# A Rating Scale to Assess Damage Caused by the 'Corn Silk Fly (*Euxesta stigmatias* Loew) (Diptera: Otitidae) on the Ears of Sweet Corn

# B.T. Scully, G. S. Nuessly, M.G. Hentz and R.L. Beiriger

Everglades Research and Education Center, Dept. of Horticultural Sciences, IFAS University of Florida, 3200 East Palm Beach Rd., Belle Glade, FL 33430

## ABSTRACT

The 'corn silk fly (Euxesta stigimatias Loew) (Diptera:Otitidae) is an agricultural pest of increasing importance on the ears of sweet corn (Zea mays L.) grown in sub-tropical and tropical production regions of North America. Adult females lay clusters of eggs on emergent corn silk and underneath the husks protecting the silk channel. Larvae hatch and begin feeding down the silk, ultimately penetrate the pericarp and feed on the developing kernel endosperm. Control practices are focused on insecticide use rather than host plant resistance. Efforts to develop sweet corn germplasm with resistance to the corn silk fly requires a damage rating method. We propose a survey type scale that includes five injury classes that range from "0" to "4". In the "0" class, damage to silk is non-existent to minimal, while category "1" indicates damage only to the silk above the ear tip. Damage categories "2", "3", and "4" reflect damage sustained from the ear tip up to the top 25% of the ear's length, up to the top 50% of the ear's length, and over 50% of the ear's length, respectively. These injury classes are assigned at roasting stage, 21 days after pollination (21 DAP). Because of the consumers near-zero tolerance for silk fly damage, this proposed rating scale is more rigorous toward the ear tip and on extruded silks. A stratified sample of 142 ears was extracted randomly from three experiments conducted over six years and included nine different sweet corn varieties. A distribution was generated for the 142 observations across the five injury classes and assessed for conformity to a Poisson distribution. A goodness-of-fit test produced Chi Square ( $\chi^2$ ) values that indicated an elevated frequency of observations in the "non-damage" category. This was likely due to the confounding of resistant ears with those that escaped infestation. The highest damage category had 46.5% of the observations, while damage categories "3", "2", and "1" had 14.1%, 10.6%, and 3.5% of the observations, respectively. The "non-damage" category had 25.4% of the observations.

#### RESUMEN

La mosca de las barbas del elote (Euxesta stigmatia Loew.) (Diptera:Otitidae) es una plaga de creciente importancia de las mazorcas de maíz dulce (Zea mays) que se cultiva en las regiones tropicales y subtropicales de Norteamérica. Las hembras adultas depositan masas de huevecillos en los estigmas emergentes por debajo de las vainas protegiendo el canal del estigma. Las larvas emergen y empiezan a alimentarse hacia la base de los estigmas y finalmente penetran el pericarpio y se alimentan del endospermo del grano en desarrollo. Los métodos de control se enfocan en el uso de insecticidas mas que en la resistencia de la planta hospedera. Esfuerzos para desarrollar germoplasma de maíz con resistencia a la mosca de las barbas del elote requieren un método de evaluación de daño. Proponemos una escala de daño que incluya 5 categorías que varíen de "0" a "4". En la categoría "0", el daño a los estigmas es mínimo o inexistente, mientras que la categoría 1 indica daño a los estigmas arriba de la punta de la mazorca. Las categorías de daño "2", "3", y "4" reflejan el daño producido a partir de la punta de la mazorca hasta un 25%, 50% y mas allá del 50% de la longitud de la mazorca, respectivamente. Estas categorías de daño se asignan 21 días después de la polinización (21 DAP). Debido a la cero tolerancia de los consumidores al daño por la mosca de las barbas del elote, esta escala propuesta es mas rigurosa hacia la punta de la mazorca y sobre las mazorcas proyectadas al exterior. Una muestra estratificada de 142 mazorcas fue extraída aleatoriamente de 3 experimentos conducidos durante 6 años e incluyó 9 diferentes variedades de maíz dulce. Se generó una distribución en las 5 categorías de daño a partir de las 142 observaciones y se evaluó de conformidad a la distribución Poisson. Los valores de Chi cuadrada ( $\chi^2$ ) indicaron una frecuencia elevada de observaciones en la categoría de no daño. Esto se debió probablemente a la confusión de las mazorcas resistentes.

Additional index words. Zea mays L., Host Plant Resistance, Lepidoptera.

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The genus Euxesta (Diptera: Otitidae) is comprised of about five dozen, mostly neotropical species. Four of these species are known crop pests in the western hemisphere, including: Euxesta annonae (F.), E. eluta (Loew), E. major (Wulp.), and the 'corn silk fly (E. stigmatias Loew) which has a wide host range encompassing vegetables, fruits and grasses (App 1938, Bailey 1940, Branco et al. 1994, Curan 1935, Evans and Zambrano 1991, Frias 1981, Painter 1955, Steyskal 1961, Wolcott 1948). To date, the corn silk fly is the only Euxesta pest found in the continental United States, and is still confined to the southern states, particularly Florida (App 1938). This insect is a primary pest, or a secondary pest in association with Lepidoptera species on sweet corn (Zea mays L.), and is a saprophyte on decaying vegetation. It has infested Florida sweet corn since before 1950 and control practices have been developed by Hayslip (1951). In the last 50 years, the sweet corn industry has grown dramatically in the subtropical and tropical regions of the Americas and the corn silk fly has become increasingly more important. Any effort to develop host plant resistance to this fly requires knowledge of its biology, resistant germplasm and a scheme to describe ear damage. The biology of E. stigimatias is known (App 1938, Seal et al. 1989, 1993, 1995, 1996), and rearing/infesting protocols are under development. Maize germplasm with resistance to Euxesta species has been identified in Brazil and Florida (Branco et al. 1994, Scully et al. 2000a). Other than a simple "damage" vs "no damage" scale used by crop inspectors for fresh market sweet corn, no rating scales are available to identify superior germplasm or discern progress from selection.

Among horticulturally important Diptera, rating schemes are available for the onion maggot (*Delia antiqua* Meigen) (Diptera: Anthomyiidae), cabbage maggot (*Hylemya brassicae* Wiedemann) (Diptera: Anthomyiidae) and the carrot fly (*Psila rosae* Fabricus) (Diptera: Psilidae). Damage by the onion maggot in production fields is assessed on a quantitative scale that measures the frequency of infestation per unit area (Carruthers et al. 1984). Damage scales for the cabbage maggot on cabbage (*Brassica oleareacea* L.) roots have been refined by Dapis and Feno (1982). Their Root Damage Index (RDI) links the infestation frequency and damage severity on a 1 to 4 scale. Cabbage maggot injury on canola (*B. campestris* L.) roots is rated on five point scale with groupings of 0%,  $\leq 25\%$ ,  $\leq 50\%$ ,  $\leq 75\%$ , and >75%, respectively (McDonald and Sears 1992). Carrot fly damage on carrot (*Dacus carota* L.) roots is calculated as a weighted average that reflects the percentage of surface area damaged and damage frequency (Ellis et al. 1978). The Hessian fly (*Mayetiola destructor* (Say) (Diptera: Cecidomyiidae) on wheat (*Aveana sativa* L.) is an important Diptera pest, and effective rating scales have relied on the counts of live and dead larvae, along with damage symptoms to improve plant resistance (Grover et al. 1989, Cartwright et al. 1994).

In addition to these pests, perhaps the most economically important Diptera on plants include about a dozen tephritid fruit flies (Diptera: Tephritidae). These insects can cause serious damage to many crops and disrupt import and export commerce. Together these flies have a host range that includes well over 200 plant species in nearly every climatic region (Christenson and Foote 1960, Bateman 1972, Robinson and Hooper 1989). As with the corn silk fly, damage caused by tephritids is cryptic and commonly shows no outward symptoms of infestation. As such, fruit fly rating scales are not based on host plant resistance or commodity damage, but rather on insect counts and percent infestation. These scales are regulatory tools that seek insect exclusion as a preemptive quarantine tactic. They are erected around a binomial rating structure that mandates zero-tolerance or very strict threshold levels.

Host plant resistance to the Dipterans has not attained levels commensurate with Lepidopteran pests, but Lepidoptera rating scales provide a framework for this effort (Ashley et al. 1989, Greany 1989, Mihm 1997). Lepidoptera damage scales on maize are available for vegetative and generative plant organs. Most damage scales for plants relate back to a 0 to 9 scheme employed by Guthrie et al. (1960) for the European corn borer (*Ostrinia nubilalis* Hubner) (Lepidoptera: Pyralidae). Currently, either identical or derivative scales are routinely used to rate damage induced by a number of Pyralidae and Noctuidae (Mihm 1997). Rating schemes for ear and kernel damage have commonly used five (Bosque-Perez et al. 1997, Wadley 1949) or six (Robertson and Walter 1963, Douglas 1947, Widstrom 1967) injury classes that are

				Expected	
Corn Silk Fly		Observed		Frequency	
Damage	Observed	Frequency	Expected	under a Poisson	
Category	Frequency	Distribution	Frequency	Distribution	X <sup>2</sup> Value
		%		%	
0	36	25.4	11.3	8.0	53.7
1	5	3.5	28.7	20.2	19.5
2	15	10.5	36.2	25.5	12.4
3	20	14.1	30.5	21.5	3.6
4	66	46.5	35.3	24.8	26.7
Totals	142	142	116.0		
Mean	2.53	2.35			
				$\gamma^2 (P \propto = 0.05) = 7.81$	

**Table 1.** Goodness of fit ( $\chi^2$ ) test for a Poisson frequency distribution of corn silk fly ear damage ratings based on an 142 ear sample and using a five category damage scale.

indicative of the percentage (%) of kernels damaged or depth of feeding penetration. These scales are used to select for resistance to ear feeding Lepidoptera, and have guided the development of resistant types of maize. Based on these Lepidoptera damage scales, we propose a scale to rate corn silk fly damage resistance in the ears and silk of sweet corn.

# MATERIALS AND METHODS

Individual ear ratings for corn silk fly damage were extracted from a field experiment planted from 1994 to 1996, and two experiment conducted from 1998 to 2000. Depending on the experiment, up to ten ears were used to compute a plot mean, but only one observation was extracted for this analysis. Fiftytwo individual ear observations were extracted from a fall armyworm corn silk fly resistance field experiment (Scully et al. 2000a). Over the three years, 52 of the 158 experimental units were sweet corn hybrids including: '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 . Plots were planted in the late spring in the Everglades Agricultural Area (EAA) adjacent to the southern shore of Lake Okeechobee. An additional 90 observations were extracted from two separate sets of experiments planted in cultivation pits at the Everglades Research and Education Center, IFAS-UF, Belle Glade, FL. Three of the four maize varieties in these two experiments had the sh2 endosperm, including 'Primetime', GSS-0966 (Novartis/Rogers-NK, Nampa, ID), and 'Shrunken Zapalote Chico' (Scully et al. 2000b). These experiments were planted in either the fall or the spring to sample differences in insect pressure. In total, 142 observations were extracted with one observation from each sh2 experimental unit at nine site-year locations. Ratings were classified in accordance to the 0 to 4 scale presented below. The observations were assigned to five discrete categories; a frequency distribution was constructed and assessed for its departure from a Poisson Series using the methods of Snedecor and Cochran (1980), and Steel and Torrie (1980).

Methodology for Rating Corn Silk Fly Damage. The female silk fly oviposits on the adaxial surface of the husk leaf adjacent to the silk or on the silk itself. Occasionally, eggs are deposited in the "bore-holes" of the fall armyworm (Spodoptera frugiperda J.E. Smith) (Lepidoptera: Noctuidae) that may have pierced the husk. Sweet corn is most susceptible during the first few days after silk emergence and prior to silk senescence. Eggs hatch in 2 to 4 days and maggots begin feeding progressively downward on the silk (App 1938, Seal et al. 1989, 1995, 1996). As the maggots feed, they massarate the silk and can ultimately penetrate the kernel pericarp and feed on the developing endosperm. This larval stage commonly lasts about 20 days, after which the larvae exit the ear, "jump-off the plant and pupate in the soil. Up to several hundred maggots can feed on a single ear, but 20 to 50 are more common on poorly protected sweet corn. Generally, damage in the silk channel and near the tip is more uniform, but as the silk fly maggots feed downwardly the damage margin becomes erratic. Damage induced by maggots entering through the "bore-holes" tends to

be more localized and less progressive.

Although grade standards are not codified in any regulatory guidelines, the Florida sweet corn industry scouts and inspects for corn silk fly damage. In production fields, spray programs are preventative, and decisions are made apriori within about a week of silk expression. Decisions are based on adult fly counts, prior to oviposition and implicitly assume all cultivars are equally susceptible. Pesticide use in the grain-fill period is guided by adult fly counts and larval damage to the developing ear. At harvest, inspectors grade food quality on a binomially distributed presence or absence (+/-) criteria, which is used to estimate the percent of infested ears. From a host plant resistance perspective this commercial "+/-" scale does not critically assess damage levels or the depth of feeding penetration. Percent damage is also misleading for crop improvement purposes, as all cultivars are hybrids and genetically identical. Thus, any differences in infestation are assumed to represent escapes and or uneven insect dispersal in the field and can confound any inference about plant resistance. However, damage to the ears is critical to assess resistance to the corn silk fly and based on this need we propose the following scale:

- 0 Damage to silk is non-existent to minimal. Silk fly maggots may have initiated silk feeding but failed to persist.
- 1 Damage occurs only to the silk above the ear tip and in the silk channel; neither the ear tip nor the top kernels sustain any damage.
- 2 Damage is sustained on the silk and/or kernels from the ear tip up to the top 25% of the ears length. This is considered intermediate damage.
- 3 Damage is sustained on the silk and/or kernels from the ear tip up to the top 50% of the ears length. This is considered heavy damage.
- 4 Damage is sustained on the silk and/or kernels from the ear tip to over 50% of the ears length. This is considered very heavy damage.

Regardless of whether silk flies are artificially or naturally infested, ears should be rated at sweet corn maturity stage, usually 21 days after pollination (21 DAP), and prior to accelerated starch biosynthesis. This coincides approximately with the end of the 20 day larval stage, but before the maggots have pupated. At this point, kernel and silk damage is near maximum, maggot numbers and size are large and crop maturity ideal. Ears that receive a rating of "0" are suitable commercially, while a rating of "1" may be marketable. Damage that occurs in the silk channel or fouls the ear tip, but fails to reach the kernels is acceptable if the ears are trimmed for the tray-pack market. Often under ambient crop stress ears may not set kernels at the cob tip, and some additional flexibility may be tolerated in category "1". Ratings of "2", "3" or "4" are not commercially acceptable, but useful for plant improvement purposes. Additionally, this scale is more rigorous near the top of the ear because consumer tolerance for silk fly damage is near-zero. It is also based on a generalized interaction between the insect and host plant; silk flies may feed differently on different hybrids. As with all subjective scales some anomalies exist and interpretive flexibility is required. For example, corn silk fly maggots may not uniformly feed down the ear; one side may be more deeply penetrated than another, which may necessitate interpretation or averaging. These types of specific interactions between the insects, plant variety and the environment could be addressed by the inclusion of  $\frac{1}{2}$  incremental ratings, resulting in a nine point scale.

## **RESULTS and DISCUSSION**

This silk fly scale is based on depth of feeding down the ear, and is analogous to Lepidopteran ear damage scales. It is classified as a survey type scale (Widstrom, 1967) and includes five injury groups similar to those employed by Wadley (1949) on corn earworm (Heliocoverpa zea Boddie) (Lepidoptera: Noctuidae) and Bosque-Perez et al. (1997) on African stalk borer species. This scale, like the Robertson and Walter (1963) scale for corn earworm damage on dent corn, employs two categories that do not involve kernel damage, and reflects the markets intolerance for silk fly damage. Many of these ear damage scales tend to have semi-logarithmic groupings, but Widstrom (1967) has argued that these type of survey scales are asymmetric and non-normally distributed. Widstrom (1967) also asserts that direct measures of organ injury are more accurate, but acknowledges these systems are slower, more tedious and often impractical when evaluating and selecting within a large germplasm base. In contrast, Mihm (1997) accepts these rating schemes, regardless of their mathematical flaws.

Although rarely articulated, most insect damage rating scales are developed and applied within the framework and assumptions of Poisson Distribution (Snedecor and Cochran 1980, Steel and Torrie 1980). This proposed corn silk fly damage scale is extrapolated from the binomial scale used commercially and is hypothesized to fit a Poisson series with five discrete categories. Poisson series are routinely used to assess quality control and is an appropriate distribution to describe rare and erratic events. Insect resistance often qualifies as a rare or erratic event. Our biological expectation was that resistance to silk fly damage would be uncommon and therefore any frequency distribution would be a progression that included few if any "resistant" or non-damaged individuals; more individuals were expected in the intermediate damage categories "1", "2", "3" reflecting partial resistance or partial damage. Numerous individuals were expected in category "4", where ears would likely sustain the severest damage and display the least resistance.

The mean damage value of the 142 ears extracted from the data set was 2.53. The mean damage value based on the expected number of observations in each damage category was 2.35 (Table 1). Ears that had damage ratings of "1", "2", '3" and "4" accounted for 74.6% (106/142) of the observed values,

and followed the expectations of a Poisson distribution, where increasing levels of resistance were progressively rarer. However, 36 ears, or 25.4 % of the total showed no damage and received a "0" rating. This is over three times larger than the expected value estimated to fit a Poisson distribution and resulted in a  $\chi^2$  value of 116.2, which was much higher than the  $\chi^2_{(0.050)} = 7.81$  needed to accept this distribution as a Poisson series. Similarly, these data failed to fit a normal distribution, where a goodness-of-fit test returned a value of  $\chi^2 = 166.83$ , compared to the needed value of  $\chi^2_{(Pec=0.050)} = 5.99$ . Additionally, in several simulated distributions the "0" category was altered in an effort to diagnose the deviation from a Poisson series. Although the  $\chi^2$  value was lowered it failed to conform to a Poisson series.

Statistical aspects notwithstanding, any host plant damage rating scale should be biologically and economically rational. It must be able to quickly and easily discern crop damage and be capable of processing and assessing the large number of crop varieties or germplasm that move through any breeding program focused on host plant resistance. With escapes and resistant individuals confounded in category "0", selection methods should be simply modified to reflect lowered expected genetic gains from selection.

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