

Differentiation of Competitive vs. Non-competitive Mechanisms Mediating Disruption of Moth Sexual Communication by Point Sources of Sex Pheromone (Part 2): Case Studies

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Abstract Eleven out of 13 disruption profiles (plots of dispenser density vs. male catch) from moth sex pheromone literature were consistent with a competitive-attraction mechanism, in which dispensers attract males and thereby divert them from females. Mean dispenser activity (D_a) across all competitive-attraction cases was 0.04 ± 0.06 (SD); values ranged from 0.0005 for a tiny laminated flake dispenser of racemic disparlure targeting gypsy moth to 0.2 for polyethylene tube dispensers used against lightbrown apple moth. A dispenser application activity (D_{Aa}) can be calculated by multiplying D_a by the number of such dispensers applied per hectare of crop. The highest dispenser application activity (D_{Aa}) values approached 200 and corresponded to >99% inhibition of catches of male moths in monitoring traps. Relative to the D_{Aa} scale, % inhibition of catches of male moths compressed and obscured large differences in D_{Aa} when % disruption exceeded 90%. For cases of competitive attraction, these two efficacy scales can be interconverted by using the formula: $D_{Aa} \approx 100/(100 \text{ minus } \% \text{ disruption})$. When disruptive point sources of pheromone were directly observed, male moths were seen approaching pheromone dispensers whose disruption profiles matched competitive attraction. Two cases fit non-competitive disruption mechanisms, which include camouflage, desensitization (adaptation and/or habituation), and sensory imbalance. In these cases, pheromone was released at rates higher than for cases of disruption by competitive attraction. Practical ramifications of the finding that competitive attraction appears to be the prevalent mechanism for moth mating

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disruption by pheromone point sources are listed. We believe that the congruence of diverse sets of mating disruption field data with explicit *a priori* predictions validates competitive-attraction theory. The analytical tools and principles governing competitive attraction that were uncovered during this study of mating disruption of moths should be generally applicable to competitive-attraction phenomena.

Keywords Mating disruption mechanisms · Profile analysis · Percent disruption · Dispenser activity · Dispenser application activity

Introduction

Miller et al. (2006, this issue) reported analytical procedures and criteria whereby mechanisms of mating disruption by point sources of sex pheromone can be differentiated into two main categories: competitive (false-plume following) vs. non-competitive (camouflage, desensitization, and sensory imbalance). In this report, we apply those procedures to case studies mainly from the published literature on mating disruption of moths. The objectives were to: (1) determine to what extent experimental disruption profiles were congruent with the various theoretical predictions detailed by Miller et al. (2006); (2) determine whether one category of disruption is more common than the other; and (3) begin to interpret the practical consequences of the results of these analyses.

Methods

We searched the literature for experimental data sets allowing graphical analysis of suppression of catches of male moths in monitoring traps vs. density of point sources of synthetic sex pheromone. A target of at least three densities of pheromone dispensers in addition to zero pheromone narrowed the prospective cases. Ideally (Miller et al., 2006), such experiments would have recorded dispenser density (D_D), trap density (T_D), relative attractiveness of traps (T), females (F), and dispensers (D), catches of males (C) over most of a moth generation, and densities of female (F_D) and male moths (M_D) (see Table 1 of Miller et al., 2006 for a key to abbreviations). However, we found no study where all these parameters were recorded. In all cases analyzed in this work, data were sufficient for valid determinations of competitive vs. non-competitive disruption. However, moth densities computed from the competitive-attraction equations of Miller et al. (2006) could be compared with measured densities in only a few cases because relative attractiveness of T , F , and D usually was not measured.

Disruption profiles were examined with three types of graphical plots: untransformed (C vs. D_D), Miller-Gut ($1/C$ vs. D_D), and Miller-de Lame (C vs. $C^* D_D$) (Miller et al., 2006). Patterns in profiles consistent with competitive vs. non-competitive disruption are summarized in Table 2 of Miller et al. (2006). Where applicable, straight lines were fit to data by weighted regression. In most cases, statistical analyses were not possible because the raw data were not accessible. For our own field experiments, statistical procedures are explained in the respective figure captions.

When estimating or interpreting male and female densities from given dosage–response profiles, we elected to first show the calculations most simply by operating on a starting premise that attractiveness of $T = F = D$. Judgments about fit of actual field data to theory should be withheld until the justification for departure from attractiveness of $T = F = D$ is

revealed and the appropriate mathematical adjustments are made. Most cases ultimately supported a competitive-attraction equation more complex than Equation (1) of Miller et al. (2006) where attractiveness of $T = F = D$.

Results and Discussion

Cases Consistent with Competitive Attraction and Inconsistent with Camouflage or Other Non-competitive Disruption Mechanisms

Oriental Fruit Moth (Grapholita molesta)

Rothschild (1975) conducted one of the most complete disruption experiments useful to the current analyses when he documented communicational disruption of oriental fruit moth (OFM) in Australian peaches. The pheromone formulation was 98% (*Z*)-8-dodecenyl acetate (2% *E*-isomer) dispensed from 1 ml closed polyethylene tubes (precursor to pheromone ropes) each releasing ca. 15 $\mu\text{g/hr}$ of pheromone. This pheromone blend did not fully match that of the female, now known to also include ca. 1% (*Z*)-8-dodecenyl alcohol (Cardé et al., 1979). In a test originally aimed at determining the number of dispensers required to prevent males from finding pheromone-baited monitoring traps, dispensers were deployed in duplicate 100 tree plots (0.4 ha) at 0, 0.5 (every other tree), 1, and 2 dispensers per tree. Twelve monitoring traps were deployed in a 3×4 grid in each plot. Trap catches were reported as the total for all 12 traps/plot over three sampling periods of 20–30 d spanning each of three moth generations (Rothschild, personal communication). Before this test began, the OFM population was quantified at 0.6–1 pupae per tree based on tree banding, and subsequently at 0.5–1 moths per tree by counting OFM adults recovered from cloths laid under trees fogged with pyrethrum. Assuming an equal sex ratio, this equates to an F_D and M_D of 0.4/tree, or about 150 moths/ha of each sex.

Rothschild's disruption outcome is scaled to 1 ha and plotted on untransformed axes in Fig. 1a, as a Miller-Gut plot in Fig. 1b, and as a Miller-de Lame plot in Fig. 1c. Although few, the data support an interpretation of competitive attraction. The untransformed plot (Fig. 1a) is more curvilinear than linear. It resembles the competitive-attraction profile in Fig. 3a of Miller et al. (2006) for a simulated moth population of about 30 moths per 72 trees (0.4/tree). The initially steep drop in catches of males with dispenser density leveled out by ca. 1 dispenser per tree. This data set is reasonably linear on a Miller-Gut plot (Fig. 1b), which then justifies derivation of secondary measures. The density of males in this experiment can be estimated (procedures of Miller et al., 2006) from Fig. 1b as: $1/\text{slope} = M_D T_D = 1/0.0004 = 2,500$. Since $T_D = 30/\text{ha}$, calculated $M_D = 83$ males/ha compared to the measured value of 150 males/ha. Allowing for some mortality and a trap efficiency of less than 100%, it is not surprising that this derived value of M_D was somewhat lower than the initial M_D directly measured by Rothschild at the start of the adult generation. Female density in this test can be derived from the absolute value of the *x*-intercept = $0.0213/0.0004 = 53 = T_D + F_D$. Because $T_D = 30/\text{ha}$, $F_D = 23$ females/ha. The derived F_D/M_D ratio is $23:83 = 1:3.6$. A time-averaged F/M ratio favoring males is expected in the kinetics of competitive attraction for OFM, whose females are not known to mate frequently, whereas males continue to seek females throughout their lives. Therefore M_D would be the more stable measure of OFM density during a given generation. However, the accuracy of such F/M ratio calculations,

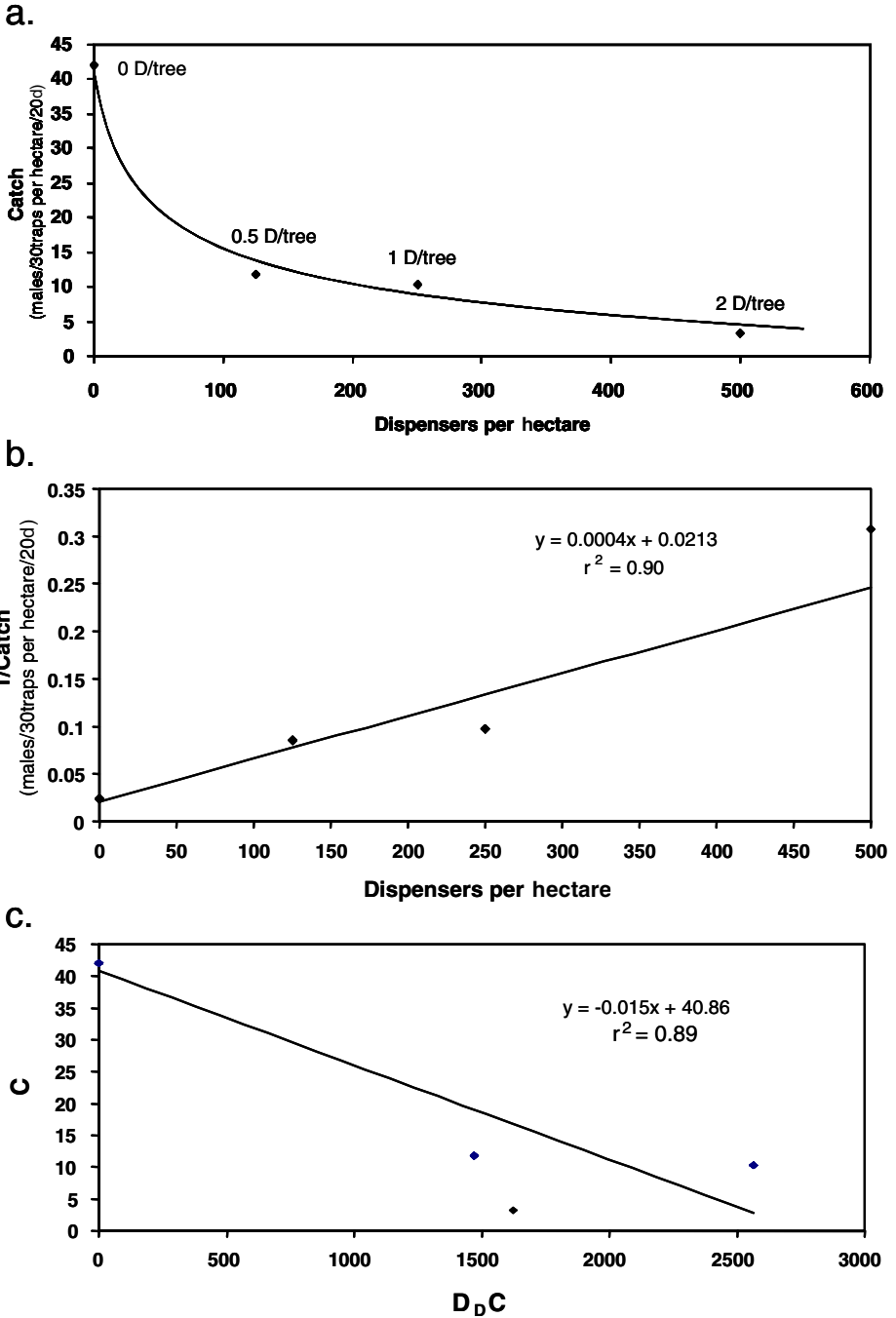


Fig. 1 Untransformed (a), Miller-Gut (b), and Miller-de Lame (c) plots of the disruption profile from Rothschild’s (1975) test deploying polyethylene tube dispensers of pheromone targeting oriental fruit moth, *Grapholita molesta*, in peaches. The curve for panel (a) was hand-drawn and fit by eye. The straight lines in (b) and (c) (as well as those in subsequent figures) were fit by weighted regression

as well as moth density calculations, are based on the untested assumption that attractiveness of $T = F = D$ in Rothschild's test. The adequate agreement between calculated and measured M_D as well as a reasonable F/M ratio in this test supports approximate equivalency in attractiveness of T , F , and D in this particular case.

The Miller-de Lame plot of Rothschild's (1975) data (Fig. 1c) is also consistent with competitive attraction. The linear fit is reasonable given the few data and the susceptibility of the lowest catches of males to sampling error. There was no convincing evidence of a consistent recurve having its inflection near $1/2y_{\max}$ like that expected for non-competitive mechanisms such as, e.g., camouflage (Fig. 7c of Miller et al., 2006). However, the current analysis would have greatly benefited by including $D_D C$ values falling between 0 and 1400. The estimate of M_D as derived from the x -intercept of Fig. 1c = $T_D M_D$ is 91 males/ha compared to 83 for the Miller-Gut plot. This translates into an estimate of 0.23 males/tree, and falls near the measured range (0.25–0.5) for M_D . F_D can be derived from the inverse of the slope of this Miller-de Lame plot: absolute value of $1/\text{slope} = (T + F) = 67$. Since $T_D = 30$, $F_D = 37$. Thus, the F/M_D ratio derived from the Miller-de Lame plot is 37:91 or 1:2.5, which is similar to the Miller-Gut estimate as required by competitive attraction theory based on Equation (1) of Miller et al. (2006).

Collectively, Rothschild's OFM data are a reasonable fit to the full set of predictions generated from our competitive-attraction model of pheromone disruption. The probability of having the data fit all these predictions by chance alone appears small. No evidence was uncovered that refuted competitive attraction for this study. Conversely, this data set is inconsistent with camouflage or other non-competitive mechanisms of pheromone disruption.

Only after fit is found consistent with competitive attraction and inconsistent with other explanatory models is it appropriate to calculate a dispenser activity (D_a) value by using procedures unique to competitive attraction. Using Equation (4) of Miller et al. (2006), D_a for Rothschild's dispenser = slope (from Fig. 1b)/ y -intercept = $0.0004/0.0213 = 0.019$. D_a , as directly revealed by the slope of Fig. 1c, was 0.015. When using the latter value and invoking Equation (6) of Miller et al. (2006), D_{Aa} for Rothschild's dispenser densities of 125, 250, and 500 dispensers/ha = 1.9, 3.8, and 7.5, respectively.

As pointed out in Miller et al. (2006), D_a values are theoretically independent of the units used to record true catches. Nevertheless, all values accumulated in Table 1 were derived by using units of male catch/trap/d, for the sake of consistency. However, valid calculations of moth densities and sex ratios must be done with catch values for actual trap densities accumulated over the total elapsed time of the test, as per the Fig. 1 example.

We (Stelinski et al., 2005c) recently quantified OFM communicational disruption in response to an emulsified wax formulation (Atterholt, 1996, 1998; De Lame, 2003) of pheromone releasing a 93:6:1 blend of (*Z*)-8-dodecenyl-acetate: (*E*)-8-dodecenyl-acetate: (*Z*)-8-dodecenol, present in the wax at a total starting concentration of 5%. This experiment was conducted in 12-tree (0.05 ha) rectangular plots of apples in Western Michigan (USA). Drops of wax (0.1 ml) were applied by syringe to branches and twigs of the trees with the aim of achieving a uniform distribution from the bottom of basal limbs to treetop. There were five replicate plots with densities of wax drops per tree of: 0, 3, 10, 30, and 100. Two sticky traps deployed in the center two trees of each plot and baited with optimized OFM monitoring lures measured male catches. A new set of wax drops was applied after each OFM adult generation, when rising trap catches revealed waning efficacy due to pheromone depletion, confirmed also by gas chromatographic analysis. During the 40 d of effective disruption per application, release rate per 0.1 ml wax drop fell from ca. 0.4 to 0.1 $\mu\text{g/hr}$ /drop.

Table 1 Comparison of Moth Communicational Disruption Outcomes Mediated by Competitive Attraction

Insect	Reference	Pheromone composition	Type of dispenser	Release rate per dispenser ($\mu\text{g/hr}$)	Experimental plot size (ha)	Maximum dispensers/ha	Maximum % disruption ^a	C_{max} (males/trap/night)	D_a (M-G slope/ \pm -intercept) ^b	D_{sa} ($D_d/\mu\text{g}$ pheromone/hr)	Maximum D_{sa} (D_a^* D_b)
Oriental fruit moth	Rothchild (1975)	Z8-12:OAc 97% E8-12:OAc 3%	Closed polyethylene tube	15	0.4 (100 trees)	500 (2/tree)	92	1.7	0.021	0.0014	11
Oriental fruit moth	Stelinski et al. (2005b)	Z8-12:OAc 93% E8-12:OAc 6% Z8-12:OH 1%	Wax drops (0.1 ml)	0.1–0.4 (0.25)	0.05 (12 trees)	27,300 (100/tree)	99.4	5.1	0.0071	0.028	194
Pink bollworm	Flint and Mertke (1983)	Z7:Z11-16:OAc 50% Z7: E11-16:OAc 50%	Rubber septa	Unknown (1 mg load)	0.03	1,821	88	78	0.0038	–	6.9
Obliquebanded leafroller	Lawson et al. (1996)	Z11-14:OAc 83% E11-14:OAc 8% Z11-14:OH 9%	Polyethylene tube	25	0.04 (12 trees)	960 (3/tree)	70	0.9	0.0022	0.00009	2.1
Obliquebanded leafroller	Deland et al. (1994)	Z11-14OAc 93% E11-14:OAc 7%	Polyethylene tube	20	0.09	2,000	90	0.3	0.0052	0.00026	10
European leafroller	Deland et al. (1994)	Z11-14OAc 93% E11-14:OAc 7%	Polyethylene tube	20	0.09	2,000	96	0.8	0.0111	0.00056	22
Fruitree leafroller	Deland et al. (1994)	Z11-14OAc 93% E11-14:OAc 7%	Polyethylene tube	20	0.09	2,000	99.4	9.0	0.0982	0.00491	196
Gypsy moth	Tchelslavskaita et al. (2005)	\pm disparlure	Laminated flakes	0.093	25	134,409	99.1	0.8	0.00052	0.00056	70
Light brown apple moth	Suckling and Angerelli (1996)	E11-14:OAc 71% E9, E11-14:OAc 3% Z9-14:OAc 26%	Polyethylene tube	10.5	0.5 (561 trees)	200	97.4	0.3	0.19	0.018	38
Beet armyworm	Shorey et al. (1994)	Z9:E12-14:OAc	2-cm-long Teflon™ capillaries	1 for 1.32 diam capillary	0.0056–0.053	6,429	98.9	18.3	0.02	0.02	129

^a % Disruption = (1 – (male catch under pheromone treatment/male catch for control)) 100.^b Number of dispensers/ha required to suppress catch by 50% is given by $1/D_a$.

Periodically during this experiment, observers positioned themselves to be able to observe and record behaviors of male moths in the vicinity of wax drops. Moth behaviors were directly observed within tree canopies on 26 total nights throughout three generations of OFM flight. In each control plot, a single wax drop was randomly deployed in one tree for observation; these plots were otherwise untreated. Two or three observers worked simultaneously, rotating among plots and conducting 20-min observational bouts per treatment, such that multiple treatments were under observation concurrently. Behavioral events were dictated into a hand-held audio recorder by the observer standing ca. 1 m from a pheromone dispenser. Observations after dusk were assisted by night-vision goggles as described by Stelinski et al. (2004a).

Male OFM were commonly observed visiting wax drops containing pheromone (Stelinski et al., 2005c). More than 75% of these males approached within 20–60 cm of the pheromone source, and over 70% moved away from wax drops within 20 sec by flying upwind. None remained near pheromone dispensers for more than 2 min (Stelinski et al., 2005c). Similar results were documented for three other tortricid pests of Michigan fruit, all of which closely (ca. 0–100 cm) and briefly (ca. 2–30 s) approached Isomate-type, polyethylene tube dispensers (Stelinski et al., 2004a, 2005a,b). We conclude that males of all these tortricids are attracted to and briefly arrested by commercially available (Pacific Biocontrol Corp., Portland, OR, USA) synthetic pheromone dispensers releasing high amounts of their respective pheromones relative to females, but in a suboptimal blend.

The OFM disruption profile for wax drops, averaged across the three moth generations per season, well fits the predictions of competitive attraction. On an untransformed plot, the disruption profile was concave rather than linear (Fig. 2a). Moreover, disruption never reached 100% despite deployment of 100 wax drops per tree. However, catches of males at 30 and 100 wax drops per tree averaged less than 1 male/trap/20 d per OFM generation. Thus, to obtain a more reliable measure of central tendency, the data for the two highest dispenser densities were combined into a mean of 65 dispensers/ha. This disruption profile was perfectly linearized by a Miller-Gut plot (Fig. 2b). Analysis of these OFM data by the Miller-de Lame (Fig. 2c) plot was compromised by not having any $D_D C$ values fall near $1/2C_{\max}$.

Female vs. male densities in these plots were not directly measured experimentally. However, M_D can be calculated from Fig. 2c via the x -intercept of 820,218. Because attractiveness of D was measured at 0.1 that of T (Stelinski et al., 2005c), the intercept of $820,218 = T_D M_D / k_D$ [Equation (10) of Miller et al., 2006]. Because $T_D = 40$ traps/ha, and $k_D = 0.1$, M_D was 2,051 males/ha, or about 9 males/tree. If $k_T = k_F$, female density can be calculated from the absolute value of the slope of Fig. 2c; $1/\text{slope} = 1/0.0087 = 115 = T_D + F_D$. Because $T_D = 40/\text{ha}$, $F_D = 75$ females/ha, yielding an F/M activity-density ratio favoring males by 1:27. We consider it likely that attractiveness of females was somewhat less than that of the optimized trap that is constantly releasing pheromone. If k_F is taken at 0.1 k_T , the calculated F/M ratio becomes 1:3 and more nearly matches the Rothschild (1975) outcome. Only after this k_F value is established experimentally can this type of calculation be made with confidence.

The D_a for wax drops, as derived from the slope of Fig. 2c, was 0.009. It was 0.007 when male catch was graphed as males/trap/d (slight difference due to rounding error judged smaller for males/trap/d). The D_{sa} for wax drops (0.028) exceeded that for Rothschild's (1975) larger dispensers (0.0014) (Table 1). Perhaps this is because our formulation was a more complete blend that included the (*Z*)-8-dodecenol. Even at a considerably greater OFM density as judged by C_{\max} (Table 1) and calculations of M_D from the above respective Miller-de Lame plots, 10 wax drops per tree disrupted as well as did

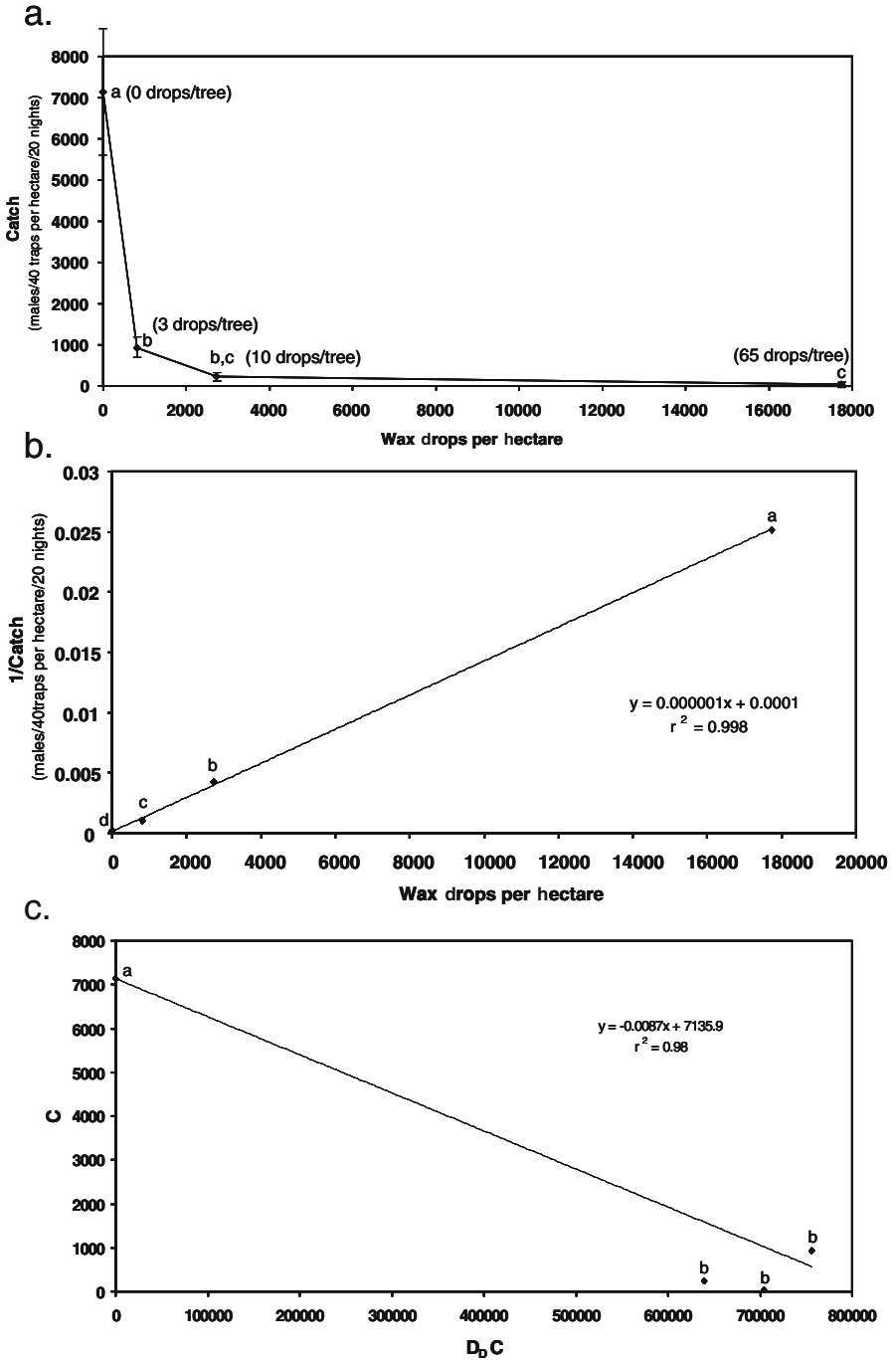


Fig. 2 Untransformed (a), Miller-Gut (b), and Miller-de Lame (c) plots of the disruption profile from Stelinski et al.'s (2005c) test deploying 0.1 ml wax drops targeting oriental fruit moth, *Graphophlita molesta*, in apples. Within a panel, means not sharing a common letter are statistically different at $P < 0.05$ by an LSD test after ANOVA on data transformed to $(x+0.5)^{1/2}$ for (a) and (c), and $\ln(x+0.5)$ for (b)

Rothschild's two polyethylene tubes per tree while using 2.5 rather than 30 μg of total pheromone/hr/tree. However, each of Rothschild's dispensers was capable of containing up to 800 mg of pheromone compared to 4 mg per wax drop. Thus the larger dispensers had the advantage of lasting longer than one adult generation.

Pink Bollworm (Pectinophora gossypiella)

Flint and Merkle (1983) quantified the relationship between inhibition of pink bollworm (PBW) traps vs. density of rubber septa deployed on stakes in 0.03 ha plots of cotton in Arizona, USA. The pheromone was gossyplure, a 50:50 mixture of 16-carbon acetates listed in Table 1. Two monitoring traps were deployed per plot to yield a T_D of 6.7/ha. The study design was complex; the various pheromone dosages were tested sequentially through time, not simultaneously. Moreover, the population of PBW increased through time. Thus, to generate a continuous disruption profile, all catches needed to be scaled to the highest catch (78 males/trap/night) via the known ratio of catch in disrupted plots relative to its paired control, also set to 78.

On untransformed axes (Fig. 3a), the disruption profile for PBW was consistent with an inverse function. Catches of males initially fell steeply, leveled off, and then appeared to approach zero asymptotically. This profile was more linear in a Miller-Gut plot (Fig. 3b) than concave as a non-competitive profile would be (Fig. 7b of Miller et al., 2006). The Miller-de Lame plot would have benefited from more data particularly at low values for $D_D C$ (Fig. 3c), but even so, the few and scattered data are not inconsistent with competitive attraction. Moth densities were not calculated for this case because the units for trapping data were unclear and the relative attractiveness of T , F , and D was not determined. D_a for these gossyplure-loaded septa was 0.0038 (slope of Fig. 3c). A D_{sa} could not be calculated because there was no report of the pheromone release rate per septum. The D_{Aa} for a D_D of 1821/ha was 6.9 (Table 1), which corresponded to 88% inhibition of catches of males relative to traps in the control plots. Disruption of PBW in response to septa loaded with gossyplure was expected to be mediated by competitive attraction, because similar dispensers had strongly attracted males. The current analysis is consistent with that expectation.

Obliquebanded Leafroller (Choristoneura rosaceana)

Lawson et al. (1996) measured obliquebanded leafroller (OBLR) disruption efficacy in 0.04-ha plots of apples in Western New York (USA) in response to four densities of polyethylene tube dispensers releasing 25 $\mu\text{g/hr}$ of total pheromone blend [83% (*Z*)-11-tetradecenyl acetate, 8% (*E*)-11-tetradecenyl acetate, 9% (*Z*)-11-tetradecenol]. This formulation was not highly disruptive; it yielded a slightly concave profile for dispenser density vs. inhibition of catch in monitoring traps (Fig. 4a) akin to that for competitive attraction under a high moth population (Fig. 3a of Miller et al., 2006). This profile yielded a straight line in Miller-Gut and Miller-de Lame plots (Fig. 4b and c), providing the strongest profile evidence for competitive attraction out of all the case studies.

Male density was not directly measured in this study. $T_D M_D$ as calculated via the x -intercept of Fig. 4c was extremely high (2,193,214) under an assumption that attractiveness of $T = F = D$. Given the T_D of 50/ha, M_D computes to 43,864 males/ha, a value incongruent with a C_{\max} of only 6,100. We suspect that attractiveness of these rope dispensers was considerably less than that for the optimized traps. If k_D were taken as 0.2 of

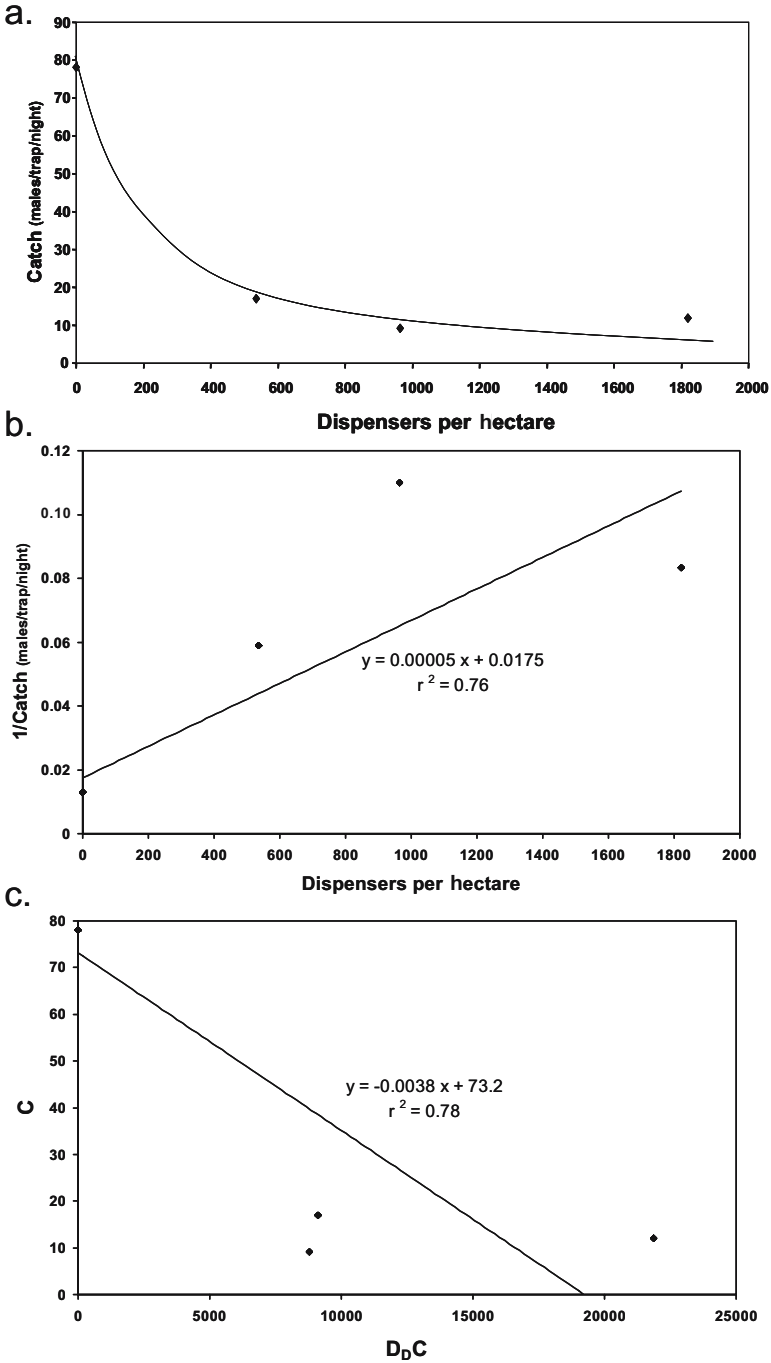


Fig. 3 Untransformed (a), Miller-Gut (b), and Miller-de Lame (c) plots of the disruption profile from Flint and Merkle's (1983) test deploying rubber septa dispensers targeting pink bollworm, *Pectinophora gossypiella*, in cotton

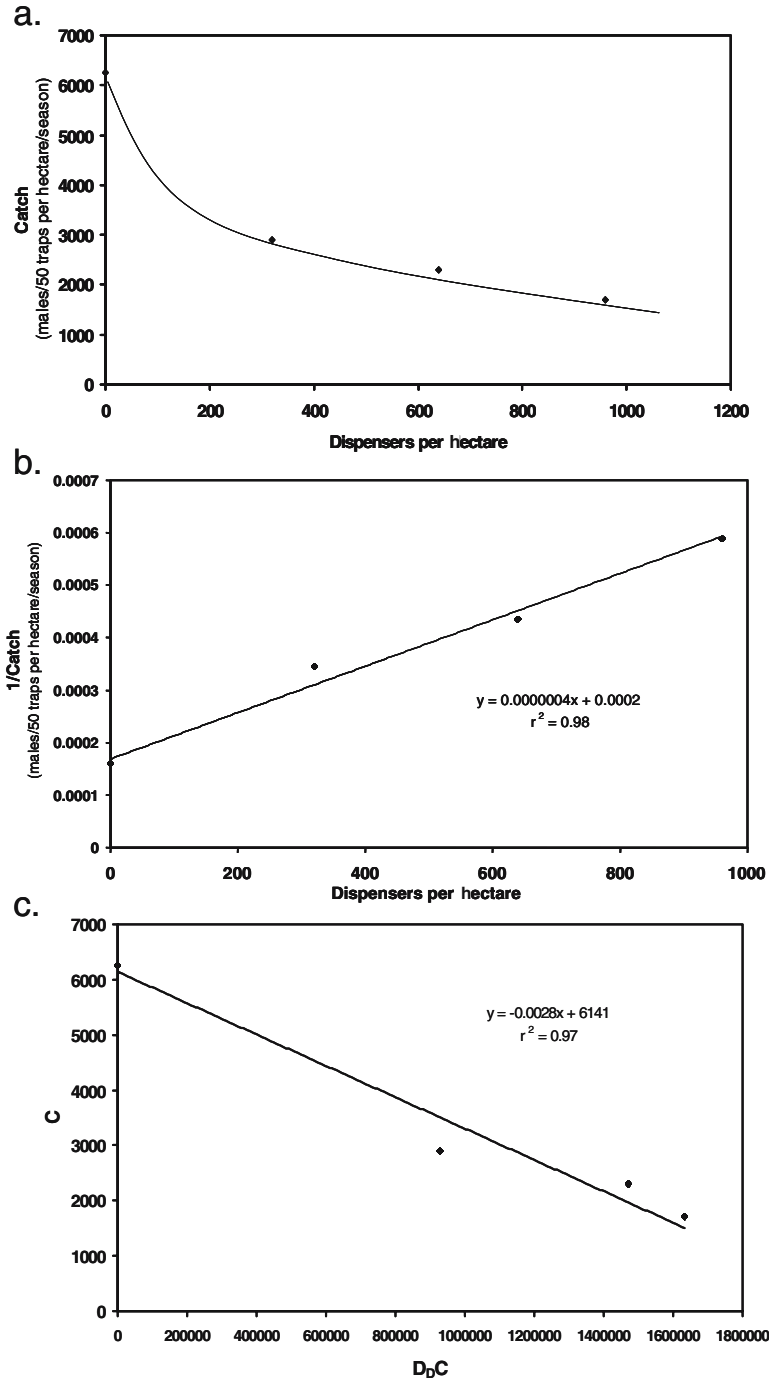


Fig. 4 Untransformed (a), Miller-Gut (b), and Miller-de Lame (c) plots of the disruption profile from Lawson et al.'s (1996) test deploying polyethylene tube dispensers targeting obliquebanded leafroller, *Choristoneura rosaceana*, in apples

k_T and k_F , the calculated M_D would be $43,864 * 0.2 = 8,800$ males/ha, which is more realistic. F_D as computed by the inverse of the Fig. 4c slope was 357 females/ha under an assumption that attractiveness of $T = F$.

D_a for OBLR responding to pheromone ropes was calculated as 0.0052 from a plot expressing catch as males/trap/d, and D_{sa} was a mere 0.0001. Thus, it is not surprising that D_{Aa} , even for 960 ropes/ha, was only 2.1 (Table 1) and efficacy as measured by % inhibition of male catch in these plots relative to that in untreated plots was only 70%.

Better disruption of OBLR has been reported in the Western United States and Canada. Deland et al. (1994) achieved ca. 90% disruption by using 1,000 and 2,000 Hamaki-con polyethylene tube dispensers per hectare. These data (plots not shown) were a better fit to competitive than non-competitive mechanisms, e.g., the Miller-de Lame plot did not recurve. D_a and D_{sa} were calculated at almost three times the corresponding values for Lawson et al.'s (1996) OBLR test (Table 1). However, whether this difference in disruption potency was attributable to the pheromone formulation or physiological/behavioral differences across OBLR races is unknown.

Deland et al. (1994) collected data for two other leafroller species along with OBLR. Disruption of both the European leafroller (*Archips rosana*) and fruittree leafroller (*A. argyrospila*) conformed to competitive attraction better than to non-competitive disruption mechanisms (plots not shown). Disruption outcomes were superior to that for OBLR (Table 1). The D_{Aa} for fruittree leafroller was a remarkable 196 at 2,000 dispensers/ha. Notably, the relative attractiveness of optimized pheromone lures in traps was found to be 3.3 times greater than male catch in traps baited with virgin females, i.e., $k_F = 0.3k_T$.

Gypsy Moth (*Lymantria dispar*)

Tchesslavskaja et al. (2005) recently reported dose–response data on gypsy moth (GM) disruption by using a 3 × 3 mm Hercon (Hercon Laboratories, Emigsville, PA, USA) Disrupt® II flake formulation of racemic disparlure, emitting at ca. 93 ng/flake/hr (Webb et al., 1988). This formulation was applied at five dosages by airplane to replicated 25 ha forest plots in Virginia. Communicational disruption was evaluated by suppression of catch in eight (+)-disparlure-baited traps per plot (0.32 traps/ha). When this test site was found to have few feral gypsy moths, ca. 150 laboratory-reared males were released in the center of each plot each week of the 6-wk test. Because gypsy moth males live ca. 2 wk (Capinera and Barbosa, 1975), the mean male population density interacting with attractant sources over this test should have stabilized around 300 males per plot (ca. 12 males/ha). Catches of ca. 270 males per plot averaged across this test suggest emigration was not substantial.

The untransformed dosage–response profile (Fig. 5a) fits competitive attraction. Male catch dropped precipitously in response to the lowest densities of flakes applied. Thereafter, efficacy increased modestly with order-of-magnitude increases in the density of flakes. Nevertheless, trap catch was suppressed to remarkably low levels (up to 99% trap inhibition). However, traps were never completely inhibited, despite the very high densities of applied flakes.

Although there was scatter in the data, the gypsy moth disruption profile was reasonably linear on Miller-Gut and Miller-de Lame plots (Fig. 5b and c). Evidence was lacking for a concavity in Fig. 5b or a recurve in Fig. 5c. D_a from a Miller-de Lame plot expressing catch as males/trap/night was 0.00052. D_{sa} was somewhat higher (0.006), because the release rate per flake was less than 1 µg/hr (Table 1). Even though the density of dispensers was 5-fold higher for GM than OFM disrupted with wax drops, D_{Aa} was lower for GM than that for the

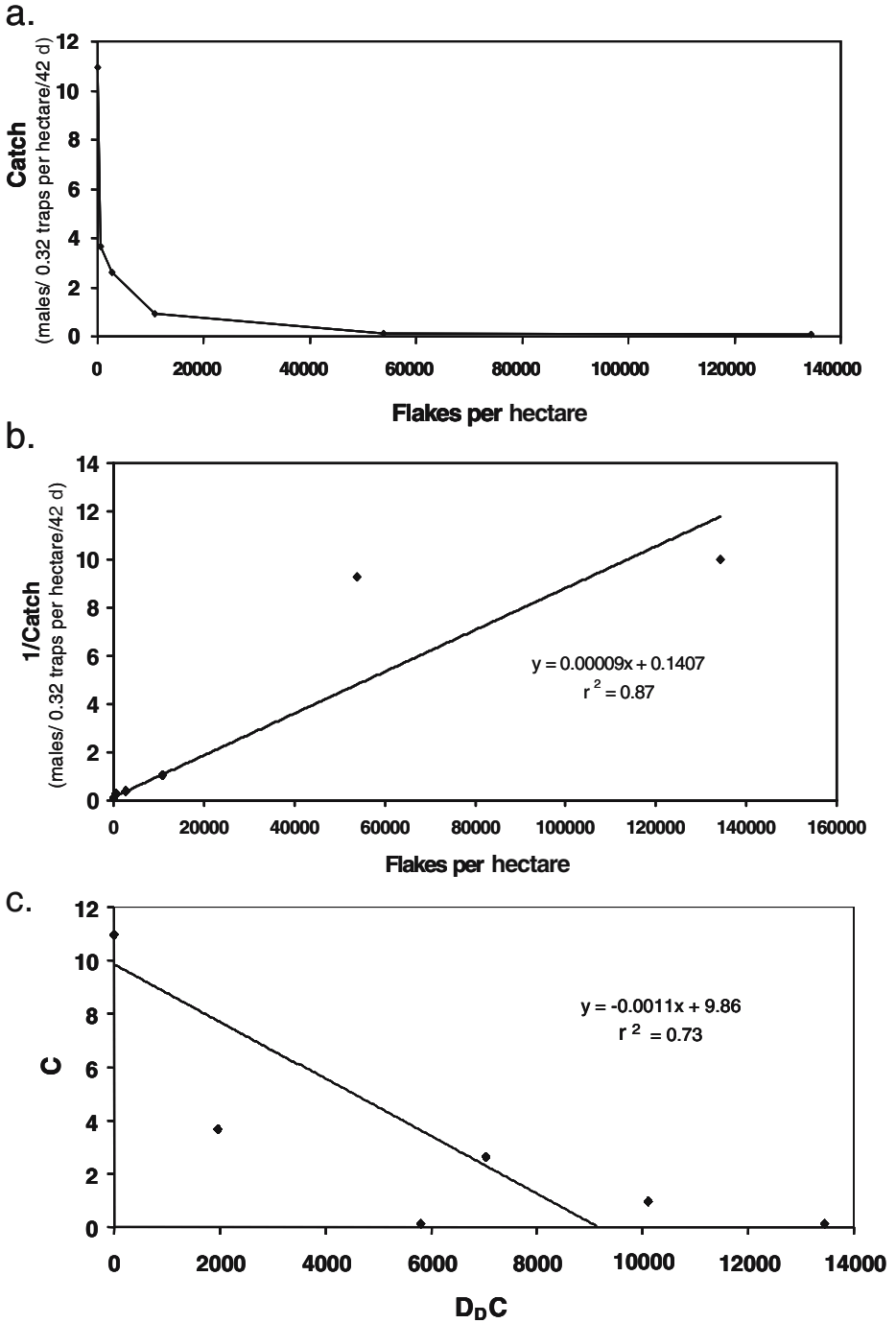


Fig. 5 Untransformed (a), Miller-Gut (b), and Miller-de Lame (c) plots of the disruption profile from Tcheslavskaja et al.'s (2005) test deploying laminated flake dispensers targeting gypsy moth, *Lymantria dispar*, in forest plots

Stelinski et al. (2005c) OFM test (Table 1) because the potency of each flake was 1/13 of that for each wax drop. It would be interesting to document how much greater D_{sa} for GM would be for flakes charged with the natural and more attractive (as well as costly) (+)-rather than (\pm)-disparlure (Miller et al., 1977).

The $T_D * M_D$ value calculated from the x -intercept of Fig. 5c was 8,964. Because $T_D = 0.32$ traps/ha in this case, M_D calculated on an assumption that attractiveness of $T = F = D$ is 28,000. This number is far higher than the 12 males/ha calculated to be in these plots based upon the male releases. The most likely explanation for this incongruence is that each flake was less attractive than a trap. We found no published data on the attractiveness of GM pheromone flakes relative to monitoring traps or females. However, by using Equation (10) of Miller et al. (2006), the k for flakes can be calculated at about 0.0004 relative to k for traps. With this adjustment, a calculated M_D becomes a reasonable 11 males/ha, or 280 males/plot. Putting this value for k_D into Equation (11) of Miller et al. (2006) yields $k_F F_D = 0.04$; so, if $k_F = 1$, $F_D =$ nearly zero, as was known to be true in this test. Thus, here is a case where attractiveness of D relative to T can be calculated with confidence from competitive attraction equations because both M_D and F_D were known, along with T_D and D_D . Although attractiveness of flakes seems trivial relative to traps, this test established that even weakly attractive (or perhaps arrestive) sources of pheromone can have important practical impact when dispensed at very high densities.

A note of caution needs to be sounded. This test was conducted in the absence of a normal density of female moths; this would inflate % inhibition of male catch and D_a . Both of these measures of disruption efficacy are sensitive to T_D and F_D , but not M_D (Miller et al., 2006). A somewhat less optimistic outcome than reported above would likely be obtained under a normal F/M ratio for GM.

Studies Varying Density of Release Sites along with Release Rate per Site

Suckling and Angerelli (1996) recorded a disruption profile for polyethylene tube dispensers targeting the lightbrown apple moth (LBAM), *Epiphyas postvittana*, in 0.5-ha plots of New Zealand apples. The pheromone formulation (Table 1) contained (*Z*)-11-tetradecenyl acetate as an “antagonist” in addition to the natural pheromone blend. This study held the overall density of sources to 200 dispensers/ha, while varying the number of release sites/ha: 0, 2, 18, and 200. This design now permits quantification of D_a and D_{Aa} when dispensers were deployed individually vs. clusters of 11 and 100 per release site.

Surprisingly, given inclusion of an “antagonist”, the disruption profile for LBAM (Fig. 6a) was consistent with competitive attraction rather than camouflage or some other non-competitive mechanism. Polyethylene tube dispensers thought to contain “antagonists” of OBLR (Stelinski et al., 2004a,b) and codling moth (CM) (Witzgall et al., 1999) have likewise been found to be attractive to males. In the LBAM study, one 100-dispenser cluster yielded 67% inhibition of male catch compared to 97% for 100 dispensers deployed individually. Thus, clustering the dispensers greatly reduced the overall treatment potency.

The Miller-Gut plot of the LBAM disruption profile was convex rather than straight (Fig. 6b). This pattern is consistent with competitive attraction where, as a group, each cluster of dispensers was more disruptive than an individual dispenser; however, the density of individual dispensers was higher than the density of clusters. We broke down the convex profile of Fig. 6b into three segments for calculations of D_a under the three different dispenser distributions. D_a for individual dispensers was 0.19 as calculated by dividing the slope of segment A–D (Fig. 6b) by its intercept [Equation (4) of Miller et al., 2006]; D_{Aa}

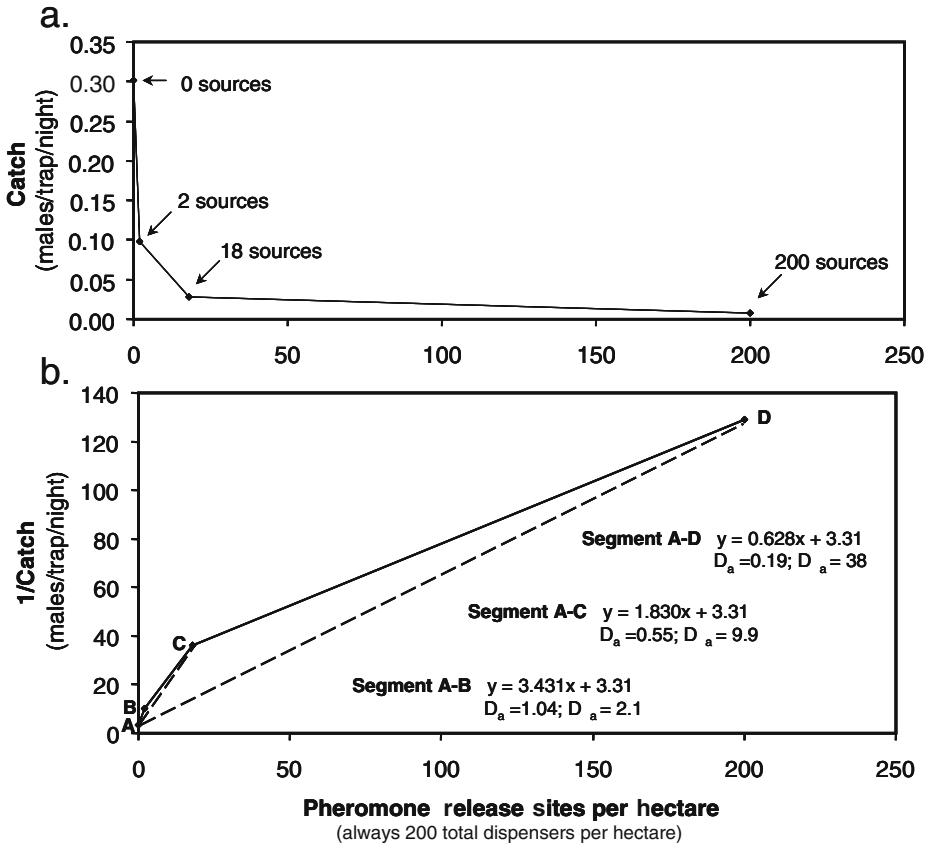


Fig. 6 Untransformed (a) and Miller-Gut (b) plots of the disruption profile from Suckling and Angerelli’s (1996) test deploying polyethylene tube dispensers targeting lightbrown apple moth, *Epiphyas postvittana*, in apples

was 38. As dispensers were clustered, D_a /source (Fig. 6b) increased by only 5.5-fold, but D_{Aa} diminished by 18-fold. This outcome confirms previous knowledge of a tradeoff between dispenser density vs. disruption strength per release site. However, the current analysis emphasizes that the loss in potency due to clumping dispensers may be greater than previously understood. Under competitive attraction, the first few dispensers deployed into a plot will dramatically decrease male catch (e.g., Fig. 2; Miller et al., 2006), except when moth density is extraordinarily high. This advantage of being the first (even though few) release sites into a plot may obscure the offsetting dramatic decline in D_a and D_{Aa} when dispensers are clumped and the density of release sites is kept low because the overall release rate of pheromone is already high.

The Miller-de Lame plot (not shown) for these LBAM was concave, as expected for competitive attraction under source clustering with a concomitant reduction in D_a per cluster. The potency of single LBAM dispensers deployed at 200 per ha was excellent ($D_a = 0.19$) and superior to that of any other dispenser in Table 1. D_{sa} of LBAM dispensers was surpassed only by that for wax drops deployed for OFM. This finding suggests that

mating disruption of LBAM would be highly effective under the approach of using thousands rather than hundreds of point sources of pheromone per hectare.

Other investigators have recorded disruption outcomes while varying pheromone release rates along with density of release sites. For example, Charlton and Cardé (1981) used 0, 668, and 1,336 release sites per ha (plot size was 0.03 ha) to measure OFM disruption in apple plots in response to Albany International Corp. (no longer available) hollow fibers releasing the three components of this moth's pheromone (see Table 1), but not in the precise natural blend. The densities of fibers per source were 0, 16, 96, and 880. These data (graphs not shown) were also consistent with competitive attraction. D_a per fiber (calculated as described above for LBAM) was 0.0019, in line with other OFM pheromone release devices (Table 1). D_a per fiber declined from 0.0019 for 16 fibers per source to 0.00034 for 880 fibers per source. This OFM research confirms that increasing pheromone release per site did not fully compensate for a reduction in the density of release sites. Reducing the density of release sites comes at a cost of reduced D_{sa} and $D_{\bar{a}}$. Both release rate per source and density of sources matter in communicational disruption mediated by competitive attraction. For LBAM and OFM within the parameter ranges tested, however, source density was more influential than overall release rate of pheromone per source.

Shorey et al. (1994) conducted an elegant study conducive to evaluation of the relationship between density of pheromone release sites and release rate per site. They arranged a test where (in our terminology) k_D and D_D were experimentally manipulated, whereas k_T and k_F , along with moth density, remained constant. This test was conducted on the beet armyworm (BAW), *Spodoptera exigua*, in plots of tomato and cotton. Only the major pheromone component, (Z9,E12)-tetradecadienyl acetate, was released from 2-cm-long Teflon™ capillary tubes varying in diameter from 0.30 to 1.32 mm. Release rate was purportedly directly proportional to capillary diameter. Single capillary tubes attached to wooden stakes were deployed into the crop, always in a 36 × 36 grid, but with variable separation between stakes: 4.6, 3.0, and 1.5 m. Therefore, plot size varied from 0.006 to 0.05 ha, and D_D varied from 0 to 6,429/ha. One monitoring trap was deployed at the center of each treatment and control plot (five replicates). Because T_D varied somewhat in these tests, we made no attempt to calculate or interpret moth densities.

With rises in k_D relative to k_T and k_F , the competitive-attraction simulations of Fig. 4c of Miller et al. (2006) predicted a series of diverging straight lines with increasing slopes on Miller-Gut plots. The Shorey et al. (1994) BAW disruption profiles fit this prediction well (Fig. 7), except for the profile for the 0.56-mm capillary, which was flatter than expected. D_a increased directly with pheromone release rate from the capillaries ($D_a = 0.018D_{sa}$; $r^2 = 0.99$), revealing that D_{sa} remained a constant 0.018, irrespective of capillary diameter. $D_{\bar{a}}$ was 129 for the largest diameter capillary (1.32 mm) releasing pheromone at 1 μg/hr and deployed at 6,429 capillaries per ha (Table 1).

Shorey et al. (1994) seem to have over-interpreted their BAW data when they concluded that release rate of pheromone point sources was much more important than density of release sites. By starting with a relatively high density of dispensers (679/ha) and progressing only upward, these investigators realized only a small gain in % disruption with additional increases in dispenser density. This probably was because their lowest treatment density occurred at a point on this disruption profile beyond the rapidly falling phase and in the asymptotic phase. On the other hand, the pheromone release rates per release site in this experiment began very low (83 ng/hr) and progressed only to 1 μg/hr. D_a increased steadily across this narrow range of release rates. However, there is no guarantee that disruption potency would have increased indefinitely with increasing

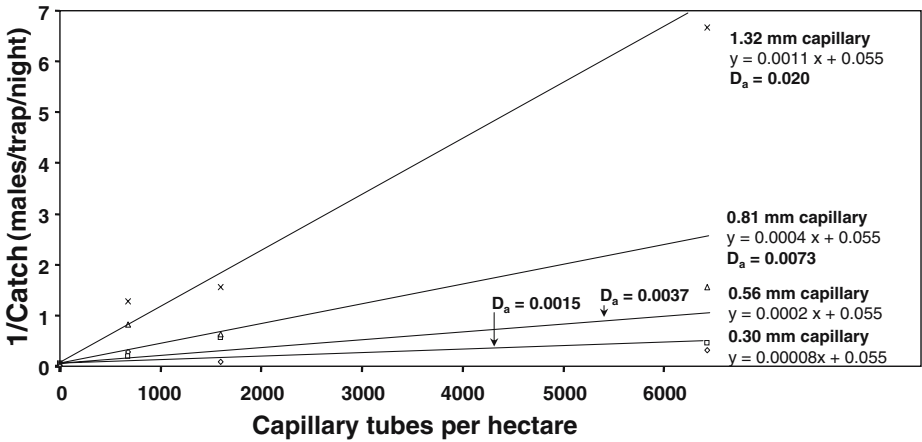


Fig. 7 Miller-Gut plot of the disruption profile from Shorey et al.’s (1994) test deploying four differently sized capillary tube dispensers targeting beet armyworm, *Spodoptera exigua*, in tomato and cotton

release rate per release site. Not realizing that this test favored showing a strong release-rate effect and a weak dispenser-density effect, these authors suggested that, for BAW, “if a certain amount of pheromone component is to be evaporated per hectare per day, it may be more effective to do so from relatively few evaporators, each releasing large amounts of pheromone and spaced far apart, than for small evaporators releasing lesser amounts of chemical and positioned correspondingly closer together” (Shorey et al., 1994). From here, it was a short step to aerosol and similar dispensers (Mafrá-Neto and Baker, 1996; Shorey and Gerber, 1996; Shorey et al., 1996; Isaacs et al., 1999) releasing very high rates of pheromone from few point sources per hectare.

A more complete picture can now be constructed from the classic data of Shorey et al. (1994) for the roles of dispenser density (D_D) and rate of pheromone release per site (k_D) in influencing disruption efficacy. Under this example of competitive attraction appearing to conform to Equation (9) of Miller et al. (2006), both factors are mutual partners interacting multiplicatively to reduce the probability of a male visit to a female from ca. 1 to $1/k_D D_D$. As such, any increase in k_D is multiplied by D_D , and *vice versa*. The mathematics indicate it is maximization of $k_D D_D$ that matters, and that this could be done variously, e.g., by using a high density of point sources, each with low attractiveness. Here, an additional benefit is that spatial coverage of the crop would be thorough. In reality, however, the pheromone release rate per dispenser cannot be permitted to fall below some minimal level required to generate an attractive plume. Conversely, few point sources releasing pheromone at high rates might theoretically generate a very high $k_D D_D$. However, deploying just a few large plumes may leave gaps in coverage, especially when fields are small. Moreover, there are usually limits to pheromone concentrations that can attract male moths. Releasing pheromone at rates too high to be attractive could nullify a competitive-attraction mechanism in favor of an alternative disruption mechanism. Maximizing $k_D D_D$ as well as D_{sa} for expensive pheromone compounds will be a worthy economic goal of applied pheromone research for the foreseeable future. It will require experiments like that of Shorey et al. (1994), but with a wider range of release rates per dispenser to reveal shifts and limits in k_D . Also, dispensers will need to be observed to determine to what degree they are attractive to males.

Summary of Case Studies Supporting Competitive Attraction

More than 10 disruption profiles were uncovered that fit the set of predictions for competitive attraction detailed by Miller et al. (2006). This set of cases can be further tested for internal consistency by correlating values for $D_{\bar{A}a}$ and % disruption (Table 1). If all cases were properly classified as competitive attraction, a consistent and interpretable

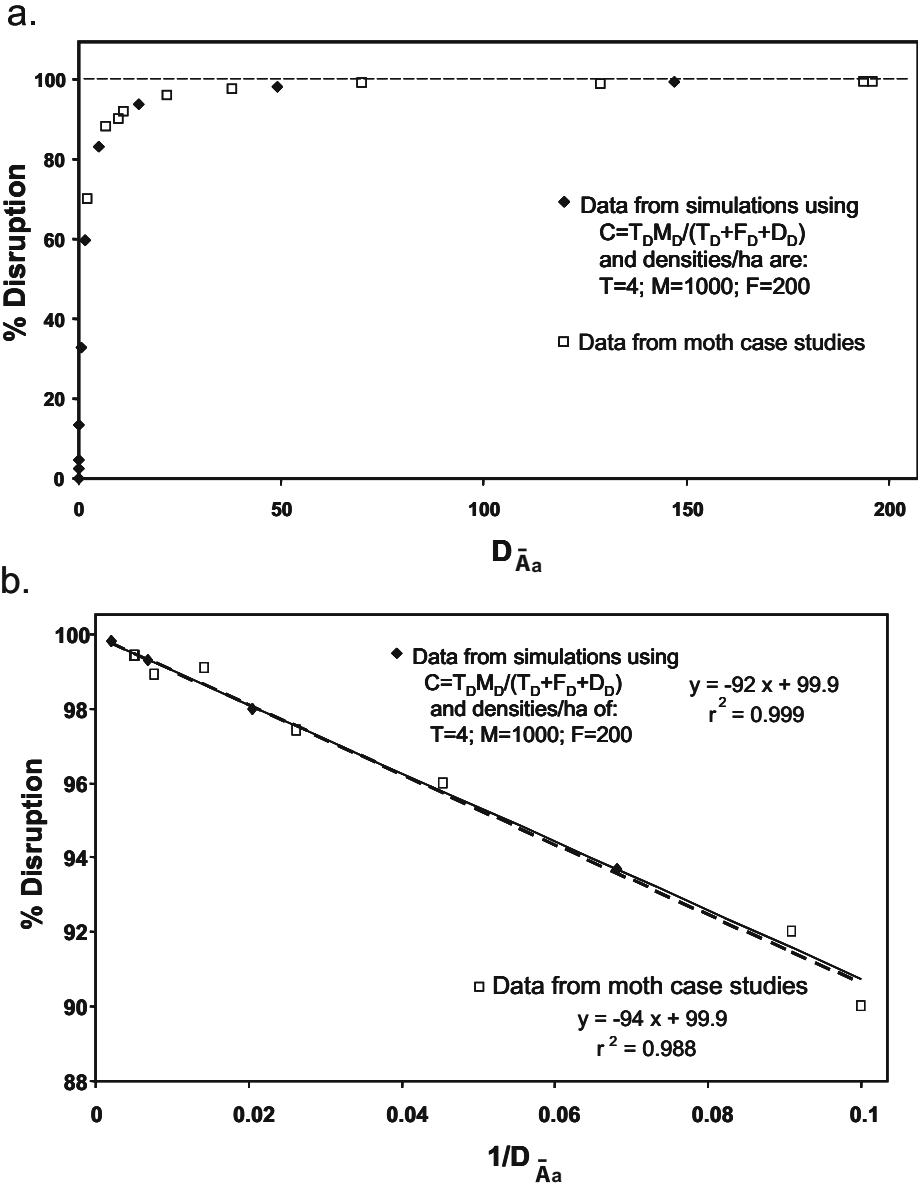


Fig. 8 Graphical comparison of measures for disruption potency arising from Table 1. (a) Relationship between % disruption vs. $1/D_{\bar{A}a}$ (b) Untransformed plot of % disruption vs. dispenser application activity ($D_{\bar{A}a}$)

relationship should be found when % disruption is plotted vs. $D_{\bar{A}a}$. This prediction is confirmed by Fig. 8a. Values of % disruption for both simulated and actual data rose dramatically with increases in $D_{\bar{A}a}$; then they rapidly tapered off and approached 100% disruption asymptotically. The fit between real and simulated data under conditions detailed in Fig. 8a was excellent.

Figure 8a demonstrates that % disruption and $D_{\bar{A}a}$ offer strikingly nonparallel measures of disruption potency. Tiny numerical differences in % disruption near 100% obscure very large differences in disruption potency as measured by $D_{\bar{A}a}$. Expansion at the bottom end and compression at the top end of the % disruption scale can distort judgments of the relative potency of disruptive formulations operating by competitive attraction. For example, an alteration in a formulation that increases disruption from 50% to 90% appears to be considerably more impressive than an increase from 98% to 99%. However, a rise from 50% to 90% inhibition of male catch in traps corresponds to an increase in $D_{\bar{A}a}$ of only 8, whereas the rise from 98% to 99% corresponds to a $D_{\bar{A}a}$ increase of 50. From the perspective of practical pest control, this suggests that % disruption values below 90% may all reflect trivial disruptive potency under competitive attraction, unless pest density is very high.

Percent disruption of male catch in traps has been a useful measure of practical outcomes of disruption experiments, but its proper interpretation requires reevaluation. We recommend that consideration be given to additional measures of disruption potency and efficacy beyond % disruption. As research fields mature, it is usual that multiple measures are devised for a given phenomenon, e.g., degrees Fahrenheit, centigrade, and Kelvin for temperature. D_a and $D_{\bar{A}a}$ can serve as alternative disruption measures for cases of disruption operating by competitive attraction. These variables seem better suited than % disruption to differentiate among the high-performance treatments (> 98% disruption) that will increasingly become the practically relevant zone of interest for pest managers and the pheromone industry.

Given that D_D occurs only in the denominator of Equation (1) of Miller et al. (2006), evidence should be found that D_D reduces catch by some consistent inverse function. Indeed, when % disruption values in Table 1 were plotted vs. the inverse of the corresponding $D_{\bar{A}a}$ values, a precise relationship was uncovered when % disruption exceeded 90% (Fig. 8b). The regression equation was: % disruption = $94(1/D_{\bar{A}a}) + 100$; the r^2 value was 0.99. This outcome was also virtually identical with that for the simulated data of Fig. 8b. Rearrangement and rounding up the slope yields (equation numbering continued from Miller et al., 2006):

$$\% \text{ Disruptions} \approx 100 - 100/D_{\bar{A}a} \quad (17)$$

Similarly, if catch in control plots is normalized to 1,

$$\text{Catch in crop receiving pheromone} \approx 1 - 1/D_{\bar{A}a} \quad (18)$$

These equations should prove useful in interconverting the mass of % disruption data already in the literature. However, Equations (17) and (18) are applicable only when disruption operates by competitive attraction and D_D values are very high so as to dominate the generative equations. Finding a consistent and precise relationship between $D_{\bar{A}a}$ and % disruption across cases classified as competitive attraction offers powerful evidence for internal consistency and hence validity of the current approach to classifying disruption mechanisms.

Cases Consistent with Non-competitive Disruption Mechanisms and Inconsistent with Competitive Attraction

Our literature search uncovered only one case supporting a non-competitive disruption mechanism. This test was conducted by McLaughlin et al. (1972) in Southern California (USA) on pink bollworm in cotton. This work pre-dated identification of the PBW's pheromone (Table 1) and used a related compound (*Z*-7-hexadecenyl acetate; hexalure) attractive to males but less so than extracts of female pheromone glands. Hexalure was released at 6 $\mu\text{g}/\text{dispenser}/\text{hr}$ from stainless steel planchets attached to wooden stakes deployed at crop height in a 10×10 array. Inter-stake distances varied (3, 10, and 30 m); thus, plot sizes varied from 0.09 to 9 ha. Disruption in each plot was evaluated from catch of males in one central sticky trap baited with several virgin females. The results were presented only as % reduction in catch relative to control plots; actual catches were not reported.

It was not apparent from the untransformed plot of McLaughlin et al.'s (1972) test (Fig. 9a) whether the data fit competitive or non-competitive disruption. Catch did not initially drop precipitously with initial increases in dispenser density, but this pattern also can be realized under competitive attraction when moth density is very high (Fig. 3a of Miller et al., 2006). However, the subsequent approach to the x -axis was quicker than expected for competitive attraction under high moth density (Fig. 9a vs. Fig. 3a of Miller et al., 2006).

Secondary plots of this disruption profile were consistent with a non-competitive disruption mechanism. The Miller–Gut plot was concave (Fig. 9b), as expected for non-competitive disruption (Fig. 7b; Miller et al., 2006). The Miller-de Lame plot was recurved and its inflection point occurred near $1/2y$ -maximum (Fig. 9c), as is diagnostic for non-competitive disruption (Fig. 7c of Miller et al., 2006). Because this PBW profile was a better fit to non-competitive than competitive disruption, the steps taken above to calculate D_a and D_{sa} could not be taken.

The release rate in this PBW study was 8.2 mg/ha/hr; it yielded 99% communicational disruption. For comparison, 6.8 mg of pheromone/ha/hr in Stelinski et al.'s (2005c) test with OFM yielded 99.4% disruption, whereas 12.5 mg of pheromone/ha/hr yielded 99% disruption in the GM test of Table 1. Thus, disruption efficiencies may not vary greatly between competitive and non-competitive disruption when rates of pheromone dispersed/ha are high as in Table 1. This finding is consistent with the simulations of Fig. 7a of Miller et al. (2006). However, these simulations suggested that efficiency of disruption by competitive attraction would be superior to that for non-competitive mechanisms, when moth and dispenser densities are moderate to low.

A previously unpublished data set of our own also fits non-competitive disruption. This test targeting OFM was conducted over the full 2003 growing season in 0.15-ha, plots (45 trees) distributed across a 3-ha, recently abandoned apple orchard in Southeastern Michigan. The pheromone blend and dispenser formulation were identical to those reported by Stelinski et al. (2005c; see Table 1). However, rather than 0.1 ml wax drops, 12-ml dollops of wax were applied to a tree branch ca. 2 m above ground level as described by De Lame (2003). Dispenser densities were 0, 50, 130, and 230/ha. The experimental design was randomized complete block with four replicates. Two monitoring traps (Stelinski et al., 2005c) spaced ca. 12 m apart and near the center of each plot assessed catch of male moths across the full 2003 growing season. Traps were checked weekly and lures were replaced at the beginning of each OFM flight (every 6–8 wk). These emulsified wax dollops were sufficiently large to remain disruptive throughout the season. Release rate per dollop slowly

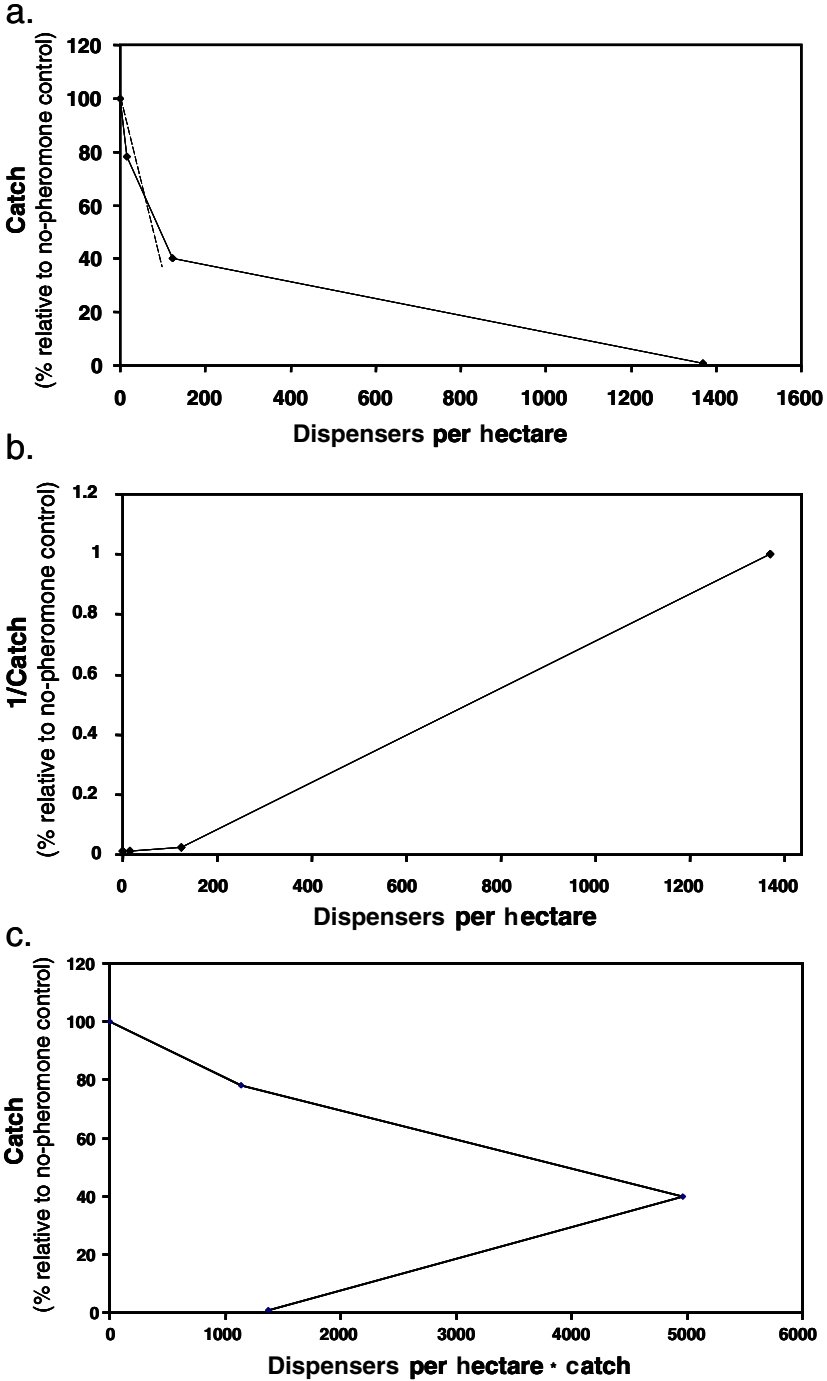


Fig. 9 Untransformed (a), Miller-Gut (b), and Miller-de Lame (c) plots of the disruption profile from McLaughlin et al.'s (1972) test deploying planchette dispensers targeting pink bollworm, *Pectinophora gossypiella*, in cotton

declined from ca. 150 to 40 $\mu\text{g/hr}$ as determined by solvent extraction (De Lame, 2003) followed by gas chromatography (Stelinski et al., 2005c).

The season-long disruption profile for this test is plotted in Fig. 10a. With initial increases in dispenser density, catch dropped almost linearly until 130 dollops/ha. However, disruption did not increase significantly with the final increment in dispenser density. Having only one datum after the slope change was insufficient for assessing whether or not the approach to total inhibition of male catch was asymptotic (Fig. 10a).

Both secondary plots of the data supported a non-competitive disruption mechanism. The Miller-Gut plot (Fig. 10b) was concave, as expected for non-competitive disruption (Fig. 7b of Miller et al., 2006). Moreover, the Miller-de Lame plot (Fig. 10c) recurved significantly ($P < 0.05$) and according to the signature of non-competitive disruption (Fig. 7c of Miller et al., 2006). Finding some cases consistent with non-competitive disruption and inconsistent with competitive attraction, as well as *vice versa*, helps to authenticate the quantitative tools proposed by Miller et al. (2006) for differentiating between the two major classes of disruption mechanisms.

Release rate is the most obvious reason for the switch from competitive to non-competitive disruption mechanisms when using 0.1-ml wax drops vs. 10-ml wax dollops to disrupt OFM. As measured by male catch in traps, attraction of OFM is strongest when sources release between 0.07 and 0.72 $\mu\text{g/hr}$ (Baker et al., 1980). Release rate from our wax dollops ($> 40 \mu\text{g/hr}$) far exceeded OFM's upper limit for attraction of males very close to a source. Graphical analysis of the resulting disruption profile suggests that attraction was nullified, and plumes were generated that disrupted moth communication by some unspecified non-competitive mechanism, such as camouflage or desensitization. During this test, we unfortunately did not yet realize the importance of observing dispensers for evidence of male attraction. Thus, critical evidence that male OFM did not orient to these wax dollops is lacking.

This experiment documented a small efficiency improvement in OFM disruption when mediated by competitive attraction vs. a non-competitive mechanism. Dollops (230) releasing pheromone at 6.4 mg/ha/hr gave 96% disruption, a questionable level for practical control. Pheromone released at 6.8 mg/ha/hr from 27,300 wax drops effected an encouraging 99.4% disruption, also under high pest pressure. However, the large wax dispensers lasted all season, whereas a new set of drops had to be applied for each moth generation.

The slope derived from the first three points of Fig. 10a was 0.055; it reflects the decline in male catch per trap per wax dollop added per hectare, when starting catch was 8.2 males/trap/night for the control plot receiving no synthetic pheromone. Thus, each dispenser diminished catch by 0.67% ($0.055/8.2$) during the linear portion of Fig. 10a. Under a pure camouflage model of pheromone disruption, Equations (14) and (15) of Miller et al. (2006) proposed that each equivalent dispenser would camouflage an equal area (x) of crop, and that the total area camouflaged would be $D_D * x$. Combining this proposition with the measured Fig. 10a slope of 0.67% suggests that each wax dollop in this OFM test operated on 0.67% of 1 ha, which equates to only 67 m^2 /dollop. Under the spacing of 225 trees/ha, 67 m^2 equates to camouflaging plume coverage of only 1.5 trees/wax dollop. Accordingly, it would take a minimum of 150 dollops to completely cover the 1 ha under a scenario of perfect packing and no plume overlaps. However, even the highest dispenser density of 230 dollops/ha allowed a catch of 4% relative to the control catch. Thus, there must be a difference between plume sizes required to impact catch vs. inhibit it completely. The current OFM experimental data suggest there were small spaces within the treated crop that were difficult to disrupt by non-competitive mechanisms. Perhaps it is the zones immediately downwind of traps and females, where their pheromone plume concentrations are highest, that are not being covered by sufficient dosages of background pheromone to effect complete non-competitive disruption.

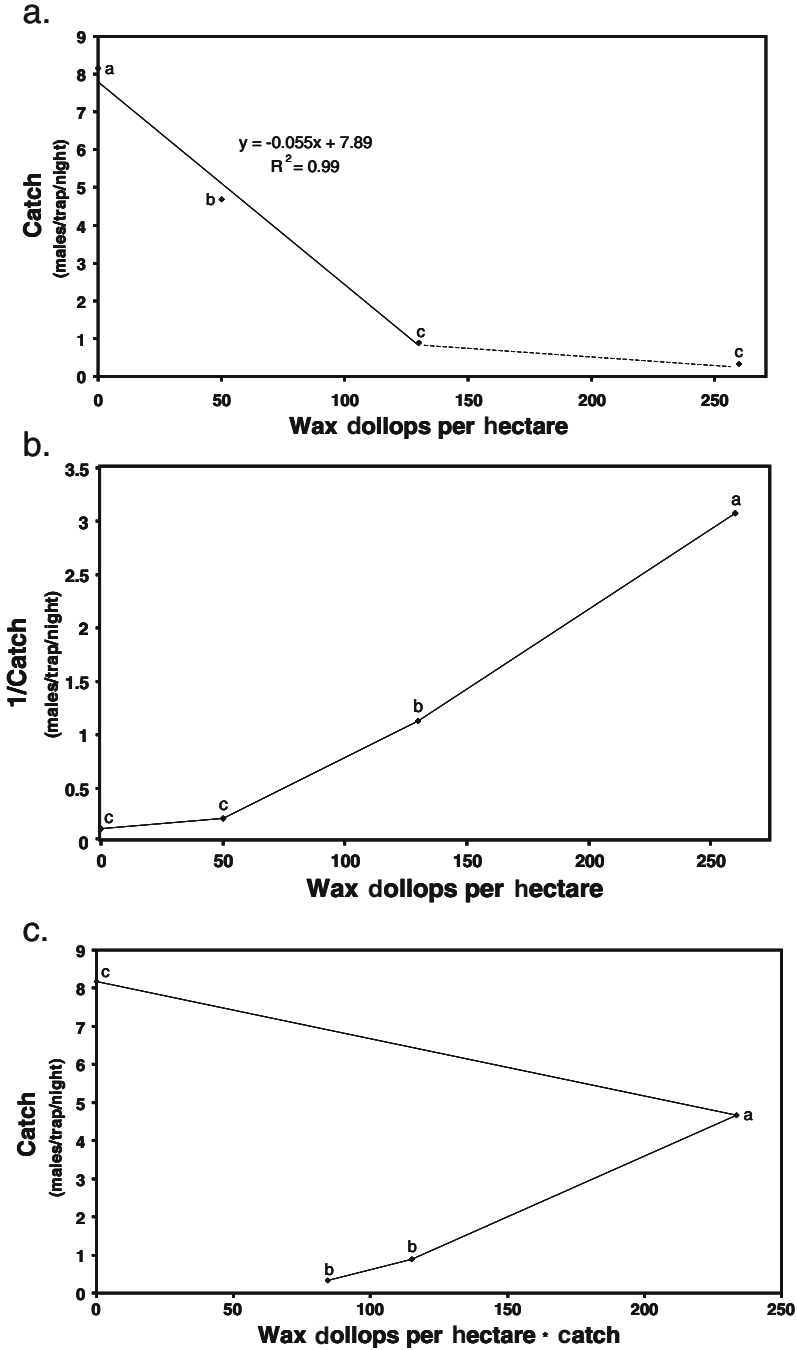


Fig. 10 Untransformed (a), Miller-Gut (b), and Miller-de Lame (c) plots of the disruption profile from our own test deploying 12 ml emulsified wax-dollop dispensers targeting oriental fruit moth, *Grapholitha molesta*, in apples. Within a panel, means not sharing a common letter are statistically different at $P < 0.05$ by an LSD test after ANOVA on data transformed to $(x+0.5)^{1/2}$ for panels (a) and (c), and $\ln(x+0.5)$ for (b)

Alternatively, even our highest density of dispensers may have been insufficient to eliminate small gaps in coverage far from dispensers.

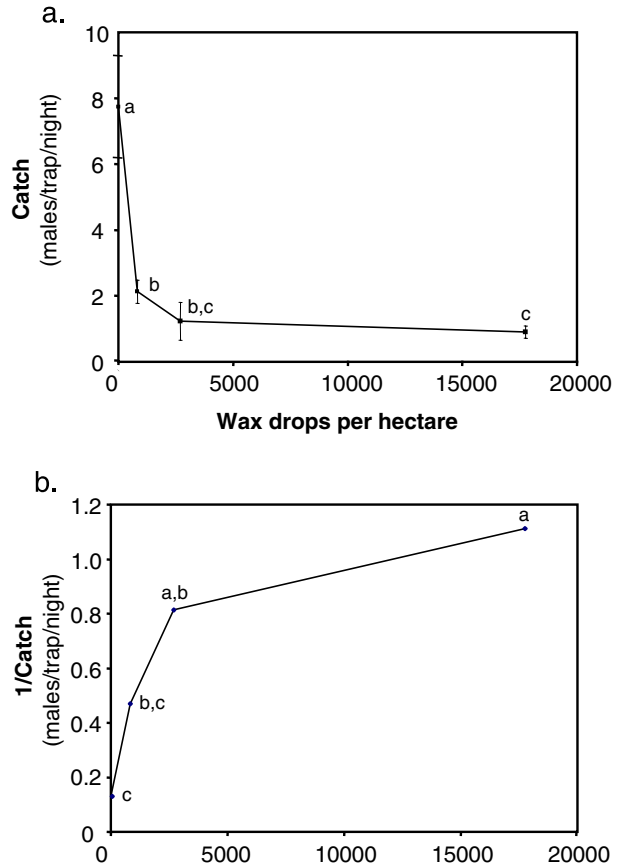
The capacity to shift from a competitive to a non-competitive disruption mechanism by using a common dispenser medium, but varying the deposit size, bodes well for emulsified wax as a flexible disruption formulation useful across a range of pest densities. By varying dispenser size and/or release rate, it might be possible to tailor the disruption mechanism for a given pest based upon its particular density, as suggested by Miller et al.'s (2006) Fig. 7a. At very high pest densities, it could be advantageous to disrupt pests by a non-competitive mechanism. On the other hand, competitive attraction appears to be the more efficient disruption mechanism for moderate to low pest populations (Fig. 7a of Miller et al., 2006). However, these decisions will also be influenced by the desired longevity of a given pheromone application. Small, but densely-distributed dispensers are likely to give the most efficient disruption for many pests, but reapplication will be required if small dispensers have limited longevity.

A Case Possibly Blending Competitive and Non-competitive Disruption Mechanisms

During the 2004 field season, we tested efficacies of varying densities of 0.1 ml emulsified wax drops in disrupting CM (*Cydia pomonella*) sexual communication. The methods were identical to those reported by Stelinski et al. (2005c) for OFM, with a few exceptions. Plots consisted of 16 rather than 12 trees, and only the major pheromone component for CM [(E8,E10)-dodecadienol] was incorporated into the wax at a starting concentration of 5%. Catch of male moths in each plot was monitored with two centrally located sticky traps baited with red rubber septa loaded with 0.1 mg of pure codlemone. This test ran for only 14 d during the first generation of moths, after which efficacy of wax drops noticeably diminished and the test was terminated. Release rate of codlemone over this period averaged 3 $\mu\text{g/hr/wax}$ drop as quantified by gas chromatography with the methods of De Lame (2003).

The resulting disruption profile for CM was a better fit to competitive attraction than to a non-competitive mechanism. The untransformed plot was sharply concave and not linear (Fig. 11a) and the Miller-de Lame plot did not recurve (data not shown). However, the Miller-Gut plot (Fig. 11b) was convex, much like that for LBAM (Fig. 6b). This is curious, because there was no clumping of wax drops as was true for ropes in Suckling and Angerelli's (1996) test. In contrast to the other competitive-attraction cases compiled in Table 1, disruption potency per dispenser declined for CM as density of wax drops rose. Emission rate of pheromone from wax drops was probably not influenced by their density, and so it is more likely that the response of CM males to monitoring traps was somehow shifted under the increasing density of pheromone plumes. One possibility is that CM males became partially desensitized to the emissions from high densities of wax drops, but not to the higher emissions from the monitoring traps, i.e., the threshold for response to pheromone point sources shifted upward (less sensitive) as the density of point sources increased. If so, visitation rate to the traps may have risen relative to weakly competing wax drops. Not having assessed the relative attractiveness of traps vs. females vs. wax drops, or deployed virgin females to assess mating, we cannot say whether disruption of females exactly reflected the disruption of traps. Disruption of females needs to be measured, as it was perhaps better or worse than disruption measured for traps. Although we are unable to fully interpret the disruption profile for this CM study, this case demonstrates the utility of these new analytical procedures in extracting potentially useful information hidden within a raw disruption profile (Fig. 11a). We expect other cases will be found

Fig. 11 Untransformed (a) and Miller-Gut (b) plots of the disruption profile from our own test deploying 0.1 ml emulsified wax-drop dispensers targeting codling moth, *Cydia pomonella*, in apples. Within a panel, means not sharing a common letter are statistically different at $P < 0.05$ by an LSD test after ANOVA on data transformed to $(x+0.5)^{1/2}$



that do not fit pure competitive or non-competitive disruption, and that more powerful analyses of these profiles will aid recognition and interpretation of mixed as well as pure disruption mechanisms.

Listing of Some Practical Ramifications When Competitive Attraction is the Leading Mechanism of Moth Mating Disruption

Pheromone Formulations for Disruption

If disruption primarily involves attraction, attractiveness of pheromone formulations should be optimized, e.g., match the natural blend of the female and optimize the release rate so as to generate plumes matching or exceeding those of females. In some cases, it may be cheaper to use an off-blend, i.e., if attractiveness of dispensers releasing abnormally high rates of a cheaper pheromone component can match attractiveness of females releasing a blend containing expensive minor components.

The density of point sources is strongly and positively correlated with the efficacy of a pheromone treatment. The higher the density of dispensers, the more effective is the treatment, provided that each dispenser remains highly attractive. However, it may be possible to apply dispensers at densities so high that they no longer attract. This could shift disruption from a competitive to non-competitive mechanism. To date, disruption efficacy per unit of pheromone used appears to be slightly higher for competitive vs. non-competitive disruption. Furthermore, it is advantageous for pheromone point sources to be thoroughly dispersed in the crop so as to avoid zones with no coverage. This is particularly important when field size is small. Very large fields may lend themselves to some reduction in dispenser density, provided pest density is low.

The release rate of individual pheromone point sources also matters; it interacts multiplicatively with dispenser density. Dispenser density and release rate should be manipulated so as to maximize $k_D * D_D$.

Formulations of pheromone to be used in disruption by competitive attraction should be flexible in the sense of offering opportunity to vary the density of dispensers and the timing of application. When pest densities are high, the number of dispensers will likely need to exceed the numbers affordable for manual application, i.e., future formulations will need to be amenable to application by machine. Desirable attributes for machine application are as follows: capability to be pumped or otherwise mechanically moved from storage reservoir to applicator; ability of the parent stock to be subdivided into particles or drops of desired sizes; transferability from applicator onto the crop; effective self-adhesion of the dispenser particles onto the crop; long-lasting release of the pheromone; avoidance of phytotoxicity or other undesired impacts of the formulation; ability to be biodegraded. An example of the type of formulation we foresee as viable for disruption that maximally exploits competitive attraction is the emulsified wax used in our OFM and CM studies. Exclusive of the active ingredients, the formulation is relatively inexpensive; it is formulable in large batches by using ordinary equipment; the product is storable in common containers; it is flowable and can be pumped to an applicator; techniques like extrusion through holes in a spinning hub can break the parent material into deposits ranging from droplets of microliter size through drops measured in hundreds of microliters, to dollops ranging up to several milliliters; this wax is self-adhering to diverse plant parts and adhesion can be increased by the addition of small amounts of benign glues; cleanup of equipment and clothing is easily accomplished using soap and water; the release rate of wax drops is surprisingly long and decreases slowly when the starting concentration of pheromone is kept below 5%; the wax carrier is not phytotoxic, as certain pheromones such as codlemone can be phytotoxic to leaves and fruits (Giroux and Miller, 2001); and these wax deposits eventually fall to the ground and biodegrade without known consequence. Other technologies with a similar set of desirable traits are the pheromone flakes (referred to above in the gypsy moth study) and pheromone fibers extensively explored several decades ago (Charlton and Cardé, 1981) and making a reappearance (Scentry Biologicals Inc., Billings, MT, USA; Knight, 2003).

Factors Influencing Performance of a Given Pheromonal Formulation

Disruption outcomes under competitive attraction are extremely sensitive to pest densities (Fig. 3; Miller et al., 2006). Generally, the density of pheromone dispensers should exceed the density of females by ca. 100-fold. Dispenser density can be somewhat lower, if attractiveness of each dispenser is commensurately greater than that of females or if a pest is

not highly fecund. We are aware of no case where an increase in the rate of pheromone released per dispenser fully compensated for reduction in dispenser density.

Measuring pest density in a crop is a required antecedent to optimizing a disruption protocol under competitive attraction.

Pheromone-baited monitoring traps might not always offer sufficient sensitivity and consistency for safely guiding real-time management decisions for mating disruption. Where attractiveness of traps equals that of females, obtaining a mean catch of one male per trap per several nights could indicate that disruption has failed, irrespective of an impressive value for % disruption. On the other hand, obtaining catches of zero in multiple traps known to be highly attractive to males constitutes the strongest evidence for success in mating disruption obtainable from traps.

Complexity, Reliability, and Sustainability of Mating Disruption

Under competitive attraction, it will probably not be possible to combine the pheromones of multiple species into one cocktail, as was explored for aerosol dispensers of pheromone (Isaacs et al., 1999). In crops like Michigan tree fruit where there are more than four key moth pests, this could translate into a substantial total number of pheromone applications over one season. Doing so will require convenient machine application of each pheromone and the capability to make applications in accordance with the appearance and abundance of each generation of each pest. This requirement adds complexity to pest management compared to application of long-lasting, broad-spectrum insecticides. Hopefully, this disadvantage for pheromones will be offset by absence of environmental hazards and sustainability of this pest management tactic.

One of the challenges to adoption of mating disruption has been reliability. In some situations, mating disruption has worked well for a given pest, whereas in other situations it has not. We suspect that widely varying pest densities explain much of this variability. With changes in formulation and application technologies, and attention paid to the principles of competitive attraction, substantial improvements in the reliability of mating disruption should be possible for many pests.

An advantage of competitive attraction is that it is already directed at forestalling the key behavior leading males to females—attraction. In contrast, when exploiting camouflage or desensitization, it will be the few males in the population capable of orienting to females despite the presence of the dispensed pheromone that will be resistant to the control measure and whose ethotype will dominate under this strong selection pressure. It may be better to sidestep this potential weakness of non-competitive mating disruption by engineering disruption to explicitly and immediately target attraction. Resistance is less of a threat under competitive attraction, if attractiveness of disruption formulations is adjusted to track the blend from authentic females over time. Nevertheless, rotating mating disruption with other management tactics is always advised.

Possible Outcomes

It is our hope that insights gained from this analysis will lead to developments that make mating disruption more practical and reliable at a time when the number of pest management tools is shrinking. Furthermore, the analytical procedures and principles arising from the study on moths may prove useful to competitive-attraction phenomena generally, be they,

e.g., use of attracticides (Eviden and McLaughlin, 2004), other kinds of insects (Polavarapu et al., 2002), competitive insect–plant interactions (Miller and Cowles, 1990), vertebrates responding to attractants (Li et al., 2002), sperm competition (Berkhead, 1998), or humans responding to competing products in the marketplace. This knowledge might also inform outcomes of the competitive interactions of inanimate homing devices involving attraction to or diversion from competing long-distance cues, e.g., <http://www.fas.org/spp/starwars/program/nmd/>).

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References

- ATTERHOLT, C. A. 1996. Controlled release of insect sex pheromones from sprayable, biodegradable materials for mating disruption. PhD dissertation, University of California, Davis, CA.
- ATTERHOLT, C. A., DELWICHE, M. J., RICE, R. E., and KROCHT, J. M. 1998. Study of biopolymers and paraffin as potential controlled-release carriers for insect pheromones. *J. Agric. Food Chem.* 46: 4429–4434.
- BAKER, T. C., CARDÉ, R. T., and MILLER, J. R. 1980. Oriental fruit moth pheromone component emission rates measured after collection by glass-surface adsorption. *J. Chem. Ecol.* 6:749–758.
- BERKHEAD, T. R. 1998. Sperm Competition and Sexual Selection. Academic Press, San Diego, CA, USA.
- CAPINERA, J. L. and BARBOSA, P. 1975. Transmission of nuclear polyhedrosis virus to gypsy moth larvae by *Calosoma sycophanta*. *Ann. Entomol. Soc. Am.* 68:493–494.
- CARDÉ, A. M., BAKER, T. C., and CARDÉ, R. T. 1979. Identification of a four-component sex pheromone of the female oriental fruit moth, *Grapholita molesta* (Lepidoptera: Tortricidae). *J. Chem. Ecol.* 5:423–427.
- CHARLTON, C. E. and CARDÉ, R. T. 1981. Comparing the effectiveness of sexual communication disruption in the oriental fruit moth (*Grapholita molesta*) using different combinations and dosages of its pheromone blend. *J. Chem. Ecol.* 7:501–508.
- DE LAME, F. M. 2003. Improving mating disruption programs for the oriental fruit moth, *Grapholita molesta* (Busck): efficacy of new wax-based formulations and effects of dispenser application height and density. M.S. thesis, Michigan State University, E. Lansing.
- DELAND, J. P., JUDD, G. J. R., and ROITBERG, B. D. 1994. Disruption of pheromone communication in three sympatric leafroller (Lepidoptera: Tortricidae) pests of apple in British Columbia. *Environ. Entomol.* 23:1084–1090.
- EVIDEN, M. and MCLAUGHLIN, J. R. 2004. Factors influencing the effectiveness of an attracticide formulation against the oriental fruit moth, *Grapholita molesta*. *Entomol. Exp. Appl.* 112:89–97.
- FLINT, H. M. and MERKLE, J. R. 1983. Pink bollworm (Lepidoptera: Gelechiidae): communication disruption by pheromone composition imbalance. *J. Econ. Entomol.* 76:40–46.
- GIROUX, P. Y. and MILLER, J. R. 2001. Phytotoxicity of pheromonal chemicals to fruit tree foliage: chemical and physiological characterization. *J. Econ. Entomol.* 94:1170–1176.
- ISAACS, R., ULCZYNSKI, M., WRIGHT, B., GUT, L. J., and MILLER, J. R. 1999. Performance of the microsprayer, with application for pheromone-mediated control of insect pests. *J. Econ. Entomol.* 92: 1157–1164.
- KNIGHT, A. L. 2003. Testing attracticide hollow-fiber formulations for control of codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae). *J. Entomol. Soc. Brit. Columbia* 100:71–78.
- LAWSON, D. S., REISSIG, W. H., AGNELLO, A. M., NYROP, J. P., and ROELOFS, W. L. 1996. Interference with the mate-finding communication system of the obliquebanded leafroller (Lepidoptera: Tortricidae) using synthetic sex pheromones. *Environ. Entomol.* 25:895–905.
- LI, W., SCOTT, A. P., SIEFKES, M., YAN, H., LIU, Q., YUN, S., and GAGE, D. A. 2002. Bile acid secreted by male sea lamprey that acts as a sex pheromone. *Science* 296:138–141.
- MAFRA-NETO, A. and BAKER, T. C. 1996. Timed, metered sprays of pheromone disrupt mating of *Cauda cautella* (Lepidoptera: Pyralidae). *J. Agric. Entomol.* 13:149–168.

- MCLAUGHLIN, J. R., SHOREY, H. H., GASTON, L. K., KAAE, R. S., and STEWART, F. D. 1972. Sex pheromones of Lepidoptera. XXXI. Disruption of sex pheromone communication in *Pectinophora gossypiella* with hexalure. *Environ. Entomol.* 1:645–650.
- MILLER, J. R. and COWLES, R. S. 1990. Stimulo-deterrent diversion: a concept and its possible application to onion maggot control. *J. Chem. Ecol.* 16:3197–3212.
- MILLER, J. R., MORI, K., and ROELOFS, W. L. 1977. Gypsy moth field trapping and electroantennogram studies with pheromone enantiomers. *J. Insect Physiol.* 23:1447–1453.
- MILLER, J. R., GUT, L. J., DE LAME, F. M., and STELINSKI, L. L. 2006. Differentiation of competitive vs. non-competitive mechanisms mediating disruption of moth sexual communication by point sources of sex pheromone: (Part 1) Theory. *J. Chem. Ecol.*, this issue.
- POLAVARAPU, S., WICKI, M., VOGEL, K., LONIERAN, G., and NIELSON, K. 2002. Disruption of sexual communication of oriental beetles (Coleoptera: Scarabaeidae) with microencapsulated formulation of sex pheromone components in blueberries and ornamental nurseries. *Environ. Entomol.* 31:1268–1275.
- ROTHSCHILD, G. H. L. 1975. Control of oriental fruit moth (*Cydia molesta*) (Busck) (Lepidoptera: Tortricidae) with synthetic female pheromone. *Bull. Entomol. Res.* 65:473–490.
- SHOREY, H. H. and GERBER, R. G. 1996. Use of puffers for disruption of sex pheromone communication among navel orangeworm moths (Lepidoptera: Pyralidae) in almonds, pistachios, and walnuts. *Environ. Entomol.* 25:1154–1157.
- SHOREY, H. H., SUMMERS, G. G., SISK, C. B., and GERBER, R. G. 1994. Disruption of pheromone communication in *Spodoptera exigua* (Lepidoptera: Noctuidae) in tomatoes, alfalfa, and cotton. *Environ. Entomol.* 23:1529–1533.
- SHOREY, H. H., SISK, C. B., and GERBER, R. G. 1996. Widely separated pheromone release sites for disruption of sex pheromone communication in two species of Lepidoptera. *Environ. Entomol.* 25:446–451.
- STELINSKI, L. L., GUT, L. J., PIERZCHALA, A. V., and MILLER, J. R. 2004a. Field observations quantifying attraction of four tortricid moth species to high-dosage pheromone rope dispensers in untreated and pheromone-treated apple orchards. *Entomol. Exp. Appl.* 113:187–196.
- STELINSKI, L. L., GUT, L. J., VOGEL, K. J., and MILLER, J. R. 2004b. Behaviors of naïve and pheromone pre-exposed leafroller moths in plumes of high-dose pheromone dispensers in a sustained-flight wind tunnel: implications for pheromone-based mating disruption of these species. *J. Insect Behav.* 17:533–553.
- STELINSKI, L. L., MILLER, J. R., and GUT, L. J. 2005a. Captures of two leafroller moth species in traps baited with varying dosages of pheromone lures or commercial mating-disruption dispensers in untreated and pheromone-treated orchard plots. *Can. Entomol.* 137:98–109.
- STELINSKI, L. L., GUT, L. J., EPSTEIN, D., and MILLER, J. R. 2005b. Attraction of four tortricid moth species to high dosage pheromone rope dispensers: observations implicating false plume following as an important factor in mating disruption. *IOBC/WPRS Bull.* 28:313–317.
- STELINSKI, L. L., GUT, L. J., MALLINGER, R. E., EPSTEIN, D., REED, T. P., and MILLER, J. R. 2005c. Small plot trials documenting effective mating disruption of significant populations of oriental fruit moth, *Grapholita molesta* (Busck), using high densities of wax-drop pheromone dispensers. *J. Econ. Entomol.* 1267–1274.
- SUCKLING, D. M. and ANGERELLI, N. P. D. 1996. Point source distribution affects pheromone spike frequency and communication disruption of *Epiphyas postvittana* (Lepidoptera: Tortricidae). *Environ. Entomol.* 25:101–108.
- TCHESLAVSKAIA, K. S., THORPE, K. W., SHAROV, A. A., LEONARD, D. S., REARDON, R. C., MASTRO, V. C., SELLERS, P., BREWSTER, C. C., and ROBERTS, E. A. 2005. Optimization of pheromone dose for gypsy moth (Lymantria: Lymantriidae) mating disruption. *Entomol. Exp. Appl.* 115:355–361.
- WEBB, R. E., TATMAN, K. M., LEONHARDT, B. A., PLIMMER, J. R., BOYD, V. K., BYSTRAK, P. G., SCHWALBE, C. P., and DOUGLASS, L. W. 1988. Effect of aerial application of racemic disparlure on male trap catch and female mating success of gypsy moth (Lepidoptera: Lymantriidae). *J. Econ. Entomol.* 81:268–273.
- WITZGALL, P., BÄCKMAN, A. C., SVENSSON, M., KOCH, U., RAMA, F., EL-SAYED, A., BRAUCHLI, J., ARN, H., BENGTSSON, J., and LÖFQVIST, J. 1999. Behavioral observations of codling moth, *Cydia pomonella*, in orchards permeated with synthetic pheromone. *Biocontrol* 44:211–237.