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Dispersal of Newly Eclosed European Corn Borer Adults (Lepidoptera: Crambidae) from Corn into Small-Grain Aggregation Plots

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ABSTRACT Genetically modified, insecticidal *Bacillus thuringiensis* (Bt) corn, *Zea mays* L., hybrids are used throughout the Corn Belt for European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), control. To slow development of Bt corn resistance, the Environmental Protection Agency requires growers to plant a refuge. Determining the appropriate distance between a refuge and Bt corn, and development of mitigation–remediation strategies such as mass releases of susceptible moths, requires an understanding of adult dispersal and mating behavior. However, much remains unknown about these behaviors. Because mating often occurs in grass near cornfields where adult *O. nubilalis* aggregate, we planted small-grain plots as aggregation sites in an attempt to retain mass-released adults. The objectives of this study were to examine influences of pheromone lure, plant density, and plant species on distributions of feral and newly emerged, laboratory-reared *O. nubilalis* among small-grain aggregation plots. Feral adults were collected in aggregation plots in relative abundance, indicating that small-grain plots were acceptable aggregation sites. In contrast, newly emerged adults that were released weekly as dye-marked pupae were rarely found in aggregation plots, with ≈ 150 –1,500-fold fewer adults captured than expected if all released adults had occupied the plots for ≥ 1 d. The majority of newly emerged adults did not colonize the aggregation plots, suggesting that recently eclosed adults leave their natal field and do not colonize the first aggregation sites encountered. Plant species significantly influenced adult distributions among aggregation plots. Mass releases of laboratory-reared pupae in the field may not be a viable remediation tactic because almost all of the newly emerged adults dispersed beyond 300 m of the release point.

KEY WORDS European corn borer, dispersal, Bt, transgenic corn

European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), is a major pest of corn, *Zea mays* L., in most of North America east of the Rocky Mountains. Yield losses and control measures associated with *O. nubilalis* in the United States have exceeded \$1 billion annually (Mason et al. 1996). Genetically modified corn hybrids that express crystal protein endotoxin genes from *Bacillus thuringiensis* (Bt) Berliner (Koziel et al. 1993) for the control of *O. nubilalis* (referred to hereafter as Bt corn) have been available commercially in the United States since 1996 and are an increasingly popular and efficacious management tool (Pilcher et al. 2002). However, there is concern that resistance to Bt corn may develop in *O. nubilalis*, which would reduce the effective life of this transgenic technology.

The insect resistance management (IRM) strategy for *O. nubilalis* on Bt corn is predicated on the expression of a high dose of Bt toxin in the plants, and the

provision of untreated, nontransgenic refugia planted within 800 m from stands of Bt corn (EPA 1998, 2001). High doses of Bt toxin increase the likelihood of mortality of larvae carrying a single resistance allele. The rationale for the refuge requirement is to facilitate mating between resistant adults that might be emerging from a Bt cornfield and susceptible adults emerging from a nearby refuge, thus diluting the frequency of resistance alleles in the *O. nubilalis* population and promoting the production of heterozygous progeny, which are likely to be susceptible to Bt corn (Taylor and Georgiou 1979, Alstad and Andow 1995, Ostlie et al. 1997, Onstad and Gould 1998, Caprio 2001). The appropriate distance between a refuge and Bt corn depends on dispersal behavior and the relative timing of mating behavior of *O. nubilalis* adults, but much remains unknown about these behaviors. If resistance to Bt corn becomes evident in an *O. nubilalis* population, manipulation of the spatial distribution of susceptible adults (wild or laboratory reared) and resistant adults to promote interbreeding may reduce the frequency of resistance alleles and slow the spread of resistance to other populations. Furthermore, knowledge of dispersal and mating dynamics of *O. nubilalis*

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will allow more realistic parameterization of IRM and integrated pest management (IPM) models.

Caffrey and Worthley (1927) first reported that *O. nubilalis* adults were found in dense vegetation outside agricultural crops during the day. Although *O. nubilalis* adults may prefer to remain in irrigated cornfields in the western Corn Belt (Hunt et al. 2001), they usually occupy areas of dense grass in the Midwest (Showers et al. 1976, DeRozari et al. 1977). These aggregation sites are where mating behaviors often occur, such as emission of pheromone and copulation (Klun 1968, DeRozari et al. 1977). Showers et al. (1976, 1980) proposed a scenario for *O. nubilalis* mating dynamics. Once *O. nubilalis* females emerge, they leave the cornfield and fly to nearby aggregation sites to imbibe freestanding water and seek mates. On the nights after mating, the females move between cornfields to oviposit and aggregation sites to rest. Conversely, according to the paradigm, males do not move as frequently as females from aggregation sites. After emerging and leaving corn, males go to nearby aggregation sites to mate, and they remain there until the female population in that site is sparse. Males then disperse to another aggregation site to seek mates.

Several factors have been proposed to govern the selection of aggregation sites, including presence of dew droplets, temperature, humidity, distance from corn stands, plant species, and plant architecture (Hodgson 1928, Showers et al. 1976, DeRozari et al. 1977, Hellmich et al. 1998, Pleasants and Bitzer 1999, Showers et al. 2001, Anderson et al. 2003, Sappington 2005). Hellmich et al. (1998) found that several grain crops including oats (*Avena* spp.) attracted moths, particularly when planted at high densities. Anderson et al. (2003) compared the preference of *O. nubilalis* to several millets and reported that proso millet, *Panicum milliaceum* L., was the most preferred by all life stages, but Siberian foxtail millet, *Setaria italica* (L.), harbored the greatest number of adults. Sappington (2005) found that aggregation sites proximal to corn harbored greater adult densities than sites not adjacent to corn. Showers et al. (2001) recovered the greatest number of marked adults from habitats consisting of a mix of smooth brome grass, *Bromus inermis* Leysser, and giant foxtail, *Setaria faberi* Herrmann, that was 65–70 cm in height and 5–20 m from the site of release. Although brome grass is an important component of aggregation sites for adults during the first flight, which occurs in spring and early summer, it senesces during midsummer and is relatively unattractive to adults of the second flight (Showers et al. 2001). Typically, giant foxtail is attractive to adults later in the summer (Pleasants and Bitzer 1999).

Learning enough about *O. nubilalis* dispersal and aggregation behavior so that it can be manipulated for IPM and IRM goals will require more thorough experimental probing. In this study, we provided a variety of potential aggregation sites to newly emerged adults by planting plots of various small grains (order Cyperales) near the natal corn plot. Our goal was to determine whether we could create conditions that would be attractive enough to retain laboratory-

reared adults near their emergence site when taken to the field as pupae. Success would provide a potential means of introducing large numbers of unmated, Bt-susceptible adults to an area where resistance alleles are increasing in frequency. We also expected that the results would provide insight into *O. nubilalis* dispersal behavior in general. The specific objectives of this study were to examine the influences of pheromone lure, aggregation site plant density, and aggregation site plant species on the distributions of released and feral *O. nubilalis* adults among aggregation plots of small grains.

Materials and Methods

Insect Culture. Adult *O. nubilalis* collected from light traps during summers 2002 and 2003 were used to establish laboratory colonies at the Corn Insects and Crop Genetics Research Unit, Ames, IA, which were the source of marked insects used in experiments in 2003 and 2004, respectively. Moths were reared following procedures similar to Raun (1961) and Guthrie et al. (1965). Standard meridic diet of wheat germ was prepared using methodology similar to Lewis and Lynch (1969). The larvae were allowed to feed ≈ 21 d on diet impregnated with either Solvent Blue 35 (Sudan Blue II, C.I. 306436, Aldrich, Milwaukee, WI), to produce blue-marked adults, or Fat Red 7B (Sudan Red 7B, C.I. 201618), to produce red-marked adults. Consumption of the dye-containing diet stains the fat bodies of *O. nubilalis* larvae, and the mark is retained through adulthood. Using two dyes allowed simultaneous moth releases in overlapping study arenas. Ostlie et al. (1984) and Hunt et al. (2000) reported that these two dyes had negligible effects on moth biology. Each dish containing the diet and larvae was overlaid with a ring of corrugated cardboard. After the ultimate larval molt, larvae colonized the cells in the cardboard rings for pupation and adult eclosion. In total, 600–800 pupae per ring are typical, and the sex ratio of females to males is $\approx 1:1$ (Guthrie et al. 1985). Larvae were reared in an environmentally controlled room at 27°C, 80% RH, and a photoperiod of 16:8 (L:D) h until pupation. Rings of pupae were placed in experimental corn plots from which marked adults emerged and dispersed. Releases were conducted during the natural adult flights. *O. nubilalis* is bivoltine in much of the Corn Belt, including Iowa (Mason et al. 1996). The first flight usually begins in mid-May, and the second flight begins in mid-July. Flights typically last 3–6 wk, with the second flight longer than the first flight.

2003 Experimental Design. Study arenas were established in Boone Co., IA, on two farms. Using recommendations for tillage and fertilization regimes for Iowa producers (Hansen 1992, Farnham 2001, Lang 2001), two 0.81-ha corn plots were planted to 'Pioneer 34B62RR' on each farm on 19 May. The density of plants in each corn plot was 69,160 plants per ha. In 2003, both farms received preemergence applications of herbicides (dicamba, glyphosate, isoxaflutole, and metolachlor) to eliminate weeds and potential adult aggregation sites from the corn plots,

Fig. 1. Schematic of the study arena at each farm for release of marked *O. nubilalis* adults from pupal rings. The numbers within the squares correspond to randomly assigned treatments in small-grain aggregation plots. Areas between aggregation plots were fallow alleyways.

and a soil insecticide (chlorpyrifos) to control corn rootworms (*Diabrotica* spp.) (Coleoptera: Chrysomelidae). The corn plots were flanked on two sides by three tiers of aggregation plots, and the distance from the center of a given corn plot to the proximal edge of each flanking tier was 37, 87, and 137, or >137 m, respectively (Fig. 1); the >137-m distance corresponds to all tiers at the farm beyond the second corn plot. Tiers were planted at different distances to examine the influence of distance from point of emergence on adult propensity to colonize aggregation plots. Each set of three tiers constituted a block, with a tier made up of four 20- by 20-m aggregation plots separated by alleyways that were 14–20 m in width (Fig. 1). Weeds in the alleyways were kept to a minimum by tilling and mowing. Within a tier, the four aggregation plots were planted to 'Oxen' spring wheat, *Triticum aestivum* L., at either the standard rate (75 kg/ha) or twice the standard rate (150 kg/ha). The tiers of aggregation plots at one farm were planted to wheat on 3 April, and the tiers at the second farm were planted on 14 April. These plots were designed to be used as aggregation sites during the time of the natural first flight of adults. One-half of the aggregation plots were spiked with a pheromone lure (Trécé Inc., Adair, OK) impregnated with a mixture of 97% *cis*-11-tetradecenyl acetate and 3% *trans*-11-tetradecenyl acetate, which attracts the Z- or *cis*-strain of *O. nubilalis*, the strain inhabiting Iowa. The pheromone lures were placed in the middle of the aggregation plots and

suspended from a metal rod 20 cm above the ground. Pheromone lures were replaced biweekly. Plots within each tier were assigned randomly to plant density and pheromone lure treatments.

Unfavorable weather conditions prevented timely planting of small grains for the second *O. nubilalis* flight of 2003. Visual appearance of the plots suggested they would not be suitable for adult aggregation because the plots were contaminated with native weed species, and the small-grain stands were not homogeneous within a plot. Few to zero marked and feral adults were collected from the second-flight aggregation plots, confirming that the condition of the plots at the time of our releases was unfavorable for *O. nubilalis* aggregation. Because the absence of feral adults (positive control) indicated an inadequate aggregation habitat, recovery data from the second *O. nubilalis* flight of 2003 were not included in any of the analyses.

During the first flight of 2003, eight cardboard rings infested with marked pupae were placed weekly in each corn plot on each farm. Two differently colored pupae were released per study arena (Fig. 1). The rings were placed near the center of the corn plots in the late afternoon. The number of pupae per ring was estimated by counting the number of pupae in two rings per color per farm on three release dates. The proportion of moths that successfully emerged during a release was estimated as the number of empty pupal cases per total number of pupae in a ring, and for releases where no emergence or pupae-per-ring data were taken, the mean of the three release dates was used. The rings were suspended ≈ 20 cm from the surface of the ground in a 61- by 61- by 61-cm wire-mesh cage, which had two 20- by 58-cm openings in the top of the sides to allow emergence of adults. Cage tops were covered with plywood or rubber mats to reduce the potential of thermal damage and desiccation of the adults induced by direct sunlight. The cages remained in the corn plots for 7 d, and five releases were made in 2003.

2004 Experimental Design. Study arenas were established in Story Co., IA, on two farms. Corn plots were planted to 'Dekalb DKC63-80RR' on each farm on 26 April. Both farms received a preemergence application of herbicides (dicamba, glyphosate, isoxaflutole, and metolachlor). The corn plots were flanked on both sides by three tiers of aggregation plots, similar to the arrangement used in 2003 (Fig. 1). However, for the second flight of 2004, the aggregation plot dimensions were 20 by 10 m, and they were situated between the 20- by 20-m aggregation plots used for the first flight. The difference in design improved planting logistics associated with preparing second-flight aggregation plots. Two 20- by 20-m plots per tier on both farms were planted on 14–15 April to Oxen spring wheat at twice the standard rate (150 kg/ha). The other two aggregation plots per tier were planted on both farms on 14–15 April to 'Jerry' oats, *Avena sativa* (L.), at twice the standard density (180 kg/ha). During the week of 27 June, the first-flight aggregation plots of senescing wheat and oats were cut and baled.

For the second flight, two 20- by 10-m wheat plots per tier on both farms were planted at twice the standard density on 9 June. The other two aggregation plots per tier on both farms were planted on 9 June to 'Manta' Siberian foxtail hay millet, *Setaria italica* L., at twice the standard density (45 kg/ha). One-half of the aggregation plots per adult flight per plant species were spiked with pheromone lure. Aggregation plots within each tier were assigned randomly to plant species and pheromone lure treatments per adult flight.

During the first and second natural adult flights of 2004, 15 cardboard rings infested with marked pupae were placed in each corn plot weekly as described for 2003. The number of pupae per ring was estimated on four release dates, and the number of rings was increased in 2004 to increase the number of released adults, and therefore improve collection rates. The proportion of adults that successfully emerged during a release was estimated for four releases. For releases where no emergence or pupae-per-ring data were taken, the mean of the four releases was used. The wire-mesh cages used to house the rings of pupae were left in the corn plots for 3 d instead of 7 d so that the released cohorts were more homogenous in age. Seven releases were made in 2004.

Data Collection. The aggregation plots were sampled 5 d/wk, beginning the day marked pupae were placed in the corn plots. Each aggregation plot was sampled with two sampling techniques: A heavy-duty, 0.38-m-diameter sweep net (Bioquip Products, Inc., Rancho Dominguez, CA) was used to collect adults so that their origin (laboratory or feral), sex, and the mating status of females (Showers et al. 1974) could be determined. Counts of feral adults were important in determining which plots were acceptable to *O. nubilalis* as aggregation sites. On each sample date, each aggregation plot was swept 30 times over a distance of ≈ 30 m before the contents of the net were assessed. Different paths were taken through the plots during sweep sampling each day to reduce damage to the grass. Second, a flush bar technique (Sappington and Showers 1983b) was used to increase the area of aggregation plots sampled and to facilitate the determination of the suitability of the small-grain plots as aggregation sites. A 1.5-m plastic rod was used to disturb the grass along a randomly selected, 20-m edge of each aggregation plot on each sample date. Flushed adults were counted on the wing, although sex and origin could not be determined.

Observations of marked adults remaining in the corn plots were taken about twice per release in 2004 ($n \approx 14$ per farm). Along the edge of each corn plot that flanked the aggregation plots, and along the middle rows of each corn plot, 50-m lengths of row were visually inspected to confirm that the marked adults were dispersing and not colonizing the corn plots at each farm.

Statistical Analyses. The number of marked adults of each color dispersing per release was estimated as the product of the number of rings placed at a farm ($n = 8$ or 15), the proportion of estimated emergence, and the estimated number of pupae per ring. The expected

number of adults per aggregation plot was calculated under the scenario that all marked adults that dispersed from the pupal rings occupied only the aggregation plots and were distributed uniformly within them. Thus, the expected mean number of released adults occupying each plot was calculated by dividing the number of marked adults of a given color released at a farm by the number of aggregation plots at the farm ($n = 36$). That quotient was multiplied by the fraction of area of an aggregation plot that was sampled with the sweep net on a sample date ($\approx 3\%$) to give the number of marked adults expected to be captured by the sweep net. An analysis of variance (ANOVA) (restricted maximum likelihood estimates, REML-ANOVA) was used to determine whether the observed mean number of marked adults swept per plot per release was different than the number of marked adults expected to be captured per plot (PROC MIXED, SAS Institute 2001). The dependent variable in the model was the mean number of adults swept, and the fixed effect was moth category (expected and observed). The random effects in the model were year, release date, sample date, block, and treatment (plant density, plant species, and pheromone lure). The moth category means were separated using the LSMEANS option (SAS Institute 2001).

To test the effect of distance of aggregation plot from point of adult release on the mean number of marked adults recovered, a mixed model was used (PROC MIXED, SAS Institute 2001). The analyses were applied to both sexes combined and to each sex separately, and the four levels of the fixed factor corresponded to the four distances of the tiers of aggregation plots from the point of adult emergence in the corn plots (distance of 37, 87, 137, and >137 m). Year, flight, and field were the random parameters in the models, and fixed effect means were separated with the LSMEANS option (SAS Institute 2001).

The influence of plant density and pheromone lure on the combined mean number of marked and feral adults collected with the sweep net in wheat plots during the first flight in 2003 was assessed with REML-ANOVA (PROC MIXED, SAS Institute 2001). The dependent variable in the model was the mean number of total adults collected per aggregation plot, and the fixed effect was treatment (standard plant density with lure, standard plant density without lure, double plant density with lure, and double plant density without lure). Block and release date were random effects in the model. The treatment means were separated using the LSMEANS option (SAS Institute 2001). Linear contrast statements were used to examine the impact of plant density, pheromone lure, and the interaction of plant density and pheromone lure. Similar models were assessed for the mean number of male *O. nubilalis* swept per aggregation plot, mean number of female moths swept per aggregation plot, and mean number of adults flushed per aggregation plot.

The REML-ANOVA models applied to the 2004 data were akin to the models used in the analyses of the first-flight data of 2003. However, for the first flight of 2004, the levels of the fixed (treatment) effect were

wheat with lure, wheat without lure, oats with lure, and oats without lure. The treatment levels of the second flight of 2004 were millet with lure, millet without lure, wheat with lure, and wheat without lure. For the data of each flight of 2004, linear contrasts were used to separate the influence of plant species, pheromone lure, and their interaction.

The relationship between the mean number of adults swept and sex was examined using REML-ANOVA (PROC MIXED, SAS Institute 2001). The dependent variable in the model was the combined mean number of marked and feral adults swept per aggregation plot, and the fixed effect was sex. The random effects in the model were year, release date, block, and treatment (plant species, plant density, and pheromone lure). The sex means were separated using the LSMEANS option (SAS Institute 2001). A similar model was used to assess the interaction between sex and treatment (plant species, plant density, and pheromone lure). The fixed effects in this case, however, were sex, treatment, and the interaction of sex and treatment. The random effects were year, release date, and block.

All of the data were transformed to $\ln(\bar{X} + 0.5)$ for all of the analyses to meet the assumptions of homoscedasticity and normality (Ott and Longnecker 2001), but the results are presented as back-transformed data. Assumptions were assessed with residual plots and normal plots (PROC UNIVARIATE, SAS Institute 2001).

Results

An estimated 215,900 artificially marked adults were released over the 2 yr of the experiment. Of these adults, 103 marked adults (0.048%) were recovered in the aggregation plots with the sweep net. Of the estimated $\approx 102,500$ red-marked adults released, 42 (0.041%) were recovered with the sweep net. Nearly 93% of the recovered red adults were males. One of the three red females recovered had not mated and two had mated within 48 h of capture, according to the presence and melanization of the spermatophore (Showers et al. 1974). Approximately 113,440 blue-marked adults were released, and 61 (0.054%) were recovered with the sweep net. Eighty-four percent of recovered blue adults were males. Two of the females recovered were unmated, and eight had mated within 48 h of collection. Observations in 2004 in the corn plots revealed no adults, suggesting that few if any of the marked adults remained in the corn instead of dispersing.

During the first flight of 2003 and the flights of 2004, feral adults were found in the small-grain aggregation plots, outnumbering marked adults by $\approx 10:1$. Over the course of the study, 1,033 feral adults were collected with the sweep net, 61% of which were males. Of the 408 females collected, 52 were unmated (13%), 348 had mated within 2 d of capture (85%), and the remaining eight (<1%) had mated >2 d previously. The presence of feral adults served as a positive control, demonstrating that the aggregation plots were ade-

Table 1. Expected and observed mean number of marked *O. nubilalis* adults recovered from aggregation plots with a sweep net within 5 d of release

Yr	Flight	Release date	Expected X/ aggregation plot	Observed X (SE)/ aggregation plot
2003	1	2 June	6.70	0.01 (0.01)
	1	9 June	6.70	0.03 (0.01)
	1	17 June	6.55	0.04 (0.02)
	1	24 June	5.50	0.01 (0.01)
	1	2 July	8.23	0.02 (0.02)
2004	1	20 May	8.15	0.03 (0.01)
	1	3 June	9.25	0.06 (0.01)
	1	8 June	6.35	0.00 (0.00)
	1	15 June	8.15	0.01 (0.01)
	2	27 July	16.20	0.09 (0.02)
	2	5 Aug.	11.63	0.00 (0.00)
	2	10 Aug.	15.80	0.01 (0.02)

quate to function as such. The densities of adults flushed from the grassy plots (Figs. 3–5) were within a factor of 10 of densities documented in natural aggregation sites in other studies (Showers et al. 1976; Sappington and Showers 1983b,c; Hellmich et al. 1998; Sappington 2005), so we conclude that the small-grain aggregation plots were generally suitable as aggregation areas.

The difference between the mean number of marked adults collected with the sweep net per aggregation plot and the expected number of adults recovered per aggregation plot was significant ($F = 53,458.6$; $df = 1, 2,077$; $P < 0.0001$). Fewer marked adults were collected by sweep net than were expected during every release, with the difference ranging from 154- to 1,580-fold (Table 1), indicating that much <1% of the newly emerged adults colonized the nearby aggregation plots for even 1 d.

Although few marked adults were recovered from the aggregation plots, we were able to test the influence of distance from release point on those that did colonize the plots. The relationship between distance from point of eclosion and the mean number of marked adults recovered was not significant (Fig. 2) for sexes combined ($F = 0.71$; $df = 3, 13.8$; $P = 0.5643$),

Fig. 2. Mean $\pm$ SE number of marked *O. nubilalis* adults collected by sweep net per aggregation plot per sample date at four distances from the point of adult emergence from pupal rings.

Fig. 3. Mean $\pm$ SE number of *O. nubilalis* collected per aggregation plot per sample date during the first flight of 2003.

males only ($F = 1.16$; $df = 3, 12.8$; $P = 0.3612$), or females only ($F = 1.39$; $df = 3, 14.4$; $P = 0.2848$).

We also examined the effect of aggregation site conditions (i.e., plant species, plant density, and pheromone lure) on the mean number of marked and feral adults (combined) collected from the aggregation plots. Over the first flight of 2003, the influence of treatment (plant density and pheromone lure) on the mean number of marked and feral adults collected with the sweep net was not significant for males ($F = 1.18$; $df = 3, 107$; $P = 0.3201$), females ($F = 1.46$; $df = 3, 107$; $P = 0.2307$), or sexes combined ($F = 2.35$; $df = 3, 107$; $P = 0.0763$) (Fig. 3). Similarly, the relationship between the mean number of adults flushed and treatment was not significant during the first flight of 2003 ($F = 2.07$; $df = 3, 84$; $P = 0.1098$) (Fig. 3).

In 2004, the relationship between the mean number of marked and feral adult males collected with the sweep net and treatment (plant species and pheromone lure) was significant during the first flight ($F = 5.46$; $df = 3, 84$; $P = 0.0018$) (Fig. 4). Linear contrasts of the first-flight data indicated that both plant species ($F = 10.08$; $df = 1, 84$; $P = 0.0021$) and pheromone lure ($F = 5.10$; $df = 1, 84$; $P = 0.0265$) significantly influenced the number of male adults collected, although

Fig. 4. Mean $\pm$ SE number of *O. nubilalis* collected per aggregation plot per sample date during the first flight of 2004.

Fig. 5. Mean $\pm$ SE number of *O. nubilalis* collected per aggregation plot per sample date during the second flight of 2004.

the interaction between the two factors was not significant ($P = 0.2760$). More males were swept from plots of oats than spring wheat plots, and pheromone-spiked plots harbored more males than plots without lure. The relationship between the mean number of females swept and treatment was not significant ($F = 2.16$; $df = 3, 89$; $P = 0.0984$) (Fig. 4). When the male and female data of first flight of 2004 were combined, the relationship between treatment and the mean number of adults collected was significant ($F = 4.87$; $df = 3, 89$; $P = 0.0035$) (Fig. 4). Linear contrasts indicated that the effect of plant species was significant ($F = 12.14$; $df = 1, 89$; $P = 0.0008$), and more adults were collected from oat plots than spring wheat plots. However, neither pheromone lure ($P = 0.1190$) nor the interaction of lure and plant species ($P = 0.9983$) was significant. The relationship between the mean number of adults flushed and treatment was not significant ($F = 1.50$; $df = 3, 84$; $P = 0.2202$) (Fig. 4).

During the second flight of 2004, the relationship between the mean number of marked and feral males collected and treatment (plant species and pheromone lure) was significant ($F = 10.04$; $df = 3, 43$; $P < 0.0001$) (Fig. 5). Linear contrasts showed that the effect of plant species was significant ($F = 29.99$; $df = 1, 43$; $P < 0.0001$), with foxtail millet being much more attractive than spring wheat. The effects of pheromone lure ($P = 0.7727$) and the interaction of lure and plant species ($P = 0.8709$) were not significant. The mean number of females was significantly affected by treatment level ($F = 12.31$; $df = 3, 43$; $P < 0.0001$) (Fig. 5), and the linear contrasts indicated significant differences between the plant species ($F = 36.91$; $df = 1, 43$; $P < 0.0001$). More females were collected from plots of foxtail millet than spring wheat plots. However, neither pheromone lure ($P = 0.9064$) nor the interaction between plant species and lure ($P = 0.9145$) was significant. When the male and female data were combined, the influence of treatment on the mean number of adults swept was significant ($F = 20.72$; $df = 3, 43$; $P < 0.0001$) (Fig. 5). Linear contrasts indicated that plant species was significant ($F = 62.10$; $df = 1, 43$; $P < 0.0001$) and the adults favored foxtail millet plots over spring wheat plots, but pheromone

lure ($P = 0.9948$) and the interaction of the two factors ($P = 0.7918$) were not significant. The relationship between the mean number of adults flushed and treatment was significant ($F = 16.27$; $df = 3, 43$; $P < 0.0001$) (Fig. 5). The linear contrast indicated that plant species was significant ($F = 48.57$; $df = 1, 43$; $P < 0.0001$), and the adults preferentially colonized foxtail millet plots over spring wheat plots. However, the mean number of adults flushed was neither affected by pheromone lure ($P = 0.8021$) nor the interaction between plant species and lure ($P = 0.6854$).

Overall, about two-fold more marked and feral male than female adults were collected with the sweep net ($F = 27.42$; $df = 1, 505$; $P < 0.0001$). When the interaction between sex and pheromone lure was examined with a type-three test of fixed effects, no significant relationship was noted ($F = 0.83$; $df = 9, 498$; $P = 0.5880$), notwithstanding that the pheromone lure mimics female-produced pheromone (Klun and Brindley 1970) and is attractive to males only.

Discussion

Dispersal behavior, and its relationship to timing of mating, has important implications for IRM modeling and strategies because patterns of dispersal directly affect spatial patterns of gene flow (Mallet 2001). The current Bt-IRM strategy for *O. nubilalis* depends critically on the assumption that any Bt-resistant adults emerging from Bt corn are likely to encounter and mate with susceptible adults emerging from nearby refugia, which growers are required to plant within 800 m of the Bt corn (EPA 2001). Despite the importance of understanding *O. nubilalis* dispersal behavior in this context, very little is known about it. Adult *O. nubilalis* have been documented traveling nearly 45 km across Lake Erie (Caffrey and Worthley 1927). Extrapolation from range expansion data suggests that adults can move 80 km annually (Chiang 1972) or 34 km per generation (Showers 1979). Likewise, Showers et al. (2001) recovered marked adults 23–49 km from their point of release and found that some adults moved >14 km within only 100 min. Yet, the proportion of a population that disperses such distances is unknown, as are the average dispersal distance per generation and its timing.

Our experiments were designed based on the same assumption inherent in the current IRM strategy for *O. nubilalis*, namely, that newly emerged adults aggregate and mate in grassy sites near the natal cornfield. However, our results were unexpected in that only a small fraction of newly emerged adults colonized nearby sites, and this raises new questions about *O. nubilalis* dispersal behavior. The small-grain species used in this study, and the dates and rates at which the aggregation plots were planted, resulted in generally suitable aggregation sites for *O. nubilalis* adults. This is evidenced by the relatively large number of feral adults collected from these sites and by the numbers of adults flushed from them, which were comparable

within an order of magnitude to the numbers reported in previous studies (Showers et al. 1976; Sappington and Showers 1983b,c; Hellmich et al. 1998; Sappington 2005). However, the natal fields from which the feral moths originated, and their distance from the study arenas, cannot be determined. We found no differences in the number of adults recovered in the grass at different distances within 300 m of the emergence site (Fig. 2). Showers et al. (2001) suggested that the distance from the point of adult moth release was a significant factor in recapture rate in pheromone traps, and that male moths were often recovered ≥ 800 m from the release point. Qureshi et al. (2005) found that distance from point of emergence of dye-marked adults within irrigated cornfields was correlated with recapture rate in pheromone and light traps, with few individuals recovered beyond 300 m. However, most of the marked adults recovered in that study were captured near the point of emergence, and their low-level recaptures at distances equivalent to the aggregation sites in our study made up the long flat tail of their fitted curves. Conversely, Hunt et al. (2001) found no differences in the number of adults captured in pheromone-baited traps at different distances within 200 m of the release site.

Despite their suitability as aggregation areas, many-fold fewer marked adults than hypothesized were recovered from the aggregation plots (Table 1). This suggests that as newly emerged adults disperse from their natal field, they do not colonize the first grassy sites encountered that are suitable for aggregation. Rather, our data indicate that most newly eclosed adults disperse beyond at least 300 m before colonizing grassy sites and mating. Showers et al. (1976) found that within 1 d, adults dispersed from grass plots where they were released. Similarly, Showers et al. (2001) noted that a small proportion of the adults released in their study remained near the point of release, but they dispersed within 1–2 d. Surveys of first-flight distribution among grassy ditches in central Iowa suggest that although some adults may colonize the grass adjacent to their presumed natal cornfields, they do not remain there long (Sappington 2005). Qureshi et al. (2005) concluded that the low recapture rate and uniform distribution of marked adults in their study suggests that most newly emerged *O. nubilalis* flew beyond their study arena and that the adults have the potential to disperse extensively. Our findings, along with evidence from previous studies (Showers et al. 1976, 2001; Qureshi et al. 2005; Sappington 2005), imply that *O. nubilalis* may undergo an obligate dispersal phase soon after emergence, as is the case for many insects (Johnson 1969). The existence and nature of such a phase will require more direct experimentation, which we are pursuing now by using flight mills.

Aggregation sites are an important component of *O. nubilalis* ecology, and it is important to understand them in an IRM context, because mating often occurs in these sites (DeRozari et al. 1977). Although few marked adults were recovered in this experiment, many feral adults were collected and differences in

spatial distributions were noted among the aggregation plots. The initial hypothesis was that within the confines of <300 m, we could manipulate aggregations nearby the natal cornfield to ultimately foster gene flow, but we found that the majority of the newly emerged adults dispersed beyond the immediate vicinity of the natal field. We were, however, able to successfully manipulate where feral adults aggregate by using different plant densities and plant species. High-density grass plots harbored the most adults numerically during the first flight of 2003 (Fig. 3). Although the influence of plant species on the number of adults swept from aggregation plots was not compared across flights because of confounding and changing moth population sizes, the effect of plant species was significant within a flight, and oats and foxtail millet were preferred as aggregation sites over spring wheat (Figs. 4 and 5). Hellmich et al. (1998) found that double density oats harbored the highest number of adults compared with regular density plots of oats and high and low densities of other small grains. It is likely that the higher density sites' microclimate is more attractive to discerning adults and that some plant species at certain densities are more suited to fostering ideal microclimatic conditions than others.

Because the reproduction strategy of *O. nubilalis*, like most Lepidoptera, is sexual (Normark 2003), the flight behavior of males and females may differ. Both sexual dimorphism and behavioral differences may lead to differences in the spatial distribution of the sexes. Showers et al. (1976, 1980) proposed that adult males spend less time in cornfields than females, and that gravid, mated females leave aggregation sites to oviposit, whereas males move among aggregation sites to find females. We found that more marked males than females colonized aggregation sites, even though we released approximately equal proportions of each sex. A similar phenomenon was observed with feral adults. Among the feral *O. nubilalis* collected, $\approx 61\%$ were males, and 84–92% of the marked adults recovered were males. The findings of Showers et al. (1976, 1980) and Sappington and Showers (1983a) parallel our findings in concluding that few females reside within aggregation sites >2 d postmating. Conversely, <1% of the females that we found in aggregation plots had been mated >48 h at the time of collection. The paucity of older females (classes 3 and 4) in the aggregation sites suggests that females leave soon after they have mated, presumably to oviposit, and that few return to the aggregation sites.

If Bt-resistant *O. nubilalis* are ever detected, mitigation-remediation tactics must be implemented to sustain the efficacy of the insecticidal, transgenic plants. Andow and Ives (2002) suggested possible tactics for reducing the frequency of resistance alleles in the field, including creating super-refugia for susceptible adults, releasing artificially reared adults into the field, manipulating spatial patterns of refugia relative to Bt corn, and using pheromone lures to modulate movements of susceptible males in and around Bt cornfields. If resistance is detected in the field and laboratory-reared susceptible adults are mass

released in that area to mate with the resistant adults, it is imperative that the former remain in the area long enough to mate. Our results indicate that mass-releases of laboratory-reared, susceptible *O. nubilalis* via placement of pupal rings in the field may not be a viable remediation tactic because almost all of the adults likely dispersed beyond the vicinity of the release point (i.e., ≤ 300 m). It will be important to more accurately clarify the spatial and temporal distribution of adults relative to the natal cornfield. Furthermore, pheromone lures did not consistently increase concentrations of males in aggregation sites as was originally hypothesized (Figs. 3–5).

Adult age or reproductive development may influence the likelihood or timing of dispersal (Johnson 1969). Although *O. nubilalis* females generally are not polyandrous, males are promiscuous (Pesho 1961, Loughner 1971, Showers et al. 1974, Elliott 1977, Royer and McNeil 1993, XiangQuan et al. 2004). Laboratory-reared, mated males may not be as prone to disperse from their point of release as were the newly emerged, unmated males in this study. If true, this would make mated males better candidates for release into an area where Bt resistance has been detected in a local *O. nubilalis* population. Releasing females that have mated may not be a viable option as a mitigation-remediation strategy because females generally will not mate again, although 20–30% multiple-mated females have been reported from light trap captures in Ontario and Pennsylvania (Elliott 1977, XiangQuan et al. 2004). Because age is correlated to their sexual maturation (XiangQuan et al. 2004), older adults may be more likely to remain in the area of release than younger adults because they are sexually mature and prepared to mate. Studies are underway to examine the influences of age and mating status on dispersal of released adults from aggregation sites.

In conclusion, we found that newly emerged, marked *O. nubilalis* adults that were released weekly as pupae were rarely found in nearby aggregation sites, suggesting that recently eclosed individuals fly away from their field of origin and do not land in the first acceptable aggregation sites encountered. The *O. nubilalis*-IRM plans for both prevention and mitigation of resistance are predicated, in part, on the unproved assumption that a preponderance of mating occurs near the natal cornfield. Our findings indicate that adults disperse at least 300 m beyond their natal field, although it remains unknown how far they disperse, and whether mating takes place before or after dispersal. Given that most mating occurs in aggregation sites (Showers et al. 1976) and that we recovered very few marked adults in nearby sites, it seems likely that most mating takes place after dispersal. Unraveling the relative timing of mating to dispersal, and a more complete characterization of dispersal behavior of *O. nubilalis*, will help scientists design better strategies for slowing the development and spread of resistance to Bt corn.

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