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Article

The Impact of Moth Migration on Apparent Fecundity Overwhelms Mating Disruption as a Method to Manage Spruce Budworm Populations

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Abstract: Aerial applications of a registered formulation of synthetic spruce budworm female sex pheromone were made in 2008, 2013 and 2014 to disrupt mating in populations of this forest insect pest in Quebec, Canada. Each year, the applications resulted in a 90% reduction in captures of male spruce budworm moths in pheromone-baited traps. A commensurate reduction in mating success among virgin females held in individual cages at mid-crown of host trees was also obtained. However, there was no reduction in the populations of eggs or overwintering larvae in the following generation (late summer and fall). The failure of this approach as a viable tactic for spruce budworm population reduction could have resulted from considerable immigration of mated females, as evidenced by high rates of immigration and emigration that caused steep negative relationships between apparent fecundity and the density of locally emerged adults.

Keywords: spruce budworm; moth; tortricidae; *Choristoneura fumiferana* (Clemens); forest protection; early intervention strategy; pheromone mating disruption; migration; dispersal

1. Introduction

The spruce budworm, *Choristoneura fumiferana* (Clemens) is an episodic tortricid defoliator of balsam fir, *Abies balsamea* (L.) Mill, and several members of the *Picea* genus, in particular white spruce *Picea glauca* (Moench) Voss, in the boreal forests of eastern North America [1,2]. Outbreaks are broadly regional, recurring every 30–40 years and lasting over 15 years [3,4]. In Canada, control methods are limited to two registered insecticides: The pathogenic bacterium *Bacillus thuringiensis* var. *kurstaki* (Btk) and the ecdysone agonist tebufenozide.

In 2007, after years of basic research, conceptual development, laboratory and field testing [5], a commercial product containing a synthetic formulation of the female sex pheromone for the spruce budworm (SBW) was registered in Canada for use against this insect: Disrupt Micro-Flakes® SBW (Hercon Environmental, Emigsville, PA, USA). This sex attractant formulation can be applied by aircraft to disrupt mating by interference with the sex pheromone emitted by females to attract males [6]. The mechanisms suggested to cause mating disruption include (1) false-plume (trail) following, (2) camouflage, (3) desensitization (adaptation and/or habituation), or (4) a combination of these [7–15].

In false-plume following, male moths are competitively attracted either to calling females or to pheromone dispensers; the latter decrease the limited search time of males and reduce mating encounters [9,14–18]. In camouflage, calling females occur within larger plumes of dispensers so that males cannot distinguish female plumes and locate the sources for mating. Desensitization includes adaptation and habituation in which high concentrations of pheromone cause neuronal fatigue so the insect becomes unresponsive to the pheromone for some time, again limiting effective search time and reducing chances of finding mates during the flight period [7,19–24].

Mating disruption (see [25] for a review) first proved useful in controlling cabbage looper moths, *Trichoplusia ni* (Hubner) [26]. It has since been used successfully on a number of insect pests and is a viable alternative to conventional insecticide programs for the control of several tortricids [27]. It offers many advantages, including reduced insecticide use, and thus conservation of natural enemies, decreased potential for the development of insecticide resistance, reduced insecticide residues on crops and in the environment, and reduced costs associated with worker protection and labor management [28].

In this paper, we report on the results of three field mating disruption trials with aerially-applied pheromone against adult spruce budworm, conducted in 2008, 2013 and 2014 in Quebec, Canada, under an array of different circumstances including forest composition, level of defoliation and the density of the target insect populations.

2. Materials and Methods

2.1. Sites

The 36 experimental sites used in these tests were located in eastern Quebec, Canada (Figure 1). Fourteen 50 ha plots were established in 2008 and were located north and east of Baie-Comeau, on the north shore of the St-Lawrence estuary. In 2013, twelve 30 ha plots were set-up on the south shore of the St-Lawrence river between Rimouski, Matane and Causapscal. In 2014, ten 100 ha plots were established in stands to the south and east of Rimouski. These sites were selected on the basis of forest composition and spruce budworm population density. Stands were mainly composed of balsam fir, white and black spruce, with a variable hardwood content (< 50% basal area) dominated by birches, aspen and maple. The North Shore area was already in a severe budworm outbreak in 2008, and was suffering its third year of defoliation. In the fall of 2007, population densities in the area of the North Shore where the mating disruption tests were conducted averaged 27.4 ± 1.1 overwintering larvae (L_2) per branch (SEM, $n = 42$). The spruce budworm populations in the Lower St-Lawrence (LSL) were at lower density in the fall of 2012, averaging 12.8 ± 2.3 L_2/branch (SEM, $n = 36$), and were either still at low density or in their first year of low to moderate defoliation in 2013. The populations in the LSL sites used in 2014 were at low density in the fall of 2013, averaging 5.8 ± 1.1 L_2/branch (SEM, $n = 150$), and had not caused significant defoliation in the years prior to the tests.

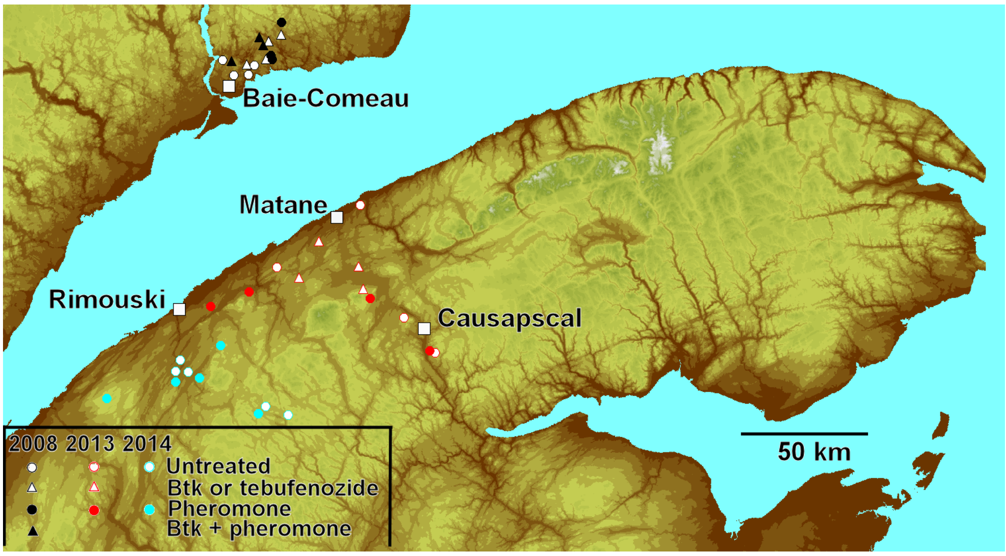

Figure 1. Elevation map of the eastern portion of southern Quebec, Canada (the Gaspé peninsula), showing the location of all plots in this study. Black: 2008. Red: 2013; Blue: 2014. Open squares: Selected city locations.

2.2. Treatments

In 2008, three treatments were applied: Btk double applications (5 days apart) in four plots, pheromone in three plots, Btk + pheromone in three plots. Four untreated plots were used as controls. The Btk (FORAY 76B, Valent BioSciences, Libertyville, IL, USA) was applied at the peak of the 4th instar at the rate of 1.5 L/ha (30 BIU/ha) at each application. At the beginning of the male moth flight season (11 and 12 July), Disrupt Micro-Flakes® SBW were applied by helicopter to three of these Btk-treated plots as well as three of the untreated plots. An ASTAR BA helicopter (Airbus Helicopter SAS, Marseille, France), equipped with the AG-NAV2® GPS navigation system (AG-NAV, Barrie ON, Canada), was used. A spreader was attached under the helicopter to apply the dry pheromone flake formulation at the label rate of 0.5 kg flakes/ha (50 g AI/ha). Both the aircraft and the helicopter flew at about 15 m above the tree canopy.

In 2013, two treatments were compared: single applications of tebufenozide (four plots), and pheromone (four plots). Four untreated plots were used as controls. Tebufenozide was applied at the rate of 70 g AI/ha (2 L/ha of Mimic 2LV®, Valent BioSciences, Libertyville, IL, USA), when larvae had reached the 5th instar (see [29] for details). Pheromone (same product, same application rate as in 2008) was applied when pheromone traps (see Section 2.3 below) reached $< 2\%$ cumulative moth catch, using a Cessna 188 (Textron Aviation, Wichita KS, USA), equipped with a flake spreader, flying about 15 m above the canopy.

In 2014, the pheromone (same product, same application rate as in 2013) was applied on five plots at the beginning of the moth flight period using the same procedure as in 2013. Five untreated plots were used as controls.

2.3. Pheromone Traps

SBW males were caught in each plot using five Multipher® (Biocom, Quebec City, QC, Canada) traps baited with the standard synthetic SBW pheromone lure (Biolure®, Contech Enterprises, Victoria, BC, Canada) in each plot. Traps were placed at the mid-crown level (8–12 m above ground) of five balsam fir trees spread across each plot at least 50 m from each other. Traps were emptied every 2 to 4 days throughout the moth flight season and the number of moths caught was counted.

2.4. Mating Success

Female spruce budworm moths do not tend to fly prior to mating, and thus it is difficult to adequately estimate mating success from samples of feral females in the field. This is why virgin females used in this study to estimate mating success were obtained from a SBW colony (Great Lake Forestry Centre, Sault Ste. Marie, ON, Canada) reared on an artificial diet [30]. We have previously demonstrated that the mating success as well as the pheromone gland content of laboratory-reared females were comparable to those of wild females [31]. Immediately after pheromone applications, 24- to 48 h-old virgin spruce budworm females were installed in individual plastic cages (Figure 2). In 2008, a single-opening cage containing a single virgin female was used (Figure 2a) [32]. In 2013 and 2014, this cage was modified by adding a second open screen funnel (Figure 2b). This modified cage is hereafter referred to as the "double-opening cage". The openings allowed males to enter the cage but were too small for the larger females to escape. Mating cages were placed at mid-crown (8–12 m above ground) in balsam fir trees using Multipher® traps as cage support. In 2008, 15 virgin-female cages were exposed in each plot at each interval. In 2013, sample size was increased to 30 cages per plot per installation, and in 2014, samples of 50 cages were used. The caged females were recovered and replaced every 3 to 4 days until the end of the male flight season. The number of males found in the cages was recorded, and females recovered from the cages were dissected to determine their mating status by the presence of spermatophores in their bursa copulatrix. In 2008, females were dissected from 7 of the 14 sample dates. In 2013 and 2014, all females were dissected.

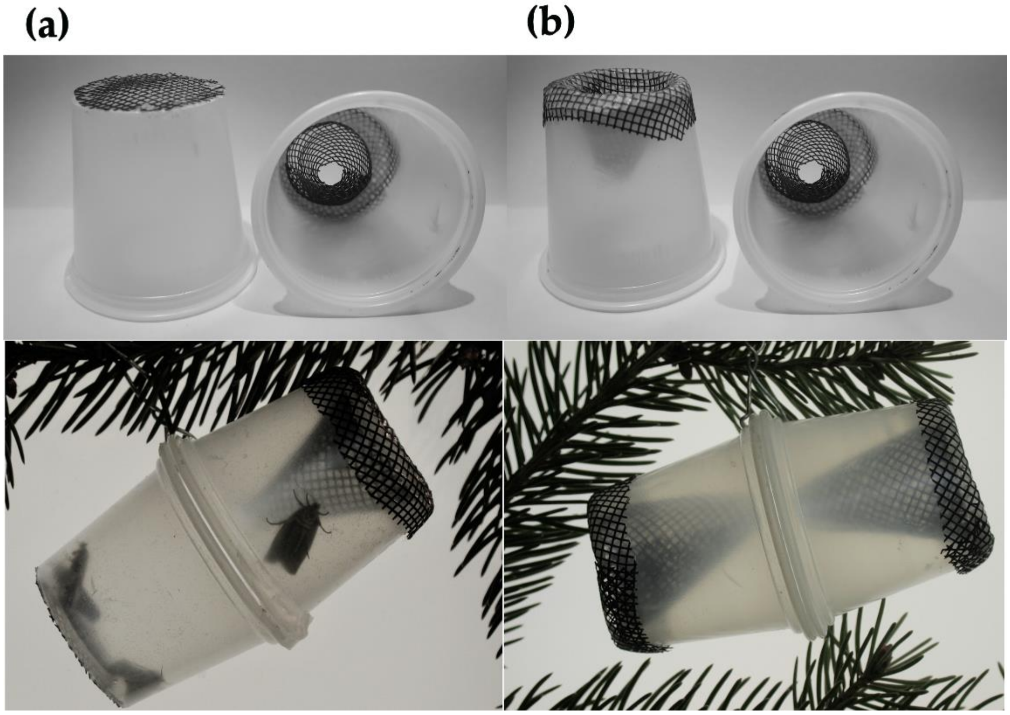

Figure 2. Cages used to assess mating success of female moths of spruce budworm. Top: Open cages; bottom: Closed cages. (**a**) Single-opening cage, used in 2008, containing one female and several trapped males. (**b**) Double-opening cage, used in 2013 and 2014. Cup diameter: 4 cm.

In 2011, under field conditions, the mating success of females held in both types of cages (single- and double-opening) was compared to that of tethered females (that we consider as close to feral females as possible). Tethered females were attached by a thin monofilament nylon wire with a drop of glue on their pronotum to a mesh wire stage about 12 cm in diameter installed on Multipher pheromone traps hung at mid-crown (8–12 m above ground) under branch tips of host trees. Three sets of 95, 128 and 126 females in single and double opening cages and tethered, respectively were available for this comparison. All were exposed simultaneously for 48 h, in three separate replicates at intervals of 8 days during the peak moth flight season.

2.5. Foliage Sampling

Foliage samples of 15 to 100 branch tips were taken in each plot from the mid-crown (8–12 m above ground) of dominant and co-dominant balsam fir and white spruce trees at several points in the insect's life cycle. In all three years, a sample of 45 cm branch tips was taken at the end of larval development (beginning of pupation). In 2013 and 2014, a second sample was taken at the end of the pupal stage, once adult emergence had started (44% emergence on average), to determine the density of emerging adults. Live pupae recovered from the foliage were reared to adult emergence to determine their survival, and the number of survivors was added to the number of emerged pupal cases found on the foliage. In 2008, a foliage sample was not taken at this stage. To obtain an estimate of emerging adult density from late-larval density measured in 2008, a linear regression between adult (A) and late-larval (P) densities measured in 2013 and 2014, was used. This regression was: $A = 0.337P - 0.0098$ ($R^2 = 0.646$, $n = 22$). A third sample of 45 to 75 cm branch tips was taken at the end of the oviposition period, once adults were no longer being caught in pheromone traps and eggs had hatched. Egg masses found on the foliage were counted. In 2013 and 2014, eggs in each mass were also counted. The number of eggs found on foliage in 2008 was estimated by multiplying the number of egg masses found by the average number of eggs per mass observed in 2013 and 2014 (17.7 eggs/mass). Current year shoots on the branches from these three foliage samples were counted, and density was expressed per shoot.

In 2008 and 2014, an additional foliage sample was taken in the fall (75 cm branch tips, about 0.16 m^2/branch among samples collected in 2008 and 2014) to measure the density of overwintering larvae (L_2). The L_2 were extracted using the NaOH washing method [33]. Shoots on these branches were not counted. To express L_2 density per shoot, the data from the egg sample collected in the LSL in 2013 were used to estimate, by linear regression, the number of shoots on balsam fir branches of 0.16 m^2 (106 shoots = 61 shoots + 281 shoots/m^2 × 0.16 m^2).

2.6. Analysis

Because the experimental designs varied from year to year (use of insecticides), and because the question we address is the impact of pheromone applications on mating success and reproduction of the target populations, we chose not to distinguish insecticide applications as distinct treatments in our analyses. Thus, assuming that insecticide applications in the early larval stages have no repercussions on surviving adults other than their density, "controls" include plots that received insecticides (either Btk or tebufenozide) and "Treated" include plots that received pheromone alone or in combination with a prior Btk application (2008). Doing this allows us to perform global analysis and make comparisons between years. However, in our Figures, we distinguish with different symbols the various treatment combinations used.

The comparison of mating success (presence or absence of a spermatophore in the recovered females) among tethered and caged females was done by logistic regression analysis using the confinement device (cage type or tethering) as factor.

The effect of pheromone treatment (T) and year (Y) on total capture of male spruce budworm moths per pheromone trap (M) was analyzed with a general linear model (GLM) using the density of emerging adults (A) as a covariate. In the absence of net migration of moths, this density is the main determinant of trap capture. No pupae were found in two plots in 2013. For analysis, these zero values were replaced by 0.00005 adults/shoot, below the observed minimum of 0.000055 pupae/shoot. The model used was:

$$\mathrm{Log}(M) = a + bY + (c + dY)T + (e + fY + gT + hYT)\mathrm{Log}(A) \tag{1}$$

The effect of pheromone treatment (T) and year (Y) on the proportion of caged virgin females that successfully mated (P) in each plot was analyzed by logistic regression using the density of emerging adults (A) as a covariate. In total, 3777 females were exposed, of which 1114 were mated (31.5%). The model used was:

$$\mathrm{Logit}(P) = a + bY + (c + dY)T + (e + fY + gT + hYT)\mathrm{Log}(A) \tag{2}$$

The effect of pheromone treatment (T) and year (Y) on the relationship between egg density and emerging adult density was tested by a GLM of the form:

$$E = a + bY + (c + dY)T + (e + fY + gT + hYT)A \tag{3}$$

where E is egg density (eggs per shoot = egg masses × mean eggs/mass) and A is emerging adult density. One missing value of E (no egg masses found in one plot in 2014) was replaced by 0.04 eggs/shoot, below the observed minimum of 0.049 eggs/shoot.

The ratio of egg density to emerging adult density, E/A, is an expression of apparent fecundity at the population level. In the absence of moth migration, apparent fecundity equals realized fecundity (the portion of their potential fecundity that moths succeed in laying before death). But when migration occurs (which is nearly always in spruce budworm), it also represents the contribution made by immigrant moths the egg population, as well as the loss of eggs that are carried away by emigrant moths (see [34] for a thorough discussion of apparent fecundity in the study of spruce budworm ecology). Apparent fecundity (E/A) was related to emerging adult density (A), year (Y) and pheromone treatment (T), as defined above, by a GLM of the form:

$$\text{Log}(E/A) = a + bY + (c + dY)T + (e + fY + gT + hYT)\text{Log}(A) \tag{4}$$

The effect of pheromone treatment (T) and year (Y) on the relationship between the density of overwintering larvae in the fall following treatment, L_2 (available in 2008 and 2014), and emerging adult density (A) was obtained by a GLM of the form:

$$L_2 = a + bY + (c + dY)T + (e + fY + gT + hYT)A \tag{5}$$

Models (1) to (5) were reduced by dropping least-significant terms one at a time until all remaining terms were significant, model consistency allowing ($\alpha < 0.05$). Residuals were tested for normality using the Anderson-Darling test [35].

3. Results

3.1. Mating Success of Caged and Tethered Females

Mating success varied significantly with holding device ($\chi^2 = 28.5$; *df*: 2346; $p < 0.001$). It was much higher among tethered females (39%, $n = 126$) than among females held in single-opening cages (12%, $n = 141$) (odds ratio 3.6, 95% confidence interval (CI): (2.5, 8.6)). In double-opening cages, females had the same likelihood of mating (33%, $n = 82$) as tethered females (odds ratio: 1.3, 95% CI: (0.7, 2.3)), and were more than 3-fold as likely to mate as females in single-opening cages (odds ratio 3.6, 95% CI: (1.8, 7.1)).

3.2. Pheromone Traps

The daily capture rate of male moths in pheromone traps was reduced by nearly 90% from the moment pheromones were applied, in all three years (Figure 3a–c). The success of caged virgin females at attracting males (Figure 3d–f) and successfully mating (Figure 3g–i) was also reduced. By contrast, insecticide applications (either Btk or tebufenozide) had no effect on capture in pheromone traps, or on mating success of virgin females, justifying that those treatments not be distinguished further in analyses.

Results of the GLM analysis (Equation (1)) indicate that total catch per pheromone trap varied significantly among years, and was reduced following pheromone applications, by the same amount (90%), each year (Table 1; $R^2 = 0.94$; Figure 4a–c). Importantly, there was no significant relationship between the total capture in pheromone traps and emerging adult density per shoot. Residuals of the reduced model were normally-distributed (Anderson-Darling $AD = 0.537$, $n = 36$, $p = 0.158$). Pheromone trap catch in the LSL was lower than expected in 2014 given the high emerging adult density per shoot (Figures 3c and 4c). It is likely that emerging adult density was overestimated because the sample providing this estimate was taken early relative to adult emergence in 2014 [36].

Table 1. Effects of emerging adult density (A), Year (Y) and pheromone treatment (T) on total capture of males per pheromone trap, Equation (1) reduced.

Source	DF	Adj SS	Adj MS	*F*-Value	*p*-Value
Year, Y	2	12.8851	6.44253	146.48	< 0.001
Pheromone, T	1	8.4613	8.46130	192.38	< 0.001
Error	32	1.4074	0.04398		
Lack-of-Fit	2	0.2451	0.12256	3.16	0.057
Pure Error	30	1.1623	0.03874		
Total	35	24.8012			

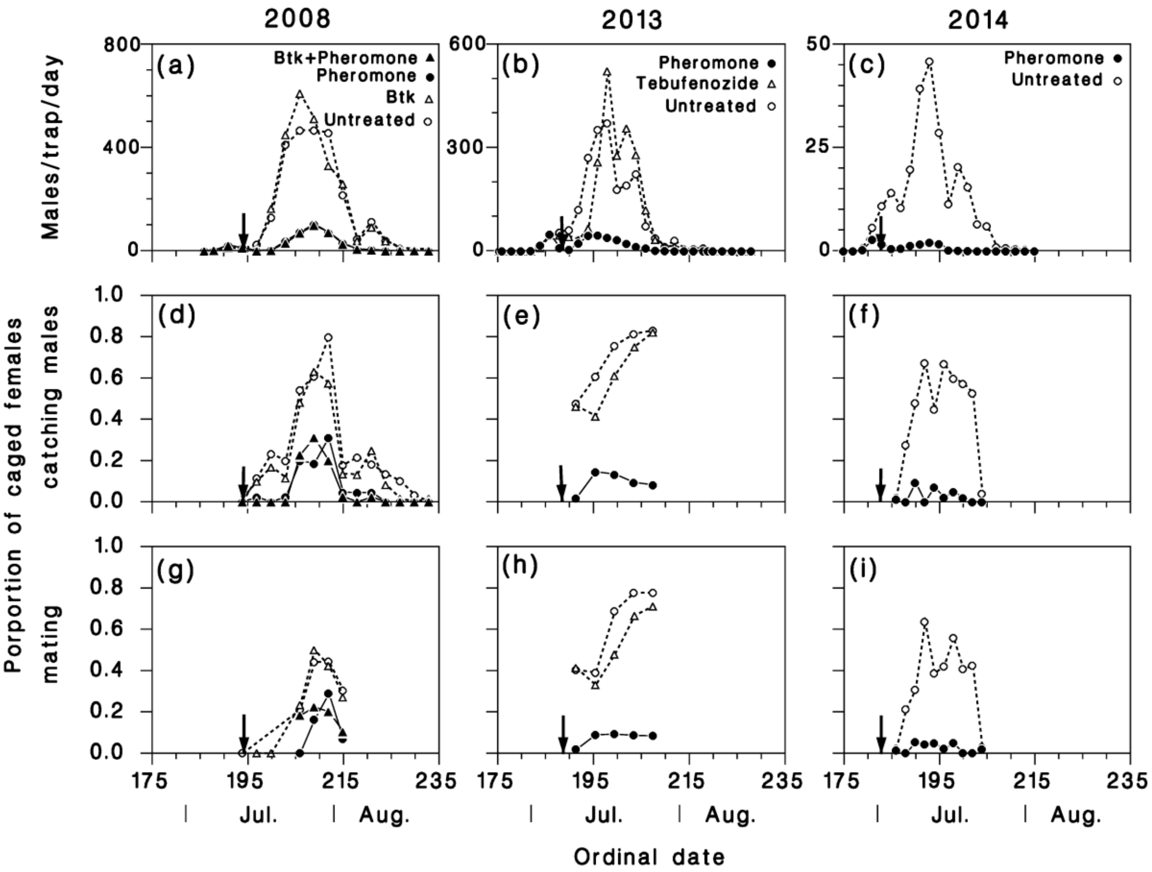

Figure 3. First row (**a**,**b**,**c**): Daily capture rates of male spruce budworm moths in pheromone traps. Second row (**d**,**e**,**f**): Success of caged virgin females at capturing males. Third row (**g**,**h**,**i**): Success of caged virgin females at getting mated. Left column: 2008. Center column: 2013. Right column: 2014.

3.3. Mating Success

The logistic regression model (Equation (2)) could not be reduced, because the 3-way interaction Log(A) × Y × T was highly significant (Table 2). The overall fit was very good (R^2 = 0.832) (Figure 4d–f). The response to pheromone treatment (T) was clear in all years, but complex in the details because of its interactions with adult density, Log(A) × T, and year, Log(A) × Y (Table 2). Overall, there was no clear relationship between mating success and emerging adult density, Log(A), as it interacted with both treatment and year.

Table 2. Results of logistic regression of mating success among caged females as influenced by adult density (A), year (Y) and treatment with pheromone (T), Equation (2), full model.

Source	DF	Adj. Mean	Chi-Square	*p*-Value
Regression	11	90.2094	992.30	< 0.001
Log(A)	1	6.4617	6.46	0.011
Year, Y	2	28.2868	56.57	< 0.001
Pheromone, T	1	2.0262	2.03	0.155
Log(A) × Y	2	7.1197	14.24	0.001
Log(A) × T	1	10.4317	10.43	0.001
Y × T	2	0.9070	1.81	0.404
Log(A) × Y × T	2	5.6419	11.28	0.004
Error	24	7.8267		
Total	35			

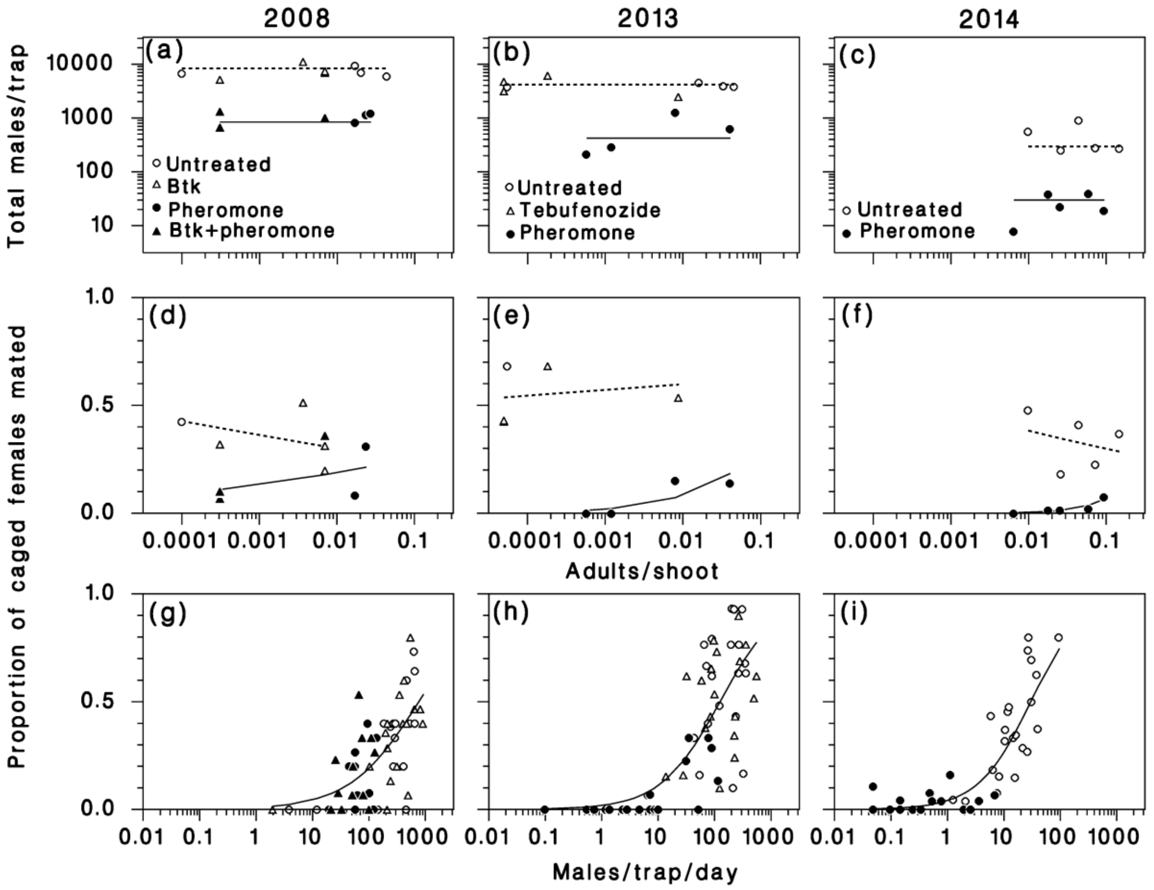

Figure 4. First row (**a**,**b**,**c**): Total capture of males per pheromone trap vs. adults per shoot, (lines: Equation 1, ——: With pheromone; ······: Without). Second row (**d**,**e**,**f**): Mating success vs. adults per shoot, (lines: Equation 2). Third row (**g**,**h**,**i**): Relationship between mating success of caged virgin females and the daily capture rate of males in pheromone traps during the exposure period (lines: Logistic regression). Left column: 2008. Center column: 2013. Right column: 2014.

The relationship between mating success among caged females and the daily capture rate of males in pheromone traps, however, corresponded closely with that reported by Régnière et al. [31] (Figure 4g–i).

3.4. Egg Density

Results of the GLM analysis (Equation (3)) indicate that egg density was unaffected by the pheromone treatment, T, and that its relationship with emerging adult density, A, varied significantly between years in both intercept and slope (Table 3; $R^2 = 0.68$; Figure 5a). Residuals of the reduced model were normally-distributed (Anderson-Darling $AD = 0.395$, $n = 36$, $p = 0.158$). Annual variations in the intercept and slope of the relationship between egg density and emerging adult density represent different levels of realized fecundity (slope) and immigration rates (intercept) (see [34] for a discussion). The immigration rate in 2008 was 0.234 eggs/shoot, the highest observed in this study (Baie-Comeau, area under full outbreak). It was intermediate (0.174 eggs/shoot) in 2013 (a high-migration year in a rising outbreak), and an order of magnitude lower (0.018 eggs/shoot) in 2014, farther to the west in the LSL region were the SBW infestation was still very patchy. Realized fecundity was highest on the North Shore in 2008 (13.4 eggs/moth), lowest during the high-migration year 2013 in the LSL (1.3 eggs/moth), and intermediate in 2014 (4.0 eggs/moth).

Table 3. General linear model (Equation (3)) of the effects of emerging adult density (A), Year (Y) and pheromone treatment (T) on the density of spruce budworm eggs. Reduced model.

Source	DF	Adj SS	Adj MS	*F*-Value	*p*-Value
Adults, A	1	0.42619	0.426188	54.08	< 0.001
Year, Y	2	0.12260	0.061300	7.78	0.002
$A \times Y$	2	0.21352	0.106761	13.55	< 0.001
Error	30	0.23641	0.007880		
Lack-of-Fit	27	0.19422	0.007193	0.51	0.855
Pure Error	3	0.04219	0.014063		
Total	35	1.26226			

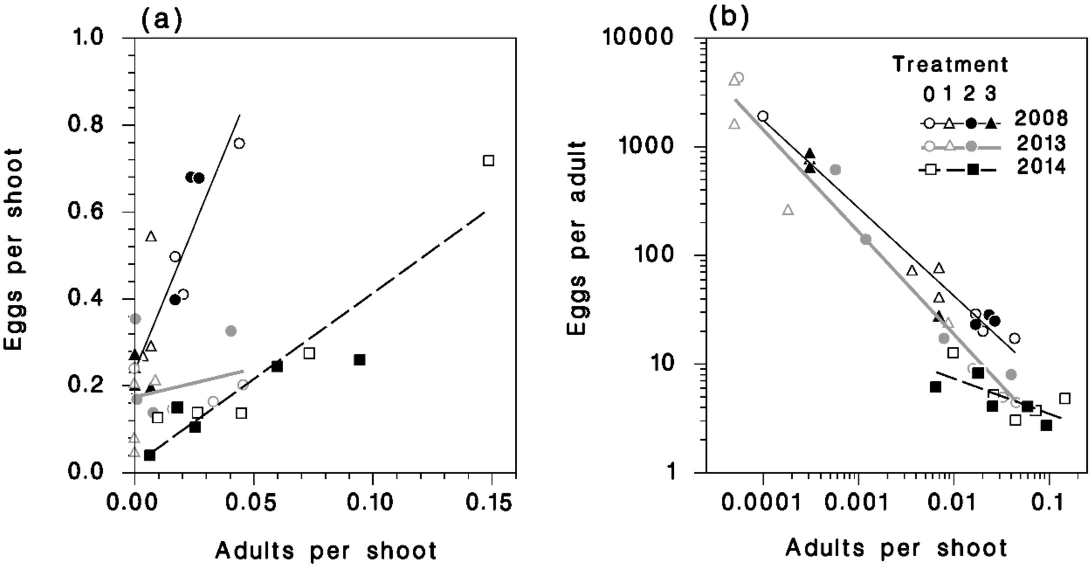

Figure 5. Relationship between adult density, and (**a**) egg density (lines: Equation (3)) and (**b**) apparent fecundity (eggs/adult) (lines: Equation (4)). Treatments: 0 is untreated, 1 is Btk or tebuzenozide, 2 is pheromone, 3 is Btk and pheromone.

3.5. Apparent Fecundity

Apparent fecundity was not affected by pheromone applications, but its relationship with adult density (Log(A)) varied significantly between years. The reduced model (Equation (4); Table 4) described the observations quite accurately (Figure 5b; $R^2 = -0.965$). Residuals were normally distributed (Anderson-Darling $AD = 0.279$, $n = 36$, $p = 0.628$).

Table 4. General linear model (Equation (4)) of the effects of emerging adult density (A), Year (Y) and pheromone treatment (T) on apparent fecundity, the ratio of eggs to adults (E/A). Reduced model.

Source	DF	Adj SS	Adj MS	*F*-Value	*p*-Value
Adult, Log(A)	1	5.7092	5.70918	173.78	< 0.001
Year, Y	2	0.4931	0.24654	7.50	0.002
Log(A) × Y	2	0.6170	0.30852	9.39	0.001
Error	30	0.9856	0.03285		
Lack-of-Fit	24	0.7913	0.03297	1.02	0.541
Pure Error	6	0.1942	0.03237		
Total	35	32.9479			

In 2013, egg density was independent of adult density and the slope of the relationship between Log(E/A) and Log(A) was −0.94 ± 0.06 (nearly −1), so that apparent fecundity was directly proportional to $1/A$ (grey line in Figure 5b), an indication of panmixis (see [34]). In 2008, the relationship between egg and adult density was significant (slope 13.4 ± 2.1 eggs/moth), with a high intercept indicating that considerable immigration was occurring in those populations (solid line in Figure 5a). Therefore, the slope of the relationship between apparent fecundity and adult density was also steep (−0.81 ± 0.04, no as close to −1 as in 2013). By contrast, in 2014, the intercept and slope of the relationship between egg and adult density were both lower than in 2008 (dashed line in Figure 5a). The slope of the relationship between apparent fecundity and adult density was consequently closer to zero (−0.32 ± 0.12). Those parameter values indicate that there was limited immigration into, but considerable emigration out of, those populations (dashed line in Figure 5b).

3.6. L_2 Density

The relationship between L_2 density and the density of emerging adults was very strong (Equation (5); $R^2 = 0.786$) (Figure 6a), and was unaffected by either pheromone applications or year (Table 5). Residuals were normally-distributed (Anderson-Darling $AD = 0.587$, $n = 24$, $p = 0.114$). Expressing the relationship in the form of the ratio L_2/A shows the same pattern as in apparent fecundity, where low-density populations have very high progeny to parent ratios when compared to higher-density populations (Figure 6b).

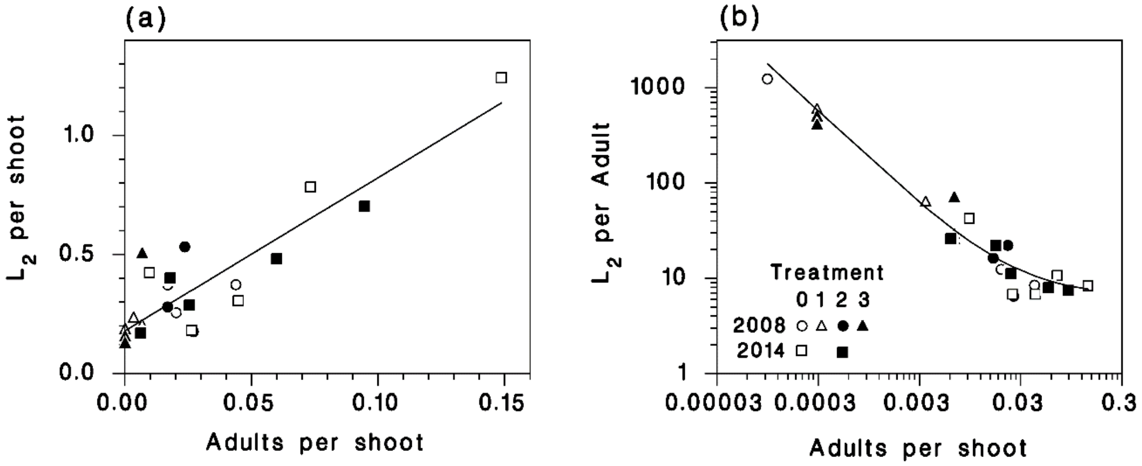

Figure 6. Relationships between adult density and (**a**) L_2 density, and (**b**) the ratio between L_2 density and adult density in 2008 and 2014. Lines: Equation (5). Treatments: 0 is untreated, 1 is Btk or tebuzenozide, 2 is pheromone, 3 is Btk and pheromone.

Table 5. General linear model (Equation (5)) of the effects of emerging adult density (A), Year (Y) and pheromone treatment (T) on L_2 density. Reduced model.

Source	DF	Adj SS	Adj MS	*F*-Value	*p*-Value
Adult, A	1	1.19871	1.19871	80.93	< 0.001
Error	22	0.32587	0.01481		
Lack-of-Fit	17	0.25502	0.01500	1.06	0.522
Pure Error	5	0.07085	0.01417		
Total	23	1.52458			

4. Discussion

Mating disruption has been successfully used against several moth pests with synthetic sex pheromone applied from the air or from the ground [18,27]. Aerially-applied pheromones incorporated in plastic laminate flakes have been successful in controlling low-density populations of the gypsy moth in the USA, even when those populations were surrounded by higher, untreated populations [37]. The facts that gypsy moth females do not fly and that migration rates in natural populations are very low have contributed to this success. Ground applications of wax-incorporated synthetic sex pheromones have also been effective in suppressing mating in the oriental fruit moth, *Grapholita molesta* [38], a tortricid.

Over the last four decades, considerable research has been devoted to understanding the concept and application of mating disruption with synthetic pheromones against the spruce budworm [5]. Most previous mating disruption trials were conducted in small plots, often with no replication (one plot per treatment) and provided no rigorous statistical reliability [5]. Thus, it is not surprising that much of this research was published in the grey literature. Trap shutdown is a common feature in most SBW mating disruption trials. However, no population reduction has been convincingly demonstrated, whether expressed as apparent fecundity (the ratio of eggs per locally-produced moth) or as reduced populations in the late summer or early fall. In contrast, our three large-scale mating disruption trials were fully replicated, with sample sizes ensuring accurate measurement of apparent fecundity (a ratio). This allows us to provide precise and unbiased estimates of the different parameters measured as a function of treatment, adult density and year. The 90% reduction in male attraction to pheromone sources (natural or synthetic) and similar reduction in the mating success of caged females were both highly promising results. However, we clearly demonstrated that pheromone applications had no impact on egg or L_2 counts or on apparent fecundity, and the use of mating disruption as a viable tool to control spruce budworm populations is doubtful.

The failure of pheromones to reduce egg or L_2 density or affect apparent fecundity could have resulted from a lack of effect on the mating success of feral females, which would suggest that mating success of caged females was not representative of the mating success of feral females. This is a hypothesis proposed in [5]. However, it is more likely that moth movement masked the limited effect of the pheromone applications. In the spruce budworm, several aspects of male and female behavior may affect the success of mating disruption with pheromones. Females calling from the top of highest trees in the canopy may still succeed in attracting males as most of the synthetic pheromone is released lower in the canopy or even on the ground [27]. We have indeed observed higher spruce budworm mating frequency in caged female placed at treetop compared to mid-crown in untreated populations (unpublished data). Daytime flight may also limit the success of mating disruption [26]. In spruce budworm, mating may occur during the daylight hours, which suggests that males can use visual cues to find females, especially when population densities are high [39]. However, other tortricid moths with daytime mating have been controlled successfully with mating disruption [27]. As a counterpart to these findings, we have seen no difference in mating success between females held in double-opening cages and tethered females. Nor has any difference in sexual attractiveness (amount of pheromone in the gland) been observed between feral and laboratory-reared females [31]. Therefore, considering the strong reduction observed in male trap captures and the mating success of caged-females, it seems unlikely that feral females in pheromone-treated plots would have succeeded in attracting mates, especially in low density populations, given the strong Allee effect associated with mate-finding in this species [31]. It is also worth noting that the positive relationship between mating success among caged females and the daily rate of capture of males in pheromone traps (Figure 4g–i) was still very apparent in pheromone-permeated air, suggesting that it is the abundance and ability of male moths to find the females that is important in determining mating success.

To the best of our knowledge, no study has provided clear evidence that the mating success of feral females is higher than that of caged or tethered females. However, even if this was the case for the spruce budworm, the huge apparent fecundities observed in low-density populations in the 2008 and 2013 trials (Figures 5b and 6b), could not possibly be attributed to egg laying by local females. Spruce budworm females are known to migrate after mating and after laying part of their egg complement [40,41] and so, given the strong negative relationship between the density of emerged adults and their apparent fecundity, moth movement is more likely the factor responsible for the failure of pheromone to reduce egg density. This failure was not the result of our treatment plots being too small. The scale of spruce budworm moth movement (in the 1000 km^2 range [40]) is of the same order as that of our test areas: in 2008 an area roughly 40 km in diameter (1250 km^2), in 2013 an area of 100 km diameter (7850 km^2) and in 2014 an area of 70 km diameter (3850 km^2). Managing a forest insect with treatment areas of such a scale is physically impossible and probably ecologically undesirable.

Pesticide applications are not expected to have a direct impact on apparent fecundity, except perhaps because of their effects on the reproductive abilities of survivors to sub-lethal doses, as documented for both Btk [42] and tebufenozide [43]. In moths that migrate following mating, successful population control by mating disruption is difficult [27]. In such cases, area-wide population management is required, and only when sources of immigrants are reduced can mating disruption succeed.

The slopes of the relationship between apparent fecundity and adult density (log (E/A) to log (A)) have significant implications in population dynamics at the regional scale. While this topic is discussed elsewhere [34,36], certain features are worth noting here. In Figure 5b, we can see the main features of apparent fecundity in the dynamics of rising SBW population: Low populations get a significant fecundity boost from immigration, while high populations emit migrants (very low apparent fecundities). Interestingly, on an operational level, our results clearly show that sampling the L_2 rather than the eggs may be sufficient (and less labor intensive) to establish a relationship between apparent fecundity (L_2/adult) and adult density (Figure 6b). Another important feature is that moth movement is a broadly regional phenomenon [40], and as a result, pheromone treatments can have very little if any effect on apparent fecundity. This should be true regardless of plot size because treated areas cannot be as large as the scale at which moths mix by migration. In large part, because of the extent and scale of moth movement, apparent fecundity may reflect the net result of migration in different population density contexts at the regional scale. For instance, in a widespread outbreak, such as on the North Shore in 2008, moths leaving a stand are likely to be replaced by immigrants as indicated by the high immigration rate and realized fecundity recorded there (0.23 eggs/shoot and 13.4 eggs/moth). In a more patchy situation, such as occurred west of the growing LSL outbreak in 2014, departing moths are less likely to be replaced, leading to very low realized fecundity (4.0 eggs/moth). Immigration rates were also much lower (0.02 eggs/shoot) among the ten plots in 2014 than they were on the North shore in 2008 (0.23 eggs/shoot) or among the LSL sites in 2013 (0.17 ± 0.03 eggs/shoot).

5. Conclusions

The outbreak in our three test areas was already widespread when our trials took place, especially on the north shore of the St-Lawrence river near Baie-Comeau in 2008 and in the Lower St-Lawrence east of Rimouski in 2013 (Figure 1). In those two trials, the risk of moth immigration was high. In 2014, the risk of immigration into the ten sites of the trial, west and south of Rimouski, was lower, but high survival rates led to high population growth rates [36]. There was little chance that mating disruption would be effective under any of those circumstances.

There may still be a faint hope that mating disruption can be of practical use as an early intervention tool against spruce budworm, if it can be applied at the first sign of an outbreak over a vast enough area, in which significant immigration is unlikely. However, given that Btk and tebufenozide applications significantly reduce SBW population growth rates compared to untreated controls or pheromone-only treatments [36,44], we question the usefulness of pursuing an approach that has so consistently failed at delivering the necessary population reduction even in the most carefully conducted trials.

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