EFFECT OF LEAF EXTRACTS OF TEOSINTE, *ZEA DIPLOPERENNIS* L., AND A MEXICAN MAIZE VARIETY, CRIOLLO 'URUAPEÑO', ON THE GROWTH AND SURVIVAL OF THE FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE)

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Abstract

The effects of leaf extracts of teosinte, *Zea diploperennis* L., and a Mexican maize variety, criollo 'Uruapeño', on the growth and survival of the fall armyworm larvae were evaluated under laboratory conditions. Hexane, methanol, and aqueous extractions were made and the extracts and residual fiber were separately incorporated into a modified Poitout & Bues meridic diet. The hexanic and methanolic extracts were concentrated in a rotary evaporator and 30 ml of each were mixed to 20 g of cellulose and then incorporated to a meridic diet. Aqueous leaf extract was not concentrated. Larval cumulative mortality, larval weight, days to pupation, pupae weight, pupae length and width, days to adult emergence were evaluated. The hexanic extract of both plants enhanced most of the FAW growth parameters. Methanolic extract and residual fiber of both plants negatively affected the pupae length and width. The aqueous extract caused 100% of larval cumulative mortality. Larvae fed on diets containing residual fiber of both plants exhibited antibiotic effects.

Key Words: *Spodoptera frugiperda*, host plant resistance, maize, corn, antibiosis

Resumen

Fueron evaluados los efectos de los extractos foliares del teosinte, *Zea diploperennis* L. y los de una variedad de maíz mexicano, criollo 'Uruapeño', sobre el crecimiento y sobrevivencia de larvas neonatas del gusano cogollero, bajo condiciones de laboratorio. Las extracciones se llevaron a cabo con hexano, metanol y agua, y éstas y la fibra residual sólida fueron mezclados dentro de dieta modificada de Poitout y Bues para gusano cogollero, por separado. Los extractos con hexano y metanol fueron concentrados en un rotaevaporador y 30 ml de cada uno fueron incorporados a 20 g de celulosa y luego incorporados a la dieta. La fase acuosa no fue concentrada. La mortalidad total larvaria acumulada, el peso larvario, los días a pupación, el peso de las pupas, la anchura y longitud de las pupas, y días necesitados por las pupas para alcanzar el estado adulto fueron determinados. La mayoría de los parámetros de crecimiento del gusano cogollero fueron favorecidos por la dietas con extracto hexánico de cada una de las plantas, el extracto metanólico y la fibra residual de ambas plantas afectaron negativamente la longitud y anchura de las pupas. Las dietas con el extracto acuoso tuvieron 100% de mortalidad larvaria acumulada. Las larvas alimentadas con dietas con fibra residual de ambas plantas mostraron efectos antibióticos sobre las larvas del gusano cogollero.

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The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), has been recognized as a polyphagous insect and important pest of many crops, particularly maize (corn), *Zea mays* L., in the southeastern region of the United States (Luginbill 1928) and the rest of the Americas (Andrews 1988).

Over the past five decades, relatively effective and inexpensive synthetic pesticides have been extensively used to protect maize from losses caused by FAW. There is a renewed interest in discovering new sources of plant resistance to the pests and in developing plant-derived insecticides (McMillian et al. 1967; Meisner et al. 1977; Smith & Fischer 1983; Binder & Waiss 1984; Benner 1993; Snook et al. 1997). Natural plant products can be of benefit in managing pest populations as well as providing leads for developing synthetic products with modes of action against the pests (Balandrin et al. 1985; Shapiro 1991; Benner 1993).

Resistance in maize to damage by the FAW has been extensively investigated (Wiseman 1985; Wiseman & Widstrom 1986; Wiseman et al. 1992; Wiseman et al. 1996; Davis et al. 1998; Williams et al. 2000). Some degree of natural resistance to FAW larvae is exhibited in some species of corn for example teosinte, *Zea diploperennis*, a wild perennial relative of corn, and centipede grass *Eremochloa ophiuroides*. The defense chemistry of maize and its relatives is associated with maysin, chlorogenic acid, caffeoylquinic acids, and other luteolin derivates (Wiseman et al. 1990; Gueldner et al. 1991; Gueldner et al. 1992).

We bioassayed leaf extracts from teosinte and a Mexican maize variety, criollo 'Uruapeño', to determine their effects on the growth and survival of the fall armyworm.

Materials and Methods

Insects

Experimental insects were from a colony derived from feral FAW larvae collected in cornfields during outbreaks in the State of Colima, México. Insects were reared on modified meridic diet for *Spodoptera frugiperda* (Poitout & Bues 1974). Neonate FAW larvae were used for all bioassays.

Teosinte and Maize Variety

Teosinte was collected in the "Reserva de la Biosfera de Manantlán", near El Terrero, Colima, México, and the Mexican maize variety, criollo 'Uruapeño', was collected in Cuauhtémoc, Colima. Plants were grown in the facilities of the Centro Universitario de Investigación y Desarrollo Agropecuario, Universidad de Colima in Tecomán, Colima using small seedbeds under nursery conditions, of 24 ± 3°C, with a photoperiod of 16:8 (L:D), and 60-75% RH.

Stage 1 plants, exhibiting a collar of 4th leaf (Hanway 1963) were selected for the extracts. Portions of leaves, about 12 cm from the whorl, were cut, and about 50 g of leaves were macerated and used as samples for the extractions. Then to each sample, 200 ml of hexane was added, and the sample was finely ground and homogenized using a Moulinex® turbomix. Samples were incubated at 25°C for approximately 2 h with stirring at 400 rpm. The solution was filtered under vacuum using Whatman No. 1 filter paper, and the residual material was washed with 100 ml of hexane. Both filtrates were combined. Methanol (200 ml) was then used to extract the residual material using the same conditions mentioned above. Finally, the residual material was extracted with 100 ml of distilled water following the procedures mentioned above and the residual material was retained. The hexane and methanol extracts were concentrated under reduced pressure on a rotary evaporator (Caframo model OB2000) to a final volume of 30 ml. Each was incorporated into 20 g of cellulose and air-dried. Water extracts were not concentrated but were directly added to the diet.

The hexane, methanol, and water extracts as well as the residual material (fiber) of both plants were added separately with 250 g of diet, and 10 ml of diet were dispensed into individual polystyrene cups (30 ml) (n = 25 cups per treatment per trial). Cups of diet were allowed to air-dry for 12 h before each cup was infested with one first instar FAW. Diets were arranged in a randomized complete block design and replicated three times. Means were separated by Student-Newman-Keuls test, $P < 0.05$. Criteria used to assess the effects of the extracts included: cumulative larval mortality, larval weight, days to prepupation and pupation, pupae weight, pupae length and width, and days required post-pupation to achieve the adult stage.

Results

The weights of sixth instar and pupae were affected by the leaf extracts of *Z. diploperennis* and maize. Heavier larvae and pupae were obtained when diets containing the hexane and methanol extracts of both plants and regular diet were used. Lighter FAW larvae and pupae were obtained when fiber (residual material) diets were used (Table 1). The 6th instars that fed on diets with hexane and methanol extracts of *Z. diploperennis* were 1.86-fold and 1.56-fold heavier, respectively, than the larvae that fed on diets containing *Z. diploperennis* fiber. The weights of 6th instars and pupae reared on diets with hexane and methanol extracts of *Z. diploperennis* and the regular diet were statistically similar. Larvae that developed on diets with hexane and methanol extracts of 'Uruapeño' were 1.58 and 1.55 fold, respectively, heavier than those larvae that developed on 'Uruapeño' fiber diets. Weights of 6th instars and pupae reared on regular diet were statistically similar to those from diets supplemented with hexane and methanol extracts (Table 1). Similar effects were observed on weights of pupae from larvae reared on diets containing hexane and methanol extracts. The mean weights ranged from 191.0 mg to 211.9 mg for *Z. diploperennis*, and from 187.5 mg to 207.4 mg for 'Uruapeño' (Table 1).

Overall, FAW growth and developmental criteria were affected by leaf extracts and residual fiber. The criteria evaluated from insects reared on residual fiber diets showed extended developmental times, diminished pupae length and width, and increased cumulative larval mortality, compared with those from insects reared on diets containing hexane and methanol extracts of both plants and regular diet (Table 2).

TABLE 1. WEIGHTS OF *SPODOPTERA FRUGIPERDA* LARVAE AND PUPAE FED ON A MERIDIC DIET SUPPLEMENTED WITH LEAF EXTRACTS OF *ZEA DIPLOPERENNIS* AND A MEXICAN *Z. MAIZE* VARIETY, 'URUAPEÑO'.

	Extracts and diets				
Strain or variety	Hexane (*n*)	Methanol (*n*)	Water (*n*)	Fiber (*n*)	Regular (*n*)
			6th instar weights (mg)		
Z. diploperennis	407.5 a (25)	343.3 ab (21)		219.0 c (16)	373.0 a (24)
Z. maize var. 'Uruapeño'	322.0 a (19)	315.0 ab (24)	—	202.8 c (14)	373.0 a (24)
			Pupae weights (mg)		
Z. diploperennis	211.9 a (16)	191.0 a (14)	—	142.5 b (12)	198.6 a (22)
Z. maize var. 'Uruapeño'	207.4 a (12)	187.5 a (15)	—	149.4 b (6)	198.6 a (22)

Means in the same row followed by the same letter are not significantly different ($P < 0.05$; Student-Newman-Keuls test).

One hundred percent larval mortality was obtained when larvae were fed diets containing water extracts. Higher larval survival was obtained when larvae were fed diets containing hexane extracts of leaves from *Z. diploperennis* (100%). Lower larval mortality was obtained with the methanol extract of 'Uruapeño' and regular diet (8.3%) (Table 2). A significant reduction was observed in the FAW survival of larvae fed on fiber diets of both plants when compared with the FAW survival of larvae fed the regular diet and diets containing hexane and methanol extracts of both plants (Table 1).

DISCUSSION

Research on maize resistance to fall armyworm and corn earworm (*Helicoverpa zea* Boddie) has attempted to correlate resistance to chemistry of the plant, plant parts, or tissues. The mechanisms of non-preference, antixenosis or tolerance, and antibiosis are involved in some or all of the manifestations of plant resistance. Some effects of antibiosis in resistant crop genotypes on insects, including extended development, decreased pupal weight, and increased mortality compared with individuals reared on susceptible hosts, were described by Painter (1951). The presence of maysin, chlorogenic acid, apimaysin and 3'-methoxymaysin in leaves or silks in corn, as possible factors of resistance to the fall armyworm and corn earworm, was reported by Gueldner et al. (1992). Maysin in corn, teosinte and centipede grass also occurs naturally and is considered as a pest bioregulator (Gueldner et al. 1991).

The allelochemical activity observed among the *Z. diploperennis* and corn extracts in this study suggests that a variety of plant chemicals may have biological activities on FAW larvae, prepupae and pupae. FAW larvae reared on diets containing water extract and residual fiber were killed or were significantly lighter than those reared on diets with hexane and methanol extracts and regular diet. Differences in larval growth and development could have caused by antibiosis as a result of the presence of maysin and its analogues (Wiseman et al. 1992; Molina-Ochoa et al. 1996), non-preference, or a combination of the two mechanisms (Williams et al. 1987). Our results are in accord with those manifestations of antibiosis observed in resistant genotypes, but we did not discount the non-preference mechanism, because it was not evaluated in this study.

We suggest that one or more non-polar compounds that enhanced the growth and development of the FAW and diminished the cumulative larval mortality may be present in the hexane fraction of both plants. In the methanol fraction of *Z. diploperennis*, the presence of polar or polar compounds affected the pupal length and width. Diets containing the aqueous fraction and fiber contained dissolved antibiotic polar compounds that negatively affected most of the growth and developmental criteria, and increased the cumulative larval mortality. The survival of larvae reared on diets containing fiber was reduced 1.84-fold, 1.46-fold, and 1.69-fold compared to survival of larvae reared on diets containing hexane and methanol extracts of *Z. diploperennis*, and regular diet, respectively. FAW survival was reduced 2.0-fold and 2.44-fold in larvae reared on fiber diets compared with larvae reared on diets containing hexane and methanol extracts of 'Uruapeño', and was reduced 2.44-fold compared with larvae reared on the regular diet, respectively. The aqueous extracts of both plants contained the antibiotic factors and reduced FAW larval survival to 0%. Larval mortality indicates that the concentration of toxic leaf factor(s) is greatly increased in extract-supplemented diets, presumably due to the removal of phago-stimulants and/or growth-promoting substances in water extracts (Smith & Fischer 1983). The higher mortality exhibited in the diets with water extracts and residual fiber of both plants indicated that both plants contained chemicals that severely reduced survival and negatively affected the growth and development of FAW larvae. Once these chemicals are removed

TABLE 2. GROWTH AND DEVELOPMENTAL CRITERIA OF *SPODOPTERA FRUGIPERDA* LARVAE FED ON MERIDIC DIET SUPPLEMENTED WITH LEAF EXTRACTS OF *ZEA DIPLOPERENNIS* AND A MEXICAN *Z. MAIZE* VARIETY, 'URUAPEÑO'.

	Extracts and diets				
Strain or variety	Hexane (*n*)	Methanol (*n*)	Water (*n*)	Fiber (*n*)	Regular (*n*)
			Days to prepupation		
Z. diploperennis	18.81 a (16)	19.80 a (15)		29.31 b (13)	18.77 a (22)
Z. maize var. 'Uruapeño'	18.92 a (19)	21.63 ab (24)	—	27.67 c (14)	18.77 a (22)
			Days to pupation		
Z. diploperennis	20.94 a (16)	21.14 a (14)		31.33 b (12)	20.77 a (22)
Z. maize var. 'Uruapeño'	20.67 a (12)	23.29 a (14)	—	28.0 b (6)	20.77 a (22)
			Pupal length (cm)		
Z. diploperennis	1.61 a (16)	1.51 b (14)		1.46 b (12)	1.64 a (22)
Z. maize var. 'Uruapeño'	1.61 a (12)	1.61 a (14)	—	1.48 b (6)	1.64 a (22)
			Pupal width (cm)		
Z. diploperennis	0.53 a (16)	0.47 ab (14)		0.43 c (12)	0.51 a (22)
Z. maize var. 'Uruapeño'	0.49 ab (12)	0.50 a (14)	—	0.46 c (6)	0.51 a (22)
			Days required by pupae to reach the adult stage		
Z. diploperennis	10.40 a (16)	11.18 a (11)	—	10.20 a (5)	11.18 a (22)
Z. maize var. 'Uruapeño'	11.17 a (12)	13.00 a (9)	—	11.50 a (4)	11.18 a (22)
			Cumulative larval mortality		
Z. diploperennis	0.0 d (24)	20.8 c(24)	100 a (24)	45.8 b (24)	8.3 c (24)
Z. maize var. 'Uruapeño'	25.0 c(24)	8.3 c (24)	100 a (24)	62.5 b (24)	8.3 c (24)

Means in the same row followed by the same letter are not significantly different ($P < 0.05$; Student-Newman-Keuls test).

through extraction, the larvae were able to use the nutrients contained in the leaves. The availability of these nutrients increased the survivorship to adulthood above those on the residual fiber and regular diet. Similar results were obtained by Bosio et al. (1990). Our results differ from those obtained by Quisenberry et al. (1988), because they did not find differences in mortality caused by diets containing water extracts of different varieties of Bermudagrass, *Cynodon dactylon* (L.). Their results suggested that the water extractable factors did not contribute to FAW resistance in the grasses evaluated.

The possibility of FAW management by foliar application of the plant tissue extracts of insect resistant corn varieties or its wild relatives could also be explored in greenhouse and field conditions. A knowledge of the compounds occurring in corn "criollo", improved varieties and wild relatives of corn would also facilitate the selection and aid the development of selective breeding procedures for varieties with an inherent resistance to FAW in tropical conditions.

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