Resistance Mechanisms of Sugarcane to Mexican Rice Borer (Lepidoptera: Pyralidae)

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ABSTRACT Larval antibiosis and adult and larval nonpreference studies were compared with field screening studies to determine the sugarcane characters and mechanisms that may confer resistance against the Mexican rice borer, *Eoreuma loftini* (Dyar). Larval antibiosis results of diet incorporation bioassays suggest the presence of antinutritional components or allelochemicals in some genotypes. Differences in adult oviposition among genotypes in laboratory, greenhouse, and field studies were slight; therefore, ovipositional preference is probably not important in conferring resistance. Laboratory experiments indicated that differences in larval establishment could be an important resistance character. Field results confirmed earlier research that sugarcane genotypes vary in their level of injury to this pest. However, field results did not always compare favorably with laboratory results, suggesting that several mechanisms of resistance may be important in this sugarcane–stalkborer system.

KEY WORDS *Eoreuma loftini, Saccharum,* plant resistance, antibiosis, nonpreference, stalkborers

TWO STALKBORING PYRALIDS, Mexican rice borer, *Eoreuma loftini* (Dyar), and sugarcane borer, *Diatraea saccharalis* (F.), are serious economic pests of sugarcane in the Lower Rio Grande Valley of Texas (Meagher et al. 1992). *E. loftini* was first detected in the Lower Rio Grande Valley in 1980 (Johnson and van Leerdam 1981) and has replaced *D. saccharalis* as the primary insect pest of sugarcane (Meagher et al. 1994). Insecticidal control of *E. loftini* has been generally unsatisfactory (Meagher et al. 1994). Classical biological control has also been unsuccessful (Browning and Melton 1987, Smith et al. 1987, Pfannenstiel et al. 1992). Lack of success with these pest management strategies has led to increased interest in plant resistance.

Plant resistance has been an important management strategy for stalkboring pyralids in many sugarcane-growing regions of the world (Mathes and Charpentier 1969, Nuss and Atkinson 1983). In Louisiana, plant resistance has been a component of the sugarcane integrated pest management program against *D. saccharalis* for many years and has been a successful management strategy when used alone or in combination with other strategies (Long et al. 1961, Bessin et al. 1990, White et al. 1993).

In Texas, the relative susceptibility of sugarcane progenitors and clones to stalk injury by *E. loftini* has been measured in field studies under natural infestation conditions. Results suggested large variability in bored internodes among progenitors such as *Miscanthus floridulus* (Labill) Warb., *Erianthus bengalense* (Retz.) Bharadw., *E. trinii* (Hack.), *Saccharum spontaneum* L., and *S. officinarum* L. (Ring and Browning 1990). Modern sugarcane is a complex polyploid with clones consisting of aneuploids developed as tri- and quadraspecific hybrids of *Saccharum* (White et al. 1993). Screening of commercial (cultivars) and noncommercial sugarcane clones showed variability in *E. loftini* injury (Pfannenstiel and Meagher 1991). Field evaluation of sugarcane germplasm for internodes bored by *E. loftini* has continued since 1989 (R.L.M., unpublished data).

Mathes and Charpentier (1969) classified components associated with stalkborer resistance as (1) unattractiveness of plants to adults for oviposition, (2) plant characters unfavorable for larval establishment in the plant, (3) plant characters that inhibit or retard larval development, and (4) plant tolerance (Painter's definition) (Painter 1951). Life history characteristics of stalkborers can provide further evidence for resistance factors in sugarcane germplasm (for review of life history characteristics, see Smith et al. 1993). The objectives of this

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research were to compare laboratory measures of larval antibiosis and adult and larval nonpreference studies with results of field injury so that mechanisms involved in conferring plant resistance to *E. loftini* can be determined.

Materials and Methods

Antibiosis Bioassays. Sugarcane leaf sheaths attached to the 5th leaf below the apical meristem were collected from mature plants of each cultivar and clone and stored at −20.0°C. Frozen leaf sheaths were then placed in a lyophilizer (Dura-Dry Condenser Module, FTS Systems, Stone Ridge, NY) for 60 h. Lyophilized tissue was passed through a 40-mesh screen using a Thomas Wiley Intermediate Mill (Thomas Scientific, Swedesboro, NJ). The resulting powder was placed in a sealed plastic bag and stored at −20.0°C. During meridic diet preparation (Martinez et al. 1988), the powder was added in the final steps at the rates of 5, 10, or 12 g/100 ml diet (=50, 100, 120 mg tissue per milliliter of diet). An additional 19.6, 39.2, and 47.0 ml of distilled water, respectively, was added to improve blending capability.

The meridic diet plus tissue was added to 25 diet cups (18.5 ml, Fill-Rite, Newark, NJ), and 1 *E. loftini* larva was added per cup. Larvae from an *E. loftini* culture collected in the Lower Rio Grande Valley and <1 yr old were used in all bioassays. Cups were placed in a growth chamber (Percival, Boone, IA) at 30°C and a 14:10 (L:D) h photoperiod. Larvae were weighed (Dexter A160, Dexter Instrument, Arvada, CO) after 14 d. After 21 d, cups were checked daily for pupal formation, and resulting pupae were weighed and their sex determined. The experiment was arranged as a randomized complete block design with 3 replications. A replicate consisted of a mean from 25 larvae.

Analysis of variance (ANOVA) (PROC GLM, SAS Institute 1987) was used to compare the 3 dependent variables larval weight, pupal weight, and days to pupation among leaf sheath tissue amounts added to meridic diet. The ANOVA model contained replication (3 levels), clone ('CP 68-350', 'CP 70-321', 'CP 70-324', 'CP 72-1210', 'CP 80-1827', 'CP 87-615', 'LCP 81-10', 'NCo 310', 'TCP 81-3058', 'TCP 83-3196', 'TCP 83-3215', 'TCP 86-3346', and 'TCP 89-4078'), tissue amount (0, 50, 100, 120 mg/ml), sex (2), and all 2nd- and 3rd-order interactions. ANOVA also was used to compare the same dependent variables among clones. However, measurements of larval weight, pupal weight, and days to pupation measurements from the diet tissue amounts (0 mg/ml) were combined and included as a "control" clone.

Nonpreference Bioassays. *Oviposition: Laboratory Choice and No-Choice Experiments.* Three separate tests were conducted to compare the ovipositional preference of moths with 2 standard sugarcane cultivars grown in Texas. Female *E. loftini* oviposit cryptically on the dried plant material near the base of the stalks by inserting the egg mass into folded leaf crevices (van Leerdam et al. 1984, 1986). To create this effect, dried sugarcane leaves were soaked in water for 5 min. One leaf edge was folded ≈7 mm inward while the entire leaf stem was pressed with heat (ironed). Four dried leaves of NCo 310 and CP 70-321 each, all 16.5 cm long, were alternately positioned vertically in a circle in soil within oviposition chambers (cardboard containers, 17 cm diameter, 17 cm deep). Test 1 contained 1 female and 2 male moths in the chambers (21 replications), and test 2 contained 5 female and 5 male moths (8 replications). Test 3 was a no-choice test in which 8 leaves of either cultivar were placed in the chambers with 5 females and males (5 replications). The chambers were placed in darkened incubators at 30°C. After 72 h, the leaves were removed, and the number of eggs on the 2 cultivars were recorded. Mexican rice borer females deposit eggs in masses ranging in number from 5 to 146; therefore, the number of eggs deposited was recorded rather than the number of egg masses (van Leerdam et al. 1986). For each test, a *t*-test for significant differences was performed (PROC T-TEST, SAS Institute 1987).

Oviposition: Greenhouse Experiments. Two tests were conducted during 1993 in large cages (1.8 m high, 1.8 m wide, 2.4 m long) within a greenhouse (fan and cooling pad design, a clear plastic covering, no shade cloth). The cages had contiguous wooden frames and were covered with white organdy cloth. Seams and joints of the cages were sealed with caulk to prevent the escape of *E. loftini.* Test 1 was a choice test that compared oviposition across 6 cultivars. Four young plants (at or nearly at the grand growth stage, ≈1.4 m tall) of each cultivar (CP 65-357, CP 70-321, CP 70-324, CP 71-1038, NCo 310, TCP 81-3058) were placed randomly within each cage. Fifty female and 15 male moths were released in the cage by placing pupae (sex determined) on a tray that was hung in the middle of the cage. Plants were sampled for eggs and number of dried, brown leaves 72 h after infestation. The test was repeated 3 times for a total of 4 trials. Data were analyzed using an ANOVA (PROC GLM, SAS Institute 1987) with trial, plant, and cultivar as class variables.

Test 2 individually compared NCo 310 versus CP 70-321, NCo 310 versus CP 70-324, and CP 70-321 versus CP 70-324. Five plants of each cultivar were randomly placed within each cage, and 30 females and 15 males were released. Three separate trials were conducted. Eggs and brown leaves were counted after 72 h. Data were analyzed using *t*-tests (PROC T-TEST, SAS Institute 1987).

Oviposition: Field Cage Experiment. Field plots were used to compare oviposition on 2 cultivars. In late 1989, NCo 310 and CP 70-321 were planted in alternating rows on the Texas Agricultural Experiment Station, Weslaco, using standard sug-

arcane planting procedures. Large cages (3.0 by 2.4 by 2.4 m) were placed over the top of both rows during May and September 1990 and May and August 1991. For each separate test, 25 female and 20 male moths were released per cage. The test was replicated 4 times by moving cages to different areas of the field. Sugarcane plants were sampled for *E. loftini* eggs and brown leaves 72 h after infestation. Data were analyzed using *t*-tests (PROC T-TEST, SAS Institute 1987).

Larvae: Laboratory Experiments. Sugarcane leaf whorl and leaf sheath tissue were used in choice tests to compare larval preference and establishment. The leaf whorl tissue test consisted of 5 cross sections (2 mm) of the standard cultivars NCo 310 or CP 70-321, and 5 pieces of a test cultivar placed alternately along the perimeter of a petri dish (9 cm diameter, 1.5 cm deep). The tested cultivars were CP 65-357, CP 70-321, CP 70-324, CP 71-1038, LCP 81-10, and TCP 81-3058. Petri dishes were filled with 2% (wt:vol) agar for moisture retention. Ten neonates were gently brushed onto the center of each petri dish and placed in a darkened incubator at 30°C. The number of larvae present on the sugarcane pieces was recorded 1, 3, 6, and 24 h after infestation (15 replications). Data were analyzed using *t*-tests (PROC T-TEST, SAS Institute 1987).

In the test of leaf sheath tissue, tissue from the 1st–4th leaf sheaths (counting from stalk bottom upward) and leaf roll tissue ≈1 mm above the apical meristem was used. Leaf roll tissue is that part of the apparent stem that consists of leaf tissue. It exists from the terminal bud to the collar of the youngest fully expanded leaf. The outer layers are primarily leaf sheath tissue and the inner portion is mostly young blade tissue. Three 5-mm cross sections of NCo 310 and CP 70-321 were placed alternately along the perimeter of a petri dish (9-cm diam.). Dishes were filled with 2% (wt:vol) agar for moisture retention. Twenty neonates were positioned in the dish center, and dishes were placed in a darkened incubator at 30°C. The number of larvae present was recorded 1, 4, and 24 h after infestation (5 replications). Data were analyzed using *t*-tests (PROC T-TEST, SAS Institute 1987).

Field Susceptibility. A comparison of 12 cultivars and clones with the Texas industry standard cultivar NCo 310 was completed using advanced breeding line evaluations from the 1989–1990 to 1994–1995 field seasons (R.L.M., unpublished data). Methods used to evaluate cultivars and clones were described by Pfannenstiel and Meagher (1991). Briefly, field plantings with 16–22 cultivars and clones were evaluated for agronomic performance and stalkborer injury in plots with 4 rows (9.1 m long) on a 1.5-m row spacing. Field layout was a randomized complete block design with 4 replications, and plantings were replicated across several Lower Rio Grande Valley locations. To evaluate stalkborer injury, 15–20 stalks were removed from the outer 2 rows of each plot at harvest. Stalks were mechanically split longitudinally and examined. The total number of internodes and the number of internodes injured were counted to calculate percentage bored internodes (Pfannenstiel and Meagher 1991, Meagher et al. 1994). Comparisons between NCo 310 and selected cultivars and clones were analyzed for each planting by ANOVA (PROC GLM–CONTRAST statement, SAS Institute 1987).

Table 1. Growth and developmental parameters for *E. loftini* fed meridic diet containing different sugarcane clone leaf sheath tissue

Tissue amount, mg/ml[a]	n^b	Wt, mg		No. days to pupation
		Larvae	Pupae	
0	924	24.6 ± 1.2a	37.9 ± 1.2a	23.0 ± 0.3a
50	945	21.1 ± 1.1b	33.2 ± 1.0b	24.1 ± 0.3b
100	872	16.3 ± 0.8c	29.6 ± 0.9c	26.2 ± 0.3c
120	802	14.5 ± 0.8c	28.8 ± 0.8c	26.5 ± 0.3c

For each variable, means ± SE followed by the same letter are not significantly different ($P > 0.05$, Waller–Duncan k ratio t-test). ANOVA for larval weight $F = 23.7$; df = 3, 200; $P < 0.0001$; for pupal weight $F = 59.4$; df = 3, 200; $P < 0.0001$; for days to pupation $F = 53.1$; df = 3, 200; $P < 0.0001$.

[a] Milligrams of sugarcane tissue per milliliter of meridic diet.

[b] Total number of larvae completing development.

Results

Antibiosis Bioassays. Adding increasing amounts of leaf sheath tissue to the meridic diet affected growth and development of *E. loftini* negatively. Larval and pupal weights were lower and days to pupation were longer when leaf sheath tissue was added to the diet (Table 1). Female larvae and pupae were heavier than those of the male (female larvae 20.1 ± 0.8 mg, male 18.0 ± 0.7 mg, $P = 0.024$; female pupae 38.9 ± 0.6 mg, male 25.7 ± 0.3 mg, $P < 0.0001$, respectively) and took longer to develop than males (female 25.7 ± 0.2 d, male 24.2 ± 0.2 d, $P < 0.0001$). The only interaction that was significant was diet amount × sex for pupal weight ($P < 0.0001$). This interaction can be explained by smaller differences between female and male pupal weights as increasing amounts of sugarcane tissue were added to the diet.

Clones expressed genotypic differences in larval and pupal weights and days to pupation (all $P < 0.0001$). Larvae fed on meridic diet (control) and diet mixed with tissue from TCP 89-4078 and NCo 310 were significantly heavier than larvae fed diet mixed with other genotypes (Table 2). Resulting pupae from the control diet were heavier than other pupae, whereas pupae from mixtures with TCP 83-3196, TCP 81-3058, CP 80-1827, and LCP 81-10 were lowest in weight. Developmental times were the shortest on control diet and mixtures with NCo 310, TCP 89-4078, and CP 70-321, whereas larvae took the longest to develop on CP 80-1827.

Table 2. Responses of *E. loftini* larvae after feeding on meridic diet containing various amounts of different sugarcane clone tissue

Clone	Wt, mg		No. d to pupation
	Larval	Pupae	
Control	24.7 ± 1.3a	37.9 ± 4.0a	22.9 ± 0.3a
TCP 89-4078	24.6 ± 2.4ab	32.7 ± 2.0bcd	23.8 ± 0.5ab
NCo 310	22.0 ± 2.1abc	33.6 ± 2.0bc	23.3 ± 0.4a
CP 70-321	19.4 ± 1.5bcd	34.2 ± 1.9b	23.9 ± 0.3ab
TCP 86-3346	19.2 ± 2.1cd	32.9 ± 1.7bcd	25.4 ± 0.6cd
TCP 81-3058	19.1 ± 1.7cd	28.3 ± 1.7ef	26.6 ± 0.6de
TCP 83-3196	19.1 ± 2.2cd	26.3 ± 1.5f	26.4 ± 0.7cde
TCP 83-3215	18.5 ± 2.3cde	29.9 ± 1.9de	25.3 ± 0.5cd
CP 72-1210	15.9 ± 1.6def	29.7 ± 2.3def	26.4 ± 0.6cde
CP 80-1827	15.7 ± 1.9def	28.5 ± 2.1ef	27.7 ± 0.6e
CP 70-324	14.4 ± 0.8def	30.7 ± 1.6cde	25.8 ± 0.6cd
CP 68-350	13.3 ± 0.8ef	30.7 ± 1.8b–e	25.2 ± 0.4bc
CP 87-615	12.0 ± 0.8f	30.3 ± 1.8cde	26.2 ± 0.6cd
LCP 81-10	11.6 ± 1.0f	28.8 ± 1.7ef	26.7 ± 0.6de

For each variable, means ± SE followed by the same letter are not significantly different ($P > 0.05$, Waller–Duncan k ratio t-test). ANOVA for larval weight $F = 4.8$; df = 12, 156; $P < 0.0001$; for pupal weight $F = 4.1$; df = 12, 156; $P < 0.0001$; for days to pupation $F = 7.3$; df = 12, 156; $P < 0.0001$.

Nutritional factors and allelochemical analysis of leaf tissue has not been investigated thoroughly in sugarcane as it has in other crops (Woodhead and Taneja 1987). Our study represents the 1st use of a diet incorporation bioassay to determine antibiosis of sugarcane tissue. Larval weight and days to pupation were not affected by the addition of leaf sheath tissue from the known susceptible genotype (NCo 310) compared with the meridic diet control. Therefore, it appears the differences between the resistant genotypes (CP 80-1827, CP 72-1210, and TCP 83-3196) and the susceptible genotypes were a result of allelochemicals in the tissue rather than from varying textures in the treatment diets (Bong et al. 1991). Of the 3 dependent variables analyzed, pupal weight explained the most variation in the models, followed by days to pupation and larval weight ($r^2 = 0.822, 0.639, 0.468$, respectively). It is not known why larval weight measured at 14 d was erratic. Even in the control or meridic diet, many larvae of the same sex were quite variable in weight but had similar pupal weights and developmental periods. Further experimentation into the physiological and behavioral requirements of *E. loftini* larvae may help to explain this variation.

Nonpreference Bioassays. *Oviposition: Laboratory Choice and No-Choice Experiments.* No significant differences were found in eggs per leaf in comparisons between NCo 310 and CP 70-321 in either choice test (test 1, $t = -0.14$, df = 31.3, $P = 0.89$; test 2, $t = 0.72$, df = 11.0, $P = 0.48$). In choice test 1, individual females laid 5.8 ± 1.4 eggs on NCo 310 leaves compared with 6.14 ± 2.1 eggs on CP 70-321. In choice test 2, 6.4 ± 1.9 eggs were laid per female per leaf on NCo 310 compared with 4.7 ± 1.0 eggs on CP 70-321. In the no-choice tests, females laid more eggs on CP 70-321 leaves than on NCo 310 leaves (10.4 ± 2.8 versus 2.9 ± 1.0, respectively, $t = -3.02$, df = 62, $P = 0.0036$).

Table 3. Ovipositional responses of *E. loftini* females to different sugarcane cultivars in a greenhouse test

Cultivar	No. eggs/plant	No. brown leaves/plant
CP 71-1038	3.5 ± 0.5a	8.7 ± 0.7a
NCo 310	1.7 ± 0.7b	5.3 ± 0.4bc
TCP 81-3058	1.3 ± 0.6b	5.1 ± 0.3c
CP 65-357	1.0 ± 0.3b	4.9 ± 0.5c
CP 70-321	0.9 ± 0.3b	6.4 ± 0.4b
CP 70-324	0.6 ± 0.1b	5.8 ± 0.2bc

For each variable, means ± SE followed by the same letter are not significantly different ($P > 0.05$, Waller–Duncan k ratio t-test). ANOVA for eggs $F = 5.4$; df = 5, 84; $P = 0.0003$; for brown leaves $F = 9.0$; df = 5, 84; $P < 0.0001$.

Oviposition: Greenhouse Experiments. CP 71-1038 contained more eggs and brown leaves per plant than others in the multicultivar experiment (Table 3). In test 2, individual comparisons showed no differences in number of eggs laid between cultivars; however, there were trends for NCo 310 to contain more eggs per plant than CP 70-321 and CP 70-324, and CP 70-324 to contain more eggs than CP 70-321 (Table 4). NCo 310 had fewer brown leaves than the other 2 cultivars.

Oviposition: Field Cage Experiment. There was a nonsignificant trend for more eggs to be collected from NCo 310 than CP 70-321 plants in the summer 1990 and fall 1991 tests (Fig. 1). The opposite trend was evident in the spring experiments of both years. The number of brown leaves per plant was similar between cultivars.

Our results suggest differences among sugarcane germplasm in number of eggs laid, although this was not necessarily related to brown, dry leaves. The amount of dry leaves available for oviposition has been suggested to be important with *Eldana saccharina* Walker (Nuss and Atkinson 1983), a stalkborer with life history attributes sim-

Table 4. Ovipositional responses of *E. loftini* females to different sugarcane cultivars in individual comparisons in a greenhouse test

Cultivar comparison	No. eggs/plant	No. brown leaves/plant
NCo 310	2.8 ± 0.6a	6.2 ± 0.3b
CP 70-321	1.4 ± 0.4a	8.0 ± 0.8a
NCo 310	3.9 ± 1.0a	6.0 ± 0.2b
CP 70-324	2.7 ± 0.3a	8.0 ± 0.5a
CP 70-321	2.0 ± 0.4a	8.1 ± 0.5a
CP 70-324	3.0 ± 0.5a	8.7 ± 0.6a

For each variable within each cultivar comparison, means ± SE followed by the same letter are not significantly different ($P > 0.05$, t-test). t-tests for NCo 310 versus CP 70-321, eggs $t = 1.83$, df = 33.7, $P = 0.077$, brown leaves $t = -2.20$, df = 38.0, $P = 0.0337$; t-tests for NCo 310 versus CP 70-324, eggs $t = 1.24$, df = 38.0, $P = 0.2215$, brown leaves $t = -3.68$, df = 38.0, $P = 0.0007$; t-tests for CP 70-321 versus CP 70-324, eggs $t = -1.37$, df = 36.3, $P = 0.1792$, brown leaves $t = -0.73$, df = 37.8, $P = 0.4726$.

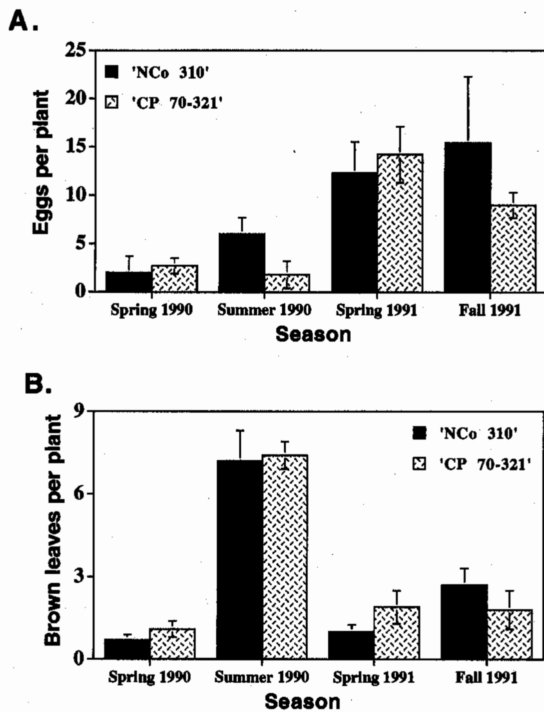

Fig. 1. Ovipositional responses of *E. loftini* females on different sugarcane cultivars in individual comparisons in field cage tests. *T*-tests for spring 1990, eggs $t = -0.39$, df = 4.3, $P = 0.7158$, brown leaves $t = -1.10$, df = 5.4, $P = 0.3168$; *t*-tests for summer 1990, eggs $t = 1.93$, df = 5.9, $P = 0.1036$, brown leaves $t = -0.16$, df = 4.0, $P = 0.8795$; *t*-tests for spring 1991, eggs $t = -0.45$, df = 5.9, $P = 0.6679$, brown leaves $t = -1.51$, df = 4.2, $P = 0.2018$; *t*-tests for fall 1991, eggs $t = 0.91$, df = 2.1, $P = 0.4552$, brown leaves $t = 0.928$, df = 3.9, $P = 0.4068$.

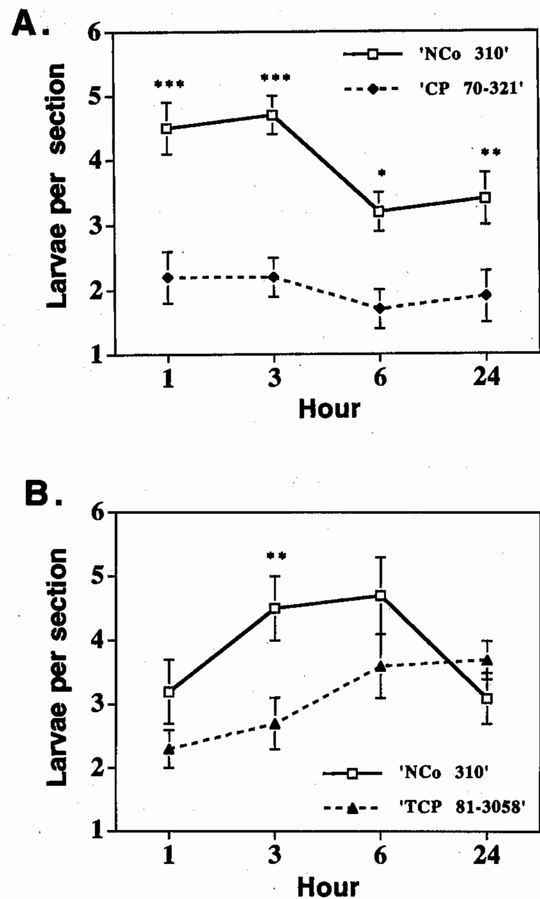

Fig. 2. Larvae of *E. loftini* found on sugarcane leaf whorl tissue from NCo 310 compared with other cultivars in laboratory choice tests. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ by PROC T-TEST (SAS Institute 1987).

ilar to those of *E. loftini* (Atkinson 1980). Generally, ovipositional resistance has not been correlated with lower stalkborer populations in sugarcane (Tucker 1933, Kyle and Hensley 1970, Fuchs and Harding 1978, David and Joseph 1984), but characters such as leaf pubescence have been shown recently to be associated with resistance against *D. saccharalis* (Sosa 1988, 1990).

Larvae: Laboratory Experiments. In tests using leaf whorl tissue, CP 70-321 had fewer larvae than NCo 310 for 1, 3, 6, and 24 h after infestation (Fig. 2a). TCP 81-3058 had fewer larvae than NCo 310 only at 3 h after infestation (Fig. 2b). In comparisons with CP 70-321, fewer larvae were collected on CP 71-1038, whereas more larvae were collected on LCP 81-10 and TCP 81-3058 (Fig. 3). The other tested cultivars contained numbers of larvae similar to NCo 310 or CP 70-321 ($P > 0.05$). In tests using leaf sheath tissue, only the 4th leaf sheath produced significant differences between NCo 310 and CP 70-321 (hour 4, 8.0 ± 0.7, 3.1 ± 1.1, respectively, $t = 3.73$, df = 6.7, $P = 0.008$; hour 24, 8.0 ± 0.9, 4.2 ± 0.7, respectively, $t = 3.17$, df = 7.5, $P = 0.014$). The 1st, 2nd, and 3rd leaf sheaths and leaf roll contained similar numbers of larvae on both cultivars ($P > 0.05$).

Larval resistance can be separated into "leaf" and "stalk" resistance. Neonate and young larvae must be able to become established within the leaves, midribs, and leaf sheaths and obtain sufficient nutrients before entering stalks. In our tests, larvae showed preferences for foliar establishment in certain genotypes, and it appears larval preference may be locationally directed among different leaf sheaths within a stalk. The lack of foliar establishment and mortality of neonate larvae has been described as a major factor of resistance (Kyle and Hensley 1970, David and Joseph 1984), with leaf sheath appression, the ability of a plant to self-trash (shed lower leaves and leaf sheaths), and leaf midrib hardness documented as specific resistant characters (Chang and Shih 1959, David and Kalra 1967, Agarwal 1969, Coburn and Hensley 1972, David and Joseph 1982). However, larval foliar establishment among cultivars as a resistance factor becomes important only if these differences persist

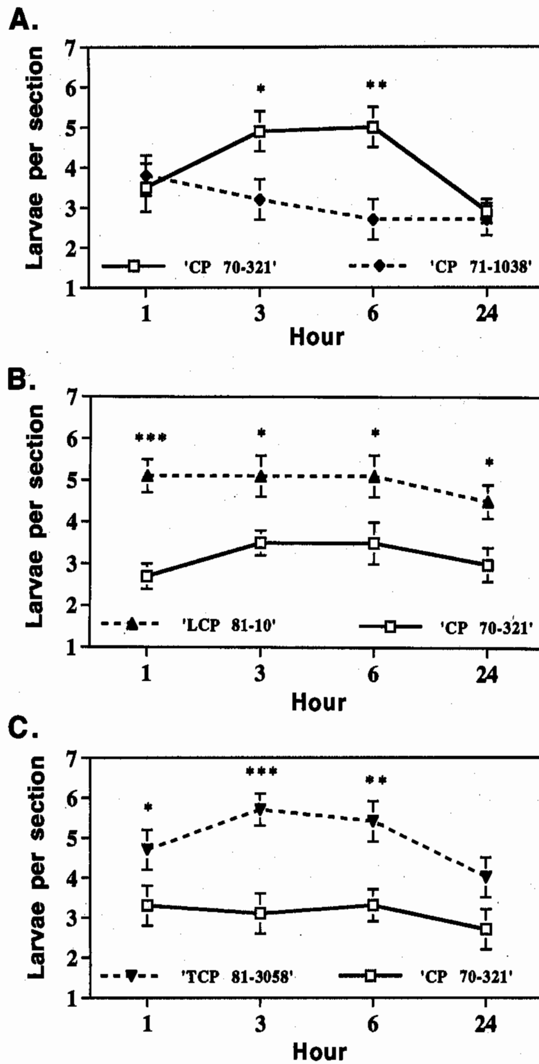

Fig. 3. Larvae of *E. loftini* found on sugarcane leaf whorl tissue from CP 70-321 compared with other cultivars in laboratory choice tests. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ by PROC T-TEST (SAS Institute 1987).

until stalks are invaded (Bernays et al. 1983); if over longer periods of development, the final level of infestation is independent of initial numbers, then differences among cultivars in establishment are not important (Chapman et al. 1983). Our study did not investigate factors specifically involved with stalk resistance. Midsized and larger larvae must be able to enter, become established, tunnel within the stalk, and gain enough nutrition to complete development and emerge as a mature, fecund adult.

Field Susceptibility. Bored internodes from the standard cultivar NCo 310 were compared with other clones in 41 field plantings. CP 70-324, TCP 89-4078, TCP 83-3196, and CP 80-1827 had significantly fewer bored internodes in 62.5% (5 of 8 comparisons), 60.0% (6 of 10), 57.1% (4 of 7), and 53.9% (7 of 13) of the plantings, respectively (Table 5). CP 70-321 (28.6%, 8 of 28), CP 72-1210 (25.0%, 5 of 20), CP 68-350 (25%, 3 of 12), TCP 81-3058 (25.0%, 1 of 4), TCP 83-3215 (14.3%, 1 of 7), and CP 87-615 (6.3%, 1 of 16) also had a significantly lower number of bored internodes, but in a fewer percentage of plantings. TCP 86-3346 (40%, 2 of 5) and LCP 81-10 (16.7%, 1 of 6) had a significantly higher number of bored internodes than NCo 310.

Table 5. Sugarcane genotypic comparison with NCo 310 of field resistance, larval antibiosis, and larval nonpreference

	Field resistance, %[a]	Leaf sheath antibiosis			Larval nonpreference[b]
		LW	PW	DP	
CP 70-324	62.5	+	−	+	−
TCP 89-4078	60.0	−	−	−	N
TCP 83-3196	57.1	−	+	+	N
CP 80-1827	53.9	+	+	+	N
CP 70-321	28.6	−	−	−	+
CP 72-1210	25.0	+	+	+	N
CP 68-350	25.0	+	−	+	N
TCP 81-3058	25.0	−	+	+	−
TCP 83-3215	14.3	−	+	+	N
CP 87-615	6.3	+	−	+	N
LCP 81-10	−16.7	+	+	+	−
TCP 86-3346	−40.0	−	−	+	N

Positive or negative (+ or −) antibiosis compared with NCo 310, using the diet incorporation variables larval weight (LW), pupal weight (PW), or days to pupation (DP). For example, CP 70-324 produced larvae with lower larval weights and longer developmental times than NCo 310 but had comparable pupal weights.

[a] Percentage of plantings where bored internodes were significantly different from NCo 310.

[b] Defines fewer larvae found compared with NCo 310; N, not tested.

Discussion

Comparisons in our studies among leaf sheath bioassays, adult and larval nonpreference studies, and field susceptibility results pose interesting questions (Table 5). The lack of antibiosis determined in laboratory tests of TCP 89-4078 and CP 70-321 indicates that larvae function well once they have entered leaf sheaths but have difficulty becoming established in leaf sheaths, entering stalks, or tunneling within stalks. Low larval numbers found on pieces of CP 70-321 leaf sheath provided evidence for low larval establishment in leaf sheaths. The opposite scenario was true for LCP 81-10, a genotype possessing high stalk injury. Diet mixtures with this genotype produced small larvae and pupae and long development times, but larval establishment, as indicated by numbers of larvae on leaf sheath pieces, was comparable with NCo 310. Perhaps stalk admittance and consumption by *E. loftini* on LCP 81-10 is more efficient than on other genotypes. CP 70-324, a genotype possessing field resistance, showed evidence for leaf sheath

antibiosis and a trend for ovipositional nonpreference (Tables 3 and 4) but provided no evidence for larval nonpreference.

Our results confirm that several mechanisms of stalkborer resistance, including antibiosis and nonpreference, are present across sugarcane genotypes. Tolerance has been suggested as a resistance mechanism in the *D. saccharalis* crop system, a conclusion based on genotypes possessing high levels of injury such as bored internodes, but low levels of damage such as dead tops, adventitious shoots, secondary tillering, and cane weight loss (White and Hensley 1987, White 1993). However, studies to determine if tolerance is a resistance mechanism in the *E. loftini* crop system have not been completed. Breeding of sugarcane for resistance to stalkborers is difficult because of hereditary characteristics of the plant (Ashraf and Fatima 1990, White et al. 1993) and limited knowledge of specific resistant characters. Sugarcane plant breeding and genetic engineering research (Gallo-Meagher and Irvine 1993) conducted to improve insect management would be enhanced by additional studies to determine resistance mechanisms and character identification precisely.

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