Examination of the Pest Status of Corn-Infesting Ulidiidae (Diptera)

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ABSTRACT Larvae of 11 species of picture-winged flies (Diptera: Ulididae) are known to feed on corn plants (*Zea mays* L.) in the western hemisphere. Larvae emerge from eggs deposited in leaf axils and corn silk to feed mostly within ears, but the primary versus secondary nature (i.e., pest status) of their infestation is not known for all of these species. Choice and no-choice tests by using a split-plot design were conducted in greenhouse and field trials to determine the pest status on sweet corn of three of these species found in Florida: *Chaetopsis massyla* (Walker), *Euxesta eluta* Loew, and *E. stigmatias* Loew. The main treatments (uninfested ears and ears experimentally infested with either *Spodoptera frugiperda* [Lepidoptera: Noctuidae] or *E. eluta* larvae) were applied at first silk. The subtreatments (*C. massyla*, *E. eluta*, *or E. stigmatias* adults caged on ears) were applied 7 d later and maintained for 10 d. All three fly species were reared from uninfested and experimentally infested ears in both choice and no-choice tests in greenhouse and field trials confirming both primary and secondary modes of ear infestation. More flies of all three species emerged from ears that were preinfested with *S. frugiperda* compared with uninfested ears suggesting either preference for or greater survival within ears previously infested by *S. frugiperda*. Fewer *E. eluta* and *E. stigmatias* emerged from ears preinfested with *E. eluta* in no-choice field tests, suggesting that previous infestation by this fly may negatively affect oviposition or that older fly larvae affect survival of neonate larvae. All three species studied here should be considered primary pests that can render unprotected sweet corn ears unmarketable.

KEY WORDS *Spodoptera frugiperda*, fall armyworm, maize, sweet corn

Sweet corn is an important vegetable crop for Florida; leading the nation in area harvested (17,037 ha) and worth an estimated US$189.2 million in 2010 (Anonymous 2011). In addition to recognized Noctuidae (Lepidoptera) whorl and ear pests such as fall armyworm [*Spodoptera frugiperda* (J. E. Smith)] and corn earworm (*Helicoverpa zea* [Boddie]), nine picture-winged fly (Diptera: Ulidiidae) species [*Chaetopsis massyla* (Walker)], *Eumecosomyia nubila* (Wiedemann), *Euxesta annonae* (F.), *E. eluta* Loew, *E. major* Wulp, *E. nitidiventris* Loew, *E. sororcula* Wiedemann, *E. stigmatias* Loew, and *E. thomae* Loew] have been found in corn (*Zea mays* L.) in North America (including central America from Mexico to Panama and Caribbean countries) (Chittenden 1911, Painter 1955, Barbosa et al. 1986, Wyckhuys and O'Neil 2007, Goyal et al. 2010). The larvae feed on tassels in the whorl and on silk, kernels, and cobs disrupting pollination and greatly reducing marketability through damage and infestation of the ears (App 1938, Bailey 1940, Seal and Jansson 1989, Hentz and Nuessly 2004). Van Zwaluwenburg (1917) first reported *E. stigmatias* as a corn pest in Puerto Rico where it damaged up to 100% of untreated corn. This fly first was discovered damaging corn in Miami, FL in 1938 (Barber 1939) and by mid century had moved north into central Florida (Hayslip 1951). This species has become a serious pest of Florida sweet corn requiring multiple insecticide applications during the ear stage to maintain a marketable crop (Mossler 2008).

Despite the damage inflicted by these flies, systematic, biological, and ecological studies of ulidiid pests of sweet corn in Florida has been complicated by incomplete systematic references, early incorrect identifications, and long periods during which these pests were not active in the major sweet corn growing areas of the state. Identification to species for specimens of United States origin largely relies on keys of foreign species (Steyskal 1968) or unpublished keys by the same author. A photograph on the front cover of a Florida Agricultural Experiment Station Circular (Hayslip 1951) was incorrectly identified as *E. stig-*

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matias and only recently recognized as *E. eluta* by a distinctive wing character visible in the image. *Euxesta stigmatias* appears to have been a continuous pest in sweet corn in extreme southern Florida (Miami-Dade County). However, based on interviews of current and retired growers by the authors, Palm Beach County growers did not experience problems with these flies from the late 1950s until early 1991 when it reemerged as a pest in eastern Palm Beach County. Infestations of *E. stigmatias* in sweet corn had again spread northward into the corn-growing region south of Orlando by 1993.

In corn surveys conducted between 2006 and 2008, four ulidiid species were reared from sweet and field corn in Florida (Goyal et al. 2011a). Two species were found throughout the state on field and sweet corn (*C. massyla* and *E. eluta*), whereas two were found only in corn grown in the southern half of the Florida peninsula (*E. annonae* and *E. stigmatias*). Although four species were collected from Florida commercial sweet corn fields, it was not initially known which of the species were primary pests that required pest management strategies to produce a profitable crop. Goyal et al. (2010) determined that *C. massyla* was a primary pest of both field and sweet corn in Florida, but *E. annonae* and *E. eluta* have not been formally studied to determine their pest status in North America.

Knowledge on the pest status of corn-infesting insects is important for the continued development of integrated pest management strategies for corn as new pests emerge. Although control strategies are required to prevent primary pests from entering ears, no treatment is required for pests with a secondary mode of attack as the presence of prior infestation is required for their feeding. Ulidiid eggs frequently have been observed within the entry and exit holes of Lepidoptera larvae on corn ears (G.G., personal observations). Scully et al. (2000) reported *E. stigmatias* and *Spodoptera frugiperda* larvae (J. E. Smith) (Lepidoptera: Noctuidae) infesting ears concurrently in southern Florida. Information on the relative attractiveness of infested versus uninfested ears may be useful for future research on the relative importance of food resources and on potential oviposition cues for these flies. Therefore, the objective of this study was to measure and compare the pest status and relative importance of prior infestation of corn ears by the three most common ulidiid species found infesting sweet corn in Florida: *E. eluta, E. stigmatias,* and *C. massyla.*

Materials and Methods

Choice and no-choice tests were conducted to measure the pest status of the flies. The studies were conducted in a greenhouse and in fields at the Everglades Research and Education Center (EREC), Belle Glade, FL (latitude: 26.666384, longitude: −80.633312).

Experimental Design. All experiments were arranged as a split-plot design with three main treatments and three subtreatments. The main treatments were designed to represent the state of ear infestation at the time of exposure to the subtreatments. Main treatments consisted of uninfested ears and ears infested with either *E. eluta* or *S. frugiperda* larvae. Five second-instar *E. eluta* larvae from a laboratory colony maintained at EREC (see details below), or three third-instar *S. frugiperda* larvae (corn strain) from a laboratory colony maintained on artificial diet at the USDA-ARS Insect Behavior and Biocontrol Research Unit, Gainesville, FL, were placed within the silk channel of an ear with the aid of a fine camel's-hair brush (size 0). Preliminary tests determined that these levels of infestation were the lowest to provide consistent levels of injury to corn ears. Second-instar *E. eluta* larvae were recognized easily by the nonoverlapping ranges of body length and width (Goyal et al. 2011b). Third-instar *S. frugiperda* larvae were recognized based on their body length, color, and exuvia (Luginbill 1928). All ears were covered with pollination bags (6.25 by 2.5 by 21.25 cm, held tightly around the middle of the ears with rubber bands) between application of the main and subtreatments to avoid unintended insect infestation.

The subtreatments were male:female pairs of *C. massyla, E. eluta,* and *E. stigmatias.* Subtreatments were initiated 7 d after application of the main treatments. In the no-choice tests, the subtreatments consisted of caging one fly species on three ears (see below), each with a different main treatment. Therefore, each fly species did not have a choice among the three main treatment effects. Each of the three fly species was introduced to a different subgroup of three ears. One no-choice test was conducted in a greenhouse and another was conducted in the field. Choice tests were conducted in the field by using groups of three adjacent plants (one with each main treatment) enclosed by small cages (see below). Subtreatments in the choice tests were applied by releasing single species of flies into a cage so that the adults were free to choose to oviposit among the ears with the three main treatments. The subtreatments for choice and no-choice tests were removed 10 d after initiation of the subtreatments. Ear cages were removed along with adults from the no-choice tests and adults were removed from caged plants in the choice tests. The corn ears were re-covered with pollination bags as discussed above In Experimental Design to protect the ears from additional infestation until the end of the test.

Late last-instar Ulidiidae larvae normally leave corn ears to pupate within the soil litter or in the dried silks at the ends of ears (App 1938). *Euxesta eluta* pupae that developed from the main treatment were removed carefully from ears 3 d after application of the subtreatment to avoid any confusion with immatures developing from the subtreatment in which *E. eluta* adults were caged on ears. *Spodoptera frugiperda* larvae take longer to develop to the pupal stage than the fly larvae and removing them from the ears would have destroyed the microhabitat at the end of the ears where the majority of ulidiid larvae develop. Therefore, the lepidopteran larvae were allowed to complete development within the ears.

The tests were terminated 17 d after initiation of the subtreatments when the top third of each ear was incised using a knife and placed individually in 1.83-liter Ziploc (S.C. Johnson & Son, Inc., Racine, WI) bags. The ears were held in the same conditions as used for colony maintenance (see below). To reduce the accumulation of excess moisture and the growth of fungi, two paper towels were added to each bag and changed every 48 h. The bags were left partially open to facilitate aeration by using box fans. Pupae were removed daily from the bags and placed on moistened filter paper (Whatman 3, Whatman International Ltd, Maidstone, England) in covered petri dishes for adult emergence. The dishes were sealed with Parafilm (Pechiney Plastic Packaging, Chicago, IL) to reduce moisture loss. Adult emergence in the petri dishes was recorded every 72 h.

No-Choice Greenhouse Tests. Studies first were conducted using potted plants in a greenhouse in December 2007 to evaluate the methods for no-choice and choice field tests planned for the following years. Sweet corn ('Obsession', Seminis Vegetable Seeds, Inc., Saint Louis, MO) was planted on 17 October 2007, in 18.9-liter (5-gal) buckets filled with Dania Muck soil collected at the EREC. Fertilizer based on the plant requirements (14–14-14 [N-P-K], 15 g per bucket) was hand-mixed with the soil in buckets one day before planting. Three seeds initially were planted in each bucket and then thinned to one seed per bucket on 5 November. Plants were irrigated as needed. The insecticide Proaxis (gamma-cyhalothrin, Dow Agro Sciences, Indianapolis, IN) was used to protect the plants from Lepidoptera larvae until tassel emergence after which no insecticide was applied. The plants were grown in a fan- and pad-cooled greenhouse maintained within 3.5°C of the ambient temperature. In total, 100 plants were grown in buckets and 72 were used for the experiment.

The corn plants were moved at tassel push into groups of nine plants, each with three subgroups of three plants, in preparation for infestation. One of each of the main treatments (uninfested ear, or experimentally infested with *E. eluta* or *S. frugiperda* larvae) was applied to an ear on one plant in each subgroup. Field-collected flies of all three species were used for the subtreatments in the greenhouse no-choice test, because laboratory colonies (see below) were not fully established for *E. stigmatias* and *C. massyla* when it was time to initiate the trial. Flies were collected from corn fields at the EREC by using sweep nets over a 6-d period to obtain enough of each species for the trial. The flies were held under laboratory conditions (see below) so that only apparently healthy and undamaged flies could be used for the tests. The flies had been held 8–14 d by the time they were used in the trial. Concurrent studies by Goyal (2010) found oviposition rates for these flies vary from 1 to 23 eggs per day on artificial diet within 5–15 d of adult eclosion. The greatest oviposition for all three species was observed on the eleventh day after adult eclosion. The preoviposition period for the three species was 10, 3, and 8 d for *C. massyla*, *E. eluta*, and *E. stigmatias*, respectively. Therefore, to attempt to compensate for expected low oviposition potential of these older, field-collected flies, five male:female pairs of flies were used in the greenhouse no-choice tests. They were caged on each ear by using 11 by 30 cm, 17-mesh bags (17 openings per linear 2.5 cm). A cotton ball dipped in 50–60% honey solution was placed in each bag as food for the adults. The open end of each bag was held tightly around the ear with a binder clip to prevent flies escaping. The mesh size of the bags was small enough to cage the flies, but large enough to allow natural pollination of the ears. The subtreatments were maintained for 10 d on the ears during which time dead adults were replaced at 48-h intervals. Eight replicates of the split-plot trials (72 plants total, 24 plants for each subtreatment) were conducted at the same time in the greenhouse.

No-Choice Field Tests. No-choice field studies were conducted during May 2008 at the EREC. Sweet corn (Obsession) was planted 17.8 cm apart on 76.2-cm row-centers on 19 March 2008 in Dania muck soil using a planter unit pulled with a tractor. Liquid starter fertilizer (11–34 −0, N-P-K) was applied in the seed furrow at planting after which supplemental fertilizer applications were managed according to local standards (Ozores-Hampton et al. 2012). Insecticides were used to protect the plants from lepidopteran larvae until tassel emergence after which no insecticides were applied. The same procedures for applying and evaluating main and sub-treatments in the greenhouse no-choice trial were used in the field no-choice trial with the following two exceptions. Flies from laboratory colonies (see below) rather than field-collected flies were used in the trial. Only one pair of 5–15 d-old adults was caged on each ear as subtreatments instead of five pairs as used in the greenhouse trial. Preliminary tests with individual lab-reared flies confirmed that females in this age bracket could deposit a greater number of eggs than the 5-pairs of flies produced earlier in the greenhouse no-choice tests based on the number of larvae that emerged from ears. Three adjacent plants with one ear each were used for each three-plant sub-group. Nine replicates of the split-plot trials (81 plants total, 27 for each subtreatment) were conducted at the same time in the field.

Choice tests. These trials were conducted in the field during December 2009 and May 2010. Sweet corn was planted on 15 and 29 September 2009 ('Garrison', Rogers Brand, Syngenta Seed, Wilmington, DE), 15 February, and 3 March 2010 (Obsession) by using the same planting arrangement and equipment on the same soil type at EREC as for the no-choice field test. Tests were initiated with the observance of first silk emergence from ear tips. Choice tests were conducted within cages (20-mesh, 2.0 m tall by 0.9 m by 0.9 m) supported internally by polyvinyl chloride pipes placed over three adjacent plants within the same plant row. The ends of the pipes were buried 0.4 m beneath the soil and ropes were placed over the tops of the cages and staked to the ground to anchor the cages in inclement weather. Cages were equipped with a full-length zipper to provide easy access to the plants. Three adjacent plants were selected and caged based on the absence of previous insect or mechanical damage to ears. The tips of ears initially were covered with pollination bags held with rubber bands to protect the ears until the main treatments were applied. The three main treatments (i.e., uninfested, experimentally infested with *E. eluta* or *S. frugiperda*) were applied to these ears as above in the no-choice tests and then recovered with the pollination bags within 24 h of caging the groups of three plants. Subtreatments were applied by releasing five pairs of 5–15-d-old laboratory-reared flies of a single species in each cage 7 d after application of the main treatments. The pollination bags were removed from ears before releasing the flies into the cages. The larger search area afforded the flies in the field cages compared with the individual ear cages used in the no-choice tests supported the decision to use five pairs of lab-reared flies in the choice tests rather than the single pair used in the no-choice field tests. No attempt was made to sample and replace flies that may have died in the cages to reduce the chances of introducing competing ulidiid species or predators into the cages. Two cotton balls dipped in a 50–60% honey solution were placed in each cage as a food source for the flies. Ears within the cages were re-covered with pollination bags 10 d after exposing them to the subtreatments. The bags were removed after 7 d and ears harvested and held for adult emergence as above for the no-choice tests.

The choice field tests were repeated four times between December 2009 and June 2010. Six replications of the split-plot trial (18 cages, 54 plants total, 18 plants for each subtreatment) were started in each of the first and second weeks of December. Two additional choice field tests also were started in May 2010, but an unequal number of subtreatment replications were established in these later trials to take advantage of all the available cages. Therefore, seven replications each with *C. massyla* and *E. eluta,* and six replications with *E. stigmatias* were started (20 cages, 60 plants total, 21 for each of the *C. massyla* and *E. eluta* subtreatments and 18 for the *E. stigmatias* subtreatments) in each of the first and second weeks of May.

Colony Maintenance. Colonies of *C. massyla, E. eluta,* and *E. stigmatias* were initiated from adults collected using sweep nets in corn fields in and around Belle Glade, FL. The flies were brought to the laboratory where they were provided with *Helicopverpa zea* Boddie (Lepidoptera: Noctuidae) artificial diet (product F9393B, Bio-Serv, Frenchtown, NJ) as an oviposition medium and larval food by using the method of Hentz and Nuessly (2004). Mature larvae leaving the diet pupated in cotton balls used to plug the end of diet tubes or in cotton sheets used to line trays where diet tubes containing developing larvae were maintained after removal from oviposition cages. The cotton balls and sheets were placed within the adult oviposition cages to allow the adults to emerge from pupae directly into the colony. All stages of all three species were maintained in an insectary room maintained at $26.5 \pm 1.0°C$, 55–70% RH, and a photoperiod of 14:10 (L:D) h. Flies used in the experiment had completed three to six generations before beginning the studies.

Statistical Analysis. Analysis of variance of the results of the choice and no-choice tests was conducted using Proc GLM (SAS Institute 2008) because of the unbalanced design of the experiments (i.e., unequal numbers of ears exposed per species). The results of the no-choice tests were conducted separately for the greenhouse versus the field tests because of differences in the environment and the number of fly pairs caged on the ears between the two tests. The main treatments (ears uninfested, or infested with either *E. eluta* or *S. frugiperda*), subtreatments (fly species), and their interactions were modeled as the independent variables in the no-choice test analyses. The number of adults that emerged per corn ear was modeled as a dependent variable in the no-choice test analyses. For the choice test analyses, experiment date and its interaction with the other independent variables were added to the list of modeled independent variables. Recent studies on the biology of these flies indicated that all three species have different reproductive potentials in terms of the number of eggs deposited during their reproductive periods (Goyal 2010). Therefore, the percentages of adults that emerged per cage from each ear were modeled as a dependent variable in the choice test analyses. The percentage of adults that emerged per main effect ear treatment was calculated by dividing the number of adults emerged per ear by the number emerged per cage. The percentages of emerged adults were transformed before analyses by using arcsine-square root to improve the normality and homogeneity of variance. Untransformed data were used for presentation purposes. Least square means were used rather than arithmetic means because of significant interaction among the independent variables. The Tukey's HSD test (SAS Institute 2008) was used for posthoc means separation with $P \leq 0.05$.

Results

No-Choice Tests. All three ulidiid species oviposited and were able to complete development from egg to the adult stage in uninfested ears, as well as in ears already infested with *S. frugiperda* or *E. eluta* larvae. In the 2007 greenhouse trial, ear infestation ($F = 2.64$; df = 2, 63; $P = 0.0794$) and fly species ($F = 15.86$; df = 2, 63; $P < 0.0001$), but not the ear infestation × fly species interaction ($F = 0.85$; df = 4, 63; $P = 0.5010$), affected the number of adults that emerged from ears. More *E. stigmatias* than *E. eluta* adults emerged from uninfested ears and ears already infested with *S. frugiperda* or *E. eluta* larvae (Table 1). More *E. stigmatias* than *C. massyla* adults emerged from *S. frugiperda*-infested ears.

In the 2008 no-choice field study, ear infestation ($F = 27.22$; df = 2, 72; $P < 0.0001$), fly species ($F = 11.34$; df = 2, 72; $P < 0.0001$), and the infestation × fly species interaction ($F = 2.93$; df = 4, 72; $P < 0.0266$)

Table 1. Least square mean ± SEM (range) number of adults that emerged per corn ear in no-choice tests conducted in a greenhouse in 2007

Subtreatment fly species[a]	Main effects treatment		
	E. eluta	*S. frugiperda*	Uninfested
C. massyla	10.6 ± 2.5cd (2–20)	11.0 ± 2.5cd (0–22)	14.0 ± 2.5bcd (6–40)
E. eluta	8.0 ± 2.5d (0–19)	13.0 ± 2.5cd (0–24)	10.1 ± 2.5cd (4–18)
E. stigmatias	16.9 ± 2.5bc (6–24)	25.4 ± 2.5a (13–38)	21.1 ± 2.5ab (12–29)

Mean ± SEM followed by different letters are significantly different (Tukey's HSD, $P < 0.05$, SAS Institute 2008).
[a] $n = 8$ per fly species per main effects treatment.

significantly affected the number of adults that emerged from ears. Significantly more adults of all the three species emerged from ears already infested with *S. frugiperda* than with *E. eluta* (Table 2). More *E. stigmatias* and *C. massyla* adults emerged from *S. frugiperda*-infested than uninfested ears. The number of *E. eluta* and *E. stigmatias* that emerged from uninfested ears were greater than from *E. eluta*-infested ears. More *E. stigmatias* and *E. eluta* than *C. massyla* adults emerged from uninfested ears. Greater numbers of *E. stigmatias* than the other two fly species emerged from *S. frugiperda*-infested ears. The mean number of adults of all three species that emerged per ear was 1.5× to nearly 2.4× greater in ears already infested with *S. frugiperda* than with *E. eluta*.

Choice Tests. All three fly species deposited eggs and completed development to the adult stage in uninfested ears and in those already infested by *S. frugiperda* or *E. eluta*. The percentage of adults that emerged per ear was significantly affected by ear infestation ($F = 63.67$; df = 2, 216; $P = <0.0001$) and ear infestation × fly species interaction ($F = 6.53$; df = 4, 216; $P = <0.0001$), but not by fly species ($F = 0.13$; df = 2, 216; $P = 0.8749$) or replicate ($F = 63.67$; df = 3, 216; $P = 0.9807$). Data from the four replicates were pooled for mean comparison of adults that emerged per ear, because replicates were not a significant source of variation in the model. Significantly greater percentages of adults of all three fly species emerged (i.e., subtreatments) from ears preinfested (i.e., main treatments) with *S. frugiperda* compared with those with *E. eluta* (Table 3). Greater percentages of *C. massyla* and *E. eluta* emerged from *S. frugiperda*-infested ears than uninfested ears. A greater percentage of *E. eluta* than *E. stigmatias* emerged from *S. frugiperda*-infested ears, whereas a greater percentage of *E. stigmatias* than *E. eluta* emerged from uninfested ears.

Discussion

All three fly species were reared from uninfested corn ears in both choice and no-choice tests, indicating that these flies attack corn as primary pests. This is the first known report of *E. eluta* as a primary pest of sweet corn in North America. All three species also oviposited and completed development in ears already infested with *S. frugiperda* or *E. eluta* larvae, indicating that they can successfully use previously damaged ears as hosts. The current results were in accordance with some of the previous studies done on the primary and secondary nature of these flies. *Chaetopsis massyla* were reared from uninfested standard sweet corn and *Bacillus thuringiensis*-enhanced sweet corn in commercial and experimental fields in southern Florida (Goyal et al. 2010). The secondary mode of attack of *C. massyla* has been reported on wetland monocots such as cattail, *Typha latifolia* L. (Typhaceae: Typhales) previously damaged by moth larvae (Lepidoptera: Noctuidae), and stems of *Carex lacustris* Willd. (Cyperaceae: Cyperales) previously damaged by larvae of *Epichlorops exilis* (Coquillett) (Diptera: Chloropidae) (Allen and Foote 1992). *Euxesta eluta* was identified as a primary pest of corn by Arce de Hamity (1986) in Jujuy, Argentina. Both Arce de Hamity (1986) and Frías (1981) in Chile reported that *E. eluta* completed development in ears damaged by other insects. *Euxesta stigmatias* has been recognized as a primary pest of sweet corn for over 90 yr (Van Zwaluwenburg 1917) and has been reported as a secondary pest of several fruits (Seal et al. 1996).

The colonization of sweet corn by these flies likely began as an extension of feeding habits that evolved along with the development of maize as a crop in Central America. Picture-winged flies have been recognized as pests of field corn in Central America for many years (Painter 1955). Field surveys in 2008 and

Table 2. Least square mean ± SEM (range) number of adults that emerged per corn ear in no choice tests conducted in the field in 2008

Subtreatment fly species[a]	Main effects treatment		
	E. eluta	*S. frugiperda*	Uninfested
C. massyla	15.1 ± 2.6d (10–23)	25.2 ± 2.6bc (8–42)	17.4 ± 2.6d (4–20)
E. eluta	17.0 ± 2.6d (4–26)	29.1 ± 2.6b (19–42)	25.4 ± 2.6bc (8–36)
E. stigmatias	18.2 ± 2.6cd (10–29)	43.3 ± 2.6a (21–58)	26.9 ± 2.6b (20–35)

Mean ± SEM followed by different letters are significantly different (Tukey's HSD, $P < 0.05$, SAS Institute 2008).
[a] $n = 9$ per fly species per main effects treatment.

Table 3. Least square mean ± SEM (range) percentage adults that emerged from corn ears in choice tests

Subtreatment fly species[a]	Main effects treatments		
	E. eluta	*S. frugiperda*	Uninfested
C. massyla	17.5 ± 3.6de (0–52.2)	57.8 ± 3.6ab (0–100)	24.7 ± 3.6cde (0–87.5)
E. eluta	16.9 ± 3.6de (0–100)	68.1 ± 3.6a (0–97.7)	14.9 ± 3.6e (0–43.8)
E. stigmatias	22.0 ± 3.8de (0–40)	43.2 ± 3.8bc (0–100)	34.7 ± 3.8cd (0–100)

Mean ± SEM followed by different letters are significantly different (Tukey's HSD, $P < 0.05$, SAS Institute 2008).
[a] $n = 24$ ears for *E. stigmatias* and 26 for *E. eluta* and *C. massyla* per treatment.

2009 confirmed that three species of Ulididae also attacked field corn during the R1 and R2 stages in Florida (Goyal et al. 2011a). Sweet corn is historically very new on the time scale of maize development. Beyond the sweetness and softer pericarp traits bread into sweet corn, additional traits for easier hand shucking have resulted in more open silk canals and looser husks. These traits likely allow easier access by ulidiid larvae into ears and results in greater numbers of larvae in the milk- and soft-dough stage kernels of sweet than field corn (Scully et al. 2000). Feeding tunnels produced by *S. frugiperda* larvae through the silk channel may further ease the access of ulidiid larvae to the cob and kernels compared with ears without substantial silk channel damage (Nuessly et al. 2007).

The results of greater numbers and percentages of flies emerging from *S. frugiperda*-infested than uninfested ears in both choice and no-choice trials indicates that oviposition by these three fly species was not deterred by prior infestation. Several genera of Ulidiidae are saprophagous with some phytophagous in nature (Hawley 1922, Chittenden 1927, Merrill 1951, Allen and Foote 1967, Kameneva and Korneyev 2010). It is possible that *C. massyla, E. eluta, and E. stigmatias* evolved their feeding habits from saprophagous to phytophagous as more accessible plant foods became available. Allen and Foote (1967) found that *Chaetopsis* spp. larvae fed on healthy tissue surrounding the entry wounds in plant stems created by Lepidoptera larvae. These flies do not penetrate through plant surfaces when they oviposit, so entry into a wider range of plants is likely provided by initial damage caused by other insects that break through the epidermis. Goyal et al. (2012) found eggs of *C. massyla, E. eluta*, and *E. stigmatias* were not able to complete their development to adults on sugarcane (*Saccharum officinarum* L.) stalks until holes were drilled into them. Two studies conducted by Frías (1981) in Chile suggested that *E. eluta* preferred Lepidoptera-infested to uninfested ears. In his first study, *E. eluta* were found only in ears infested with *H. zea* larvae; however, *E. eluta* and *E. annonae* were found in both *H. zea*-infested ears and undamaged ears in his second study.

The results of our trials may indicate the presence of attractive olfactory cues in ears damaged by Lepidoptera larvae that results in significantly greater oviposition in infested compared with uninfested ears. Rojas (1999) found that *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae) deposited significantly more eggs on cabbage plants previously damaged by locusts than on undamaged plants. However, significantly fewer *E. eluta* and *E. stigmatias* adults emerged from ears that were already infested with *E. eluta* larvae compared with uninfested ears in the 2008 no-choice field tests. It is possible that females of these flies were deterred from depositing eggs in ears infested with fly larvae. Prokopy (1975) determined that female *Rhagoletis fausta* (Osten Sacken) (Diptera: Tephritidae) deposited a pheromone on sour cherries (*Prunus cerasus* L. [Rosales: Rosaceae]) that deterred repeated oviposition attempts by the same species. Alternatively, older larvae already infesting the ears (main treatment) may have negatively affected the development or survival of the neonate larvae that emerged from eggs (subtreatment). In several experiments conducted by Crombie (1944) on inter- and intraspecific competition with two grain-infesting species, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) and *Rhizopertha dominica* (Fabricius) (Coleoptera: Bostrichidae), survival was greatest for whichever species or larvae of the same species infested the grain first. Chemical or physical changes in plants because of herbivory, as well as competition for the same resource, may be affecting outcome of these interactions among the four species tested in the current study.

Differences in the experimental designs of the 2007 and 2008 no-choice tests may have resulted in more flies emerging from ears in the second than first trial. Although fewer pairs of flies were used in the no-choice field than greenhouse trial, the former used older, field-collected flies compared with the younger, lab-reared flies in the latter trial. In general, it would be expected that field-collected insects perform better than lab reared ones if other variables (e.g., age, sex) were equal. Glas et al. (2007) showed that field-collected *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) deposited more eggs than laboratory-reared individuals on maíze and vetivar grass [*Vetiveria zizanioides* (L.) Nash]. Laboratory-reared *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) had lower larval emergence and a shorter preoviposition period compared with those of field-collected flies (Rössler 1975). However, our studies on the development and reproduction of *C. massyla* and *Euxesta* spp. later determined that egg deposition increases to its maximum around 10–13 d after adult eclosion and then decreases toward the end of their adult life (Goyal 2010). Therefore, even with the assumption of a uniform age distribution with the subsample of field-collected flies used in 2007, these adults likely produced fewer eggs than flies within their peak reproductive period obtained from our laboratory colony.

In summary, all three picture-winged fly species deposited eggs and developed readily on uninfested ears as well as on those previously attacked by Diptera and Lepidoptera pests. More flies of all three species emerged from ears that were infested with *S. frugiperda* larvae than uninfested ears in choice tests, suggesting either preference for or greater survival within ears previously infested with such larvae. The results suggest that presence of *S. frugiperda* damage in corn fields could attract flies resulting in a greater population of flies and even greater damage to ears than in fields without army- or earworm damage. Interviews with local pest control advisors confirmed that fields heavily infested with *S. frugiperda* before tassel push are more likely to experience greater numbers of picture-winged flies than fields with low levels of these Lepidoptera pests (G. N., unpublished data). Therefore, control measures for *S. frugiperda* before the sensitive ear stage may help to reduce the relative attraction of flies to sweet corn fields. However, our experience also indicates that ears in fields of standard, as well as *Bacillus thuringiensis*-enhanced sweet corn varieties, with no or very low levels of *S. frugiperda* feeding damage can still become 100% infested by these flies if not treated with insecticides in southern Florida. Likewise, secondary ears left on plants at harvest to be eventually harvested as silage provide untreated reservoirs of ears (both infested and uninfested with army- and earworms) for ulidiid population development. *Chaetopsis massyla*, *E. eluta*, and *E. stigmatias* should be considered primary pests of sweet corn.

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