

**Experimental pheromone applications using
Disrupt Micro-flakes SBW® for the control of the
spruce budworm populations: Quebec mating
disruption trials 2008**

Richard Trudel¹, Alain Dupont¹, Alain Bélanger¹, and
Jacques Régnière² – ¹SOPFIM ; ²NRCan, SCF

Final Report

Submitted to SERG-International

Submitted by:

Richard Trudel, Ph.D.

Société de protection des forêts
contre les insectes et maladies
(SOPFIM)

May 19, 2011

Abstract

A trial was conducted during the summer 2008 in the Baie-Comeau region, on the Quebec North Shore, to evaluate the efficacy of a newly registered pest management tool against the spruce budworm (SBW). This pest management tool consists in the aerial application of pheromone micro-flakes to disrupt mating and limit the reproductive potential of SBW females during the egg-laying period.

The experimental design included 4 treatments: blocks treated (1) with *Bacillus thuringiensis* var *kurstaki*; (2) with pheromones; (3) a combination of both treatments; and (4) untreated control blocks. The application of pheromone micro-flakes was carried out at the beginning of the moth flight period to disrupt communication between males and females and limit mating success. The observations made in July and August showed that SBW male moths were significantly less successful at finding pheromone traps and caged virgins females in the zones treated with the pheromones compared to the control zones. The mean number of captured moths per Multipher traps per 3-day periods in the treated zones was 57, compared to 402 male moths in the control zones (a 7-fold difference). Thus, males could locate pheromone traps and virgin females even in blocks treated with pheromone, but in considerably smaller numbers.

As measured by the density of egg masses and second instars (L₂), the treatments had no impact. On average, a total number of 29 egg masses were collected per block (on 15 branches) per 3-day period in the pheromone treated zones compared to 27 egg masses in the control blocks. For the L₂, mean numbers of 1891,8 and 1531,5 larvae were recorded in the blocks with and without pheromones, respectively. In forest stands in outbreak situations, the presence of high concentrations of synthetic pheromones in the air did not disrupt mate location sufficiently to prevent mating and reduce apparent fecundity. This control method may present more promising results if used as an “early intervention management strategy”, under low SBW population density.

Résumé

Applications expérimentales de Disrupt Micro-flakes SBW® pour le contrôle des populations de la tordeuse des bourgeons de l'épinette: Essais de confusion reproductive – Québec, Côte-Nord 2008

Un essai de terrain a été réalisé au cours de l'été 2008 dans le secteur de Baie-Comeau, sur la Côte-Nord du Québec, afin d'évaluer l'efficacité d'une nouvelle stratégie de lutte homologuée contre la tordeuse des bourgeons de l'épinette (TBE). Cette stratégie de lutte consiste en l'application aérienne de micro-flocons de phéromone sexuelle afin de perturber l'accouplement et ainsi nuire au potentiel de reproduction des femelles en période de ponte.

Le dispositif expérimental était composé de 4 traitements, soient des blocs traitées (1) au *Bacillus thuringiensis* var *kurstaki* (Btk); (2) aux phéromones; (3) traitement combiné Btk/phéromones; et (4) des blocs témoins. L'application des micro-flocons de phéromones a

été réalisée au début de la période de vol des adultes de TBE afin de perturber la communication entre mâles et femelles et ainsi limiter les chances d'accouplement. Les résultats obtenus au cours des mois de juillet et août ont permis de constater que les papillons mâles de TBE étaient significativement plus désorientés durant la période d'accouplement dans les zones traitées aux phéromones comparativement aux zones témoins. Le nombre moyen de papillons capturés dans les pièges Multipher par période de trois jours dans les zones traitées était de 57, comparativement à 402 papillons mâles dans les zones témoins (presque 7 fois plus). Cette donnée nous permet de constater que malgré l'application de flocons de phéromones, il est possible pour les mâles de localiser les pièges à phéromone (qui eux simulent une femelle), mais en quantité significativement moins grande.

De plus, les différents traitements n'ont eu aucun impact sur la mesure de la densité de masses d'œuf et des larves de stade 2 (L_2). En moyenne, un nombre total de 29 masses d'œuf ont été récoltées par bloc (sur 15 branches) par période de trois jours dans la zone traitée aux phéromones, comparativement à 27 masses d'œufs dans la zone témoin. Pour ce qui est des larves, des nombres moyens de 1891,8 et 1531,5 L_2 ont été enregistrés respectivement dans les blocs avec et sans phéromones. Dans les peuplements forestiers en situation épidémique, la présence d'une concentration élevée de phéromone synthétique dans l'air n'a pu empêcher les mâles de repérer les femelles et ainsi prévenir l'accouplement et réduire la fécondité. Cette stratégie de lutte pourrait obtenir de meilleurs résultats si elle était utilisée comme « méthode d'intervention hâtive », et ce, dans des peuplements de basse densité de TBE.

Table of contents

ABSTRACT	2
RESUME.....	2
TABLE OF CONTENTS.....	4
1. INTRODUCTION.....	5
2. METHODOLOGY.....	7
2.1 SITE SELECTION.....	7
2.2 EXPERIMENTAL DESIGN	7
2.3 APPLICATION OF TREATMENTS	7
2.4 TREATMENT EFFICACY EVALUATION.....	11
2.4.1 <i>SBW population densities</i>	11
2.4.2 <i>Egg mass and hibernating larvae survey (L₂) sampling</i>	11
2.4.3 <i>Mating success</i>	11
2.4.4 <i>Field life of the product</i>	12
2.4.5 <i>Statistical analysis</i>	12
3. RESULTS.	13
4. DISCUSSION.....	19
4.1 APPLICATION OF THE DRY FORMULATION INSTEAD OF APPLYING IT WITH THE STICKER	23
4.2 REINVASION OF THE STUDY SITES BY ALREADY MATED FEMALES	24
4.3 HIGH LEVEL OF SBW POPULATION IN THE STUDY AREA.....	24
5. CONCLUSION	25
6. RECOMMENDATIONS	25
REFERENCES CITED	27

1. Introduction

The spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae), is the most destructive insect defoliator of mixed boreal forest stands in eastern North America (Blais, 1983; Sanders, 1991). The insect feeds primarily on white spruce, *Picea glauca* (Moench) Voss, and balsam fir, *Abies balsamea* (L.) Mill, which is the most vulnerable host species to *C. fumiferana* (MacLean, 1980). During major outbreaks, tens of millions of hectares can be severely defoliated by this insect. Most control methods mentioned in the recent literature involve the use of biological insecticides, primarily *Bacillus thuringiensis* var. *kurstaki* (Btk). Through a combination of annual surveys, prediction models, targeted control strategies and proper forestry practices, it is now possible to reduce economic losses caused by spruce budworm outbreaks. At present, a number of *Bt* products and Mimic are the only control products commercially available. It would be necessary and prudent to have additional, complementary and alternative pest management technologies in place that can provide options to forest managers.

Semiochemicals, also called behavior-modifying chemicals, are chemicals emitted by organisms to transmit information to other individuals (Thorpe et al., 2006). Allelochemicals (e.g., allomones, which benefit the species emitting the signal, and kairomones, which benefit the receiving species) are a subset of semiochemicals that operate interspecifically, whereas pheromones are a subset of semiochemicals that operate intraspecifically. Pheromones that act as attractants cause an organism to move towards the chemical source. Insect pheromones that act as sex attractants show promise for suppressing pest populations through mating disruption. The potential for modifying an insect pest's behaviour through the use of pheromones to control its impact on a crop has been investigated widely in the last 30 years. The idea behind mating disruption is to create interference with the sex pheromone emitted by the female to a level at which the male has difficulty locating her.

The term mating disruption has been applied to methods using synthetic pheromone dispensers without traps to confuse and disrupt communication (Byers 2007). The mechanisms suggested to cause mating disruption include (1) false-plume (trail) following, (2) camouflage, (3) desensitization (adaptation and/or habituation), and (4) combinations of these (Shorey 1977, Bartell 1982, Cardé 1990, Valeur and Löfstedt 1996, Cardé et al. 1998, Evenden et al. 2000, Gut et al. 2004, Miller et al. 2006a, b).

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 (Daterman et al. 1982, Cardé 1990, Mani and Schwaller 1992, Stelinski et al. 2004, Miller et al. 2006a, b). In the mechanism of camouflage, calling females occur within larger plumes of dispensers so that males cannot distinguish female plumes and locate females for mating. Desensitization includes adaptation and habituation in which high concentrations of pheromone cause fatigue of neurons so the insect becomes unresponsive to pheromone for some time, again limiting effective search time and reducing chances of finding mates during the flight period (Bartell and Roelofs 1973, Shorey 1977, Kuenen and Baker 1981, Baker et al. 1989, Figueredo and Baker 1992, Rumbo and Vickers 1997, Stelinski et al. 2003, Judd et al. 2005).

Mating disruption first proved to be successful in controlling cabbage looper moths, *Trichoplusia ni* (Hubner) (Shorey et al. 1967), and since then has been used successfully on a number of insect pests (Cardé & Minks 1995). In addition, it has been proven to be a viable alternative to conventional insecticide programs for the control of several Tortricid pests (Cardé and Minks 1995). 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 fruit and in the environment, and reduced costs associated with worker protection and labor management (Thomson et al. 2001).

Over the past decade, research projects were conducted to develop a new green product for use in SBW early intervention integrated pest management programs. This research was oriented on the development of a SBW pheromone blend and an appropriate carrying system for its use in large forest stands. Decades of work by many researchers has finally culminated in a pheromone product being registered by the Pest Management Regulatory Agency (PMRA) for SBW suppression in early 2007.

During this 2008 field trial, we intended to evaluate the efficacy of this promising control tool in an infested SBW area that is located on the North Shore of the province of Quebec. Knowing that mating disruption is an early intervention strategy, the main objective of this project was to evaluate the potential of this control method during a SBW outbreak. The specific objectives were: (1) Carry out and evaluate a demonstration trial with the newly registered spruce budworm management tool DISRUPT Micro-Flake[®] SBW; (2) evaluate its potential role in spruce budworm mitigation programs; and (3) evaluate the impact of DISRUPT Micro-Flake[®] SBW and *Bt* on spruce budworm populations when used alone and in combination.

2. Methodology

2.1 Site selection

This experiment was conducted in the North Eastern part of Quebec, more precisely in the North Shore region, near Baie-Comeau (Figure 1). The public forest in this sector, which is mainly composed of coniferous trees, is affected since few years by a spruce budworm outbreak. The dominant tree species present in these sites are balsam fir, black spruce, and white spruce, which are sometimes mixed with white birch and quaking aspen. The sites selection for this study was based on stand age classes (30-50 year-old trees), 2007 defoliation mapping, aerial pictures of the area, and on a ground evaluation of L₂ trough branch sampling (Fall 2007).

2.2 Experimental design

This study required the use of 16 fifty-hectare blocks to compare the four different treatments, using four replicates per treatment (Table 1). In each of the blocks, 15 survey trees were selected systematically in three lines for data collection and traps installation.

2.3 Application of treatments

To obtain optimum synchronization of treatments, the seasonal development of the TBE was monitored every two (2) days from the 2nd instar to the moth stage. Using this information, the aerial spraying of the Btk commercial formulation occurred from June 13 to June 15 2008. Cessna 188 aircrafts were used for the application of the biological pesticide, which were fitted with 4 AU-4000 Micronair atomizers (RPM = 7000-8000) and an AGNAV-GPS guidance system. The flow rate was calibrated to deliver a volume of 1.5 L/ha in a swath width of 30 m. The aircraft flew at 190 km/h and at about 15 m above the tree canopy.

The pheromone flakes aerial application was realized from July 11 to July 12 2008 with a helicopter ASTAR BA+ equipped with a GPS navigation system AG-NAV®2. Since the texture of the flake formulation with all its additives was too thick, a dry formulation was used for the application of the pheromone treatment. A spreader was attached under the helicopter to apply the dry pheromone formulation (Figure 2A, 2B). The flow rate was calibrated to deliver a volume of 700 g/ha in a swath width of 15 m. The aircraft flew at 85 km/h and at about 15 m above the tree canopy.

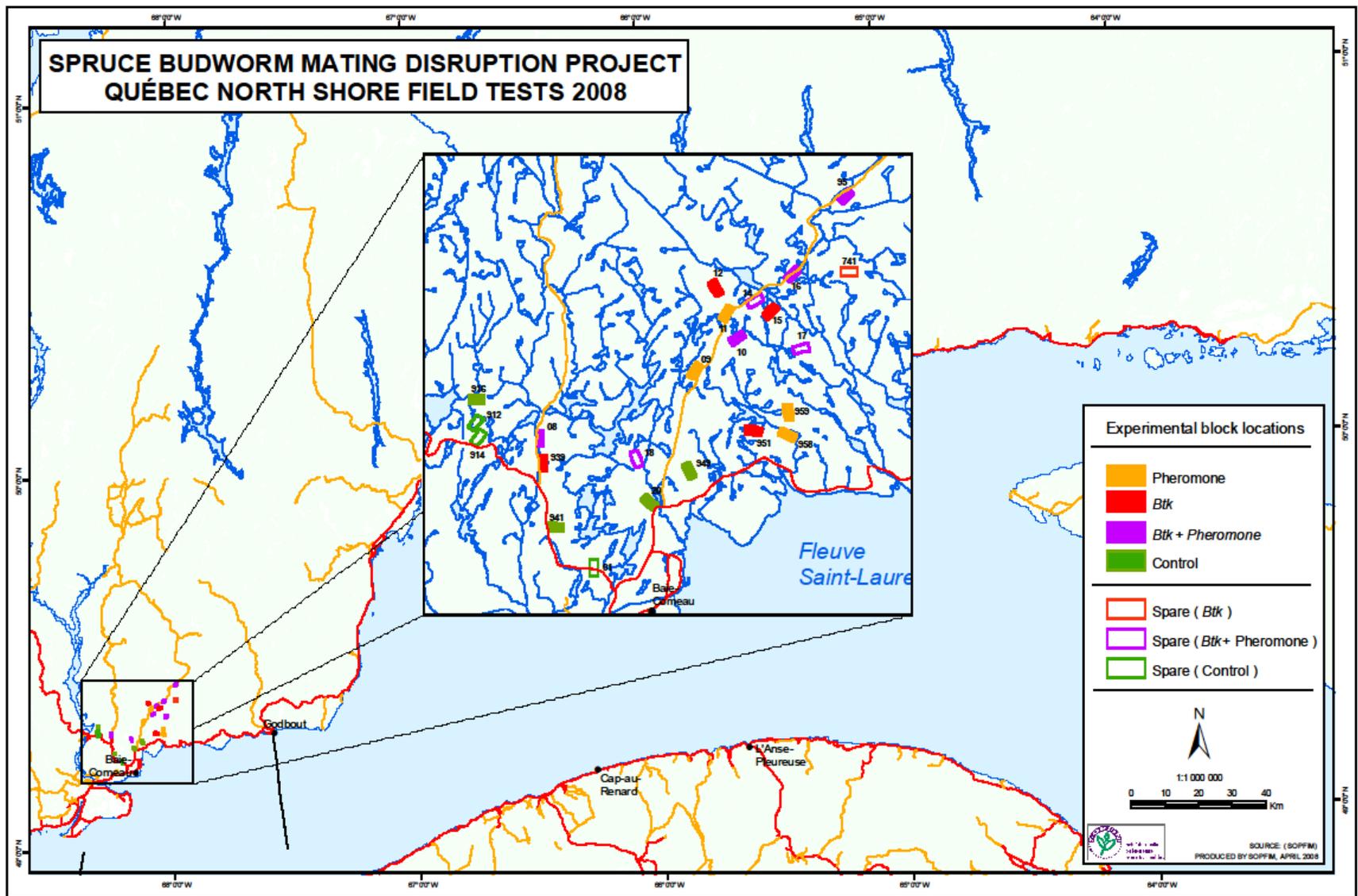


Figure 1. Localisation of the study area on Quebec North shore, near Baie-Comeau

Table 1. Experimental design and treatment description

BLOCK #	AREA (ha)	TREATMENTS	PRODUCTS	DOSE / HA	SENTINELLE TREES / BLOCK	2007 L ₂ SURVEY	
						L ₂ / Br.	L ₂ / 10 m ²
5	50	Pheromone	SBW Disrupt	1 X 35 g a.i.	15	18	948
95	50	Pheromone	SBW Disrupt	1 X 35 g a.i.	15	15	933
958	50	Pheromone	SBW Disrupt	1 X 35 g a.i.	15	15	948
959	50	Pheromone	SBW Disrupt	1 X 35 g a.i.	15	19	1274
Total	200				Mean	16,8	4103
15	50	Btk	Btk	2 x 30 BIU	15	26	1493
18	50	Btk	Btk	2 x 30 BIU	15	41	2314
741	50	Btk	Btk	2 x 30 BIU	15	83	4674
951	50	Btk	Btk	2 x 30 BIU	15	34	1752
Total	200				Mean	46	10233
8	50	Btk + Pheromone	Btk + SBW Disrupt	2 x 30 BIU + 35 g a.i.	15	27	1563
9	50	Btk + Pheromone	Btk + SBW Disrupt	2 x 30 BIU + 35 g a.i.	15	79	4770
10	50	Btk + Pheromone	Btk + SBW Disrupt	2 x 30 BIU + 35 g a.i.	15	19	1000
12	50	Btk + Pheromone	Btk + SBW Disrupt	2 x 30 BIU + 35 g a.i.	15	12	731
Total	200				Mean	34,3	8064
20	50	Control	-----	Control	15	27	1426
914	50	Control	-----	Control	15	20	1278
941	50	Control	-----	Control	15	24	1251
949	50	Control	-----	Control	15	22	1267
Total	200				Mean	23,3	5222



Figure 2. Equipment used for the dry application of the pheromone flakes. A) the spreader;
B) The helicopter under which was attached the spreader.

2.4 Treatment efficacy evaluation

2.4.1 SBW population densities

Spruce budworm population density assessments have been conducted on a periodical basis:

- Before *Btk* 1st application (4th instar)
- Five (5) days after the first *Btk* application
- Five (5) days after the second application
- At the end of the feeding period (75% pupal stage)
- Egg mass survey
- L₂ survey

2.4.2 Egg mass and hibernating larvae (L₂) survey sampling

Samples were collected every three (3) days from 15 balsam fir trees (75 cm branch), beginning before the pheromone application and for a 20-day period after the treatment, in all blocks. In addition, each egg mass was characterized as fertile, infertile, parasitized or emerged.

During the fall, 75 cm branch samples were collected on fifteen (15) balsam fir trees in each experimental block, and processed by the NaOH extraction method to provide counts of overwintering larvae / branch.

2.4.3 Mating success

Fifteen “virgin female baited” (VFB) cages (Delisle 2008, Figure 3A) were used to document the mating success of females according to the different treatments. The 24-48 hour virgin females were installed uniformly in each block on the sentinel trees using a Multiplier trap as cage support. The females and cages were collected and replaced every 3 days. Males in the cages were counted and females were dissected for the presence of spermatophores (Figure 3B).

Following the pheromone flake applications, 5 baited Multiplier traps installed in the experimental blocks were used to assess the effect of pheromone application on male capture levels and to document the moth stage seasonal activity pattern. All the traps were emptied every 3 days until the end of the flight period. The lures in the Multiplier traps were replaced during the first week of July.

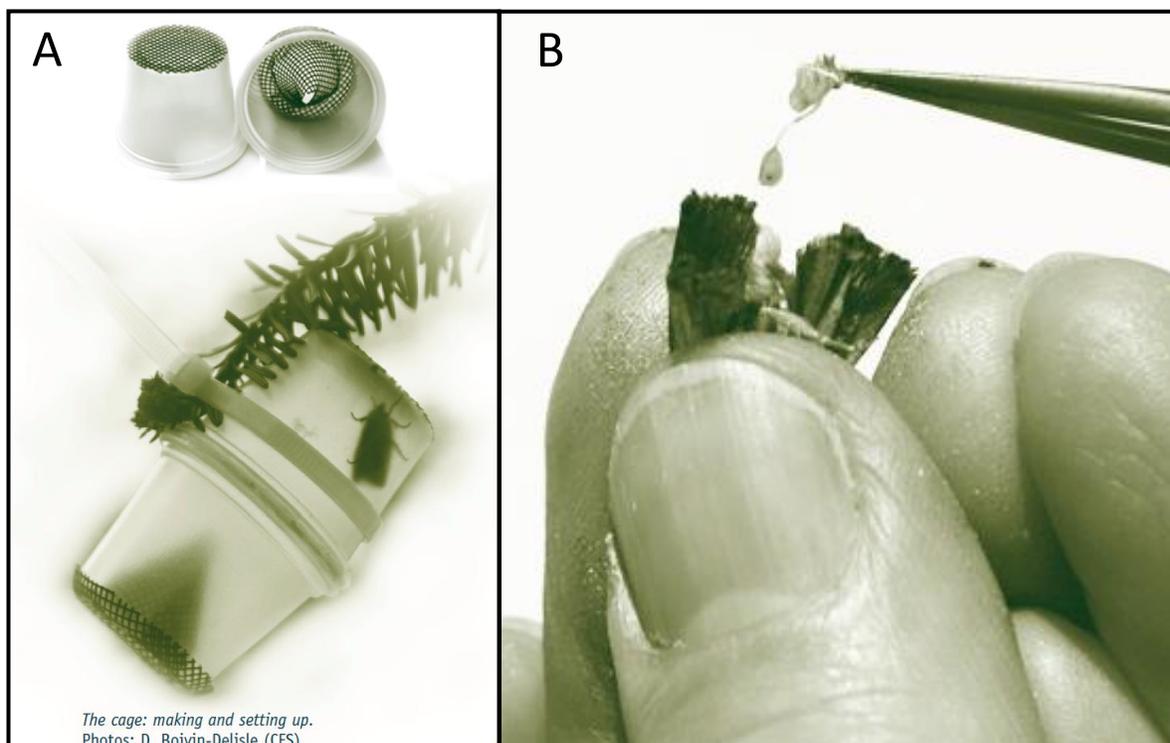


Figure 3. (A) VFB cages (Delisle 2008) ; (B) Extraction of the female reproductive system

2.4.4 Field life of the product

The half-life of the Hercon Disrupt Micro-Flakes under field conditions was evaluated using periodical sampling of ~20 micro-flakes on a screen after the application (1 hour, day 1 to day 20). The frozen samples were sent to CFS-AFC in New Brunswick for analysis. Data from these analyses were not yet available at the writing of this report.

2.4.5 Statistical analysis

Parameters evaluated during this study were subjected to an analysis of variance using PROC GLM and contrasts for multiple means comparisons. Percent data were subjected to arcsin square root transformation before analysis. Normality and variance homogeneity were verified using PROC UNIVARIATE. Further analyses were performed to (1) establish the relationship between number of males caught in the cages of virgin females and capture of males in pheromone traps (moth density), and (2) establish the relationship between number of males caught in the cages and the probability of a female being mated. Symbols and indices used repeatedly in the following paragraphs are defined here. Let n_{ijk} be the number of virgin females exposed in treatment i ($i=1,2,3,4$) and replicate j ($j=1,2,3,4$) at time k ($k=1, \dots, K$), s_{ijk} the number

of females mated, and c_{ijk} the number of cages in which at least one male was caught. Capture or mating success π_{ijk} are estimated by c_{ijk} / n_{ijk} or s_{ijk} / n_{ijk} , and were analyzed by logistic regression using the following initial model:

$$\text{Logit}(\pi_{ijk}) = a_i + b_i \ln(M_{ijk})$$

where a_i and b_i are the treatment-specific intercepts and slopes, and M_{ijk} is the number of male moths caught per day per pheromone trap in trial ijk with c_{ijk} or $s_{ijk} \in \text{Binomial}(n_{ijk}, \pi_{ijk})$.

Parameters were estimated by maximum likelihood, and the models (one for capture success, one for mating success) were reduced on the basis of Akaike's Information Criterion ($\text{AIC} = 2k - 2\ln L$) where L is maximum likelihood and k is the number of parameters in the model being estimated.

3. Results.

The mean dose used for the pheromone treatment was about 686 g of pheromone flakes per hectare (Table 2). Because large quantities of pheromone flake formulation were required during the calibration process, only 6 blocks out of 8 could be treated for mating disruption. Abiotic parameters measured during the different spraying sessions are presented in Table 2.

The effect of the pheromone application showed up clearly in pheromone-trap moth capture reduction, which varied from 80 to 99% (Figure 4, 5a). During the peak flight period (end of July), we captured 3500 male moths per period of 3 days per trap in the control blocks. Even after 39 days, a 95% male catch reduction was observed in the treated zone compared to the control area (Figure 4). However, the double applications of *Bacillus thuringiensis var kurstaki*, at the rate of 1.5 L/ha, had little detectable effect on moth capture in pheromone traps (Fig. 5a). Nevertheless, assuming that the *Btk* applications had some population reduction effect, it is possible that the interventions' efficacy was not detected in pheromone trap catches because of re-invasion of sprayed plots by males from neighbouring untreated areas.

Significantly fewer females from the pheromone treated blocks succeeded to attract males in the VFB cages when compared to females in the control blocks (Table 3; Figure 5b). The proportion of virgin-female cages in which at least one male was found was very strongly related to the moth capture rate in pheromone traps (Figure 6). The best model estimated a common regression line for the controls, *Btk*, and Disrupt treatments, and a somewhat distinct

line for the Btk+Disrupt treatment (Figure 6). The final model, with 4 parameters, had an AIC of 1779.4, compared to 1784.2 for a model where no treatments are distinguished. The final model, for controls, Btk and Disrupt alone was:

$$\text{Logit}(\pi) = -4.8 \pm (0.2) + 1.7(\pm 0.1) \ln(M)$$

and:

$$\text{Logit}(\pi) = -7.6 \pm (1.4) + 3.3(\pm 0.7) \ln(M)$$

for the Btk+Disrupt treatment. Thus, the Btk+Disrupt treatment may have had a more drastic effect of shutting down capture in the VFB cages than the other treatments, at low male moth densities. However, in view of the very wide scatter (variability) of the capture success data and the narrow range of male moth densities (measured by pheromone traps), this difference is not believed to have biological significance.

When females succeeded to attract males, significantly fewer males were found in the VFB cages from the treated blocks compared to cages in the control blocks (Tables 3). The relationship between number of males caught per cage and mating success (averaged on a trial basis), suggests a significant probability of male escape (Figure 7). In fact, 53 of the 214 mated females obtained during these trials had no male in their cage at the time of recovery from the field. However, once the males succeeded to reach the females in the cages, the same percentage of mated females was observed among the different treatments (Table 3).

The effect of the pheromone flakes application on mating success is also fairly obvious (Figures 1c, 8). But by our analysis, this effect did not go beyond that recorded with the reduction of catches in pheromone traps. The full, 8-parameter model had an AIC=855.6, while the best model (AIC=848.6) was also the simplest (2 parameters), where treatment effects were accounted for through pheromone trap catch reduction (disorientation):

$$\text{Logit}(\pi) = -4.7 \pm (0.5) + 1.6(\pm 0.2) \ln(M)$$

Once again, the effect of the pheromone treatment was clear, through the reduction in pheromone trap catch.

Finally, it is in the blocks that were treated with Btk and pheromone that we obtained significantly fewer number of egg masses compared to the other treatments (Table 4). However, there was no difference between treatments when we compared the number of L₂ per branch at the end of the summer.

Table 2. Data recorded during the aerial application of the pheromone micro-flakes

Date of treatment	Period	Block #	Product	Hectares	Dose/ha (g)	Beginning time	End time	Temperature (°C)	Relative humidity (%)
2008-07-11	AM	12	Disrupt MF SBW	52	1119,2	05:53	06:56	15	88
2008-07-11	PM	958	Disrupt MF SBW	50,75	699,5	18:52	19:40	19	53
2008-07-12	AM	8	Disrupt MF SBW	50,93	574,9	04:19	05:16	11	76
		959	Disrupt MF SBW	46,19	574,9	05:34	06:23	14	72
		95	Disrupt MF SBW	51,75	574,9	07:02	08:04	17	63
		10	Disrupt MF SBW	53,07	574,9	08:19	09:23	19	57

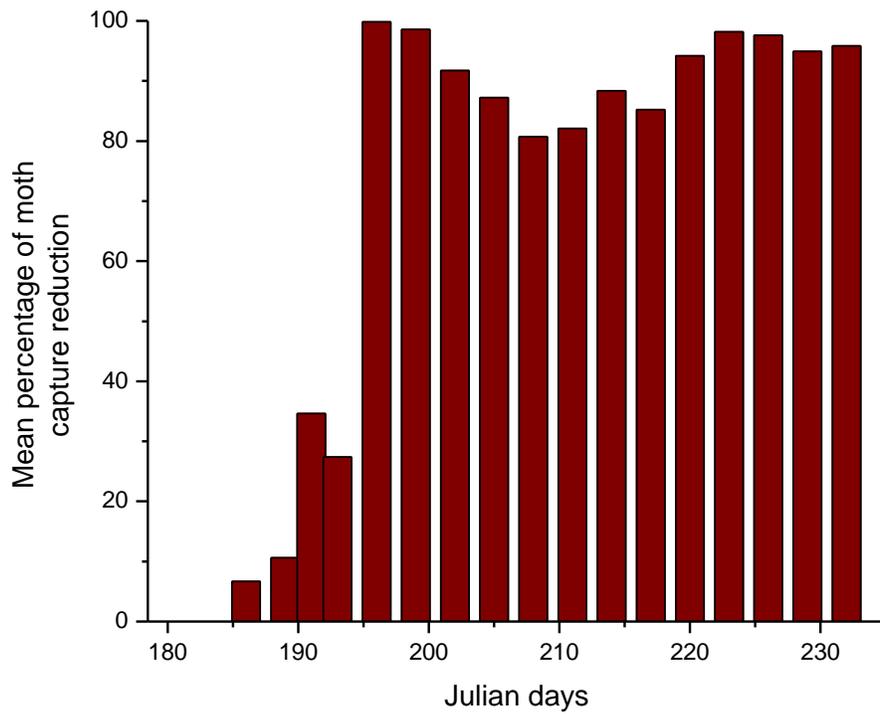


Figure 4. Impact of the pheromone treatment on moth capture following its application on Julian day 193. (% moth capture reduction = $[(\text{control}-\text{phero})/\text{control}] * 100$)

Table 3. Success of females from the different treatments to attract males in the VFB cages (mean \pm SE)

Parameters evaluated	Without pheromone		With pheromone		P value
	Control	Btk	Pheromone	Btk	
% cage with males	55.8 \pm 6.8 ^a	50.1 \pm 6.2 ^a	15.3 \pm 4.5 ^b	18.9 \pm 5.2 ^b	< 0.0001
No. male/cage	2.3 \pm 0.5 ^a	1.6 \pm 0.4 ^a	0.3 \pm 0.08 ^b	0.4 \pm 0.1 ^b	0.0002
% mated female	48.7 \pm 9.0 ^a	64.5 \pm 8.2 ^a	62.3 \pm 12.9 ^a	67.1 \pm 12.9 ^a	0.6056

Table 4. Impact of the pheromone treatment on the total mean number of egg masses per block and mean number of L₂ observed on branches (mean \pm SE)

Parameters evaluated	Without pheromone		With pheromone		P value
	Control	Btk	Pheromone	Btk	
No. egg masses/block	29.7 \pm 4.2 ^a	23.5 \pm 2.8 ^{ab}	38.6 \pm 5.6 ^a	19.5 \pm 2.6 ^b	0.0087
No. of L ₂ /branch	29.8 \pm 3.3 ^a	21.6 \pm 2.2 ^a	34.8 \pm 4.8 ^a	28.0 \pm 3.8 ^a	0.0621
No. of L ₂ /10m ² of foliage	1751.4 \pm 191.9 ^a	1312.9 \pm 139.8 ^a	2181.6 \pm 286.1 ^a	1602.4 \pm 213.1 ^a	0.0609

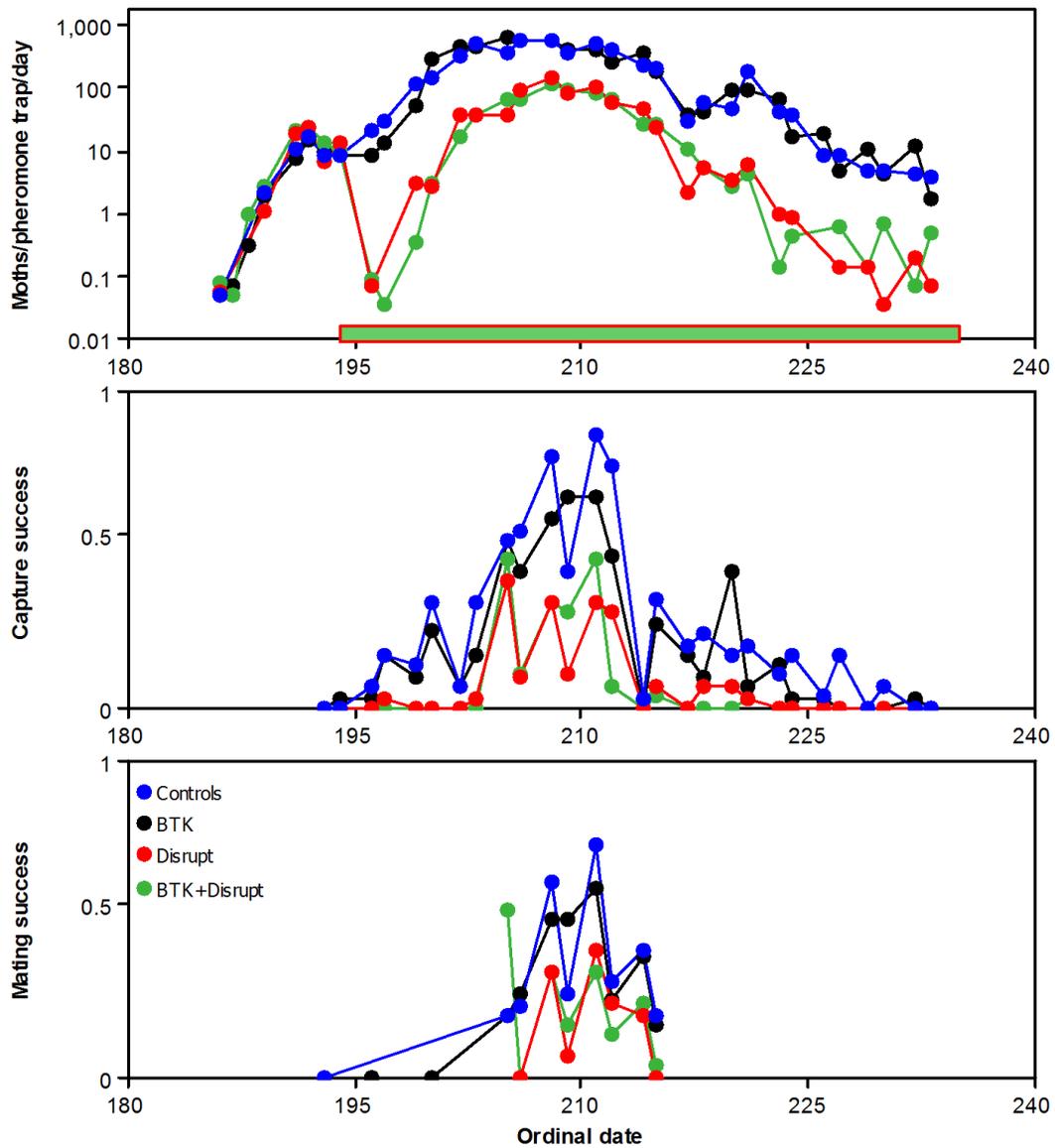


Figure 5. (a) Capture of male spruce budworm moths in pheromone traps (log sale) (green bar: period when pheromone flakes were present in treated plots). (b) Male capture success in virgin-female cages. (c) Mating success of caged females. ●: controls; ●: Btk; ●: Disrupt; ●: Btk+Disrupt.

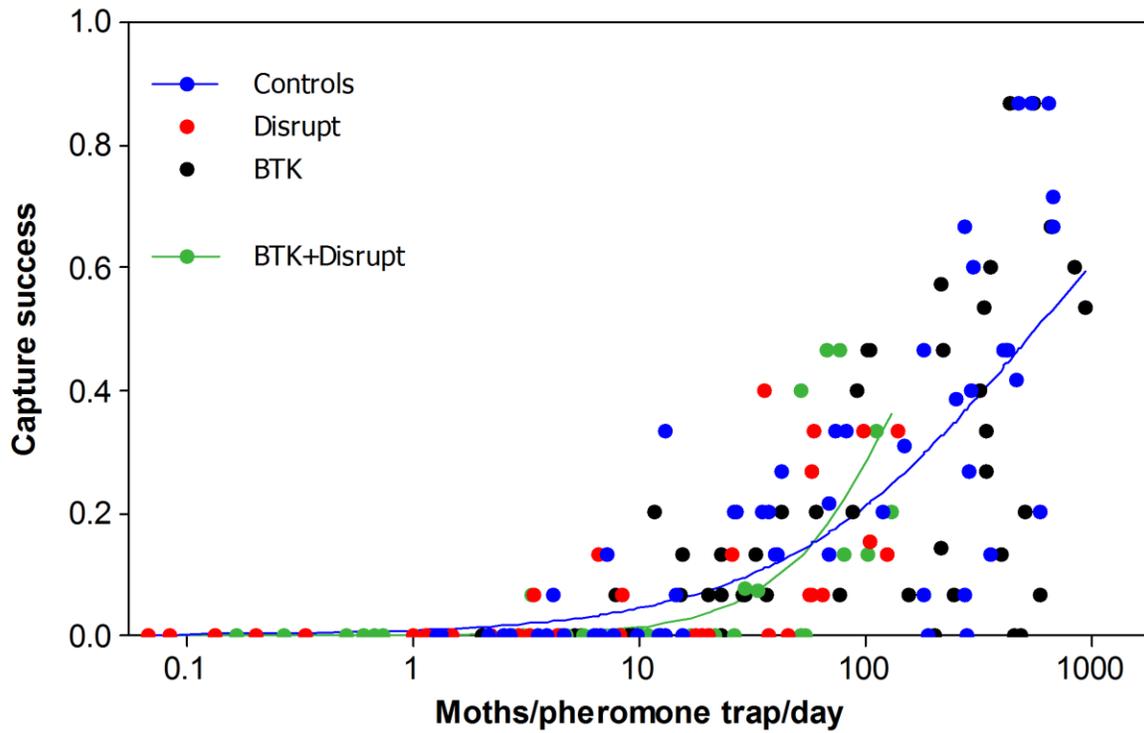


Figure 6. Male-capture success in virgin-female cages (proportion of cages containing at least one male), as a function of the capture rate of males in Multipher pheromone traps. ●: controls; ●: Btk; ●: Disrupt; ●: Btk+Disrupt. —: Regression line for controls, Btk and Disrupt. —: Regression line for Btk_Disrupt.

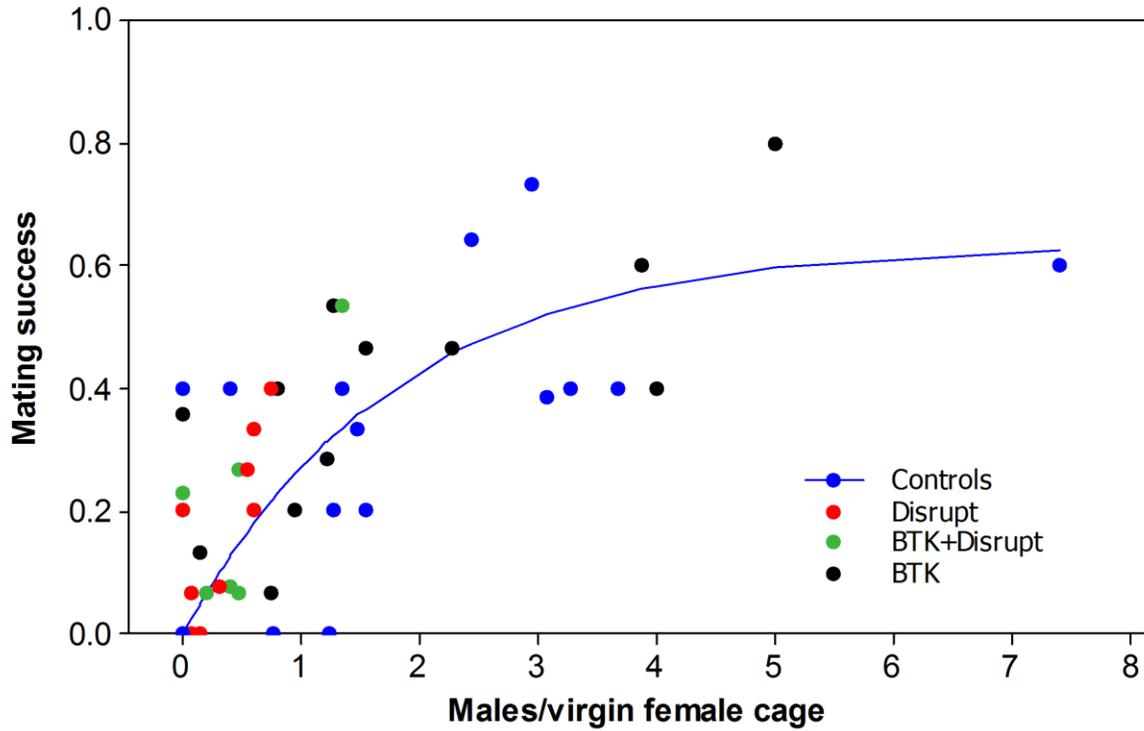


Figure 7. Mating success (proportion of mated females) as a function of average number of males caught per virgin-female cage. ●: controls; ●: Btk; ●: Disrupt; ●: Btk+Disrupt. —: Regression line for all treatments combined.

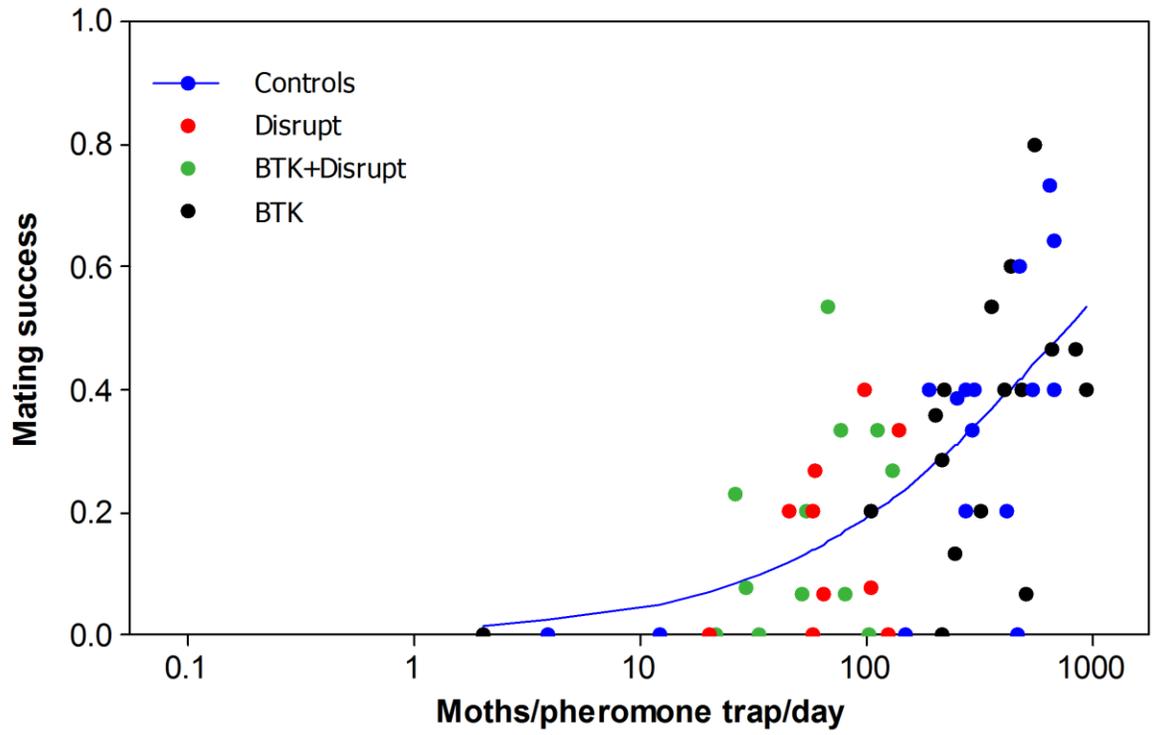


Figure 8. Mating success (proportion of mated females) as a function of the capture rate of males in Multiplier pheromone traps. ●: controls; ●: Btk; ●: Disrupt; ●: Btk+Disrupt. —: Regression line for all treatments combined.

4. Discussion

This field trial demonstrated that the aerial application of DISRUPT Micro-Flake SBW® could disrupt communication between male and female SBW, as shown by capture results in the Mutlipher traps and the VBF cages. Even if a dry formulation was used, it was possible to permeate the air in the treated blocks and induce a communication disruption effect. Moreover, further analyses on the relationship between male moth captures (population density) and capture success or mating success also revealed a significant impact. However, the main goal of that control strategy is to significantly reduce the SBW population level at the egg or L₂ stages, and ultimately limit defoliation and tree mortality in pheromone treated forest stands. During this field trial, in an outbreak situation, the use of pheromone applications alone over the 50 ha treated blocks did not to produce sufficient mating disruption in the SBW population to achieve this objective. Even though important differences were observed in moth catches due to treatment, the the dry application of the pheromone flakes was not sufficient to prevent mating within the wild population. Some hypotheses can be proposed to explain the lack of success of this control method during this field experiment:

4.1 Application of a dry formulation instead of using a sticker

The application of the pheromone flakes with glue, as prescribed, would have allowed to increase the number of flakes in the top part of the canopy (first third), where mating occurs most often. During this study, most of the flakes reached the ground with the dry application. This lack in the protocol might be a part of the reason why we did not reach the level of success desired.

Thorne et al. (2006) observed that trap catches and mating success of deployed females were higher in plots treated with flakes without sticker compared to the plots in which sticker was used, but the differences were not statistically significant. During that experiment, trap catch was reduced by 67 percent compared to controls in plots treated with flakes without sticker and by 90 percent in plots treated with flakes with sticker. Finally, mating success was reduced by 89 percent compared to controls in plots treated with flakes without sticker and by 99.5 percent in plots treated with flakes with sticker (Thorne et al. 2006).

4.2 Reinvasion of the study sites by already mated females

Another possibility to explain our results is that females that were mated outside the pheromone treated blocks would have later invaded the pheromone-treated blocks.

The migration ability of mated female SBW is discussed a lot among the scientific community but not much information is available in the literature to document this behavior. However, Régnière et al. (1999, pers. com.) conducted an experiment in the Outaouais region (Quebec) in which they evaluated the impact of female migration, calculated from egg masses found in relation to population density. The Outaouais SBW population level in these experimental blocks during this project was evaluated at 0.25 L/bud, that is quite similar to the SBW population levels on Quebec North Shore, which was 0.22 L/bud. In conclusion, this experiment revealed that the immigration rate observed in the Outaouais was 0.1 ± 0.02 eggs/bud, meaning that very few SBW females reinvaded the treated zones – following Btk applications. Although, in the present study it is possible that adult's movement had annihilate all effects on mating success and that mating success had not been pronounced enough to show through in the evaluation of egg masses and L₂ densities.

4.3 High level of SBW population in the study area

The last hypothesis is that the level of SBW populations in this trial was too high to demonstrate a population effect of mating disruption. The density of moths was so high that even with a successful disorientation due to the presence of high concentrations of artificial pheromone in the air of the treated plots, males could still find the calling virgin females and mate them. The disrupting effect of the pheromone application was not strong enough to sufficiently reduce the pheromone trap catch (an expression of the female-finding “pressure” applied by males in the plots). This control method was meant, as presented by Kettela and Silk (2006), “For use in early intervention management strategies”. In the present study, on the Quebec North Shore, the pheromone treated sites had an average of 28.5 larvae per branch, when surveyed in the 2007 fall. Study sites with lower levels of SBW infestation (eg < 10 larvae/branch) might provide better results. Further studies should be conducted to verify these hypotheses.

5. Conclusion

This project allowed us to test the efficacy of the Disrupt Micro-flakes SBW®, a new green product that was developed over the past decade to induce mating. The main goal of developing this new product was to provide additional pest management strategies for the repression of SBW populations. Mating disruption is a widely used and efficient control method that is applied everywhere around the world against several insect pests from different orders. However, the “SBW outbreak context” in which this control strategy was tested here does not represent an ideal situation for the use of mating disruption.

Although mating disruption trial failed from the point of view of population reduction, the mating success data do show a clear degree of mating disruption. These results are important from two points of view. First, they confirm that spruce budworm mating success is strongly density dependent, and that mating success can be expected to drop considerably in populations where the pheromone trap capture rate drops below 10 moths per trap per day.

The second finding is that the application of pheromone as a mating disruption tool can be efficacious under appropriate population circumstances. When considered as an early intervention tool, this control strategy should be exploited in low density SBW populations. Used in such manner, it might allow maintaining SBW populations at endemic levels and protect trees from severe defoliation and growth loss. In addition, it would be complementary with microbial larvicide spaying operations since these control strategies are occurring at different periods of the season. Indeed, it would be possible to concentrate control efforts on larvae in the month of June in forest stands with severe SBW outbreaks and latter in July, consider conducting mating disruption operations against adult as an early intervention strategy in low SBW population sites. This pest management strategy would constitute a powerful tool for forest pest managers in reducing the impacts of SBW and improving forest productivity.

6. Recommendations

Even though no reduction in egg mass or L₂ SBW populations were obtained in this first large scale operational trial, mating disruption should be submitted to additional experiments.

This experiment allowed us to identify a strong relationship between mating success and SBW population density. This phenomenon provides an indication of the level of population reduction and sexual disorientation required to achieve successful mating disruption. Interventions, whether through efficacious pesticides or pheromone applications (or both) show promise in considerably reducing mating success, as long as the density of the male moth populations (as measured with pheromone traps) is dropped sufficiently low, and there is a low risk of surrounding populations (males or females) re-invading the treated area.

We believe these conditions can be met in the early stages of outbreak development when high populations occur in isolated areas (“hot spots”). Thus, if additional mating disruption efficacy tests are to be carried out, these conditions should be met. However, even once efficacy has been demonstrated (population reduction in the egg or L₂), it is not clear how this tactic could be usefully deployed in an integrated population management strategy.

The main issue that needs to be resolved is whether “hot spot” control would present any advantage over the current foliage protection strategy. Could it slow the development of an outbreak? Could it prevent an outbreak from developing further? To answer these questions we need to understand the processes that lead to outbreak development: natural enemies, mating success and moth immigration (males and females) as determinants of population growth rates in rising outbreaks. In other words, what makes a budworm population emerge from the endemic state and whether that process is reversible through population management.

References cited

- Baker, T. C., B. S. Hansson, C. Löfstedt, and J. Löfqvist. 1989.** Adaptation of male moth antennal neurons in a pheromone plume is associated with cessation of pheromone mediated light. *Chem. Senses* 14: 439-448.
- Bartell, R. J. 1982.** Mechanisms of communication disruption by pheromones in control of Lepidoptera: a review. *Physiol. Entomol.* 7: 353-364.
- Bartell, R. J., and W. L. Roelofs. 1973.** Inhibition of sexual response in males of the moth *Argyrotaenia velutinana* by brief exposures to synthetic pheromone or its geometric isomer. *J. Insect Physiol.* 19: 655-661.
- Byers, J. A. 2007.** Simulation of mating disruption and mass trapping with competitive attraction and camouflage. *Environ. Entomol.* 36: 1328-1338.
- Cardé, R.T. 1990.** Principles of mating disruption, pp. 47-71. *In* R. L. Ridgway and R. M. Silverstein (eds.), *Behaviormodifying chemicals for pest management: applications of pheromones and other attractants*. Marcel Dekker, New York.
- Cardé, R. T., and A. K. Minks. 1995.** Control of moth pests by mating disruption: successes and constraints. *Annu. Rev. Entomol.* 40: 559-585.
- Cardé, R. T., R. T. Staten, and A. Mafrá-Neto. 1998.** Behavior of pink bollworm males near high-dose, point sources of pheromone in field wind tunnels: insights into mechanisms of mating disruption. *Entomol. Exp. Appl.* 89: 35-46.
- Daterman, G. E., L. L. Sower, and C. Sartwell. 1982.** Challenges in the use of pheromones for managing western forest Lepidoptera, pp. 243-254. *In* B. Leonhardt and M. Beroza (eds.), *Insect pheromone technology: chemistry and applications*. American Chemical Society, Washington, DC.
- Delisle, J. 2008** A tool for evaluating mating disruption in spruce budworm: CFS forges ahead. Canadian Forest Service, Laurentian Forestry Centre, Quebec QC, Canada. *Branching out* 43.
- Evenden, M. L., G.J.R. Judd, and J. H. Borden. 2000.** Investigations of mechanisms of pheromone communication disruption of *Choristoneura rosaceana* (Harris) in a wind tunnel. *J. Insect Behav.* 13: 499-510.
- Figueredo, A. J., and T. C. Baker. 1992.** Reduction of the response to sex pheromone in the oriental fruit moth, *Grapholitha molesta* (Lepidoptera: Tortricidae) following successive pheromonal exposures. *J. Insect Behav.* 5: 347-362.

- Gut, L. J., L. L. Stelinski, D. R. Thompson, and J. R. Miller. 2004.** Behaviour-modifying chemicals: prospects and constraints in IPM, pp. 73-121. *In* O. Koul, G. S. Dhaliwal, and G. W. Cuperus (eds.), *Integrated pest management: potential, constraints, and challenges*. CABI Publishing, Cambridge, MA.
- Judd, G.J.R., M.G.T. Gardiner, N. C. Delury, and G. Karg. 2005.** Reduced antennal sensitivity, behavioural response, and attraction of male codling moths, *Cydia pomonella*, to their pheromone (*E,E*)-8,10-dodecadien-1-ol following various pre-exposure regimes. *Entomol. Exp. Appl.* 114: 65-78.
- Ketella, E.G. and P.J. Silk. 2006.** Development and testing of pheromone formulations for use in early intervention management strategies of the spruce budworm. *In* the Proceeding of the SERG International and USDA Forest Service Workshop, Banff, Alberta, February 13-16, 2006, pp. 19-26.
- Kuenen, L.P.S., and T. C. Baker. 1981.** Habituation versus sensory adaptation as the cause of reduced attraction following pulsed and constant sex pheromone pre-exposure in *Trichoplusia ni*. *J. Insect Physiol.* 27: 721-726.
- Mani, E., and R. Schwaller. 1992.** Results of 12 years experience to control codling moth, *Cydia pomonella* L. by mating disruption. *IOBC/WPRS Bull.* 15: 76-80.
- MacLean, D.A. 1980.** Vulnerability of fir-spruce stands during uncontrolled spruce budworm outbreaks: a review and discussion. *Forestry Chronicles*, 56, 213–221.
- Miller, J. R., L. J. Gut, F. M. de Lame, and L. L. Stelinski. 2006a.** Differentiation of competitive vs. non-competitive mechanisms mediating disruption of moth sexual communication by point sources of sex pheromone (part). *Theory. J. Chem. Ecol.* 32: 2089-2114.
- Miller, J. R., L. J. Gut, F. M. de Lame, and L. L. Stelinski. 2006b.** Differentiation of competitive vs. non-competitive mechanisms mediating disruption of moth sexual communication by point sources of sex pheromone (part 2): case studies. *J. Chem. Ecol.* 32: 2115-2143.
- Nakamura, K., and K. Kawasaki. 1977.** The active space of the *Spodoptera litura* (F.) sex pheromone and the pheromone component determining this space. *Appl. Entomol. Zool.* 12: 162-177.
- Rumbo, E. R., and R. A. Vickers. 1997.** Prolonged adaptation as possible mating disruption mechanism in oriental fruit moth, *Cydia (Grapholita) molesta*. *J. Chem. Ecol.* 23: 445-457.

- Sanders, C.J. 1991.** Biology of North American spruce budworms. Tortricid Pests, Their Biology, Natural Enemies and Control (ed. by L. P. S. van der Geest and H. H. Evenhuis), pp. 579–620. Elsevier Science Publishers BV, The Netherlands.
- Shorey, H. H. 1977.** Manipulation of insect pests of agricultural crops, pp. 353-367. *In* H. H. Shorey and J. J. McKelvey, Jr. (eds.), Chemical control of insect behaviour: theory and application. Wiley, New York.
- Shorey H. H., L. K. Gaston, and C. K. Saario. 1967.** Sex pheromones of noctuid moths. XIV. Feasibility of behavioral control by disrupting pheromone communication in cabbage loopers. *J. Econ. Entomol.* 60: 1541-1545.
- Stelinski, L. L., J. R. Miller, and L. J. Gut. 2003.** Presence of long-lasting peripheral adaptation in the oblique-banded leafroller, *Choristoneura rosaceana* and absence of such adaptation in redbanded leafroller, *Argyrotaenia velutinana*. *J. Chem. Ecol.* 29:405-423.
- Stelinski, L. L., L. J. Gut, A. V. Pierzchala, and J. R. Miller. 2004.** Field observations quantifying attraction of four tortricid moths to high-dosage pheromone dispensers in untreated and pheromone-treated orchards. *Entomol. Exp. Appl.* 113: 187-196.
- Thomson, D., J. Brunner, L. Gut, G. Judd, and A. Knight. 2001.** Ten years implementing codling moth mating disruption in the orchards of Washington and British Columbia: starting right and managing for success. *IOBC/ WPRS Bull.* 24: 23-30.
- Thorpe K., R. Reardon, K. Tcheslavskaja, D. Leonard, and V. Mastro. 2006.** A Review of the Use of Mating Disruption to Manage Gypsy Moth, *Lymantria dispar* (L.). U.S. Department of Agriculture – Forest Service, Forest Health Technology, Enterprise Team – Morgantown. Report No. FHTET-2006-13.