Resistance in Maize to *Euxesta stigmatias* Loew (Diptera: Otitidae)[1,2]

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Abstract The corn silk fly, *Euxesta stigmatias* Loew (Diptera: Otitidae), and related otitid species can cause severe crop losses to sweet corn, *Zea mays* L., grown in tropical and subtropical regions. In Florida, the fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), and *E. stigmatias* are two debilitating insect pests on the sweet corn ear that are considered the most costly and difficult to control. Our purpose was to search for resistance to the corn silk fly in a diverse set of maize germplasm that included sweet, floury, field and popcorn, and to determine if any empirical relationship existed between *E. stigmatias* damage and *S. frugiperda* infestation in corn ears. The overall means across two sites, 3 yrs and 16 genotypes was 1.77 for corn silk fly damage, rated on a 0 to 4 prototype scale, and 30.1% for fall armyworm infestation. Clear differences existed between the sweet and popcorn types when compared to the field and floury types. The field and floury corns sustained significantly less damage by the corn silk fly than the sweet and popcorn types (0.91 vs 3.33). Five of these genotypes, CEW-R58, DDSB, GT-RI4, Mp704 and 'Zapalote Chico 2451', had both lower corn silk fly damage ratings and lower levels of fall armyworm infestation. Across this broad germplasm base no statistical relationship was identified between corn silk fly damage and fall armyworm infestation, suggesting that each insect species responds independently to different maize genotypes. Husk extension was partly related to reduced *S. frugiperda* infestation, and tip tightness was partly related to reduced *E. stigmatias* damage. These results indicate that field corn could possibly serve as source of resistance to the corn silk fly for the improvement of sweet corn.

Key Words Corn, land race, fall armyworm, *Spodoptera frugiperda, Zea mays*

The corn silk fly, *Euxesta stigmatias* Loew (Diptera: Otitidae), is a crop pest that ranges from Paraguay to Florida and southern Texas (App 1938). The genus *Euxesta* is comprised of about 64 taxa of mostly neotropical origin with only a few species native to U.S. temperate regions (Curran 1935, Wolcott 1948, Steyskal 1961). As a saprophyte, the corn silk fly has a wide host range that includes grasses, vegetables and fruits. On sweet corn, *Zea mays* L., this insect behaves as a primary pest and as a secondary pest behind or in association with lepidopteran species (Hayslip 1951). In Puerto Rico, *E. stigmatias* was first reported feeding and attacking sweet corn in 1917 by Van Zwaluwenberg (Bailey 1940), but it is now known that a number of fly

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species infest developing corn ears in the Carribean region (Barbosa et al. 1986). Other destructive otitid flies reported on maize include *E. major* (Wulp) in Guatemala (Painter 1955), *E. eluta* (Loew) and *E. annonae* (F.) in Brazil (Branco et al. 1994), Ecuador (Evans and Zambrano 1991) and Chile (Frias 1981). The corn silk fly arrived in Florida sometime prior to development of procedures for chemical control in 1951 (Hayslip 1951). With the geographic expansion of the sweet corn industry over the last 10 to 15 yrs, *E. stigmatias* has concomitantly expanded its range from southern to northern Florida and into Georgia (B. Johnson, pers comm.).

The life cycle of this fly on sweet corn is well documented. White oblong eggs less 1.0 mm long are laid in groups of 8 to 40 during the late morning and early afternoon (Seal and Jansson 1993, Seal et al. 1996). The preferred oviposition site is just inside the husk tip on silks that range from 1 to 6 d old (App 1938, Seal et al. 1995). Eggs hatch in 2 to 4 d and the maggots feed and move down the silk channel into the developing ear. The larval stage generally lasts less than 20 d depending on temperature. As mature larvae exit the ear they "jump" to the soil and pupate. Adults emerge in 5 to 9 d. A small percentage of the flies will pupate inside the husks (Seal and Jansson 1989, Seal et al. 1995). The life cycle of the corn silk fly is usually completed in less than 3 wks, but reproductive adults can live up to 4 wks.

Control strategies in Florida have been tailored to corn silk fly biology and behavior (Nuessly et al. 1999). Eggs are protected within the silk channel, and larvae are protected by husks while feeding on the ear. With no commercially available *shrunken-2* sweet corn cultivars known to have silk fly resistance, insecticides must be applied to the exposed adult stage. Emergent adults tend to congregate on the upper leaves and in the tassel region from early evening to early morning. Egg-laying females converge around the corn ears to oviposit from mid-morning to mid-afternoon (Seal et al. 1996). The flies are attracted to corn fields as tassels emerge, and insecticides are applied beginning with silk emergence. In southern Florida this insect is ubiquitous and insecticides are applied to control adults starting as early as tassel push and continuing until the silks have dried. While most oviposition occurs during silking, *E. stigmatias* continues to deposit eggs into the silk channel and in the husk tunnels left by lepidopteran larvae or bird feeding for another 2 wks after the approximately 7-d silking period. Therefore, sweet corn ears are susceptible to damage from silking until harvest, while field corn is susceptible to damage through dough stage. Due to physical and time constraints associated with treating large areas of corn with ground machinery, pesticides for the silk fly are usually applied aerially. Organophosphates (e.g., chlorpyriphos and methyl parathion) and pyrethroid pesticides are labeled for the control of adult silk flies (Nuessly et al. 1999). In order for these contact pesticides to have the greatest effect, it is recommended that they be applied during the early morning or evening when most of the flies are exposed above the leaf canopy. Fields with large edge to total area ratios can be rapidly recolonized, necessitating frequent scouting and repeated pesticide applications, often daily (Nuessly et al. 1999).

Host plant resistance to *E. stigmatias* has not been identified in maize, although resistance to an unknown species of *Euxesta* was identified in two sweet corn test hybrids developed in Brazil (Branco et al. 1994). There is also anecdotal evidence that field corn is less susceptible than sweet corn to the corn silk fly. One purpose of this study was to assess the differences in susceptibility to *E. stigmatias* between field and sweet corn across a small but diverse maize germplasm base. In this initial search to identify corn silk fly resistance, field corn germplasm with varying mechanisms and levels of resistance to the fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), and the corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), were included. In southern Florida both *E. stigmatias* and *S. frugiperda* often infest corn ears concurrently and resistance to both species is desirable. The second objective was to determine if any empirical relationship exists between resistance to fall armyworm and corn silk fly in maize.

Materials and Methods

Genetically diverse maize genotypes and accessions including dents, flints, floury, sweet and popcorn germplasm were rated for damage by the corn silk fly and infestation by fall armyworm. The sweet corn types included the *shrunken 2* hybrids 'Snow White' (Harris-Moran Seed Co., Nampa, ID), 'SS 8102' (Abbott & Cobb Seed Co., Feasterville, PA), 'Fla. XP-7' (Scully et al. 1997) as standards, plus experimental hybrids UFW 4 and UFB 43, and the *sugary 1* hybrid 'Walters White'. The field corn germplasm included the standard hybrids 'XL-678' (DeKalb Seed Co., Dekalb, IL) and 'PX-304C' (Pioneer Hybrid Seed, Des Moines, IA), which are common silage hybrids grown in southern Florida, along with inbreds Mp704 (Williams and Davis 1982) and Mp707 (Williams and Davis 1984) developed in Mississippi, and synthetic/composite populations GT-CEW-R58 (Widstrom et al. 1975), GT-DDSB (Widstrom et al. 1988), GT-FAW (Widstrom et al. 1993), and GT-RI4 (Widstrom et al. 1984) developed in Georgia. The materials acquired from Mississippi and Georgia were developed with varied levels of resistance to *H. zea,* southwestern corn borer (*Diatraea grandiosella* Dyar) (Lepidoptera:Pyralidae) and *S. frugiperda* In addition, the floury land race 'Zapalote Chico 2451' (Anderson 1959, Straub and Fairchild 1970) and the popcorn line PI 340856 (Wilson and Wiseman 1988, Wiseman et al. 1992) were included because of their known resistance to lepidopteran insects conferred by the flavone glycoside, maysin, and its chemical analogues (Ellinger et al. 1980, Snook et al. 1995).

Three randomized complete blocks (RCB) were planted over a 3-yr period from 1994 to 1996 in the Everglades Agricultural Area, south of Lake Okeechobee in Florida. The first planting included 10 accessions in a five replicate randomized complete block that was seeded on 28 March 1994 adjacent to a commercial sweet corn field in Canal Point, FL, with a history of high corn silk fly infestation. Each plot was a single row 7.62 m long by 0.76 m wide with plants spaced every 20 cm. The soil type at this site is a Torry Muck (Euic, hyperthermic, Typic Medisaprist) located ≤100 m from Lake Okeechobee. No fertilizer was applied to this site, but Thimet 20G (phosphorodithioate) was incorporated at planting using the maximum label rate for the control of soil insects. All other cultural practices were standard for the region, although insecticide and fungicide use were terminated prior to tassel expression.

In 1995 and 1996 this experiment was moved to the Everglades Research and Education Center in Belle Glade, FL on a Pahoke Muck (Euic, hyperthermic, Lithic Medisaprist). Preplant fertilizers were broadcast in accordance with soil test results and the preplant soil insecticide Mocap 20G (ethoprop) and Counter 15G (terbufos) were applied at label rates in 1995 and 1996, respectively. No fungicides were applied at the Belle Glade site. Foliar insecticides Lannate LV (methomyl) and Larvin 3.2 (thiodicarb) were sprayed as needed at the rate of 1.2 L $\cdot$ ha^{-1} to control heavy leaf feeding by fall armyworm larvae. As tassels emerged insecticide applications ceased. Each plot was again a 7.62 m long single row, but 0.91 m wide with plants spaced every 20 cm at both sites. In 1995, 12 accessions were planted in a four replicate RCB design starting on 1 March and staggered through 13 March. In 1996, the same 12 accessions were planted in a four replicate RCB design with planting dates that ranged from 5 March to 23 March. The staggered planting dates helped synchronize silk and tassel expression among the genotypes, compensated for any temporal distortions in the silk fly population and allowed the insect roughly equal choice among the host genotypes. Planting dates were chosen toward the end of the commercial production season in the Everglades Agricultural Area to maximize infestations. Populations of both *E. stigmatias* and *S. frugiperda* are dramatically elevated toward the end of the sweet corn harvesting season and with the onset of summer in southern Florida.

A 7.15-m section of each plot was harvested at roasting stage, normally 21 d after pollination, but hot weather compressed this duration to just over 17 d in some plots. Between 15 and 25 ears were collected from each plot and stored at 7.0°C until all were harvested. Husks were carefully removed from 10 ears in each plot with measurements taken on husk extension, tip tightness, damage caused by the *E. stigmatias* maggots and percent infestation by *S. frugiperda* larvae. Data were analyzed on the basis of plot means derived from the 10 ear sample using general linear models in SAS (SAS Institute 1982). The tightness of the husk beyond the tip of the ear was measured on a 1 to 5 scale, while husk extension (cm) measured the length of husk beyond the ear tip (Kaukis and Davis 1986, Scully et al. 1994). Fall armyworm damage was assessed as percent of ears infested using a random 10-ear sample from each experimental unit.

For maize, no rating scale exists to assess the damage caused by the corn silk fly or any other related *Euxesta* taxa. In this experiment a prototype damage scale was developed and applied at roasting stage, 17 to 21 d after pollination. It specifically focuses on corn ears and has five categories ranging from 0 to 4 as follows: 0—No Damage to the silk or ear; 1—Damage only to the silk above the ear tip; no damage to the ear tip; 2—Damage to the ear tip and/or the upper 25% of the ear; 3—Damage to the upper 50% of the ear; 4—Damage to over 50% of the ear.

Usually, as the number of silk fly maggots in an ear increases, the further down the silk and ear they progress. Kernels are often penetrated at a single point leaving the pericarp relatively undamaged. Downward migration patterns are not commonly uniform, but this working scale is inherently conservative and based on the farthest movement of any maggot. Similarly, this scale is more refined and rigorous toward the distal end of the ear, and less critical basally. Although, this scale is set up for research purposes, food quality standards for commercially grown sweet corn would not normally allow damage ratings above 1.0; ears with ratings above 1.0 would automatically be culled.

Results and Discussion

Maggot damage by the corn silk fly varied significantly among this diverse maize germplasm (Table 1). Across the 158 experimental units planted over the 3-yr period the distribution of silk fly damage ratings was inverse to normal (Fig. 1A). A total of 61 plots received a commercially acceptable rating of <1.0, while 43 plots received susceptible ratings of ≥3.5. This distribution (Fig. 1A) reflected the general differences between the more susceptible sweet corns and the less susceptible field corn germplasm. However, reliance on natural infestation suggests that genetic resistance

Table 1. Genotype means (±SE) for ear damage by the corn silk fly and ear infestation by the fall armyworm over a diverse set of maize genotypes with different endosperm types

Maize genotype	Endosperm phenotype	Corn silk fly damage (0 to 4 scale)	Fall armyworm infestation (%)
MP 704	Starchy	0.18 ± 0.09	26.7 ± 4.4
GTRI4	Starchy	0.38 ± 0.11	13.3 ± 4.4
Zap. Chico	Floury	0.51 ± 0.19	16.0 ± 3.6
DDSB	Starchy	0.76 ± 0.28	25.3 ± 7.4
MP 707	Starchy	0.89 ± 0.26	38.6 ± 9.5
PX 304C	Starchy	1.11 ± 0.38	40.7 ± 7.9
FAW	Starchy	1.16 ± 0.29	45.0 ± 7.5
CEW R58	Starchy	1.47 ± 0.27	25.5 ± 5.6
XL 678	Starchy	1.70 ± 0.39	44.0 ± 9.3
PI 340856	Popcorn	3.04 ± 0.72	28.6 ± 12.4
SS 8102	*shrunken 2*	3.06 ± 0.26	46.7 ± 10.0
Walters White	*sugary 1*	3.11 ± 0.24	18.5 ± 4.2
Snow White	*shrunken 2*	3.13 ± 0.44	41.3 ± 10.1
Fla. XP-7	*shrunken 2*	3.28 ± 0.27	20.8 ± 5.1
UFW-4	*shrunken 2*	3.66 ± 0.11	8.0 ± 4.9
UFB-34	*shrunken 2*	3.74 ± 0.11	34.0 ± 8.1
Overall Means		1.768 ± 0.12	30.1 ± 2.0
LSD ($P \leq 0.05$)		0.657	17.4

was partly confounded by plants that may have escaped damage due to an uneven silk fly distribution in the experiments, variation among years and phenological differences in silk expression. The distribution of the fall armyworm infestation was positively skewed (Fig. 1B.) with 54 plots having 10% or less infestation and very few having 80% or more infestation. As with the silk fly damage ratings, those plots with lower armyworm infestations included genetic resistance, escapes and reflect differences in insect preference given reasonably equal choice among genotypes.

The analysis of variance revealed that the differences between the maize genotypes and years were highly significant ($P \leq 0.001$) for silk fly damage ratings and infestation by the fall armyworm. No significant differences existed among the blocks, but the interaction between genotypes and years was significant for both insects. For the fall armyworm, average yearly infestations were 38.8% and 34.7% in the first and second year, but significantly lower (13.2%) in the third year. The average corn silk fly damage rating was highest at the Canal Point site (2.76) in the first year, and lower (1.54 and 0.99) in the second and third years at the Belle Glade site. Corn silk fly and

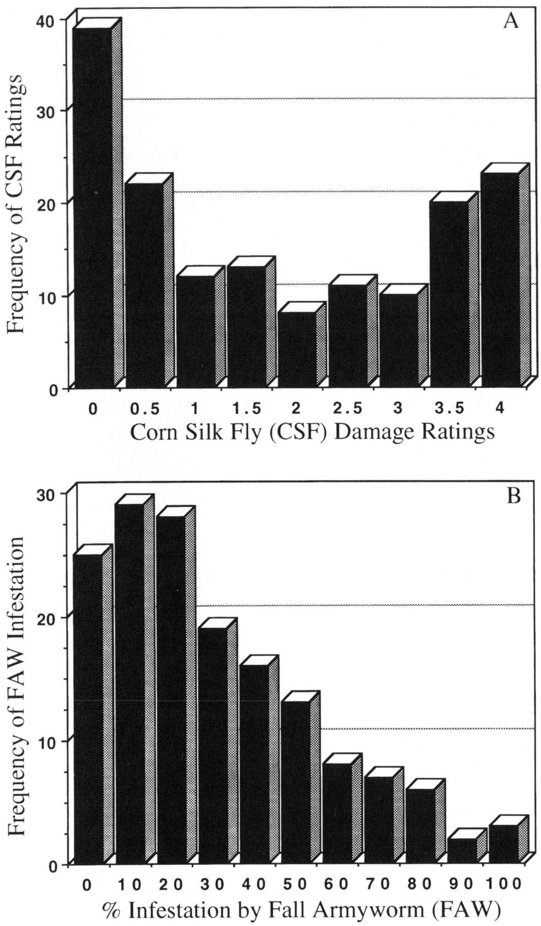

Fig. 1. A) The frequency of corn silk fly (CSF) damage ratings on the silk and ears of corn based on a 0.0 to 4.0 rating scale; and B) the frequency of fall armyworm (FAW) infestation.

fall armyworm pressure throughout the region was generally much lower in the third year of this study. This annual flux in these pest populations induced a differential response by maize genotypes that resulted in this significant interaction component. Differences among the maize genotypes for both corn silk fly damage and fall armyworm infestation were significant (Table 1), but not correlated (Fig. 2). Although not detected statistically, fall armyworm infestations were seemingly altered by an unknown interaction with the corn silk fly. It appeared that under heavy silk fly infestation the fall armyworm was somehow discouraged from colonizing corn ears, or possibly that severe silk fly damage masked evidence of armyworm infestation, particularly in the silk channel and on the ear tip.

The mean corn silk fly damage rating and the fall armyworm infestation frequency

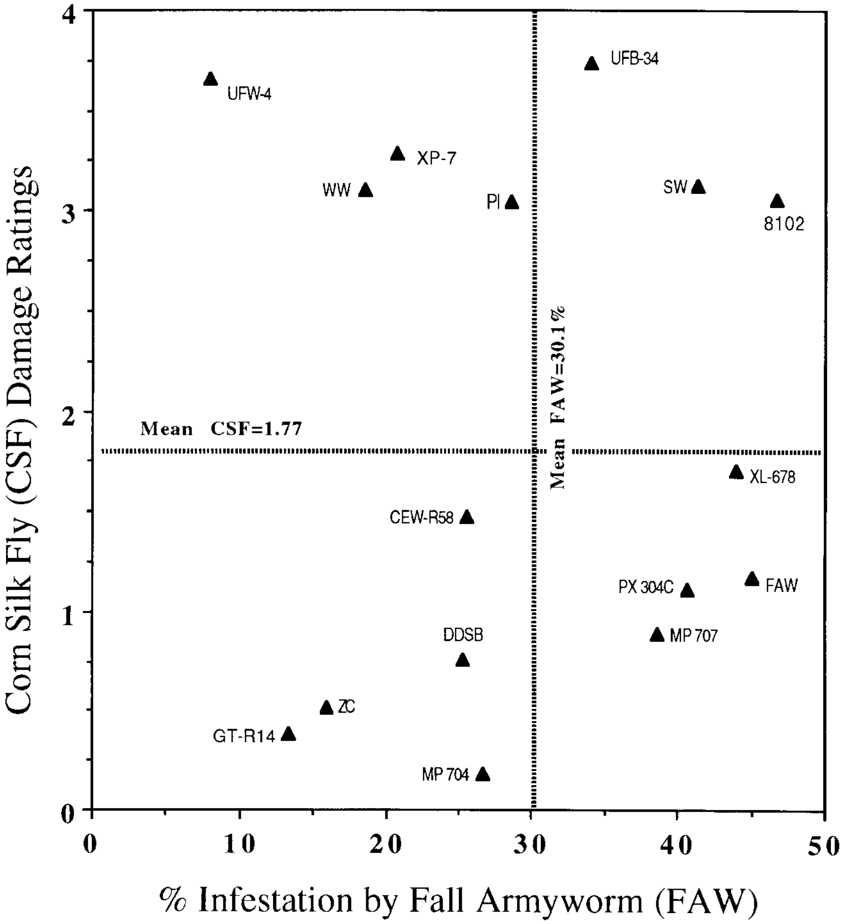

Fig. 2. The association between the means for corn silk fly damage ratings (0.0 to 4.0 rating scale) and percent fall armyworm infestation on the silk and ears of 16 corn accessions referenced against the overall experiment means for corn silk fly damage and fall armyworm infestation. (Note that PI = PI 340856; SW = 'Snow White'; WW = 'Walters White'; ZC = 'Zapalote Chico 2451'; with the remaining entries uncoded).

for all genotypes across sites, blocks and years was 1.77 and 30.1%, respectively (Table 1, Fig. 2). No meaningful correlation was apparent among the genotype means for silk fly damage and armyworm infestation, but a pattern among these means allowed the sweet corn to be clearly distinguished from the field corn (Table 1, Fig. 2). Above the 1.77 mean for corn silk fly damage all the sweet corns clustered together regardless of endosperm type, along with the popcorn line PI 340866 (PI). Below this mean the flint/dent germplasm and the floury landrace 'Zapalote Chico 2451' (ZC) clustered together indicating resistance to damage by the corn silk fly in comparison to the sweet and popcorns. The silk fly damage ratings for the sweet corn group ranged from a low of 3.04 in the PI accession to a high of 3.74 in the test hybrid UFB-34. The field corns had silk fly damage ratings that were spread from 0.18 in the Mississippi inbred 'Mp 704' to 1.70 in the commercial hybrid 'XL-678'. Resistance to the fall armyworm among the genotypes generally followed expectations. Field corn lines such as GT-FAW (FAW) (Widstrom et al. 1993), Mp 707 (Williams and Davis 1984) and the popcorn line PI 340866 (PI) (Wilson and Wiseman 1988) were expected to have lower than the average armyworm infestation because they were selected for greater resistance to lepidopteran insects. Conversely, the susceptible sweet corn hybrids 'Fla. XP-7' and UFW 4 were expected to have much higher levels of armyworm infestations because they were selected for horticultural quality rather than insect resistance. But, these two hybrids deviated from expectation. The cause of this lowered rate of infestation was not known, although heavy damage by *E. stigmatias* may have masked armyworm damage. The *sugary-1* hybrid 'Walters White' (WW) was originally developed to have mechanical resistance to lepidopterans based on long tight husks (Widstrom, pers. comm.), and performed as expected (Fig. 2). For breeding purposes CEW R58, DDSB, GT RI4, Mp 704 and 'Zapalote Chico 2451' (ZC) reflected a level of resistance to both insects that were better than the experiment-wide averages (Fig. 2). The results for these five lines were also consistent and repeatable over the 3-yr experiment period. Three sweet corn hybrids 'SS8102' (8102), 'Snow White' (SW) and test hybrid UFB-34 were highly susceptible to both insects. Unfortunately, 'SS8102' (8102) and 'Snow White' (SW) are prominently grown in Florida by the fresh market sweet corn industry.

The relationship between plant morphological traits such as longer husk extensions and tighter tips have been implicated as mechanisms of resistance to the corn earworm (Douglas 1947, Wiseman et al. 1978). Across this germplasm base, the relationship between fall armyworm infestation and husk extension fit a model defined by $Y = 0.5 - 0.04X$ with a coefficient of determination (r^2) of 0.28 ($P = 0.035$) (Fig. 3A). These data followed the results of Wiseman et al. (1978) and suggested that increasing husk extension tended to reduce the frequency of armyworm infestation, particularly for husk extensions longer than 6.0 cm. However, no significant regression model fit fall armyworm infestation as a function of tip tightness (Fig. 3B). Given the germplasm used in this experiment, tip tightness was an inconclusive predictor of fall armyworm infestation.

For the corn silk fly, extended husks and tighter tips failed to impede damage caused by the fly maggots. Statistically significant models described the relationship between silk fly damage and husk extension as $Y = -1.05 + 0.6X$ with an $r^2 = 0.49$ ($P = 0.003$) (Fig. 3C), and a quadratic function best described the association between silk fly damage and tip tightness as $Y = -13.6 + 12.7X - 2.3X^2$ with an $R^2 = 0.69$ ($P = 0.0004$) (Fig. 3D). The regression equation for tip tightness better predicted levels of corn silk fly damage than did the equation for husk extension, but a positive slope for the husk extension model suggested that as husk lengthened silk fly damage worsened. Furthermore, it is difficult to develop a biologically meaningful explanation to support the quadratic function that best described the relationship between tip tightness and silk fly damage. Endosperm type proved to be a better predictor of corn silk fly damage than either morphological trait. For both husk extension and tip tightness the sweet corn group sustained greater silk fly damage than did the field corn (Fig. 3C, 3D). Clear patterns between the sweet corn and the field corn groups were discernible for silk fly damage with two clusters sharply delineated above and

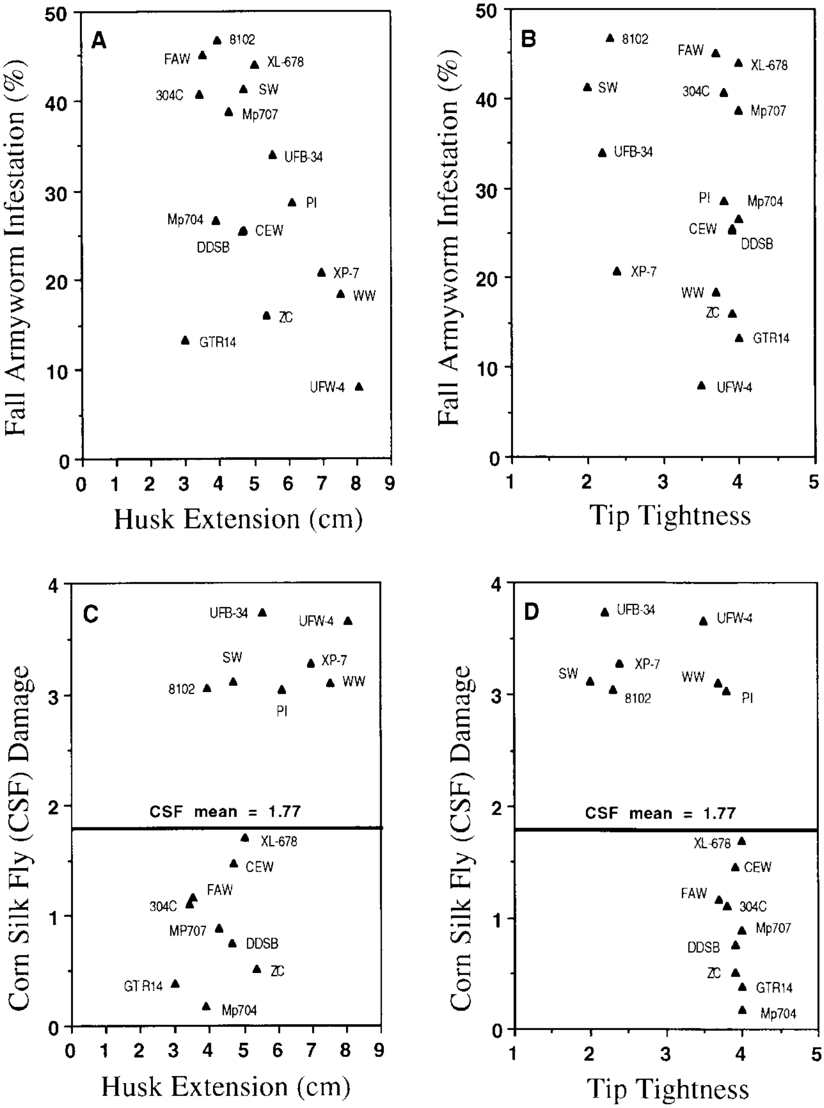

Fig. 3. The percent fall armyworm infestation as function of: A) mean husk extension beyond the tip of the ear (cm); and B) infestation as function of mean tip tightness on the silk and ears of 16 corn accessions followed by C) the association between the means for corn silk fly damage ratings (0.0 to 4.0 rating scale) as function of mean husk extension beyond the tip of the ear (cm); and D) between the means for CSF damage as a function of mean tip tightness measured on a 1 to 5 scale and referenced against the overall means for corn silk fly damage (Fig. 3C, 3D). (Note that PI = PI 340856; SW = 'Snow White'; WW = 'Walters White'; ZC = 'Zapalote Chico 2451'; with the remaining entries uncoded).

below the mean of 1.77 (Fig. 3C, 3D). The sweet corns also had a wider dispersion for both husk extension and tip tightness than did the field corns (Fig. 3C, 3D). The sweet corn group expressed husk extensions that averaged from 5.5 to 8.1 cm, while the field corn had 3.0 to 5.3 cm of husk extension (Fig. 3C). For tip tightness ratings, the sweet corns were spread across a range of 2.0 to 3.8 (Fig. 3D). In contrast, the tip tightness averages for the field corn were confined to the 3.7 to 4.0 range. This finding probably led to the better fit of the quadratic model for silk fly damage as a function of tip tightness (Fig. 3D). Regardless of husk morphology, the field corns did have a relatively greater range for silk fly damage, but all were below the mean of 1.77 (Fig. 3C, 3D).

Overall, these data indicate that sweet corn and field corn are dissimilarly susceptible to the corn silk fly, and that no empirical relationship exists between silk fly damage and fall armyworm infestation. Although these trends are evident and significant, a different set of maize germplasm in a different environment could yield different results. It may be premature to categorically state that field corns are "resistant" to the corn silk fly and sweet corns not, or that no conceivable relationship exists between silk flies and armyworms cohabitating on the same ear of corn. However, among these genotypes and across these sites and years, four field corn lines and 'Zapalote Chico 2451' consistently resisted damage by the silk fly. These five genotypes should have merit as a source of silk fly resistance for the improvement of sweet corn. If the heightened susceptibility of sweet corns to the corn silk fly can be lowered, the role of the endosperm mutant genes and their pleiotrophic effect on silk biochemistry needs to be understood, along with the effect of genes closely linked to these various endosperm mutant alleles. Additionally, something should be known about the genetic basis and the mechanisms of resistance to the corn silk fly in the corn ear.

Historically, the corn silk fly has been confined to southern Florida and considered a local pest of secondary importance behind the lepidopterans. Over the past decade the importance of *E. stigmatias* as a corn pest has risen and is expected to continue this rise. A now continuous market demand for fresh sweet corn has required the use of sequential plantings that move northward from the southern tip of Florida into Georgia. These agricultural and marketing practices facilitate the movement and spread of the silk fly into new and more temperate production regions. As the silk fly disperses into these new regions it must interact with different flora and fauna and adapt to cooler weather patterns. Indeed, these adaptations are apparently underway. The importance of the corn silk fly may further shift as transgenic resistance to lepidopterans is incorporated into sweet corn hybrids. Transgenic resistance should reduce insecticticide use on the fall armyworm, and subsequently relax control pressures on the corn silk fly increasing the need for specific control strategies and host plant resistance.

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