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

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Abstract

A research project was conducted during the summer 2008 in the Baie-Comeau region, on the Quebec North shore, in order to evaluate the efficacy of a new pest management tool that was registered against the spruce budworm (SBW). This pest management tool consists in the aerial application of pheromone micro-flakes in order 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) control blocks. The application of pheromone micro-flakes was carried out in the beginning of the moth flight period in order to disrupt communication between males and females and then limit mating success. The results obtained during the months of July and August allowed to observe that SBW male moths were significantly more disoriented during the mating period 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). These data showed that even though pheromone flakes were applied in the treated blocks, it has been possible for males to localize pheromone traps (which simulated females), but in significantly lesser quantity.

Concerning the egg masses and second instar (L_2) surveys, these data showed a lot less differences. In 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 L_2, 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, as observed in the different treated blocks, adults did not need chemical signals (pheromones) to locate each other since there were so many moths flying during the mating period. This control method would present more conclusive results when use 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 projet de recherche 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 (pratiquement 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.

Pour ce qui est du suivi des masses d'œuf et des larves de stade 2 (L_2), ces données présentent beaucoup moins de différences. En moyenne, un nombre total de 29 masses d'œuf ont été récolté 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 des peuplements forestiers en situation épidémique, la très grandes quantités de papillons volant à proximité les uns des autres durant la période d'accouplement fait en sorte qu'ils n'ont pas besoin de signaux chimiques (phéromones) pour se localiser. 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.

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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 in order 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 survival when used alone and in synchrony.

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 L_2 trough branch sampling (Fall 2007).

2.2 Experimental design

This study required the use of 16 fifty-hectare blocks in order 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

In order to obtain optimum synchronization of treatments, the seasonal development of the TBE was monitored every two (2) days from the 2^{nd} 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 in order 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.

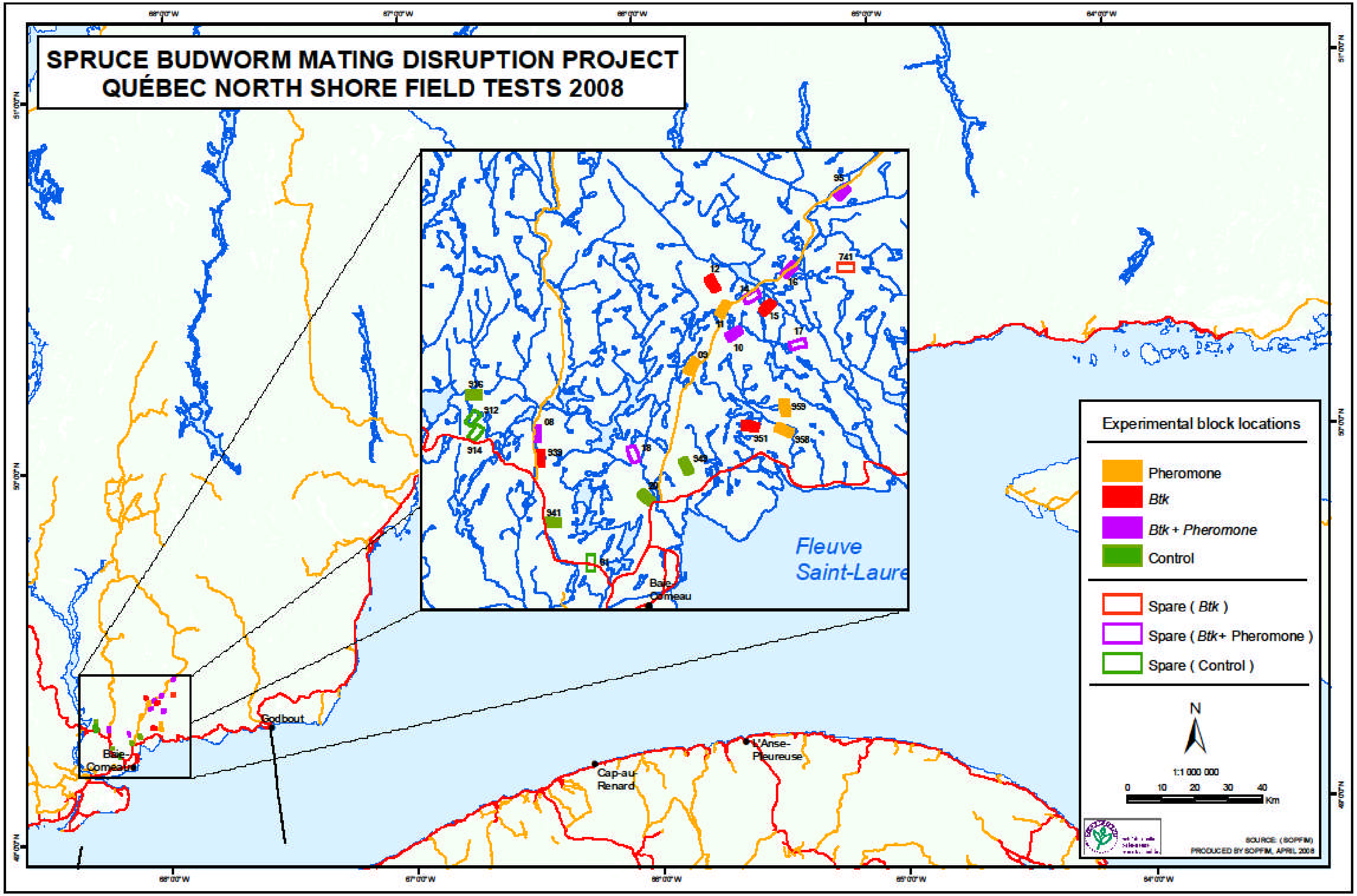

Figure 1. Localisation of the study area on Quebec North shore, near Baie-Comeau

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 speader; 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% pupae stage)
- Egg mass survey
- L_2 survey

2.4.2 Egg mass and hibernating larvae survey (L_2) 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 were characterized as fertile, unfertile, parasitized or emerged.

During the fall season, 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 (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 Multipher trap as cage holder. The females and cages were collected and replaced every 3 days for the evaluation of male presence and for spermatophore frequency analysis. Collected females from VFB cages were brought back to the lab and each of them were individually frozen for further mating status analysis (Figure 3B).

Following the pheromone flake applications, 5 baited Multipher traps installed in the experimental blocks were used to evaluate the level and the persistence of the communication disruption according treatments. All the traps were emptied at each 3 days until the end of the flight period to assess the effect of pheromone application on male capture levels and to document the moth stage seasonal activity pattern. The lures in the Multipher traps were replaced during the first week of July.

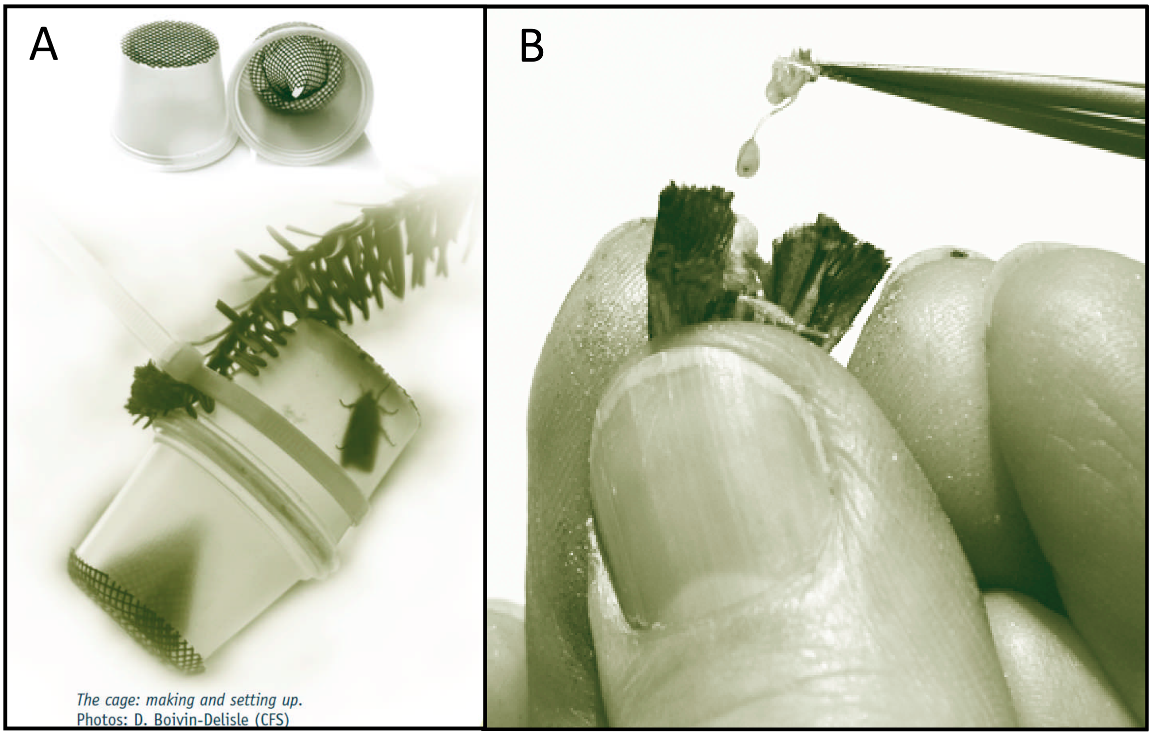

Figure 3. (A) VFB cages (Delisle 2008) ; (B) Extraction of the female reproductive system

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. Information on the analyse procedures were not available at the writing of the report

3. Results.

The mean dose used for the pheromone treatment was about 686 g of pheromone flakes per hectare (Table 2). Since greater quantities of pheromone flake formulation were required for the calibration process, only 6 blocks out of 8 were treated for mating disruption. Abiotic parameters measured during the different spraying sessions are presented in Table 2. A major decrease in male moth catches were observed after only few days following the application of the pheromone treatment (Figure 4). During this experiment, the percentage of moth capture reduction due to the pheromone application varied from 80 to 99% (Figure 5). It is in the peak flight period that we obtained the greater number of moths in the baited traps that were placed in the experimental blocks. During this period (end of July), we captured 3500 male moths per period of 3 days in a single baited traps 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 3).

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). In addition, 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). 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).

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 more difference between treatments when we compared the number of L_2 per branches 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

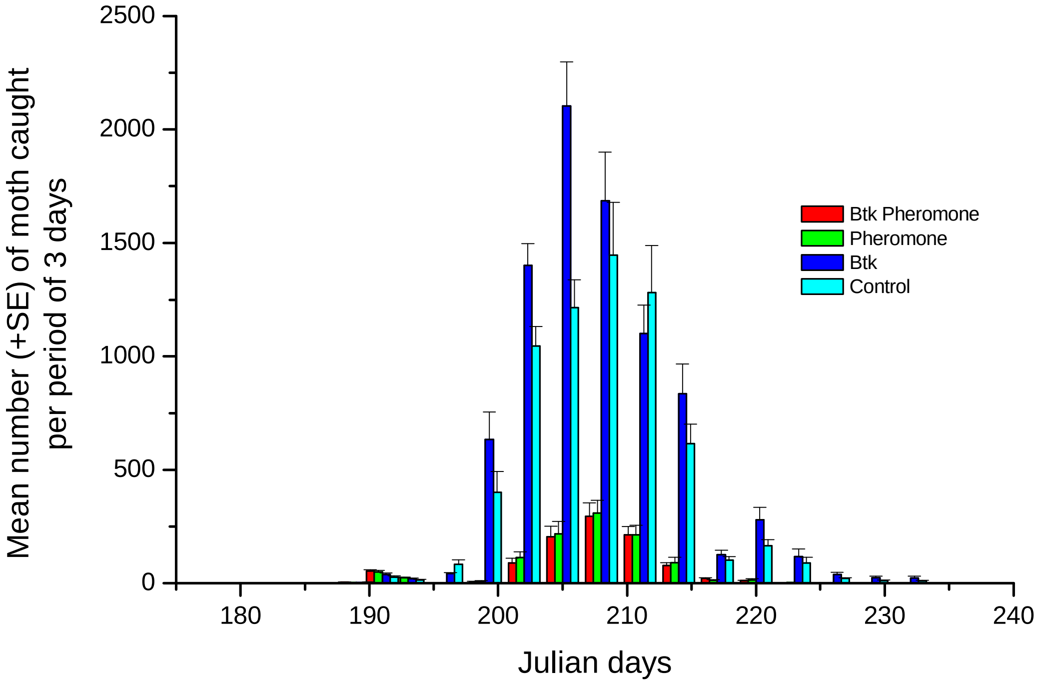

Figure 4. Moth captures in baited Multipher traps according to treatments over the experimental period – pheromone application on Julian day 193

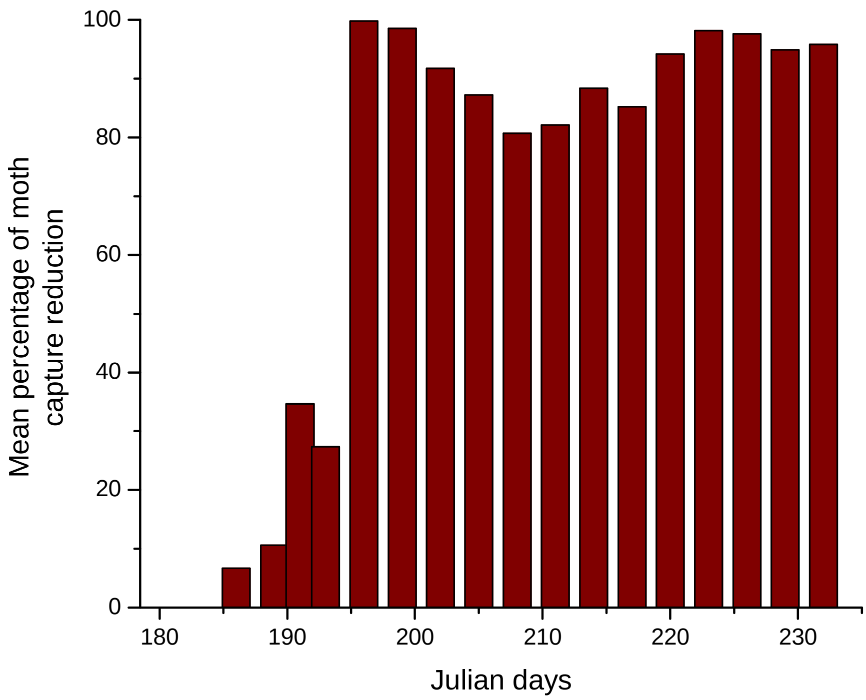

Figure 5. Impact of the pheromone treatment on moth capture following its application on Julian day 193. (% moth capture reduction = [(control-phero)/control]*100)

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

Pamameters evaluated	Without pheromone		With pheromone		*P* value
	Control	Btk	Pheromone	Btk	
% cage with males	55.8 ± 6.8[a]	50.1 ± 6.2[a]	15.3 ± 4.5[b]	18.9 ± 5.2[b]	< 0.0001
No. male/cage	2.3 ± 0.5[a]	1.6 ± 0.4[a]	0.3 ± 0.08[b]	0.4 ± 0.1[b]	0.0002
% mated female	48.7 ± 9.0[a]	64.5 ± 8.2[a]	62.3 ± 12.9[a]	67.1 ± 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 L_2 observed on branches (mean ± SE)

Pamameters evaluated	Without pheromone		With pheromone		*P* value
	Control	Btk	Pheromone	Btk	
No. egg masses/block	29.7 ± 4.2[a]	23.5 ± 2.8[ab]	38.6 ± 5.6[a]	19.5 ± 2.6[b]	0.0087
No. of L2/branch	29.8 ± 3.3[a]	21.6 ± 2.2[a]	34.8 ± 4.8[a]	28.0 ± 3.8[a]	0.0621
No. of L2/10m^2 of foliage	1751.4 ± 191.9[a]	1312.9 ± 139.8[a]	2181.6 ± 286.1[a]	1602.4± 213.1[a]	0.0609

4. Discussion

This field trial succeeded to demonstrate 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 has been possible to saturate the air in the treated blocks and induce a communication disruption effect. However, the main goal of that control strategy is to significantly limit the increase of the SBW population level, by reducing L_2 populations, 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 seem to produce mating disruption in the SBW population. Even though a lot of differences were observed in moth catches according to treatments, the pheromone permeated air as produced by the dry application of the flakes was not enough to prevent mating among the population. Some hypotheses were raised in order to be able to explain the lack of success of this control method during this field experiment:

4.1 Application of the dry formulation instead of applying it with the sticker

The application of the pheromone flakes with the glue, as it was prescribed, would have allowed to increase the number of flakes in the top part of the canopy (first third), where mating occurs mainly. 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.

However, Thorne et al. (2006) observed that trap catches and mating success of deployed females were higher in the plots treated with flakes without sticker compared to the plots in which sticker was used, but the differences were not statistically significant. During this 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 these results would be the reinvasion impact of mated females in the study sites, meaning that females that were mated outside the pheromone treated blocks would have later invaded these 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, what 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. For another Trotricid moth, the European grapevine moth, *Lobesia botrana*, it was demonstrated that females could disperse at a distance of 80 m once mated (Schmitz et al. 1996). Furthermore, a mating disruption trial was conducted by Trudel et al. (2006) against the spruce seed moth, *Cydia strobilella* (Leppidoptera: Tortricidae), in a white spruce seed orchard near Drummondville (Quebec). In this experimental design, the pheromone treated block was about 100 m away from the control block in the seed orchard and a significant reduction of 62.1% of cone damage was recorded in the pheromone treated block, when compared to the control block (Trudel et al. 2006). This result allow us to assume that very few females moved from the control block to the treated block, once mated, in their search for an oviposition site.

4.3 High level of SBW population in the study area

The last hypothesis, the high level of SBW population in the pheromone treated blocks, would appear to be the most responsible for that lack of success. With so high SBW population levels, as we observed in our different treated blocks, adults do not even need chemical signals (pheromones) to locate each other since there are so many moths flying all around. 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. Using study sites with lower level of SBW infestation (< 10 larvae/branch) might allow obtaining better results. Further studies like this one should be repeated in order to verify these hypotheses.

5. Conclusion and Recommendations

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 in order to induce mating disruption among pheromone treated populations. The main goal behind these efforts in developing a new pesticide 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 and against several insect pests from different orders. However, the "SBW outbreak context" in which this control strategy was tested during this study appeared to do not represent an ideal situation to obtain optimal results for the use of mating disruption.

As considered as an early intervention tool, this control strategy should be exploited in low density SBW populations. Used in such manner, it would allow maintaining SBW populations at endemic levels and protect trees from severe defoliation and growth losses. Furthermore, 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.

Even though no conclusive results were obtained in reducing SBW population – in terms of egg masses and L_2 – for this first large scale operational trails, considerations should be given to mating disruption for further demonstration experiments. This pest management strategy constitutes a powerful tool that could help forest pest managers to reduce the impacts of SBW and contribute to improve forest productivity.

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