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Influencing European Corn Borer (Lepidoptera: Crambidae) Aggregation Sites in Small Grain Crops

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Abstract

Reliable methods to attract European corn borer, *Ostrinia nubilalis* (Hübner), adults to small grain crops could be used to aggregate moths into small well-defined areas for control purposes or could be used in a resistance management program for delaying potential *O. nubilalis* resistance to transgenic corn. The objective of this research was to determine whether small-grain crops could be managed to influence *O. nubilalis* aggregation behavior. In farmer-managed oat, *Atena sativa* (L.), fields, more *O. nubilalis* adults were attracted to high-density patches of oat compared with standard patches of oat; no difference was found between patches of high-density oat and brome, *Bromus* spp. Numbers of *O. nubilalis* moths found in 6 barley, *Hordeum vulgare* (L.), and legume treatments (1995), and 4 oat/legume treatments (1996) were significantly different. The highest number of *O. nubilalis* adults were observed in barley planted with alfalfa, *Medicago sativa* (L.), followed by barley planted with crimson clover, *Trifolium incarnatum*(L.), barley planted with berseem, *Trifolium alexandrinum* (L.), barley planted with black medic, *Medicago lupulina* (L.), barley alone, and barley planted with lespedeza, *Lespedeza stipulacea* (Maximowicz). Double-planted oat attracted the highest number of *O. nubilalis* adults followed by oat planted with crimson clover, oat planted with alfalfa, and single-planted oat. Each study suggests that there is a positive correlation between moth aggregation and canopy area. Suggestions are made that timing canopy closure of a small-grain crop with peak *O. nubilalis* flight should maximize *O. nubilalis* aggregation and should thereby increase the efficacy of any control measures.

Keywords

European corn borer, refuge, trap crop, aggregation, legume, resistance management

Disciplines

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Influencing European Corn Borer (Lepidoptera: Crambidae) Aggregation Sites in Small Grain Crops

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ABSTRACT Reliable methods to attract European corn borer, *Ostrinia nubilalis* (Hübner), adults to small grain crops could be used to aggregate moths into small well-defined areas for control purposes or could be used in a resistance management program for delaying potential *O. nubilalis* resistance to transgenic corn. The objective of this research was to determine whether small-grain crops could be managed to influence *O. nubilalis* aggregation behavior. In farmer-managed oat, *Avena sativa* (L.), fields, more *O. nubilalis* adults were attracted to high-density patches of oat compared with standard patches of oat; no difference was found between patches of high-density oat and brome, *Bromus* spp. Numbers of *O. nubilalis* moths found in 6 barley, *Hordeum vulgare* (L.), and legume treatments (1995), and 4 oat/legume treatments (1996) were significantly different. The highest number of *O. nubilalis* adults were observed in barley planted with alfalfa, *Medicago sativa* (L.), followed by barley planted with crimson clover, *Trifolium incarnatum* (L.), barley planted with berseem, *Trifolium alexandrinum* (L.), barley planted with black medic, *Medicago lupulina* (L.), barley alone, and barley planted with lespedeza, *Lespedeza stipulacea* (Maximowičz). Double-planted oat attracted the highest number of *O. nubilalis* adults followed by oat planted with crimson clover, oat planted with alfalfa, and single-planted oat. Each study suggests that there is a positive correlation between moth aggregation and canopy area. Suggestions are made that timing canopy closure of a small-grain crop with peak *O. nubilalis* flight should maximize *O. nubilalis* aggregation and should thereby increase the efficacy of any control measures.

KEY WORDS European corn borer, refuge, trap crop, aggregation, legume, resistance management

AGGREGATION BEHAVIOR of European corn borer, *Ostrinia nubilalis* (Hübner), adults has intrigued investigators for many years. Adults "seek shelter during the day underneath leaves of weeds, grasses, and cultivated crops" (Caffrey and Worthley 1927) and that "they are often found lurking in the grass headlands adjacent to fields of corn and other crops." These aggregation areas have been termed "action sites" because much of the mating behavior of *O. nubilalis* moths occurred in or near them (Showers et al. 1980).

Reliable methods to attract *O. nubilalis* adults to agronomic crops are important for 2 reasons. First, crops can be used to aggregate moths into small well-defined areas for control purposes (Showers et al. 1980). Trap-cropping *O. nubilalis* moths followed by a precise application of chemicals or biological agents would reduce treated acreage. Second, these crops can be used in resistance management programs for delaying corn borer resistance to transgenic corn (Gould 1986, 1994; Mallet and Porter 1992; Tabashnik 1994). Spatial and temporal location of *O. nubilalis* aggregation sites can encourage panmixis of *O. nubilalis* adults. Also, many crops support larval growth and could provide important refuge (i.e., nontransgenic hosts) for larvae that are susceptible to transgenic corn (R.L.H., unpublished data).

Aggregation sites for *O. nubilalis* adults typically consist of brome grass, *Bromus* spp. (May–June) or foxtail, *Setaria* spp. (July–August) (Showers et al. 1976); however, other weeds and crops such as oat, *Avena sativa* (L.), also are suitable. The presence of free water combined with physical factors for mating (temperature, relative humidity, and illumination) contributes to the aggregation of adults (DeRozari et al. 1977). Density (45% covered by vegetation) of plants within a site also contributes to the formation of this microclimate (Showers et al. 1976). Potential use of agronomic crops as aggregation sites for *O. nubilalis* has not been evaluated. The objective of this research was to learn whether small-grain crops alone or in combination with legumes influence *O. nubilalis* aggregation behavior. Moth attraction to various plantings of small grains was evaluated in 3 experiments. Different densities of oats were compared with brome, a plant known to attract *O. nubilalis*, in the 1st experiment. Barley interplanted with 6 types of legumes were evaluated in a 2nd experiment; and 2

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densities of oats and oat interplanted with alfalfa or crimson clover were evaluated in a 3rd experiment.

Materials and Methods

Brome Grass and Oat. Ten commercial spring-planted oat fields in Story and Boone counties in central Iowa were selected as research sites. In each field, areas of differing plant density were classified as high-density or standard oat plots. Brome grass bordered each of these fields. Portions of these borders also were designated as plots. Criteria used to select plots included proximity to corn (<100m), patch size (>100 m^2), canopy (>45%) and height (50–120 cm) (Showers et al. 1976, 1980). Canopy of high-density oat >75%; canopy of standard oat was between 45 and 75%. Brome canopy, in most cases, was greater than 75%. Canopy percentage was estimated by holding a square (1/4 m^2) above the plants and visually assessing the portion of the ground covered with vegetation. Adult sampling consisted of sweeping a 1-m aluminum bar through the plant canopy in 10 consecutive meter-long sweeps (Derrick and Showers 1990). Adults were counted as they were flushed out of the plants; 2 or 3 subsamples each of standard oat, dense oat, and brome were made at 10 locations (6 Story County, 4 Boone County). Each location was sampled between 14 and 20 June 1995; counts were taken before 0930 hours CDST. Sampling dates corresponded with 1st-generation *O. nubilalis* adult flight (Mason et al. 1996); oat plots were at late boot stage to early flowering.

Barley and Legume Interplantings. Barley, *Hordeum vulgare* (L.), and barley/legume mixtures were planted 25 April 1995 at the Iowa State University Burkey Farm located 15 km west of Ames. Eighty-four plots (1.5 by 7.6 m) were established beside a corn field. These plots consisted of barley (variety Stander, 400 seeds per square meter) and barley interplanted with 6 types of legumes. A randomized complete block design was used with each of the 7 treatments replicated 3 times within each of 4 blocks. Legumes evaluated were alfalfa, *Medicago sativa* (L.) (variety CMF101); crimson clover, *Trifolium incarnatum* (L.) (variety unstated); berseem, *Trifolium alexandrinum* (L.) (variety Multicut); black medic, *Medicago lupulina* (L.) (variety George); lespedeza, *Lespedeza stipulacea* (Maximowičz.) (variety Kobe); and sweet clover, *Melilotus lupulina* (L.) (variety Hubam), at the rate of 17, 23, 14, 14, 23, and 14 kg/ha, respectively. Legume seeds, inoculated with proper *Rhizobium* strains, were broadcast before drilling of barley seed: 34 kg/ha of nitrogen was broadcast before planting. *O. nubilalis* adults were flushed and counted as before (by 0930 hours CDST) over 7.6 m^2 of vegetation. Samples were taken on 21 June. In addition, height of barley, height of legume, and percent canopy measures were taken for each plot after sampling.

Oat and Legume Interplantings. Oat/legume mixtures were planted 24 April 1996 at the Iowa State University Johnson Farm located 2 km south of Ames and 2 May 1996 at the Iowa State University Sorenson Farm located 10 km west of Ames. Twenty-four plots (1.5 by 10 m) were established next to a corn field at each farm. These plots consisted of oat (variety Bay, 325 seeds per square meter), oat double planted (650 seeds per square meter), oat (325 seeds per square meter) interplanted with alfalfa (variety CMF101, 17 kg/ha), and oat (325 seeds per square meter) interplanted with crimson clover (variety unstated, 23 kg/ha). A randomized complete block design was used with each of the 4 treatments replicated 6 times at each farm. Legume seeds, inoculated with proper *Rhizobium* strains, were broadcast before drilling of oat seed. *O. nubilalis* adults were flushed and counted as before (by 0930 hours CDST) over 10 m^2 of vegetation. Flush samples were taken 6 times, twice a week, from 10 to 28 June. In addition, height of barley, height of legume, and percent canopy measures were taken for each plot 12 June.

Data Analyses. Flush-bar data from the oat patches and brome were compared using 1-way analysis of variance (ANOVA) (SAS Institute 1985). Waller–Duncan *K*-ratio *t*-tests (Waller and Duncan 1969) were used to separate flushed-moth means for standard oat, dense oat, and brome. Flushed-moth data from the barley and legume experiment were analyzed by least squares ANOVA (PROC GLM, SAS Institute 1985) in a randomized complete block design. Sweet clover was not included in the analysis because the plants were defoliated by the sweetclover weevil, *Sitona cylindricollis* Fåhraeus, before flushing observations. Waller–Duncan *K*-ratio *t*-tests or LSD tests (STDERR = PDIFF, SAS Institute 1985) were used to separate treatment means from the barley and legume experiment. Percent canopy, height of barley, and height of legume were run as covariates. A 3-level factorial design was used for analyzing moth counts from the oat and legume experiment. Location, blocks, and treatments were used as grouping factors. Waller–Duncan *K*-ratio *t*-tests were used to separate treatment means. Percent canopy, height of oat, and height of legume were run as covariates only for observations taken from 10 to 14 June. These were the sampling dates that were closest to the 12 June plant measures used as covariates.

Results

Brome Grass and Oat. Number of moths flushed from the brome aggregation sites and oat sites were significantly different (F = 18.71; df = 2, 18; P < 0.0001). Dense-oat and brome sites means were significantly higher than the standard-oat site mean; brome and dense-oat site means were not significantly different (Table 1).

Barley and Legume Interplantings. Numbers of moths flushed from the barley and barley/legume plots were significantly different (F = 6.77; df = 5, 15; P < 0.002) (Table 2). Numbers of *O. nubilalis* moths flushed from 7.6 m^2 of barley interplanted with either alfalfa or crimson clover were significantly more than barley alone; no difference between crimson and ber-

Table 1. Mean ± SEM number and range of *O. nubilalis* moths flushed from 3 types of vegetation in or adjacent to 10 fields in Story and Boone counties

Plant type	*n*	Range of location means	No. adult *O. nubilalis*
Brome	10	5.0–23.0	15.6 ± 1.8a
Oat (dense)	10	8.3–43.0	21.9 ± 3.9a
Oat (standard)	10	0–5.3	2.5 ± 0.6b

Means with the same letter not significantly different (Waller–Duncan, $P < 0.05$)

seem clover was found (Table 2). Numbers of moths found in barley and legume interplantings that included berseem clover, black medic, and lespedeza were not significantly different from the number of moths found in barley alone. Height of barley ($F = 0.89$; df = 1, 47; $P = 0.351$) and height of legume ($F = 1.56$; df = 1, 47; $P = 0.217$) were not significant covariates. Percent canopy was a significant covariate when it was added to the ANOVA ($F = 4.53$; df = 1, 47; $P < 0.04$; estimate of slope 0.092 ± 0.043 [mean ± SE]). Numbers of moths observed in the plants increased as percent canopy increased for all the legumes except lespedeza (Fig. 1). After adjustment for plant canopy, the alfalfa/barley treatment was significantly higher than all the other treatments ($F = 4.16$; df = 5, 22; $P < 0.009$) (Table 2).

Oat and Legume Interplantings. More *O. nubilalis* moths were flushed from 10 m^2 of double-planted oat than 10 m^2 of oat interplanted with crimson clover, oat interplanted with alfalfa, or single-planted oat when all weeks were combined (Table 3). Oat and crimson clover, and oat and alfalfa had more *O. nubilalis* than the single-planted oat, but only the oat and crimson clover treatment was significantly different (Table 3). Significantly more *O. nubilalis* were flushed at Johnson Farm compared with Sorenson Farm ($F = 7.10$; df = 3, 30; $P < 0.012$). When data from each week of the experiment were analyzed separately, double-planted oat had higher numbers of *O. nubilalis* adults during the 1st (10–14 June) and 2nd (17–21 June) weeks (Table 3); however, data from the 2nd wk should be qualified because there was a significant location by treatment interaction ($F = 14.37$; df = 3, 30; $P < 0.0001$). When these data were analyzed separately by location, differences between treatments were found only at the Johnson Farm (Table 3). No differences among treatments were found during the 3rd wk. For the remainder of the analysis, data from the 10–14 June samplings were considered. Height of oat ($F = 1.61$; df = 1, 29; $P = 0.214$) and height of legume ($F = 3.62$; df = 1, 29; $P = 0.067$) were not significant covariates, although height of legume is notable. Percent canopy was a significant covariate ($F = 12.65$; df = 1, 29; $P < 0.0013$; estimate of slope 0.092 ± 0.032). Moths observed in plants increased as percent canopy increased for all treatments (Fig. 2). When the model was adjusted for canopy covariance there were no significant differences between treatments (Table 3).

Discussion

Scouting and properly timed applications of insecticide can be used to manage 1st-generation *O. nubilalis* larvae (Lynch et al. 1977, Moffat 1991, Mason 1996). Treatments are most effective from egg hatch to the 2nd instar, because 3rd–5th instars are unaffected by insecticides after they bore into the corn plant (Mason 1996). However, there are no consistent, economically effective tactics for managing the 2nd generation of this pest; timing of insecticide applications is difficult because of a prolonged oviposition period.

Showers et al. (1980) suggested using aggregation plants as trap crops for adult *O. nubilalis*. This method places control emphasis on adults rather than larvae. *O. nubilalis* larval damage to corn was significantly reduced when *O. nubilalis* aggregation sites located next to corn fields were treated with carbaryl (Showers et al. 1980). The aggregation sites were composed primarily of giant, *Setaria faberi* R. Herrmann, and green foxtail, *Setaria viridi* (L.) P. Beauvois, and many of them were near waterways. This practice was not promoted after reports surfaced that producers were spraying *O. nubilalis* aggregation sites in waterways. This often leads to chemical contamination of surface water (L. C. Lewis, personal communication). Unfortunately, a less persistent insecticide, methomyl, was not as effective in reducing *O. nubilalis* damage (Derrick and Showers 1991).

Advantages of using an agronomic crop for aggregating *O. nubilalis* moths are that they can be located

Table 2. Mean number and canopy adjusted means of *O. nubilalis* moths flushed from 7.6 m^2 vegetation in barley and legume plots

Plant type	*n*	No. adult *O. nubilalis* means ± SEM[a]	Canopy adjusted no. adult *O. nubilalis* LS means ± SEM[b]
Alfalfa and Barley	4	4.75 ± 0.90a	5.03 ± 0.55a
Crimson clover and Barley	4	3.92 ± 0.98ab	1.30 ± 1.30b
Berseem and Barley	4	2.92 ± 0.44bc	2.23 ± 0.62b
Black medic and Barley	4	1.83 ± 0.65c	2.24 ± 0.57b
Lespedeza and Barley	4	1.42 ± 0.52c	2.56 ± 0.75b
Barley	4	1.50 ± 0.48c	2.98 ± 0.86b

[a] Means with the same letter not significantly different (Waller–Duncan, $P < 0.05$)
[b] Means with the same letter not significantly different (SAS, LSD/STDERR PDIFF, $P < 0.05$)

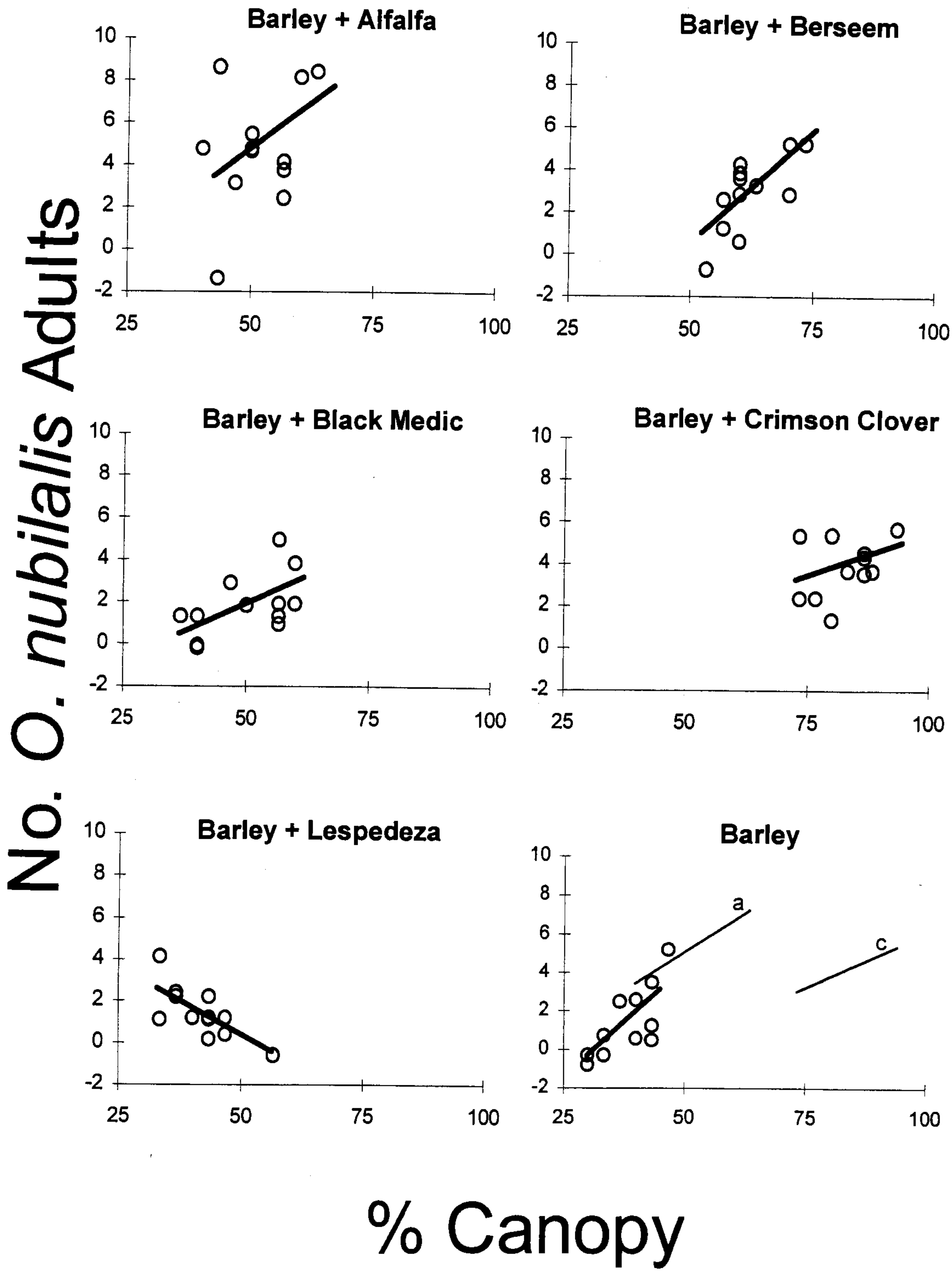

Fig. 1. Mean number (adjusted for block effects) of *O. nubilalis* adults flushed from 7.6 m^2 of vegetation with a 1-m bar plotted against percent canopy for 6 treatments: barley planted with alfalfa (estimate of slope 0.131 ± 0.101 [mean ± SE]), berseem (0.209 ± 0.113), black medic (0.092 ± 0.071), crimson clover (0.083 ± 0.150), lespedeza (−0.140 ± 0.106) and no legume (0.237 ± 0.137). In the control plot, "a" and "c" represent alfalfa and crimson clover regression lines, respectively.

Table 3. Mean ± SEM number of *O. nubilalis* moths flushed from 10 m^2 vegetation in oat and legume plots for all dates (10–28 June), week 1 (10–14 June), week 2 (17–21 June) by location, week 3 (24–28 June), and canopy adjusted means for week 1

Dates in June	Single Oat (n = 13)	Double Oat (n = 12)	Oat + Alfalfa (n = 12)	Oat + Crimson (n = 11)	F	$P > F$
All	3.65 ± 0.36c	7.72 ± 0.72a	4.49 ± 0.39bc	4.76 ± 0.29b	26.13	0.0001
10–14	2.35 ± 0.48b	4.92 ± 0.63a	3.13 ± 0.59b	3.27 ± 0.45b	8.15	0.0004
17–21 Johnson[a]	6.25 ± 0.77b	20.33 ± 0.92a	9.08 ± 1.57b	8.42 ± 1.24b	28.42	0.0001
17–21 Sorenson[a]	3.00 ± 0.77	5.08 ± 0.83	2.83 ± 0.51	2.53 ± 1.08	2.16	0.14
24–28	4.04 ± 1.25	5.54 ± 1.40	4.29 ± 1.18	5.09 ± 1.14	2.35	0.092
10–14 adjusted	3.07 ± 0.49	3.74 ± 0.60	3.56 ± 0.46	3.27 ± 0.46	0.32	0.81

Means with the same letter not significantly different (Waller–Duncan, $P < 0.05$).

[a] Johnson Farm, n = 6 for each treatment; Sorenson Farm, n = 7, 6, 6, and 5 for single oat, double oat, oat/alfalfa and oat/crimson clover treatments, respectively; df = 3, 15 when analyzed by location otherwise df = 3, 30.

away from waterways, and that the planting date can be adjusted to attract 1st or 2nd moth flights. Studies are underway to understand the relationships between various plant phenologies and relative attractiveness of 1st and 2nd generation moths. The goal is to develop a variety of cropping methods for attracting moths from 1st and 2nd flights that corn producers can adapt to local conditions and existing equipment. These methods could be used in an integrated pest management program (IPM) to reduce chemical insecticide usage. Treating *O. nubilalis* adults in aggregation sites permits a more judicious use of insecti-

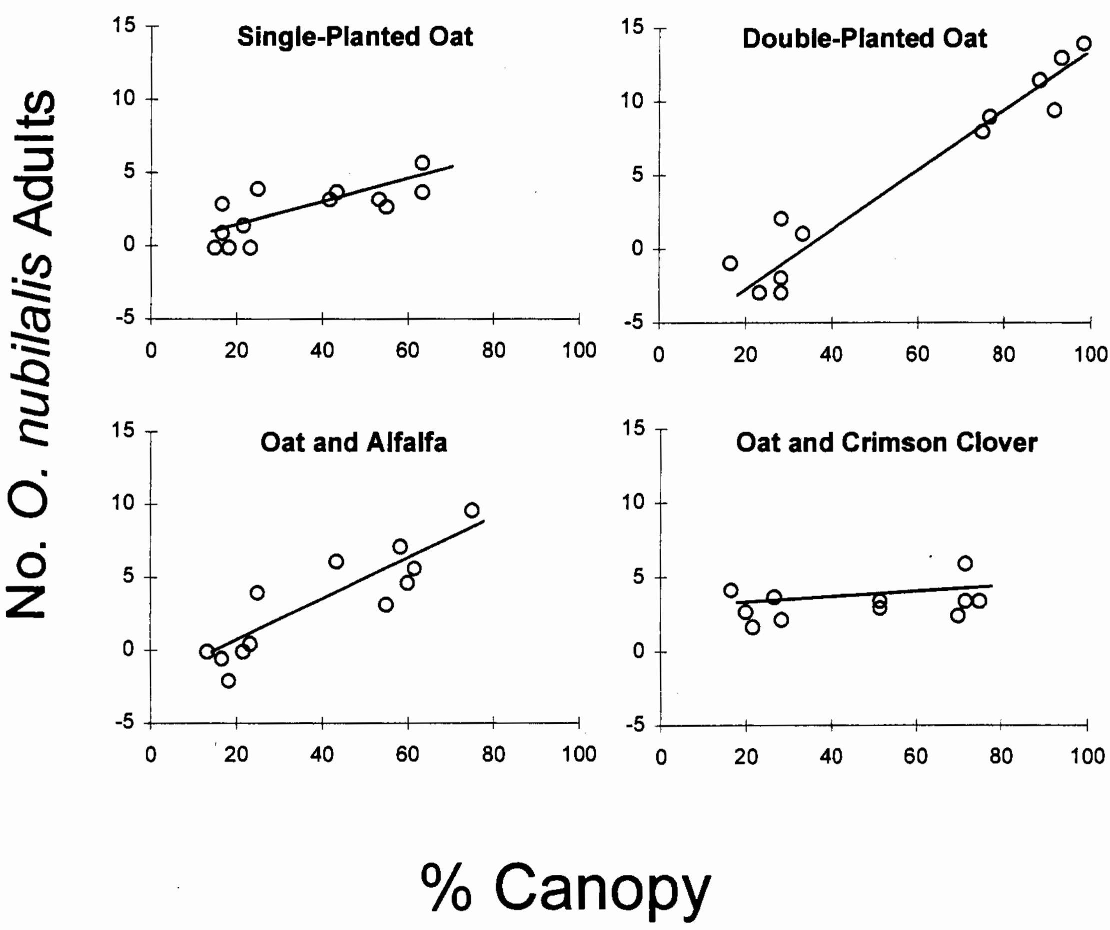

Fig. 2. Mean number (adjusted for block and location effects) of *O. nubilalis* flushed from 10 m^2 of vegetation with a 1-m bar plotted against percent canopy for four treatments: single-planted oat (estimate of slope 0.072 ± 0.068 [mean ± SE]), double-planted oat (0.193 ± 0.064), oat planted with alfalfa (0.147 ± 0.063), and oat planted with crimson clover (0.017 ± 0.061).

cides. Also, these areas could be targeted for biological control agents and promoted as nurse plots.

Aggregating *O. nubilalis* moths to well-defined sites is a 1st step toward controlling this pest. Cropping methods can be used to influence where *O. nubilalis* adults aggregate, by increasing canopy percentages and plant densities to attract high numbers of moths. We have selected crops that producers can easily incorporate into a management scheme. For example, oat is commonly planted in turn rows or set-aside land in Iowa (Iowa Agricultural Statistics Service 1995). Other small grain crops such as barley, wheat, or millet also potentially could be used to manipulate adult *O. nubilalis* populations.

Results from the 1995 field season suggested that canopy density was an important factor for aggregating *O. nubilalis* adults. In 1996, attempts to increase canopy percentages were made by selecting a wide-leaf forage variety of oat (Bay) and by double planting oat. This strategy was effective. More *O. nubilalis* adults were observed in the double-planted oat treatment than any other 1996 treatment. The 1995-data also suggested that alfalfa exhibited an unknown trait, beyond the canopy effect, that attracted moths.

Double-planted oat reached canopy closure at the Johnson Farm near the peak of *O. nubilalis* flight, the 2nd wk. During this time, the double-oat treatment attracted 2–3 times more *O. nubilalis* than the other treatments that had not yet attained canopy closure. Canopy closure for double-planted oats at the Sorenson Farm occurred $\approx$ 1 wk later. The highest number of *O. nubilalis* at the Sorenson Farm was found during the 3rd wk, which was past the peak of *O. nubilalis* flight. This suggests that timing canopy closure of a small-grain crop with peak *O. nubilalis* flight would maximize *O. nubilalis* aggregation and would help increase the efficiency of any control procedures. Microhabitat formed from canopy closure, or near canopy closure, is conducive to *O. nubilalis* aggregation. Showers et al. (1976) and DeRozari et al. (1977) found similar results when they were investigating *O. nubilalis* aggregation sites in foxtail.

A growing consensus from the scientific community is that effective refuges will play a critical role in managing insect resistance to transgenic plants (Gould 1986, Mallet and Porter 1992, Tabashnik 1994). A refuge refers to one or more alternative hosts, or nontransgenic crop, that will support the growth of susceptible insects. The premise is that susceptible insects from the refuge, if present in sufficient numbers, will mate with potentially resistant insects and dilute resistance genes. Crops that attract *O. nubilalis* adults could have 2 important roles in managing *O. nubilalis* potential resistance to transgenic corn hybrids. First, *O. nubilalis*-aggregation crops could promote panmixis. Aggregation sites could be placed strategically to encourage rare resistant moths to mate with susceptible moths. Currently, parameters for determining spatial and temporal arrangements of aggregation sites are not known. Understanding basic *O. nubilalis* mating biology (e.g., distances *O. nubilalis* males and females fly before and after mating) is necessary before recommendations can be made. Second, noncorn crops could provide important sources of refuge. Studies are underway to evaluate the fitness of *O. nubilalis* that develop on oat and several other crops that attract *O. nubilalis* moths. If crops that support high numbers of *O. nubilalis* are identified, then they could be incorporated into IPM and resistance management strategies.

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