A NUTRITIONAL PERSPECTIVE OF SUGARCANE RESISTANCE TO STALK BORERS AND SAP FEEDERS

By

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Abstract

TWO RELATIVELY new key species in Louisiana that conform to the plant stress hypothesis are the Mexican rice borer, *Eoreuma loftini* (Dyar) and the sugarcane aphid, *Melanaphis sacchari* (Zehntner). High performance liquid chromatography differentiated insect resistant and susceptible sugarcane cultivars based on nutritional profiles involving free amino acids (FAAs). For *E. loftini* susceptible cultivar LCP 85-384, concentrations of essential and nonessential FAAs in whole leaf tissue samples were more than twice as high as in the resistant cultivar HoCP 85-845. Similarly, *M. sacchari* susceptible L 97-128 exhibited more than three fold higher concentrations of essential FAAs in the phloem sap compared to the resistant cultivar, HoCP 91-555. Two essential FAAs, histidine and arginine, were detected only in the sap of L 97-128. A novel approach to evaluate insect resistant and susceptible cultivars is proposed.

Introduction

The Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae) and sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) conform in their herbivore-plant interactions to the plant stress hypothesis (White, 1969). In this relationship, host plants stressed from lack of water, increased salt, reduced soil fertility, or excessive damage are more suitable hosts enhancing one or more aspects of herbivore bionomics (Reay-Jones *et al*., 2003, 2005b, 2007a). *M. sacchari* and *E. loftini* are relatively new pests to Louisiana sugarcane (White *et al*., 2001, Hummel *et al*., 2008). In contrast, the more established invasive species, the sugarcane borer, *Diatraea saccharalis* (F.) conforms to the plant vigour hypothesis where the biology is enhanced by an association with vigorously growing plants (Price, 1991).

Introduced from Mexico into the Lower Rio Grande Valley of Texas in 1980 (Johnson, 1985), *E. loftini* is the major insect pest of sugarcane representing more than 95% of stalk borer populations in that area (Reay-Jones *et al*., 2005a). The insect spread northward and north-eastward into Louisiana at a rate of 16.5–23 km/year (Reay-Jones *et al.*, 2007b). *E. loftini* has been cited as having a potential economic impact on the Louisiana sugarcane industry of up to $220 million/annum (Reay-Jones *et al*., 2008). Cryptic oviposition sites on dried sugarcane leaves, larval mining of midribs and leaf sheaths, boring into stalks within 3–7 days after egg hatch, and pupation in frass-packed tunnels render *E. loftini* inaccessible to control by biological and chemical agents (Reay-Jones *et al*., 2005a).

M. sacchari is the most abundant aphid species in Louisiana sugarcane. It vectors sugarcane yellow leaf virus (SCYLV). SCYLV was discovered in Louisiana in 1999, and in 2002, 48% of

fields were infected throughout the industry (McAllister *et al*., 2008). Disease management of SCYLV is facilitated using a seed cane certification program conducted by the Louisiana Department of Agriculture and Forestry (McAllister *et al*., 2008). Spread and incidence of SCYLV depends mainly on the spring migration of *M. sacchari* into the field (McAllister *et al*., 2005). This indicates potential for using resistant cultivars to prevent disease spread, since these cultivars will presumably attract fewer aphids and/or negatively affect their biology.

The *M. sacchari*-resistant cultivar (HoCP 91-555) and experimental lines with *E. loftini* resistance similar to the commercial resistant HoCP 85-845 demonstrate potential for managing these two invasive pests based on host plant resistance. This paper highlights some of the biochemical relationships involving free amino acids (FAAs) associated with *Saccharum* spp. plant resistance to *E. loftini* and *M. sacchari.*

Eoreuma loftini

Screening and finding genetic sources of resistance to *E. loftini* is an important component of the variety development program of the Louisiana State University AgCenter. Commercial and experimental cultivars are evaluated yearly in replicated trials under natural infestations with heavy *E. loftini* pressure at Ganado in Jackson County, TX. Initial studies at this location conducted in 2002 helped identify *E. loftini* susceptible (LCP 85-384) and resistant (HoCP 85-845) cultivars that are used as standards (Table 1). Even though the older cultivar CP 70-321 demonstrated significantly better resistance than HoCP 85-845 under heavy pressure, studies at Weslaco in Hidalgo County with lighter *E. loftini* infestations, showed that HoCP 85-845 would make a better standard (Reay-Jones *et al*., 2003). Assessing resistance to *E. loftini* requires determination of percentage of bored internodes and per hectare moth production based on frequency of adult (moth) emergence holes (Table 1).

Table 1—Injury (± SEM) by *E. loftini* to six sugarcane cultivars, resultant survival (± SEM) of older larvae inside the stalks, and moth production (± SEM) at Ganado, Jackson County, TX, 2002 (Reay-Jones *et al.,* 2003).

Cultivar	% Bored internodes	Relative survival[a]	Moth emergence x10^3 /ha[b]
LCP 85-384	67.5 (5.7)a	0.225 (0.065)a	112.3 (37.5)a
HoCP 96-540	62.5 (6.8)a	0.200 (0.030)a	105.6 (7.9)a
HoCP 91-555	57.5 (3.4)ab	0.363 (0.144)a	165.1 (65.4)a
HoCP 85-845	47.2 (4.9)bc	0.150 (0.039)a	62.7 (17.0)a
NCo 310	36.2 (3.1)cd	0.166 (0.035)a	53.1 (13.4)a
CP 70-321	28.3 (1.9)d	0.171 (0.023)a	39.1 (5.5)a
F[c]	34.01	1.27	2.12
P	< 0.0001	0.316	0.106

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

Further studies were conducted to assess nutritional differences between these cultivars using high performance liquid chromatography (HPLC). Whole leaf tissue sample analyses revealed differences in FAA concentrations. As shown in Table 2, the total amount of essential FAAs was more than twice as high in susceptible LCP 85-384 compared with HoCP 85-845.

Table 2—Selected free amino acid (FAA) accumulations (nmol/10 µL juice) in sugarcane leaves (5 node stage)n from *E. loftini* susceptible LCP 85-384 and resistant HoCP 85-845, greenhouse oviposition studies at Weslaco, TX 2003-2004 (Reay-Jones *et al*., 2007a).

Free amino acid	HoCP 85-845	LCP 85-384
Arginine	14	31
Histidine	92	251
Methionine	0	3.8
Threonine	42	176
Valine	56	125
[a]Sum	273	612
*Proline	459	1680
*Aspartic acid	252	379
*Serine	177	416

[a]Total of selected 'essential free amino acids,' as a necessary component in insect diet, critical for growth and development (Chapman, 1998).
*Nonessential amino acids are not critical for growth but are needed for optimal growth of insect (Chapman, 1998).

These studies also showed a strong ovipositional preference for cultivars and plant conditions which had higher amounts of essential FAAs. Methionine, an essential amino acid was only detected in the leaves of the susceptible LCP 85-384. Ovipositional preference of *E. loftini* indicated positive associations between egg masses per plant and both essential FAAs (arginine, phenylalanine, and threonine) and dry leaves, and between eggs per plant and both essential FAAs (methionine and threonine) and dry leaves (Reay-Jones *et al.*, 2007a). It is also pointed out that this increased nutritional quality is not necessarily limited to just FAAs.

Melanaphis sacchari

Greenhouse studies on biotic potential of *M. sacchari* on commonly grown commercial sugarcane cultivars in Louisiana have shown significant cultivar effects on aphid reproductive rates. The cultivar with the least biotic potential (0.06) was HoCP 91-555 compared to the maximum (0.16) on L 97-128, showing greater than 2.6-fold differences in the number of offspring per female per day (Fig. 1).

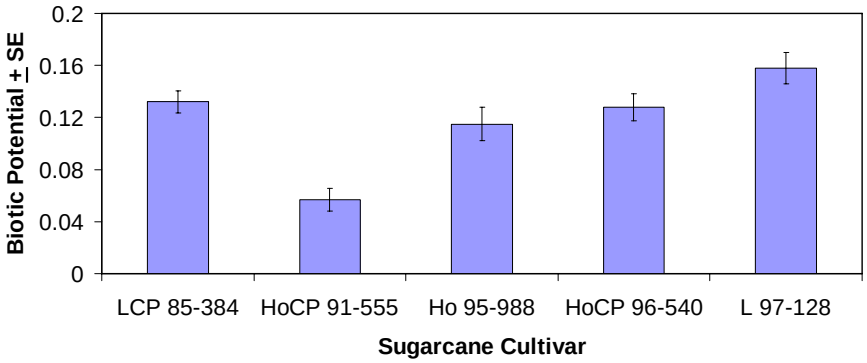

Fig. 1—*Melanaphis sacchari* biotic potential on Louisiana sugarcane.

FAAs in the phloem sap indicated that free histidine and arginine were present only in susceptible cultivar L 97-128 (Table 3). The total amount as well as the percentage of essential FAAs in the phloem sap of L 97-128 was also higher when compared to that in the sap of the resistant cultivar HoCP 91-555. In contrast, the phloem sap of HoCP 91-555 was dominated by nonessential FAAs. On-going analyses of honeydew from aphids feeding on both cultivars is

revealing that certain FAAs are detected only in the honeydew of the susceptible cultivar L 97-128 suggesting that the aphid is able to synthesise these FAAs while feeding on this cultivar.

Table 3—Free amino acid concentrations in the whole leaf tissue (nmoles/10 µL juice) and phloem sap (picomoles/ µL solution) of the *E. loftini* and the *M. sacchari* resistant and susceptible sugarcane cultivars.

	E. loftini		*M. sacchari*	
	Resistant	Susceptible	Resistant	Susceptible
Free amino acid	HoCP 85-845	LCP 85- 384	HoCP 91-555	L 97-128
Alanine	447	1442	268	168
Arginine	14	31	0	14
Aspartic acid	252	379	148	89
Glutamic acid	238	96	167	117
Glycine	43	0	46	48
Histidine	92	251	0	65
Isoleucine	12	9	0	0
Leucine	24	13	0	0
Lysine	0	0	0	0
Methionine	0	4	0	0
Phenylalanine	33	4	0	0
Proline	459	1680	0	0
Serine	177	416	114	115.5
Threonine	42	176	23	26
Tyrosine	144	18	0	0
Valine	56	125	16	0
Total	2033	4643	781	642
Total essential[a]	273	612.4	39	105
Total nonessential	1760	4030	742	536
% essential	13.4	13.2	5.0	16.4
% nonessential	86.6	86.8	95	83.6

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

Discussion

Population growth and in turn the pest population on many host plants relates to the plant's nutritional status, and often the availability of plant FAAs (McNeil and Southwood, 1978; Showler, 2004) in susceptible versus resistant cultivars. Resistant and susceptible cultivars to *E. loftini* and *M. sacchari* are showing association with the presence of certain amino acids (Table 3), consistent with the concept that their absence can prevent insect growth (Chapman, 1998). We are finding that some essential amino acids can be synthesised by the sugarcane aphid, and this can help increase their biotic potential on some cultivars lacking those FAAs.

Moths utilise contact chemoreceptors on their antennae, proboscis, tarsi, and ovipositor to assist in completing the behavioural responses essential to accepting or rejecting a host plant (Kogan, 1994). Assuming that *E. loftini* can detect host plant FAA levels and that such levels influence oviposition preference, levels of essential FAAs may help explain variability in ovipostion. *E. loftini* oviposition was associated with arginine (egg masses per plant) and aspartic acid (eggs laid per plant), which accumulated under water deficit stress (Reay-Jones *et al*., 2007a). Thus, host plant foliar FAAs likely affected the oviposition preference of *E. loftini,* a devastating insect pest of sugarcane.

Aphids feed on phloem sap that largely consists of sugars and is limited in nitrogenous compounds. Essential amino acids in the phloem sap are generally less than 25% of the total amino acid concentration with some less than 0.2% (Chapman, 1998). A close association exists between host plant nutritional status including nutrient ratios and development of aphids (Dixon, 1985, Febvay *et al.,* 1988). Our study suggests that the differences in biotic potential on L 97-128 and

HoCP 91-555 are associated with differences in composition and concentration of FAAs detected in the sap.

The preferred feeding site of *M. sacchari* is the lower senescing leaves of sugarcane plants, and one reason for this may be the higher levels of FAAs in these leaves (Reay-Jones *et al*., 2005b). *E. loftini* prefers to oviposit on senescing leaves of sugarcane that are associated with higher FAAs levels. Therefore, reducing sugarcane drought-stress with irrigation is cited as a way of decreasing *E. loftini* oviposition and possibly *M. sacchari* build up by decreasing both the nutritional value of the crop for these insects and the number of ovipositional sites (i.e., dry leaves) for *E. loftini*. The practical value of these fundamental studies is the potential use of a non-bioassay approach to evaluate resistance and susceptibility of sugarcane cultivars to stalk borers and sap feeders. This would allow screening and selection of insect resistant cultivars in the absence of invasive species, a technique of value in proactive research when the pest has not yet invaded.

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UNE PERSPECTIVE NUTRITIONNELLE DE LA RESISTANCE DE LA CANNE À SUCRE AUX FOREURS DES TIGES ET AUX INSECTES PIQUEURS-SUCEURS

Par

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MOTS CLES: *Eoreuma loftini, Melanaphis sacchari, Saccharum* spp., Acides Aminés Libres, Cultivar Résistant.

Résumé

PARMI LES principaux insectes ravageurs attaquant la canne à sucre en Louisiane, le foreur des tiges *Eoreuma loftini* (Dyar) et le puceron *Melanaphis sacchari* (Zehntner) se comportent similairement dans leurs interactions avec leur plante hôte. Ces deux insectes suivent la « plant stress hypothesis », hypothèse selon laquelle un herbivore préfèrerait une plant stressée. La technique de chromatographie liquide à haute performance a été utilisée pour différencier les cultivars résistants et sensibles à ces insectes en se basant sur les profiles des acides aminés libres (AAL) présents. Dans le cultivar sensible a *E. loftini*, LCP 85-384, les concentrations en AAL essentiels et non-essentiels étaient plus du double de celles mesurées dans le cultivar résistant, HoCP 85-384. De même, L 97-128, un cultivar sensible a *M. sacchari*, avait des concentrations en AAL essentiels plus de trois fois supérieures a celles mesurées dans la sève élaborée du cultivar résistant HoCP 91-555. Deux AAL essentiels, l'histidine et l'arginine, n'ont été détectés que dans la sève de L 97-128. A partir de ces résultats, la possibilité d'une approche évaluant la résistance des cultivars de canne à sucre à certains ravageurs sans recourir à des infestations d'insectes est discutée.

UNA PERSPECTIVA NUTRICIONAL DE LA RESISTENCIA DE LA CAÑA DE AZUCAR A BARRENADORES DEL TALLO Y CHUPADORES

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PALABRAS CLAVE: *Eoreuma loftini, Melanaphis sacchari, Saccharum* spp., Amino Acidos Libres, Cultivos Resistentes.

Resumen

DOS PLAGAS relativamente nuevas, el barrenador mexicano del arroz, *Eoreuma loftini* (Dyar) y el áfido gris de la caña de azúcar, *Melanaphis sacchari* (Zehntner), son claves en Luisiana para probar la hipótesis del estresamiento de la planta. A través de la técnica de la cromatografía líquida de alta eficacia, se pudo diferenciar cultivares de caña de azúcar resistentes y susceptibles al ataque de insectos, basados en perfiles nutricionales que involucran amino ácidos libres (FAAs). Para el cultivar LCP 85-384 susceptible a *E. loftini,* las concentraciones de los FAAs esenciales y no esenciales en las muestras de todo el tejido de la hoja fueron superiores en más de dos veces que las encontradas en el cultivar resistente HoCP 85-845. Similarmente, L 97-128 susceptible a *M. sacchari* presentó concentraciones tres veces más altas de FAAs en la savia del floema al compararlo con el cultivar resistente, HoCP 91-555. Dos FAAs esenciales, histidina y arginina, se detectaron solo en la savia de L 97-128. Se propone este enfoque novedoso para evaluar cultivares resistentes y susceptibles.