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Relationship of European Corn Borer Resistance in Sorghum to HCN-p and DMBOA Content in Leaf and Sheath-Collar Tissue

W. D. Guthrie
Iowa State University

Francis A. Haskins
University of Nebraska-Lincoln, fhaskins@neb.rr.com

Herman J. Gorz
United States Department of Agriculture

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RELATIONSHIP OF EUROPEAN CORN BORER[1] RESISTANCE IN SORGHUM TO HCN-p AND DIMBOA CONTENT IN LEAF AND SHEATH-COLLAR TISSUE[2]

W. D. Guthrie[3], F. A. Haskins[4], and H. J. Gorz[5]

Abstract: Both high- and low-HCN-p genotypes of sorghum, *Sorghum bicolor* (L) Moench, were resistant to the European corn borer (ECB), *Ostrinia nubilalis* (Hübner). It was not proven that HCN-p is or is not a chemical factor conditioning resistance to leaf feeding by first-generation ECB and resistance to sheath-collar feeding by second-generation ECB in sorghum. If HCN-p is a resistance factor, however, it is effective at very low levels because levels in the low-HCN-p genotypes were very low.

DIMBOA is not a chemical factor conditioning resistance in sorghum to the ECB because midwhorl leaves and sheath-collar tissue of both high- and low-HCN-p genotypes contained no DIMBOA.

Key Words: *Ostrinia nubilalis*, sorghum, host plant resistance, dhurrin, DIMBOA.

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During the period of egg deposition by first-generation European corn borers (ECB), *Ostrinia nubilalis* (Hübner), sorghum *Sorghum bicolor* (L) Moench, is in the whorl stage of plant development. Most larvae feed on leaf tissue in the moist area deep in the whorl of sorghum plants through 9 d after egg hatch. Most first-generation larval mortality of the ECB occurs during the first few days after egg hatch. Resistance to first-generation ECB on sorghum as in maize, *Zea mays* (L), is, therefore, leaf-feeding resistance; i.e., high antibiosis against first and second instars (Dharmalingam et al. 1984).

During the 1960's, F. F. Dicke (unpublished data) evaluated several varieties of sorghum under a low level of artificial ECB infestation (75 eggs/plant). During 1981-1983, Guthrie et al. (1985) evaluated 208 sorghum hybrids under a very high level of artificial ECB infestation (750 eggs/plant). All genotypes of sorghum were resistant to leaf feeding by first-generation ECB. The leaves on sorghum had pinholes (Fig. 1) indicating that some larvae fed for a short time on leaf tissue.

Beck and Lilly (1949) found that cyanogenetic content in whorl leaf tissue of sorghum plants is responsible, at least in part, for the high resistance of sorghum to ECB larvae during the whorl stage of plant development. One objective of our study was to determine if sorghum genotypes high in content of dhurrin [p-hydroxy-(S)-mandelonitrile-β-D-glucoside] and, thus, in hydrocyanic acid potential (HCN-p) in whorl leaf tissue are resistant to ECB and, conversely if sorghum genotypes with low levels of HCN-p are susceptible. DIMBOA (2, 4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one) is a chemical factor that conditions resistance in some genotypes of maize to leaf feeding by first-generation ECB (Tseng et al., 1984). A second objective of this study was to determine if whorl leaves of the sorghum genotypes contain DIMBOA.

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[3] USDA-ARS and Department of Entomology, Iowa State University, Ankeny and Ames.

[4] Department of Agronomy, University of Nebraska, Lincoln, Nebraska 68583.

[5] USDA-ARS and Department of Agronomy, University of Nebraska, Lincoln, Nebraska 68583.

Published in the Journal of Agricultural Entomology (January 1988) v. 5, no. 1

Fig. 1. A class 2 visual leaf rating showing pinhole feeding, typical of a highly resistant reaction to first-generation ECB on sorghum.

During the period of egg deposition by second-generation ECB, sorghum is in various stages of anthesis. Most larvae feed on sheath-collar tissue through 35 d after egg hatch. Resistance in sorghum as in maize, therefore, is resistance to sheath-collar feeding (Guthrie et al. 1984). Sorghum genotypes vary in degree of resistance-susceptibility when an infestation occurs during anthesis. Some genotypes are highly susceptible (Atkins et al., 1983; Ross et al. 1982). On susceptible genotypes of maize (infested during anthesis), ECB larvae tunnel throughout the whole plant. In contrast, ECB larvae rarely enter sorghum stalks below the peduncle, and only peduncles and heads are damaged. A third objective of our study was to determine if genotypes of sorghum containing different amounts of HCN-p and DIMBOA in sheath-collar tissue are resistant to second-generation ECB.

MATERIALS AND METHODS

The genotypes of sorghum evaluated were: (1) BKS8, a low-HCN-p (24 ppm, dry leaf tissue) parental line; (2) BN32, a high-HCN-p (858 ppm) parental line; (3) F_3-1, a line from a high-HCN-p (788 ppm) F_2 plant (28-4) from the cross, BSK8 × BN32; (4) F_2-2, a line from a low-HCN-p (33 ppm) F_3 plant (28-9) from the cross, BKS8 × BN32; (5) F_3-3, a line from a high-HCN-p (927 ppm) F_2 plant (36-7) from

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the cross, BN32 × BKS8; (6) F_3-4, a line from a low-HCN-p (18 ppm) F_2 plant (36-10) from the cross, BN32 × BKS8; and (7) Check #2 (used only in 1986), an experimental grain sorghum hybrid known to be resistant to first-generation ECB and susceptible to second-generation ECB. The HCN-p values given are from plants grown at Lincoln, Nebraska, in 1984. A single major gene pair is primarily responsible for the large difference in HCN-p content between BKS8 and BN32. There were no obvious maternal effects, and F1's were generally intermediate in HCN-p level between the two parents, indicating that neither high nor low HCN-p was completely dominant (Gorz et al. 1986).

The genotypes were planted in single-row plots in two different experiments (randomized complete-block design with four replications for each experiment) at Ankeny, Iowa. Plots were planted 15 May 1985 and 17 May 1986. Rows were 3.3 m long, and distance between rows was 100 cm; stand was thinned to ca. 10 cm between plants when plants were ca. 15 cm high.

In the first experiment, six plants in each plot were artificially infested with 30 egg masses (ca. 750 eggs)/plant in five applications of six masses each spaced 1 d apart during the midwhorl stage of plant development. The same number of plants and egg masses were used for infestation during anthesis in a second experiment. Infestation and egg production techniques were reported by Atkins et al. (1983) and Guthrie et al. (1960, 1971).

In the first experiment, midwhorl leaves from six uninfested plants in each plot were taken for HCN-p analysis, and six plants were used for DIMBOA analysis; plants were cut above the growing point. For HCN-p analysis, whole leaves with midribs removed were used. For DIMBOA analysis, midwhorl leaves with 10 cm of tips removed were used. In the second experiment, sheath-collar tissue from each plot (at anthesis) was taken for HCN-p (six plants) and DIMBOA (six plants) analyses.

In the first experiment, leaf-feeding damage was rated on a plot basis 21 d after egg hatch, as described by Guthrie et al. (1960). In a 1-to-9 rating scale, classes 1 and 2 (Fig. 1) are highly resistant, classes 3 and 4 are resistant, classes 5 and 6 are intermediate, and classes 7 to 9 (Fig. 2) are susceptible. In the second experiment, cavity counts (cm of damage in peduncles and heads) were made from six plants in each plot 60 d after egg hatch as described by Atkins et al. (1983).

For HCN-p analysis, whorl leaves and sheath-collar tissue were cut into 2.5-cm pieces and dried for 4 h at 75°C. The dried samples were ground with a Wiley mill fitted with a 1-mm screen. A weighed portion from each plot was first extracted with water at room temperature to obtain dhurrin (the cyanogenic glucocide). An aliquot of each extract was treated with sodium hydroxide to hydrolyze the dhurrin, releasing cyanide into the solution. Cyanide content was then determined with the colorimetric reagents of Lambert et al. (1975) as described by Gorz et al. (1986).

For DIMBOA analysis, midwhorl leaves (75 cm in extended leaf height) and sheath-collar tissue from six plants in each plot were placed in plastic bags and frozen at -23°C until used. The frozen leaf and sheath-collar tissue were thawed, dried in an oven at 48°C, and ground into a fine powder for DIMBOA analysis. The chemical determinations were actually for MBOA (6-methoxybenzoxazolinone), expressed as milligrams of MBOA per gram of plant tissue. We used a modification of the procedures reported by Klun and Robinson (1969). Because DIMBOA is

Published in the Journal of Agricultural Entomology (January 1988) v. 5, no. 1

chemically labile and decomposes stoichiometrically to MBOA, DIMBOA concentrations can be determined by chemical analysis of dried plant tissue for MBOA. Details of the MBOA extraction procedure were reported by Tseng (1984).

Fig. 2. A class 9 visual leaf rating showing numerous elongated lesions, typical of a highly susceptible reaction to first-generation ECB on maize.

For analysis of variance of plot means, total sum of squares for leaf-feeding ratings, cavities in peduncles and heads, HCN-p of leaf tissue, and HCN-p of sheath-collar tissue for each year were partitioned into components for replications (3df), genotypes (5 df in 1985, 6 df in 1986), and error (15 df in 1985, 18 df in 1986). LSD ($P < 0.05$) values were calculated as described by Steel and Torrie (1960) to determine the level of significance of differences between means.

RESULTS AND DISCUSSION

The analysis of variance showed no significant difference in leaf-feeding damage among genotypes in 1985. In 1986, BN32 and check #2 had significantly

Published in the Journal of Agricultural Entomology (January 1988) v. 5, no. 1

less leaf-feeding damage than did the other five genotypes, but the difference was of little practical importance because, during the 2-year period, both high- and low-HCN-p genotypes of sorghum rated highly resistant (class 2) or resistant (class 4) to leaf feeding by first-generation ECB (Table 1). As in previous studies (Guthrie et al., 1985), whorl leaves of the sorghum genotypes in the present study had pinholes (Fig. 1) indicating that some larvae fed for a short time on leaf tissue, similar to those on resistant genotypes of maize. None of the genotypes had numerous elogated lesions typical of susceptible genotypes of maize (Fig. 2).

Table 1. ECB leaf-feeding damage, ECB peduncle and head damage, and HCN-p levels in whorl leaves and sheath-collar tissue in seven genotypes of sorghum, Ankeny, Iowa.

	Leaf feeding ratings*		HCN-p in leaf tissue‡		Cavities (cm)†		HCN-p in sheath collar tissue‡	
Genotype	1985	1986	1985	1986	1985	1986	1985	1986
1. BKS8	2.3	4.0	31	45	1.5	1.0	45	18
2. BN32	2.0	2.0	752	465	11.0	6.0	239	65
3. F_3-1	2.0	4.0	689	300	2.8	1.4	298	104
4. F_3-2	2.0	4.0	95	69	3.0	3.4	54	12
5. F_3-3	2.0	3.5	711	302	7.5	4.9	214	73
6. F_3-4	2.0	4.0	38	49	2.3	6.6	52	12
7. Check #2		2.3		475		15.4		60
LSD 0.05		0.8	94	76	4.5	2.6	52	26

* Leaf-feeding damage was rated on a 1-to-9 scale, with 1 indicating no damage and 9 indicating extensive damage to leaf tissue 21 d after egg hatch.

† Cavities (cm of damage in peduncles and heads) were determined 60 d after egg hatch. There were no cavities in stalks below the peduncle.

‡ mg HCN-p per kg of dry midwhorl leaf or sheath-collar tissue.

As expected, there were large differences among genotypes for HCN-p of midwhorl leaf tissue. Midwhorl leaves of most genotypes had higher HCN-p in 1985 than in 1986, but the differences between high- and low-HCN-p genotypes in both years were great (Table 1).

On genotypes of sorghum susceptible to second-generation ECB, the larvae survive on sheath-collar tissue (Fig. 3) through 35 d after egg hatch (Guthrie et al. 1984) and then enter peduncles and heads, causing extensive damage (Fig. 4). Cavity counts in peduncle and heads can be used, therefore, to measure resistance-susceptibility. Cavities (cm of damage) in peduncles and heads of high- and low-HCN-p genotypes of sorghum ranged from 1.5 to 11.0 in 1985 and from 1.0 to 6.6 in 1986. The susceptible check contained 15.4 cm of damage in 1986 (Fig. 4).

Genotypes with high levels of HCN-p in midwhorl leaves also had relatively high levels in sheath-collar tissue, and genotypes with low HCN-p levels in midwhorl leaves had low levels in sheath-collar tissue. Midwhorl leaves had more than twice the HCN-p of sheath-collar tissue. Sheath-collar tissue of all genotypes was higher in HCN-p in 1985 than in 1986 (Table 1).

We did not prove that HCN-p is or is not a chemical factor conditioning resistance to leaf feeding by first-generation ECB and resistance to sheath-collar feeding (as measured by damage in peduncles and heads) by second-generation

Published in the Journal of Agricultural Entomology (January 1988) v. 5, no. 1

ECB. If HCN-p is, however, a resistant chemical factor, as indicated by Beck and Lilly (1949), it is effective at levels that are no higher than those observed for the low-HCN-p sorghum genotypes.

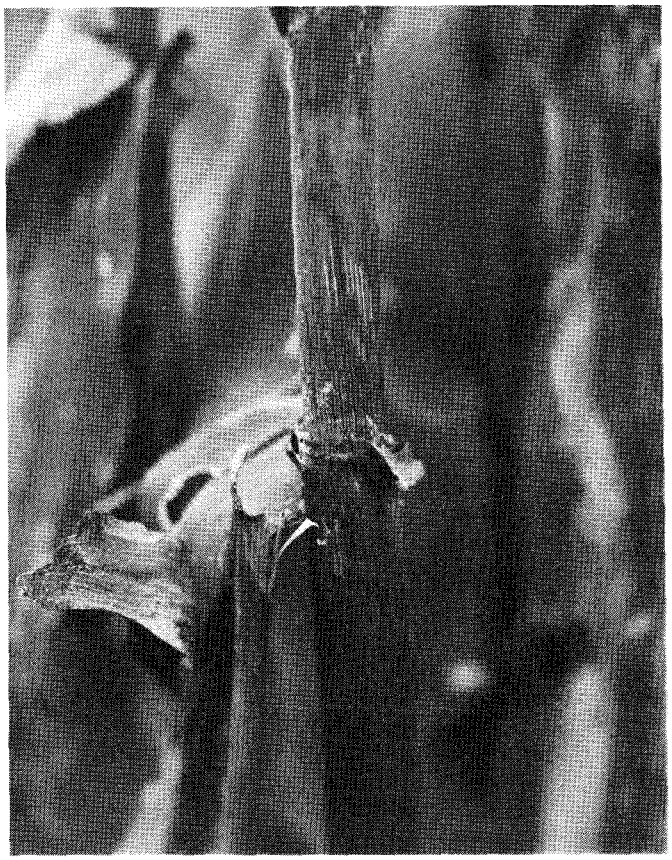

Fig. 3. Sheath-collar feeding damage by second-generation ECB on sorghum when infested at anthesis.

DIMBOA is not a chemical factor conditioning resistance in sorghum to the ECB because midwhorl leaves and sheath-collar tissue of both high- and low-HCN-p genotypes contained no DIMBOA. Guthrie et al. (1985) also found no DIMBOA in midwhorl leaves of four sorghum hybrids.

Published in the Journal of Agricultural Entomology (January 1988) v. 5, no. 1

Fig. 4. Peduncle and head damage on a susceptible genotype of sorghum caused by second-generation ECB when infested at anthesis.

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