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Characterization of Corn Earworm Larval Growth on Soybean Terminals [1]

Harbans L. Bhardwaj [2], **Muddappa Rangappa, Ali I. Mohamed,** and **Anwar A. Hamama.** Agricultural Research Station, Virginia State University, Petersburg, VA 23806.

ABSTRACT

A comparison of weights of larvae, raised for 14 days on 30 soybean cultivars (five each from six maturity groups), identified Gnome-85, Essex, and Padre to be susceptible and Newton, Walters, and Colquitt to be resistant to foliar feeding by corn earworm (*Helicoverpa zea* Boddie). In general, the larvae grew better on cultivars of northern maturity groups (II, III, and IV) as compared to cultivars of southern maturity groups (V, VI, and VII). The larvae raised on susceptible cultivars had higher lipid contents (fresh weight basis) as compared to those raised on resistant cultivars. Differences existed for contents of saturated and unsaturated fatty acids in larvae raised on susceptible or resistant cultivars. These results indicate that chemical composition of terminals might be involved in soybean resistance to corn earworm and that it might be possible to develop resistant soybean cultivars based on comparisons of chemical constituents in the terminals.

INTRODUCTION

Corn earworm (*Helicoverpa zea* Boddie) is a devastating pest of row crops causing major damage on corn, cotton, peanut, and soybean. Kogan (1980) found that corn earworm had the greatest economic impact on crop production in the United States of America. It causes primary damage to soybean pods before seed enlargement begins which affects yield and seed quality (Biever et al. 1983). Although, most serious damage to soybean, *Glycine max* (L.) Merr., occurs from southern Virginia to Alabama (Stinner et al. 1980), other southern production regions may also suffer damage (Turnipseed and Kogan, 1987).

Concerns for environmental quality and cost of insect control have resulted in concerted efforts aiming to develop host plant resistance in field crops, including soybean. Rufener et al. (1987) developed a larval antibiosis screening technique to screen soybean for resistance to mexican bean beetles (*Epilachna varivestis* Mulstant). This laboratory technique is based on larval maturity and mortality after 10 days of feeding on excised leaflets of various soybean lines. High mortality or slow larval development was used to indicate high level of antibiosis resistance. A comparison of larval weights after 14 days feeding on excised leaves in the

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2 Send correspondence to: Harbans L. Bhardwaj, Agricultural Research Station, Virginia State University, Box 9152,Petersburg, VA 23806.

laboratory was successfully used by Bhardwaj et al. (1987) to characterize bollworm resistance in cotton lines. Larval weights after 5-8 days of feeding on resistant and susceptible cultivars were demonstrated to be an acceptable measurement of soybean resistance to various insect pests by Beach and Todd (1988). Javaid et al. (1991) and Joshi et al. (1991) used weights of corn earworm larvae when raised on terminals of soybean lines to successfully identify corn earworm-resistant lines.

Three plant introductions (PI-229358, PI-171451, and PI-227687) have been identified as sources of resistance to foliar feeding insects in soybean (Van Duyn et al. 1971; Clark et al. 1972). Even though progress has been made to genetically enhance corn earworm resistance in soybean, progress has been slow in developing insect-resistant cultivars with acceptable yields (Javaid et al. 1991).

Our objective was to screen recently released, high yielding soybean cultivars for resistance to corn earworm foliar feeding. The intent was to identify resistance in these agronomically desirable cultivars rather than in unadapted genotypes with unacceptable yield potential that would have to be improved. Variation in larval weights was used to characterize soybean cultivars for their ability to support corn earworm larval growth. The cultivars on which the larval growth was minimal were classified as resistant whereas those on which larvae attained significantly more weight were classified as susceptible. The contents of various fatty acids in larvae raised on resistant and susceptible cultivars were also compared.

MATERIALS AND METHODS

During 1992, 285 soybean cultivars representing 12 maturity groups (OO, O, I, II, III, IV, V, VI, VII, VIII, IX, and X) were planted on May 14, 1992, at Randolph Farm of Virginia State University for seed multiplication and observations. Each cultivar was planted in a single row. The distance between rows was 75 cm, and the plants were spaced approximately 5 cm apart within rows. Recommended cultural practices for soybean production in Virginia (Reese, 1992) were followed. Thirty soybean cultivars, five each from six maturity groups (II, III, IV, V, VI, and VII), were selected for inclusion in the present study based on availability of sufficient foliage, most recent release date, and availability of resources. These thirty cultivars were registered from 1988 to 1992 except for Essex which was registered in 1973.

Terminals (approximately upper 15cm of the plants) from 56 to 63 days old soybean plants were harvested from the field and brought to the laboratory. Eight to ten terminals were placed on a 12.5cm diameter Whatman filter in a disposable petri dish. Approximately 5 newly hatched corn earworm larvae were placed in each petri dish. The larvae were reared from eggs obtained from the Insect Rearing Laboratory, USDA-ARS, Stoneville, MS 38776. The filter papers were kept moist with tap water during the duration of the experiment to keep soybean terminals from drying. Six petri dishes were used for each cultivar. The petri dishes were arranged in the laboratory in a randomized complete block design with six replications. On the eleventh day of these experiments, old terminals were replaced with fresh terminals, mainly to clean the larvae of feces, etc. and to facilitate larval handling. These larvae were weighed on an electronic scale on the fourteenth day. If significant differences for larval weight existed among cultivars within a maturity group, the cultivar on which the highest larval weight was observed was classified as susceptible whereas the cultivar on which the lowest larval weight was observed was classified as resistant to corn earworm feeding. The terms susceptible and resistant are derived from comparative growth of corn earworm larvae on terminals of respective soybean cultivars in the laboratory and may not relate to actual losses caused by corn earworm under field conditions.

Upon recording of larval weights on the fourteenth day, all larvae from six petri dishes of a cultivar were composited to form one sample. These samples were immediately frozen. The lipids were extracted from frozen corn earworm larvae by hexane-isopropanol (3:2 v/v) according to the method of St. John and Bell (1989). The fatty acid methyl esters (FAME) were prepared from extracted lipids as described by Mohamed and Rangappa (1992). The FAME were analyzed on Supelcowax 10 capillary column (30m x 0.25 mm i.d. and 0.235 m film thickness) in a Hewlett-Packard model 5890 gas chromatograph equipped with a flame ionization detector (FID). Helium was used as a carrier gas at a flow rate of 1.85 ml/minute with split ratio of 1:100. The oven temperature was isothermal at 270°C with injector and deflector temperature set at 250 and 260°C, respectively. Identification of FAME was based on comparison of retention time of unknown peaks to fatty acid methyl ester standards. Quantification of various fatty acids was done by the aid of heptadecanoic acid (17:0) as an internal standard. At least three injections were made for each sample. Individual fatty acids were expressed as a relative weight percentage of total fatty acids. Comparisons were made for fatty acid profiles between larvae raised on resistant and those raised on susceptible cultivars as identified by comparison of larval weights.

All data were analyzed using Analysis of Variance and Duncan's Multiple Range Test at a 5% level of significance (SAS, 1989).

RESULTS AND DISCUSSION

Significant differences in larval weights were observed only when corn earworm larvae were raised on cultivars of maturity groups II, V, and VII (Table 1). Based on these comparisons, Gnome 85 (MG II), Essex (MG V), and Padre (MG VII) were classified as susceptible and Newton (MG II), Walters (MG V), and Colquitt (MG VII) were classified as resistant to corn earworm feeding.

A comparison of the lipid content in larval bodies (Table 2) indicated that corn earworm larvae fed on susceptible cultivars accumulated more lipid in their bodies as compared to those fed on resistant cultivars. In maturity group V, the differences were significant at the 5% level. The overall mean lipid content in corn earworm larvae fed on susceptible cultivars was approximately three times greater than for larvae fed on resistant cultivars (97 vs. 284 μg/g). We did not analyze the soybean foliage but it seems that chemical composition of terminals of susceptible cultivars might be different from that in resistant cultivars since the diet of corn earworm larvae in this study consisted only of soybean terminals.

Further evaluations revealed differences for fatty acids among corn earworm larvae fed on resistant and susceptible cultivars. The fat in the bodies of larvae fed on susceptible cultivars contained long chain fatty acid ($C_{22:0}$) whereas the fat in the bodies of larvae fed on resistant cultivars lacked this fatty acid (Table 2). The larvae fed on susceptible cultivars also had lower contents of $C_{18:0}$ fatty acid as compared to those fed on resistant cultivars. However, this situation was reversed

TABLE 1. Weights of 14 day old corn earworm larvae fed on soybean terminals.

Maturity Group	Cultivar	Larval Weight(mg)		Rating
II	Gnome 85	225.5	a*	Susceptible**
II	Amcor 89	202.9	ab	-
II	Chapman	196.0	ab	-
II	Hoyt	145.2	bc	-
II	Newton	123.3	c	Resistant
III	Kunitz	135.6	a	-
III	Dunbar	124.1	a	-
III	Hayes	117.9	a	-
III	Sprite 87	106.6	a	-
III	Hobbit	102.2	a	-
IV	Pixie	174.7	a	-
IV	Ripley	168.2	a	-
IV	Spry	112.4	a	-
IV	Hamilton	110.5	a	-
IV	Delsoy 4900	85.8	a	-
V	Essex	192.7	a	Susceptible
V	Hartwig	157.0	b	-
V	TN5-85	117.3	c	-
V	Hutcheson	91.9	cd	-
V	Walters	72.6	d	Resistant
VI	Asgrow 6785	89.8	a	-
VI	Twiggs	79.1	a	-
VI	Sharkey	70.4	a	-
VI	Bryan	65.3	a	-
VI	Lloyd	45.7	a	-
VII	Padre	137.2	a	Susceptible
VII	Stonewall	112.5	ab	-
VII	Thomas	99.9	ab	-
VII	Hagood	63.9	bc	-
VII	Colquitt	42.2	c	Resistant

*Means followed by similar letters within maturity groups are not different according to Duncan's Multiple Range Test (P = 0.05).

** Rating of host plant reaction to corn earworm feeding. If significant differences existed among cultivars within a maturity group, the cultivars with extreme larval weights were classified as resistant or susceptible.

TABLE 2. Comparison of lipid profiles of corn earworm larvae fed on resistant and susceptible soybean cultivars.

Fatty Acid(%)	Mat. Group = II		Mat. Group = V		Mat. Group = VII		Overall	
	Res[a]	Sus	Res	Sus	Res	Sus	Res	Sus
16:0	18.4[b]	16.1	16.8	12.1*	20.3	24.5	18.5	17.6
16:1	3.1	1.4	3.0	1.1**	2.5	5.8	2.9	2.8
18:0	20.1	14.9**	17.3	17.3	16.0	12.6*	17.8	14.9*
18:1	19.9	17.2	20.4	16.5**	14.4	17.0	18.3	16.9
18:2	14.5	16.5	17.4	11.7**	11.5	6.9*	14.4	11.7
18:3	17.6	19.5	21.8	30.5**	28.1	27.2	22.5	25.7
20:0	0.8	1.0	1.8	1.4	0.8	0.8	1.1	1.1
$\mu 1$[c]	5.5	3.8	1.6	3.8	6.5	3.9	4.5	3.9
22:0	0.0	0.4	0.0	1.2*	0.0	0.0	0.0	0.5*
$\mu 2$[d]	0.0	9.3**	0.0	4.4	0.0	1.3	0.0	5.0*
Total Lipids (μg/g)	100.6	272.4	88.6	288.1*	102.3	292.1	97.1	284.2**

[a]Corn earworm larvae raised on resistant (Res) or susceptible (Sus) soybean cultivars.
[b]Percent content of fatty acids in total lipids based on fresh body weight.
[c]Unsaturated C_{20} fatty acid. The number of double bonds is unknown.
[d]Unsaturated C_{22} fatty acid. The number of double bonds is unknown.
*,**The mean fatty acid contents differed significantly in corn earworm larvae fed on resistant or susceptible soybean cultivars within maturity groups (P = 0.05 and0 .01, respectively).

with respect to linolenic acid ($C_{18:3}$), where the larvae fed on susceptible cultivars had higher content. Since the corn earworm larvae were raised under controlled conditions from a common genetic stock, lack of genetic differences among larvae can be safely assumed. Hence, the differences in fatty acids among corn earworm larvae fed on susceptible and resistant cultivars must be associated with differences in their diet i.e. soybean terminals. These studies indicate a need for chemical analysis of soybean terminals in order to relate variation in chemical composition to corn earworm larval growth.

The essential fatty acids, which include linoleic acid and linolenic acid, serve several physiological functions in vertebrates. The differences in lipid and fatty acid contents between corn earworm larvae raised on resistant and those raised on susceptible soybean cultivars indicate that a strategy to block lipid accumulation in corn earworm might be a potential means of corn earworm resistance in soybean.

The larvae of many species are important food items in many parts of the world (DeFoliart, 1991). The analyses of the nutritional value of 22 species of caterpillars revealed that kcal/100 grams dry weight averaged 457, ranging from 397 to 543, and crude protein content averaged 63.5%, ranging from 45.6% to 79.6% (Malaisse and Parent, 1980). Most species were also observed to be excellent sources of iron. The considerable proportion of 18:0, 18:1, 18:2, and 18:3 fatty acids in bodies of corn earworm larvae indicates the possibility that a soybean cultivar could be identified to raise corn earworm larvae to provide a source of these fatty acids for human or animal nutrition.

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