoreuma loftini* end-of-season injury and yields

3.2.1 2013 Field experiment

Multivariate analysis of end-of-season *E. loftini* injury and yield in 2013 showed that at least one injury (percentage of bored internodes, no. adult emergence holes) or yield (fresh stalk weight, sucrose concentration, ethanol productivity) parameter changed with N rate (Wilks' Lambda = 0.1736; $F = 3.77$; $df = 15, 63.9$; $P < 0.0001$) and sorghum cultivar (Wilks' Lambda = 0.0004; $F = 20.44$; $df = 4, 10$; $P = 0.0052$). The effect of N was generally consistent across cultivars, as shown by the absence of an interaction (Wilks' Lambda = 0.3026; $F = 1.09$; $df = 30, 94$; $P = 0.3646$). Higher N rates were associated with increases in the percentage of bored internodes (Table 1). At 0 kg N/ha, the percentage of bored internodes averaged $2.9 \pm 0.6\%$ across all cultivars, and no increase in injury was detected at 45 kg N/ha. The percentage of

bored internodes at 0 kg N/ha increased 1.7 and 2.9-fold when N was increased to 90 and 135 kg/ha, respectively. At 45 kg N/ha, increases in the percentage of bored internodes were only present when the N rate was increased to 135 kg/ha (2.2-fold). The percentage of bored internodes varied by cultivar across all N rates (Table 1), with ES 5140 expressing a 1.4-fold increase in injury compared to M81E. Although no difference in emergence per stalk was detected between N rates of 0 and 45 kg/ha, emergence per stalk increased 7.4-fold when N was increased from 45 to 135 kg/ha and 3.1-fold when N was increased from 90 to 135 kg/ha (Table 1). *Eoreuma loftini* adult emergence per stalk was 3.4-fold greater in ES 5200 than in M81E. Despite no interaction in the multivariate analysis, the univariate analysis detected an N rate by cultivar interaction for emergence holes per stalk (Fig. 1a). High-biomass sorghum cultivars responded similarly to increasing N, with emergence per stalk decreasing numerically at 45 kg N/ha, and then increasing at 90 kg N/ha. In M81E, emergence per stalk was identical at 45 and 90 kg N/ha. In ES 5200 and ES 5140, emergence per stalk increased significantly at 135 kg N/ha when compared to 90 kg N/ha; however, this spike in emergence was not as high in M81E.

Increased N had a positive impact on fresh stalk weight across all cultivars (Table 1), ranging from 0.22 kg/stalk at 0 kg N/ha to 0.34 kg/stalk at 135 kg N/ha. Pairwise comparisons failed to detect differences in stalk weight between plots subjected to 0 and 45 kg N/ha, or 90 and 135 kg N/ha; however, stalk weights at 135 kg N/ha were 1.5 and 1.4-fold greater than at 0 and 45 kg N/ha, respectively. Stalk weight varied by cultivar (Table 1), with that of ES 5200 being 2.6 and 5.2-fold greater than that of ES 5140 and M81E, respectively. No difference in stalk weight was detected between ES 5140 and M81E. Sucrose concentration across all cultivars was not impacted by N rate, but varied by cultivar. Sucrose concentration in M81E was 1.2 and 1.4-fold greater than in ES 5200 and ES 5140, respectively (Table 1). A positive response to N was

detected for ethanol productivity (Tables 1) and ranged from $12.2 \pm 1.7 \times 10^3$ liter/ha at 0 kg N/ha to $18.9 \pm 1.8 \times 10^3$ liter/ha at 135 kg N/ha. Ethanol productivity remained unchanged between 0 and 45 kg N/ha, but increased 1.5-fold when N was increased from 45 kg/ha to 135 kg/ha (Table 1). Ethanol productivity also varied by cultivar (Table 1), with ES 5200 yielding 2.6 and 4.9-fold more ethanol than in ES 5140 and M81E, respectively.

Results from linear regression detected a positive relationship between N rate and the percentage of bored internodes ($F = 47.77$; $df = 95$; $P < 0.0001$) (Fig. 2a) across all cultivars in 2013. An increase in N by 1 kg/ha corresponded to an increase of 0.04 ± 0.01 in the percentage of bored internodes. Associations between the percentage of bored internodes and fresh stalk weight, sucrose concentration, and ethanol productivity were not detected (Fig 2b–2d).

3.2.2 2014 Field experiment

Multivariate analysis of end-of-season data in 2014 showed that at least one of the injury or yield parameters changed with N rate (Wilks' Lambda = 0.2313; $F = 2.98$; $df = 15, 63.9$; $P = 0.0012$) and cultivar (Wilks' Lambda = 0.0012; $F = 11.39$; $df = 4, 10$; $P = 0.0158$). The effect of N was similar to data from 2013, as shown by the absence of an interaction (Wilks' Lambda = 0.2245; $F = 1.42$; $df = 30, 94$; $P = 0.1041$). The percentage of bored internodes varied among N rates (Table 2). Similarly with data from 2013, the percentage of bored internodes increased with higher rates of N, ranging from 1.2% at 0 kg N/ha to 3.9% at 135 kg N/ha. Injury in the absence of N increased 3.5-fold when N was applied at 90 and 135 kg/ha. Differences in the percentage of bored internodes were not detected among cultivars. Despite a 2.2-fold numerical increase in *E. loftini* adult emergence per stalk between 0 and 135 kg N/ha, variations in emergence per stalk among N rates or cultivars were not detected (Table 2, Fig. 1b).

In 2014, fresh stalk weights were lowest at 0 kg N/ha, increasing 1.4-fold at 135 kg N/ha, although differences in stalk weights were not detected between 0, 45, 90 kg N/ha. Consistent with 2013, sucrose concentration varied among cultivars but not N rates (Table 2). Sucrose concentration in M81E and ES 5200 were 1.2 and 1.3-fold greater than in ES 5140. Differences in sucrose concentrations among N rate by cultivar were detected ($F = 2.45$; $df = 6,33$; $P = 0.0451$), with the greatest sucrose concentrations being detected at 90 and 135 kg N/ha for ES 5200 ($5.2 \pm 0.3\%$), 135 kg N/ha for ES 5140 ($4.4 \pm 0.3\%$), and 0 kg N/ha for M81E ($5.6 \pm 0.3\%$). Sucrose concentration in M81E was negatively impacted by increasing N, decreasing 1.4-fold at 135 kg N/ha when compared to yields at 0 kg N/ha. Ethanol productivity, though numerically less than in 2013, had a positive response to N (Table 2), ranging from $5.1 \pm 1.4 \times 10^3$ liter/ha at 0 kg N/ha to $7.6 \pm 1.4 \times 10^3$ liter/ha at 135 kg N/ha across all cultivars. Ethanol yields numerically increased 1.3-fold between 0 and 45 kg N/ha, but did not change after increasing N to 90 kg/ha. Increasing N to 135 kg/ha increased ethanol productivity 1.5-fold when compared to plants under the 0 kg N/ha regime.

Results from linear regression detected a positive relationship between N fertilization rate and the percentage of bored internodes ($F = 28.23$; $df = 95$; $P < 0.0001$) (Fig. 2a) across all cultivars in 2014. An increase in N by 1 kg/ha corresponded to an increase of 0.02 ± 0.004 in the percentage of bored internodes. In addition, a negative relationship was detected between the percentage of bored internodes and sucrose concentration ($F = 6.46$; $df = 95$; $P = 0.0127$) (Fig. 2c), but not fresh stalk weight or ethanol productivity (Fig. 2b and Fig. 2d). Sucrose concentration decreased by $0.1 \pm 0.04\%$ w/w for every unit increase in the percentage of bored internodes.

4. Discussion

Nitrogen fertilization had a direct impact on *E. loftini* infesting sorghum cultivars bred as bioenergy feedstocks. The association between increased levels of *E. loftini* injury and increased N fertilization documented in this study is consistent with Showler (2015), where high-rate applications of compost increased the percentage of *E. loftini* bored internodes in sugarcane. Increases in applied N in sugarcane have also been associated with greater infestation levels and survival of the African sugarcane stalk borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae) (Atkinson and Nuss, 1989). In addition, associations between stem borer infestations and N fertilization have been documented for *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in grain sorghum (Van den Berg and Van Rensburg, 1991; Addo-Bedaiko and Thanguane, 2012) and corn (Arshad et al., 2013).

The association between N fertilization and *E. loftini* injury and adult emergence could result from the association between N fertilization and host-plant nutritional quality, specifically free amino acids (Showler and Moran, 2014; Showler, 2015). Increases in free-amino acids essential for insect growth and development (Nation, 2002) could have increased host preference for oviposition in *E. loftini* females, thus increasing injury. An additional environmental factor that might increase host preference and subsequent injury, as demonstrated with *E. loftini* in sugarcane, is drought stress, which has been associated with increased concentrations of free amino-acids (Reay-Jones et al., 2005; Showler and Castro, 2010a). Fields used in this study were not irrigated, which could have increased *E. loftini* infestations through enhancement of nutrients. Furthermore, drought stress would also increase the occurrence of dry and folded leaf tissue, which is known to be a preferred site for *E. loftini* oviposition, and has been associated with higher concentrations of free amino acids (Reay-Jones et al., 2005; 2007b; Showler and

Castro, 2010a; 2010b). Recorded rainfall from May through October was ≈ 432 mm in 2013 and ≈ 838 mm in 2014 (National Weather Service, 2015), indicating that higher infestations observed in 2013 could have been due to lower rainfall.

Elevated numbers of *E. loftini* emergence holes in response to N fertilization in 2013 indicates that N can impact moth production, thus having the potential to influence area-wide populations. This same association has been demonstrated in sugarcane (Showler, 2015). Because *E. loftini* has the ability to infest numerous crop and non-crop hosts (Showler et al., 2011; 2012; Beuzelin et al., 2011; Showler and Reagan, 2012), minimizing moth production is key in reducing area-wide impacts. The lack of a detectable increase in adult emergence in 2014 may have been caused by reduced pest pressure that year, evident from lower levels of injury in our study and lower *E. loftini* pheromone trap catches across east Texas in 2014 compared to 2013 (Way et al., 2013; 2014).

Reductions in yield might have occurred from increased *E. loftini* injury under higher N rate; however, this damage was likely masked by increased plant growth. Although *E. loftini* injury is capable of reducing yields in high-biomass and sweet sorghum (VanWeelden et al., 2015), our results suggest N fertilization exerts a greater influence on yield. The reduction of sucrose concentration in 2014 from *E. loftini* injury indicates reductions in sucrose concentration are less likely to be off-set by increased N fertilization rate as sugar content in sorghum is not as heavily impacted by N (Erickson et al., 2012), especially in non-irrigated environments (Han et al., 2012) such as those in this study. The positive association between N rate and stalk biomass demonstrated in this study is consistent with results from previous studies (Erickson et al., 2012; Han et al., 2012), although this trend is not always linear. Under non-irrigated conditions, production of biomass may increase at the expense of sugar (Han et al., 2012), which may

explain discrepancies between stalk weight and sucrose concentration relative to N rate.

Although stalk samples collected for yield analysis were relatively small and may have not been adequate to determine precise yield estimates, each stalk sampled for injury was processed for yield determination to accurately assess the relationship between injury and yield.

The high biomass and moderate concentrations of sucrose exhibited in ES 5200 give this cultivar the greatest potential for production of ethanol. Results in 2013 demonstrated that even with no added N, ES 5200 was estimated to produce more ethanol than ES 5140 or M81E under high N rates, justifying ES 5200 as a candidate for low-input biofuel production. In addition, production of ES 5200 under low to moderate N fertilization rates will reduce infestations and subsequent production of *E. loftini* adults, lessening any negative impacts from planting bioenergy sorghum in close proximity to conventional crops.

The sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), occurred in experimental plots during both years of this study and might have impacted assessment of sorghum yield losses from *E. loftini*. *Melanaphis sacchari* has been reported as a pest of grain sorghum in Asia and Africa (Blackman and Eastop, 2000) and of sugarcane in Florida and Louisiana (Mead, 1978; White et al., 2001). However, the aphid emerged as a severe pest of grain sorghum in the United States in 2013 (Villanueva et al., 2014), and infestations on high-biomass and sweet sorghum cultivars have been reported (VanWeelden et al., 2015). *Melanaphis sacchari* infestations in this study were controlled using multiple applications of flonicamid (Carbine 50 WG, FMC Corporation, Philadelphia, PA, USA) from early summer through the time of harvest, but might have had an impact on ethanol productivity in all cultivars. Rates of N fertilizer have been directly related to aphid abundance, as exhibited with greenbug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), on grain sorghum (Archer et al., 1982); however,

further studies will need to be conducted to determine whether this trend applies to *M. sacchari* on bioenergy sorghum.

Current N fertilization recommendations for sweet sorghum by the Louisiana State Agricultural Center range from 45 to 90 kg/ha (Viator et al., 2010); however, estimates as high as 90 to 110 kg/ha have been recommended for optimal use in production of sweet sorghum for biofuel (Erickson et al., 2012). Additional costs of increasing N applications from 90 kg/ha to the maximum rate examined in the study (135 kg/ha) would average \$55/ha (Deliberto et al., 2015), which would allow for greater ethanol yields from lignocellulosic biomass but little or no additional yields from sucrose. Results from this study suggest N recommendations of 45 to 90 kg/ha would allow for a balance of higher yields and lower *E. loftini* injury and moth production, which in turn, would increase the compatibility of bioenergy sorghum in conventional cropping systems in the Gulf Coast region by reducing area-wide impacts on *E. loftini*.

Acknowledgments

The authors thank Dr. David Blouin for statistical consultation. We thank Narinder Heer, Tyler Torina, Najib Mahmud, Rachel Anderson (LSU AgCenter), Becky Pearson, Caleb Verret, and Caleb Marshall (Texas A&M AgriLife Research and Extension at Beaumont) for technical assistance in the field. We thank Randy Eason (Texas A&M AgriLife Research and Extension at Beaumont) for preparation of fields for planting. This research was funded by the USDA-NIFA Agricultural and Food Research Initiative (AFRI) Sustainable Bioenergy program grant 2011-67009-30132. This paper has been approved by the Director of the Louisiana Agricultural Experiment Station for publication.

References Cited

- Addo-Bediako, A. and N. Thanguane. 2012.** Stem borer distribution in different sorghum cultivars as influenced by soil fertility. *Agr. Sci. Res. J.* 2: 189–194.
- Archer, T. L., A. B. Onken, R. L. Matheson, and E. D. Bynum, Jr. 1982.** Nitrogen fertilizer influence on greenbug (Homoptera: Aphididae) dynamics and damage to sorghum. *J. Econ. Entomol.* 75: 695–698.
- Arshad, M. J., S. Freed, S. Akbar, M. Akmal, and H. Tahira. 2013.** Nitrogen fertilizer application in maize and its impact on the development of *Chilo partellus* (Lepidoptera: Crambidae). *Pakistan J. Zool.* 45: 141–147.
- Atkinson, P. R. and K. J. Nuss. 1989.** Associations between host-plant nitrogen and infestations of the sugarcane borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Bull. Ent. Res.* 79: 489–506.
- Beuzelin, J. M., A. Mészáros, T. E. Reagan, L. T. Wilson, M. O. Way, D. C. Blouin, and A. T. Showler. 2011.** Seasonal infestations of two stem borers (Lepidoptera: Crambidae) in noncrop grasses of Gulf Coast rice agroecosystems. *Environ. Entomol.* 40: 1036–1050.
- Blackman, R. L. and V. F. Eastop. 2000.** The aphids, pp. 297-298. *In* R. L. Blackman and V. F. Eastop [eds.], *Aphids on the world's crops-an identification and information guide*, 2nd ed. John Wiley and Sons, West Sussex, England.
- Blade Energy Crops. 2012.** Blade energy crops product manual. Blade Energy Crops, Thousand Oaks, CA, US.
- Broadhead, D. M., K. C. Freeman, and N. Zummo. 1981.** ‘M81E’---A new variety of sweet sorghum. Mississippi Agricultural and Forestry Experiment Station Information Sheet 1309.

- 417 **Deliberto, M. A., M. E. Salassi, and B. M. Hilburn. 2015.** Projected costs and returns: crop
 418 enterprise budgets for sorghum production in Louisiana. Louisiana State University
 419 Agricultural Center. A. E. A. Information Series No. 303.
- 420 **Dyar, H. G. 1917.** Seven new crambids from the United States. *Insecutor Inscitiae Menstruus*. 5:
 421 84–87.
- 422 **Erickson, J. E., K. R. Woodward, and L. E. Sollenberger. 2012.** Optimizing sweet sorghum
 423 production for biofuel in the Southeastern USA through nitrogen fertilization and top
 424 removal. *Bioenerg. Res.* 5: 86–94.
- 425 **Gash, A. F. J. 2012.** Wheat nitrogen fertilisation effects on the performance of the cereal aphid
 426 *Metopolophium dirhodum*. *Agron.* 2: 1–13.
- 427 **Han, K. J., W. D. Pitman, M. W. Alison, D. L. Harrell, H. P Viator, M. E. McCormick, K.**
 428 **A. Gravois, M. Kim, D. F. Day. 2012.** Agronomic considerations for sweet sorghum biofuel
 429 production in the South-Central USA. *Bioenerg. Res.* 5: 748–758.
- 430 **Honek, A. 1991.** Nitrogen fertilization and abundance of the cereal aphids *Metopolophium*
 431 *dirhodum* and *Sitobion avenae* (Homoptera: Aphididae). *Zeitschrift fur Pflanzenkrankheiten*
 432 *und Pflanzenschutz*. 119: 655–660.
- 433 **Johnson, K. J. R. 1984.** Identification of *Eoreuma loftini* (Dyar) (Lepidoptera: Pyralidae) in
 434 Texas, 1980: Forerunner for other sugarcane boring pest immigrants from Mexico? *Bull.*
 435 *Entomol. Soc. Amer.* 30: 47–52.
- 436 **Johnson, K. J. R. and M. B. van Leerdam. 1981.** Range expansion of *Acigona loftini* into the
 437 Lower Rio Grande Valley of Texas. *Sugar y Azucar* 76: 34.

- 438 **Legaspi, J. C., B. C. Legaspi, Jr., E. G. King, and R. R. Saldaña. 1997.** Mexican rice borer,
439 *Eoreuma loftini* (Lepidoptera: Pyralidae) in the Lower Rio Grande Valley of Texas: its
440 history and control. Subtrop. Plant Sci. 49: 53–64.
- 441 **Mattson, W. J. Jr. 1980.** Herbivory in relation to plant nitrogen content. Ann. Rev. Ecol. Syst.
442 11: 119–161.
- 443 **Mead, F. W. 1978.** Sugarcane aphid, *Melanaphis sacchari* (Zehntner)—Florida-New continental
444 United States record. Coop. Plant Pest Rep. 34: 475.
- 445 **Nation, J. L. 2002.** Insect Physiology and Biochemistry. CRC Press, Boca Raton, Florida, USA.
- 446 **National Weather Service Weather Forecast Office. 2015.** Beaumont, Texas climate graphs.
447 Web. 14 April 2015. <http://www.srh.noaa.gov/lch/?n=bptclimategraph>.
- 448 **Osborn, H. T. and G. R. Phillips. 1946.** *Chilo loftini* in California, Arizona, and Mexico. J.
449 Econ. Entomol. 39: 755–759.
- 450 **Reay-Jones, F. P. F., L. T. Wilson, A. T. Showler, T. E. Reagan, M. O. Way. 2007a.** Role of
451 oviposition on preference in an invasive crambid impacting two graminaceous host crops.
452 Environ. Entomol. 36: 938–951.
- 453 **Reay-Jones, F. P. F., L. T. Wilson, M. O. Way, T. E. Reagan, and C. E. Carlton. 2007b.**
454 Movement of Mexican rice borer (Lepidoptera: Crambidae) through the Texas rice belt. J.
455 Econ. Entomol. 100: 54–60.
- 456 **Reay-Jones, F. P. F., M. O. Way, M. Sétamou, B. L. Legendre, T. E. and Reagan. 2003.**
457 Resistance to the Mexican rice borer (Lepidoptera: Crambidae) among Louisiana and Texas
458 sugarcane cultivars. J. Econ. Entomol. 96: 1929–1934.

- 459 **Reay-Jones, F. P. F., A. T. Showler, T. E. Reagan, B. L. Legendre, M. O. Way, and E. B.**
460 **Moser. 2005.** Integrated tactics for managing the Mexican rice borer (Lepidoptera:
461 Crambidae) in sugarcane. *Environ. Entomol.* 34: 1558–1565.
- 462 **SAS Institute. 2008.** User's Manual, Version 9.2. SAS Institute, Cary, NC, USA.
- 463 **Sétamou, M., F. Schulthess, N. A. Bosque-Pérez, and A. Thomas-Odjo. 1995.** The effect of
464 stem and cob borers on maize subjected to different nitrogen treatments. *Entomol. Exp. App.*
465 77: 205–210.
- 466 **Showler, A. T. 2015.** Effects of compost and chicken litter on soil nutrition, and sugarcane
467 physiochemistry, yield, and injury caused by the Mexican rice borer, *Eoreuma loftini* (Dyar)
468 (Lepidoptera: Crambidae). *Crop Prot.* 71: 1–11.
- 469 **Showler, A. T. and B. A. Castro. 2010a.** Influence of drought stress on Mexican rice borer
470 (Lepidoptera: Crambidae) oviposition preference and development to adulthood in
471 sugarcane. *Crop Prot.* 29: 722–727.
- 472 **Showler, A. T., and B. A. Castro. 2010b.** Mexican rice borer (Lepidoptera: Crambidae)
473 oviposition site selection stimuli on sugarcane, and potential field applications. *J. Econ.*
474 *Entomol.* 103: 1180–1186.
- 475 **Showler, A. T. and P. J. Moran. 2014.** Associations between host plant concentrations of
476 selected biochemical nutrients and Mexican rice borer, *Eoreuma loftini*, infestation. *Entomol.*
477 *Exp. Appl.* 151: 135–143.
- 478 **Showler, A. T. and T. E. Reagan. 2012.** Ecology and tactics of control for three sugarcane
479 stalk-boring species in the Western Hemisphere and Africa, pp. 1–15. In: Goncalves, J. F.
480 and K. D. Correia, (Eds), *Sugarcane: Production and Uses*. Nova, New York, New York,
481 USA, pp. 1–15.

- 482 **Showler, A. T., J. M. Beuzelin, and T. E. Reagan. 2011.** Alternate crop and weed host plant
483 oviposition preferences by the Mexican rice borer (Lepidoptera: Crambidae). *Crop Prot.* 30:
484 895–901.
- 485 **Showler, A. T., B. E. Wilson, and T. E. Reagan. 2012.** Mexican rice borer injury to corn
486 greater than to sorghum and sugarcane under field conditions. *J. Econ. Entomol.* 105:
487 1597–1602.
- 488 **Tew, T. L., R. M. Cobill, and E. P. Richard, Jr. 2008.** Evaluation of sweet sorghum and
489 sorghum × sudangrass hybrids as feedstocks for ethanol production. *Bioenerg. Res.* 1:
490 147–152.
- 491 **United States Department of Energy Bioenergy Technologies Office. 2013.** Theoretical
492 ethanol yield calculator and biomass feedstock composition and property database. Web. 10
493 Nov 2013. http://www.afdc.energy.gov/fuels/ethanol_feedstocks.html.
- 494 **United States Environmental Protection Agency. 2007.** Summary of the Energy Independence
495 and Security Act. Web. 21 November 2013.
- 496 **Van den Berg, J. and J. B. J. Van Rensburg. 1991.** Infestation and injury levels of stem borers
497 in relation to yield potential of grain sorghum. *S. Afr. J. Plant Soil.* 8: 127–131.
- 498 **Van Zwaluwenburg, R. H. 1926.** Insect enemies of sugarcane in western Mexico. *J. Econ.*
499 *Entomol.* 19: 664–669.
- 500 **Van Zwaluwenburg, R. H. 1950.** The insects affecting sugar cane in Mexico. *Proc. Int. Soc.*
501 *Sugar-cane Technol.* 7: 373–377.
- 502 **VanWeelden, M. T., B. E. Wilson, J. M. Beuzelin, T. E. Reagan, and M. O. Way. 2015.**
503 Yield response to Mexican rice borer (Lepidoptera: Crambidae) injury in bioenergy and
504 conventional sugarcane and sorghum. *J. Econ. Entomol.* DOI: 10.1093/jee/tov190.

- 505 **Vasilakoglou, I., K. Dhima, N. Karagiannidis, T. Gatsis. 2011.** Sweet sorghum productivity
 506 for biofuels under increased soil salinity and reduced irrigation. *Field Crop Res.* 120: 38–46.
- 507 **Viator, S., W. Alison, D. Harrell, K. Han, J. Griffin, D. Stephenson, J. Whatley, A. Hogan,**
 508 **M. Salassi, M. Deliberto, and T. Mark. 2010.** Production of sweet sorghum in Louisiana as
 509 a biofuel feedstock crop. Department of Agricultural Economics Staff Report No. 2010-03.
 510 Louisiana State University Agricultural Center, Baton Rouge, LA, USA.
- 511 **Villanueva, R.T., M. Brewer, M.O. Way, S. Biles, D. Sekula, E. Bynum, J. Swart, C.**
 512 **Crumley, A. Knutson, P. Porter, R. Parker, G. Odvody, C. Allen, D. Ragsdale, W.**
 513 **Rooney, G. Peterson, D. Kerns, T. Royer, and S. Armstrong. 2014.** Sugarcane aphid: A
 514 new pest of sorghum. Texas A&M AgriLife Extension: Ento-035. College Station, TX, USA.
- 515 **Wale, M., F. Schulthess, E. W. Kairu, and C. O. Omwega. 2006.** Cereal yield losses caused
 516 by the lepidopterous stemborers at different nitrogen fertilizer rates in Ethiopia. *J. Appl.*
 517 *Entomol.* 130, 220–229.
- 518 **Way, M. O., T. E. Reagan, and J. M. Beuzelin. 2013.** Trapping for the Mexican rice borer.
 519 2013 Annual Report, Texas A&M AgriLife, College Station, TX, USA.
- 520 **Way, M. O., T. E. Reagan, and J. M. Beuzelin. 2014.** Trapping for the Mexican rice borer.
 521 2014 Annual Report, Texas A&M AgriLife, College Station, TX, USA.
- 522 **Way, M. O., F. P. F. Reay-Jones, and T. E. Reagan. 2006.** Resistance to stem borers
 523 (Lepidoptera: Crambidae) among Texas rice cultivars. *J. Econ. Entomol.* 99: 1867–1876.
- 524 **White, W. H., T. E. Reagan, and D. G. Hall. 2001.** *Melanaphis sacchari* (Homoptera:
 525 Aphididae), a sugarcane pest new to Louisiana. *Fla. Entomol.* 84: 435–436.
- 526 **Wilson, B. E., A. T. Showler, T. E. Reagan, and J. M. Beuzelin. 2012.** Improved chemical
 527 control for the Mexican rice borer (Lepidoptera: Crambidae) in sugarcane: Larval exposure, a

novel scouting method, and efficacy of a single aerial insecticide application. J. Econ.

Entomol. 105: 1998–2006.

Wilson, B. E., T. N. Hardy, J. M. Beuzelin, M. T. VanWeelden, T. E. Reagan, R. Miller, J.

Meaux, M. J. Stout, and C. E. Carlton. 2015a. Expansion of the Mexican rice borer into
rice and sugarcane in Louisiana. Environ. Entomol. 44: 1–10.

Wilson, B. E., M. T. VanWeelden, J. M. Beuzelin, T. E. Reagan, M. O. Way, W. H. White,

L. T. Wilson, and A. T. Showler. 2015b. A relative resistance ratio for evaluation of
Mexican rice borer (Lepidoptera: Crambidae) susceptibility among sugarcane cultivars. J.
Econ. Entomol. DOI: 10.1093/jee/tov076.

Table 1. *Eoreuma loftini* injury and yield parameters (LS means $\pm$ SE) in high-biomass and sweet sorghum at varying N fertilization rates, Beaumont, TX, 2013.

Treatment	Percent Bored Internodes	Adult Emergence/Stalk	Weight/Stalk (kg)	Sucrose Concentration	Ethanol Productivity (10 ³ liter/ha)
N Rate					
0 kg/ha	2.9 $\pm$ 0.6 c	0.07 $\pm$ 0.05 b	0.22 $\pm$ 0.03 b	5.7 $\pm$ 0.03	12.2 $\pm$ 1.7 b
45 kg/ha	3.7 $\pm$ 0.6 bc	0.07 $\pm$ 0.05 b	0.25 $\pm$ 0.03 b	5.7 $\pm$ 0.03	12.5 $\pm$ 1.7 b
90 kg/ha	5.0 $\pm$ 0.6 b	0.17 $\pm$ 0.05 b	0.27 $\pm$ 0.03 ab	5.7 $\pm$ 0.03	14.1 $\pm$ 1.8 ab
135 kg/ha	8.4 $\pm$ 0.6 a	0.52 $\pm$ 0.05 a	0.34 $\pm$ 0.03 a	5.9 $\pm$ 0.03	18.9 $\pm$ 1.8 a
<i>F</i> ¹	19.69	27.19	26.99	0.19	4.18
<i>P</i> > <i>F</i>	<0.0001	<0.0001	0.0054	0.9025	0.0150
Cultivar					
ES 5200	5.1 $\pm$ 0.5 ab	0.31 $\pm$ 0.05 a	0.52 $\pm$ 0.03 a	5.7 $\pm$ 0.03 b	27.4 $\pm$ 2.1 a
ES 5140	5.8 $\pm$ 0.5 a	0.21 $\pm$ 0.05 ab	0.20 $\pm$ 0.03 b	4.9 $\pm$ 0.03 c	10.4 $\pm$ 2.1 b
M81E	4.1 $\pm$ 0.5 b	0.09 $\pm$ 0.05 b	0.10 $\pm$ 0.03 b	6.7 $\pm$ 0.03 a	5.5 $\pm$ 2.1 b
<i>F</i> ²	3.48	5.75	44.25	30.49	30.42
<i>P</i> > <i>F</i>	0.0356	0.0404	<0.0001	<0.0001	0.0001

¹ df = 3,81; 3,27; 3,27; 3,32.9; 3,26.5, respectively

² df = 2,81; 2,6; 2,9; 2,32.9; 2,8.9, respectively

For the N rate and cultivar main effects, means followed by the same letter are not significantly different (*P* > 0.05, Tukey's HSD). No pairwise testing was done when *P* > *F* was greater than 0.05.

Table 2. *Eoreuma loftini* injury and yield parameters (LS means $\pm$ SE) in high-biomass and sweet sorghum at varying N fertilization rates, Beaumont, TX, 2014.

Treatment	Percent Bored Internodes	Adult Emergence/Stalk	Weight/Stalk (kg)	Sucrose Concentration	Ethanol Productivity (10 ³ liter/ha)
N Rate					
0 kg/ha	1.1 $\pm$ 0.5 b	0.05 $\pm$ 0.04	0.13 $\pm$ 0.03 b	4.8 $\pm$ 0.2	5.1 $\pm$ 1.4 b
45 kg/ha	2.6 $\pm$ 0.5 ab	0.06 $\pm$ 0.04	0.16 $\pm$ 0.03 ab	4.4 $\pm$ 0.2	6.6 $\pm$ 1.4 ab
90 kg/ha	3.8 $\pm$ 0.5 a	0.09 $\pm$ 0.04	0.16 $\pm$ 0.03 ab	4.7 $\pm$ 0.2	6.9 $\pm$ 1.4 a
135 kg/ha	3.9 $\pm$ 0.5 a	0.11 $\pm$ 0.04	0.18 $\pm$ 0.03 a	4.5 $\pm$ 0.2	7.6 $\pm$ 1.4 a
<i>F</i> ¹	9.86	1.45	3.18	0.78	5.53
<i>P</i> > <i>F</i>	<0.0001	0.2340	0.0367	0.5142	0.0034
Cultivar					
ES 5200	2.3 $\pm$ 0.5	0.06 $\pm$ 0.03	0.14 $\pm$ 0.03	5.1 $\pm$ 0.2 a	6.8 $\pm$ 1.3
ES 5140	3.2 $\pm$ 0.5	0.10 $\pm$ 0.03	0.17 $\pm$ 0.03	4.0 $\pm$ 0.2 b	6.5 $\pm$ 1.3
M81E	3.0 $\pm$ 0.5	0.08 $\pm$ 0.03	0.16 $\pm$ 0.03	4.7 $\pm$ 0.2 a	6.4 $\pm$ 1.3
<i>F</i> ²	1.87	1.20 $\pm$ 0.03	1.29 $\pm$ 0.03	12.7	0.38
<i>P</i> > <i>F</i>	0.1705	0.3056	0.2887	<0.0001	0.6862

¹ df = 3,33; 3,81; 3,33; 3,33; 3,33

² df = 2,33; 2,81; 2,33; 2,33; 2,33

For the N rate and cultivar main effects, means followed by the same letter are not significantly different (*P* > 0.05, Tukey's HSD). No pairwise testing was done when *P* > *F* was greater than 0.05.

Figure 1. *Eoreuma loftini* adult emergence in high-biomass and sweet sorghum cultivars at four N fertilization rates, (a) 2013 and (b) 2014, Beaumont, TX.

Differences in adult emergence / stalk were not detected among treatments in 2014.

Figure 2. Simple linear regressions estimating relationships among N rate, *E. loftini* injury, and yield, Beaumont, TX, 2013–2014. Regressions include (A) N rate vs percentage of bored internodes, (B) percentage of bored internodes vs fresh weight per stalk, (C) percentage of bored internodes vs sucrose concentration, and (D) percentage of bored internodes vs ethanol productivity.

Data from 2013 and 2014 were analyzed separately.