



## Use of *Trichogramma* and *Bt* for control of *Pieris rapae* in cabbage on Prince Edward Island

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### ABSTRACT

Organic cabbage growers on Prince Edward Island have limited means to control *Pieris rapae* (Lepidoptera: Pieridae), their main lepidopteran pest in cabbage. The use of *Bt* (*Bacillus thuringiensis* subsp. *kurstaki*) or spinosad products (e.g., Entrust) are common practice but repeated applications can become expensive, while parasitoid wasps (*Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae)) could provide an economically feasible alternative if shown to be effective. Eight plots were established in an organic cabbage field and encircled with a fence to reduce dispersion between treatments. Four treatments (control, *Bt*, *Bt*+*Trichogramma* release, and *Trichogramma* release) were set up in these plots during the 2014 growing season. The proportion of marketable cabbages was significantly reduced in control plots compared with other treatments. Further, *Bt*+*Trichogramma* treatment resulted in significantly higher proportion of marketable cabbages than *Trichogramma* alone. No significant differences were observed among treatments for cabbage head weight or size. Economics and potential of *Trichogramma* in an integrated pest management strategy for organic cabbage production are discussed.

### RÉSUMÉ

Les producteurs de choux organiques de l'Île-du-Prince-Édouard ont peu de moyens pour contrôler *Pieris rapae* (Lepidoptera : Pieridae), leur principal lépidoptère ravageur du chou. L'utilisation de *Bt* (*Bacillus thuringiensis* ssp. *kurstaki*) ou de produits à base de spinosad (par exemple, Entrust) est pratique courante, mais des applications répétées peuvent devenir coûteuses, alors que des guêpes parasitoïdes (*Trichogramma brassicae* Bezdenko (Hymenoptera : Trichogrammatidae)) pourraient offrir une alternative économiquement viable si leur efficacité pouvait être démontrée. Huit parcelles ont été établies dans un champ de choux organiques et furent entourées d'une clôture dans le but de réduire la dispersion entre les traitements. Quatre traitements (contrôle, *Bt*, *Bt* + libération de *Trichogramma*, et libération de *Trichogramma*) ont été mis en place dans ces parcelles pendant la saison de croissance 2014. La proportion des choux commercialisables a été significativement réduite dans les parcelles témoins par comparaison aux autres traitements. De plus, le traitement *Bt* + *Trichogramma* a produit une proportion significativement plus élevée de choux commercialisables que *Trichogramma* seul. Aucune différence significative n'a été observée entre les traitements en lien avec le poids ou la taille des têtes de chou. Les aspects économiques et le potentiel de *Trichogramma* dans une stratégie de gestion intégrée des ravageurs pour la production de chou biologique sont discutés.

### INTRODUCTION

*Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae) is the main lepidopteran pest affecting cabbage production on Prince Edward Island (PEI) and organic producers have limited means to protect their crop. Other pests attacking cabbage are the cabbage looper (*Trichoplusia ni* Hübner) (Lepidoptera: Noctuidae), diamondback moth, (*Plutella xylostella* L.) (Lepidoptera: Plutellidae), thrips (*Thrips tabaci* Lindeman) (Thysanoptera: Thripidae) and cabbage

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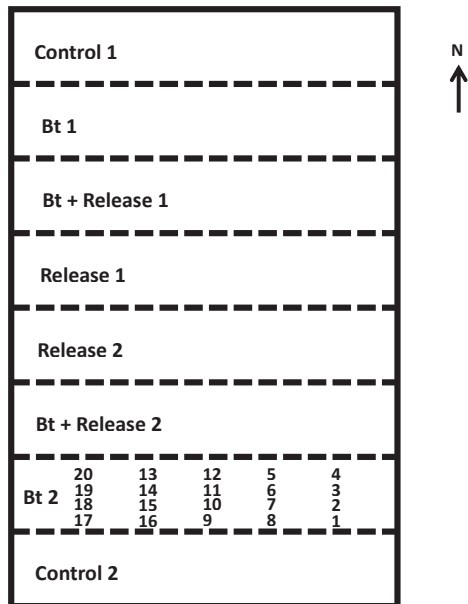
flea beetle (*Phyllotreta albionica* LeConte) (Coleoptera: Chrysomelidae), with *Pieris rapae* causing the most damage. Without a control, 80% of cabbage heads can be rendered unmarketable due to lepidopteran feeding. Throughout this study, *Trichoplusia ni* and *Plutella xylostella* were only encountered a couple times, therefore, results reported here will focus on *Pieris rapae* only. Application of *Bt* (*Bacillus thuringiensis* subsp. *kurstaki*) (i.e., Dipel 2X DF), or products containing spinosad (i.e., Entrust) are the two main controls available to organic producers. Dipel 2X (*Bt*) provides good control but does not last long in the field while Entrust provides longer term benefit but is more expensive to use. Some growers use a combination of Entrust and Dipel 2X to protect their crop. Effective and economic alternative control methods are desired to ensure sustainability of production. While *Trichogramma* wasps (Hymenoptera: Trichogrammatidae) are an ecologically sound and sustainable biological control method in many parts of the world, *Trichogramma* has not been used by cabbage producers on PEI. *Trichogramma* spp. have been effectively used for control of *Helicoverpa* sp. (Lepidoptera: Noctuidae) in tomato, okra or chili in India (Krishnamoorthy 2012) and against *Erinnyis ello* (L.) (Lepidoptera: Sphingidae) in cassava in Brazil (Soares et al. 2014). In Germany, *Trichogramma brassicae* Bezdenko is used against European corn borer (*Ostrinia nubilalis* Hübner) (Lepidoptera: Crambidae) on 11,000 hectares of corn; *Trichogramma cacoeciae* Marchal and *Trichogramma dendrolimi* (Matsumura) are used against codling moth (*Cydia pomonella* L.) (Lepidoptera: Tortricidae) and plum moth (*Grapholita funebrana* Treits.) (Lepidoptera: Tortricidae) in tree fruits; *Trichogramma evanescens* Westwood is used against cabbage pests; and, other *Trichogramma* species are used against vine moths (*Lobesia botrana* (Den. & Schiff.) and *Eupoecilia ambiguella* (Hübner) (Lepidoptera: Tortricidae)) on grapes (Zimmerman 2004). In China, nearly 4 million hectares of corn are managed for Asian corn borer (*Ostrinia furnacalis* Guen.) (Lepidoptera: Crambidae) using *Trichogramma dendrolimi*, *Trichogramma chilonis* Ishii and *Trichogramma ostrinae* Pang and Chen (Wang et al. 2014). In cabbage, Lundgren et al. (2002) evaluated *Trichogramma brassicae* in the USA and more recently, Stoleru et al. (2012) evaluated *Bt* and *Trichogramma evanescens* in Romania. Results were promising but showed variety and planting timing to influence results. Integration of *Bt* with *Trichogramma* was shown to be effective against tomato fruitworm (*Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae)) and European corn borer, reducing damage

by 6% and 20%, respectively, over control plots (Oatman et al. 1983 and Losey et al. 1995). Cabbage producers on PEI were interested in evaluating *Trichogramma* as a method to control lepidopteran pests. The objective of this work was to evaluate the use of *Trichogramma brassicae* through application in an organic cabbage field as follows: *Trichogramma* release+*Bt*, *Trichogramma* release, and *Bt* alone for control of *Pieris rapae*.

## METHODS

The 1.0-ha field site was located in Greenvale, Prince Edward Island (46°20'53.40"N, 63°18'31.96"W). Cabbage plants, variety Lennox, were transplanted on 9 July 2014. An application of Entrust 80W, Dow Chemical, (108.7 g/ha) was applied to the field on 5 August to remove any *Pieris rapae* larvae prior to the study. Eight treatment plots were established on 7 August running across the width of the field, each plot measuring 14.5 m x 8.5 m containing 10 rows of cabbage (Figure 1). Rows were spaced ca. 86 cm apart and cabbages spaced 30 cm apart within the rows. Cabbage plants, 20/plot, were monitored for *Pieris rapae* throughout the season; they were flagged on 7 August and cleaned of any larvae. The 20 flagged cabbages per plot were selected as follows: 4 cabbages per row from rows 4-8, with 4 cabbages located between flagged cabbages within the row. Flagged cabbages were surveyed for eggs and larvae twice per week until harvest on 14 October. Eggs were counted and circled during each survey, not removed between surveys and not counted if empty. Larvae were counted but not removed between survey dates. There were 2 replicates of each treatment: control (no *Bt*, no *Trichogramma*), *Bt* only, *Bt* + *Trichogramma*, and *Trichogramma* only. Treatments were arranged to reduce interference from other treatments, reduce contamination of the control and *Bt* plots, and for ease of *Bt* application for the grower. As such, the control plots were on either edge of the field, with treatment plots in the following order moving into the field: *Bt* only, *Bt* + *Trichogramma*, and *Trichogramma* only (centre of the field, see Figure 1). The enclosures around each plot were ca. 120 cm high and consisted of Tyvek house wrapping stapled to 5 cm x 5 cm posts spaced ca. 150 cm apart. Each plot was fully enclosed and separated from the others using this fencing. The fences were installed to reduce movement of *Trichogramma* between plots as PEI can be windy and *Trichogramma* are known to be affected by strong winds (Yu et al. 1984; Fournier and Boivin 2000; Lundgren et al. 2002). *Trichogramma* cards (Tricho-Gard) were obtained from Anatis BioProtection (278 rang Saint-André, Saint-

**Figure 1:** Layout of the plot showing treatments and placement of the sampled cabbages (1-20). Although shown only in the Bt 2 plot, the same cabbage placement was used in each treatment plot.



Jacques-le-Mineur, Quebec), placed into plots starting 14 August and replaced every 7-10 days until 29 September. Each *Trichogramma* and *Trichogramma* + *Bt* plot received 1 card based on the recommendation of Anatis BioProtection where 1 card is used for 200 m<sup>2</sup> and each card contained 8000 *Trichogramma brassicae* eggs. Dipel 2X DF, Valent Canada, was applied to *Bt* and *Bt* + *Trichogramma* plots at a rate of 544 g/ha on 29 August, 4 September, and 11 September. At harvest, all 20 cabbage heads were visually inspected for damage and qualitatively categorized as either marketable or non-marketable. Cabbages evaluated as non-marketable displayed obvious feeding damage from *Pieris rapae*. Marketable heads were weighed and measured.

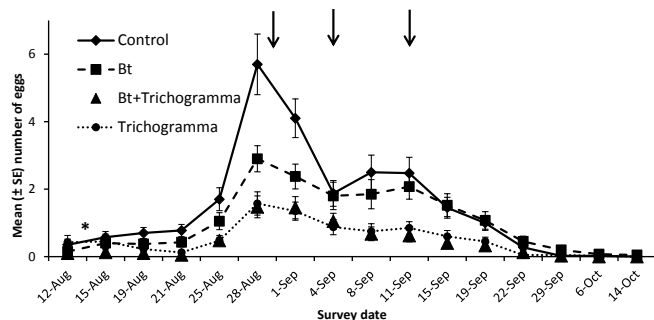
All analyses were performed using R statistical software (R Core Team, 2012). Given the non-randomized layout of the treatment plots within the field, analysis was conducted to determine if there was a pre-treatment bias in egg counts across the plots. We wanted to establish whether adult butterflies had equal access to all cabbages within the study, or if the enclosures affected their access to parts of the field. As the same cabbages were surveyed repeatedly over time, egg counts were treated as a repeated measures, Poisson-distributed time series variable analysed using a generalized linear mixed model function (GLMM) in R (glmer) with sampling date and plot as random variables and with treatment as a fixed variable. The treatment

chosen to be the comparative or base case was the control. The model was restricted to the first 6 sampling dates (12 – 28 August) before the first application of *Bt* on 29 August. Pairwise comparisons of the coefficients were carried out using Tukey's HSD (multcomp package) with  $\alpha=0.05$ . Coefficient estimates from the analysis were exponentiated to give estimates of the mean densities per treatment. Larval counts were treated the same as egg counts but with no restriction on sampling dates. Results of the egg count analysis showed a statistically significant difference between the control plot egg counts compared to the other treatments (Table 1). Closer inspection of the data revealed that 75% of the outliers occurred in the first 2 rows of plot 'control 1'. To offset the effects of the bias in the control plots, the larval analysis was carried out using egg counts as a covariate. Dispersion values for the egg and larval models were 1.13 and 1.08, respectively, so we considered that no correction for over dispersion was required. Marketable and unmarketable cabbage heads were pooled across plots and analysed using a 2x4 contingency analysis on the proportions followed by Fisher's pairwise comparison with Bonferroni adjustment. For the marketable cabbages, head weight and size were compared between treatments using an ANOVA. The economics of using *Trichogramma* was evaluated by comparing the cost per hectare when using *Trichogramma* cards compared with the current controls of Entrust or Dipel 2X. Due to the bias in the pre-treatment egg counts, the authors recognize that the following results should be interpreted with caution.

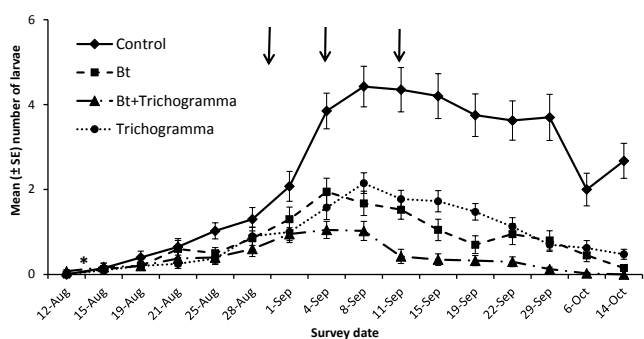
## RESULTS

Adult *Pieris rapae* were observed flying throughout all plots over the course of the study. *Pieris rapae* eggs and larvae were surveyed from 12 August through 14 October inclusive (Figures 2 and 3). The pattern of oviposition during the first 6 survey dates (prior to Dipel 2X application) showed a bias towards the edge plots (controls) (Figure 2 and Table 1). Eggs were significantly more abundant in the control plot than in either the *Bt*+*Trichogramma* or *Trichogramma* alone plots (GLMM, Table 1). The number of eggs in the *Bt* alone plot was not significantly different from the control plot or either of the other two treatments. Similar results were obtained for larval counts; significantly higher counts were observed in the control plot than the other treatment plots. The lowest number of larvae were counted in the *Bt*+*Trichogramma* treatment (GLMM, Table 2). For both egg and larval counts, numbers tapered off starting in early September with minimal eggs and larvae found on the cabbages by early October. At harvest, percentage of

**Figure 2:** Mean ( $\pm$  SE) number of *Pieris rapae* eggs found on cabbage heads over the growing season in 2014. Arrows denote application of Dipel 2X (Bt) on 29 Aug, 4 Sept, and 11 Sept. \* denotes first introduction of *Trichogramma* cards.



**Figure 3:** Mean ( $\pm$  SE) number of *Pieris rapae* larvae found on cabbage heads over the growing in 2014. Arrows indicate application of Dipel 2X (Bt) on 29 Aug, 4 Sep and 11 Sep. \* denotes first introduction of *Trichogramma* cards.



**Table 1:** Regression table of fixed effect coefficients from generalized linear mixed model analysis of *Pieris rapae* eggs on cabbages grown on PEI within plots for the first six survey dates. Random effects variance (standard deviation): Date – 0.16 (0.39), Location – 0.71 (0.84).

	Estimate (log)	Expected count*	P value
Control (intercept)	-0.00049	0.99	0.999 a
Bt	-0.59335	0.55	0.144 ab
Bt+Trichogramma	-1.47938	0.23	0.0003 b
Trichogramma	-1.07747	0.34	0.008 b

\*Expected counts indicate the relative change in egg counts within each treatment. For example, an increase of 1 egg count in the field will result in a 0.99 increase of eggs in the Control and a 0.34 increase in eggs in the *Trichogramma* plot.

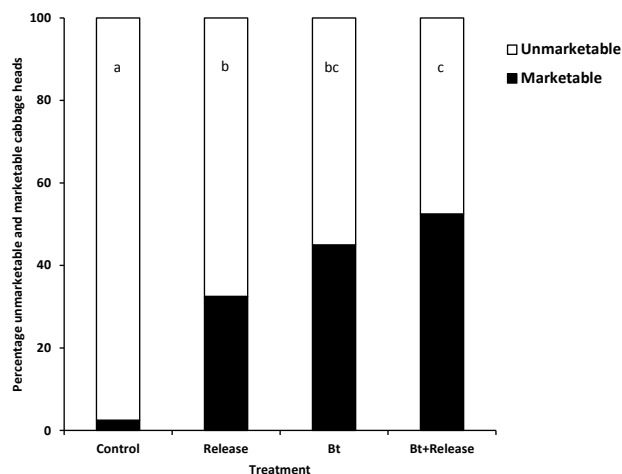
marketable heads was lowest and significantly so for the control plots compared with all other treatment plots (Figure 4). The highest percentage of marketable heads was obtained from the *Bt+Trichogramma* plots, significantly greater than the control and the *Trichogramma* alone

**Table 2:** Regression table of fixed effect coefficients from general linear mixed model analysis of *Pieris rapae* larval counts with egg as a covariate on cabbages grown on PEI. Random effects variance (standard deviation): Date – 1.28 (1.13), Location – 0.11 (0.35), Eggs – 0.002 (0.013).

	Estimate (log)	Expected count*	P value
Control	0.39	1.48	0.295 a
Bt (intercept)	-1.03	0.36	0.003 b
Bt+Trichogramma	-1.71	0.18	1.2E <sup>-6</sup> b
Trichogramma	-0.98	0.37	0.005 b

\*Expected counts indicate the relative change in larval counts within each treatment. For example, an increase of 1 larvae in the field will result in an increase of 1.48 larvae in the Control plots and an increase of 0.37 larvae in the *Trichogramma* plot.

**Figure 4:** Percentage of unmarketable and marketable cabbage heads in plots treated with *Trichogramma* or *Bt* or both during 2014 in an organic cabbage field. The proportion of marketable heads was significantly different among treatments ( $\chi^2=65.6$ ,  $P<0.0001$ ). Bars with the same letter not significantly different (Fishers's pairwise test using Bonferroni adjustment).



plots. Use of *Trichogramma* alone was not significantly different from using *Bt* alone. There was no significant impact of any treatment on head weight or size (Table 3). It is worth noting that there was only 1 marketable cabbage from the control plots and it was approximately 30% of the weight of cabbages from the other plots.

## DISCUSSION

Our results are consistent with an earlier study by Lundgren et al. (2002) who found the use of *Trichogramma* to be less effective than *Bt* in controlling lepidopterans. *Trichogramma* was released into those plots when, on average, 10 *Pieris rapae* larvae per cabbage head were present. Our study introduced *Trichogramma* when there were less than 2 larvae per cabbage head present.

**Table 3:** Mean head weight and dimensions (polar and equator) of marketable cabbage heads. There were no significant differences between treatments ( $F_{3,47}=0.79$ ,  $P=0.50$ ,  $F_{3,48}=0.79$ ,  $P=0.50$ ,  $F_{3,48}=0.45$ ,  $P=0.72$  for head weight, polar and equator circumference, respectively).

Treatment	Head weight /g	Polar diam. /cm	Equator diam. /cm
Control	392.0	7.0	6.0
Bt	1064.7	15.4	12.9
Bt + <i>Trichogramma</i>	1070.6	15.4	12.9
<i>Trichogramma</i>	1086.4	15.4	13.1

Lundgren used cards containing ~ 5,400 parasitized eggs in their 9 m x 12 m plots, while this study had cards containing ~8,000 eggs per 8 m x 14 m plot. While these differences may be subtle, they may have been significant enough to cause the difference in results obtained. Lundgren et al. (2000) found larval levels in *Trichogramma* plots to equal those found in the control, which is counter to our results where *Bt+Trichogramma* did reduce larval populations when compared with the control. Lundgren also showed no increase in marketable cabbage heads with the use of *Trichogramma*.

Stoleru et al. (2012) evaluated *Bt* and *Trichogramma evanescens* in organic cabbage fields in Romania. The mid-season varieties yielded high harvest weight when treated with *Trichogramma evanescens* at 120,000 individuals/ha while the late season varieties showed no benefit of *Trichogramma evanescens* when compared with the other treatments. Given that Lennox is a late season variety, and PEI has a short growing season, it is possible that *Trichogramma* experienced less optimal conditions for foraging in the field toward the end of the field season. *Trichogramma* spp. require appropriate temperature and plant structure to provide optimal control. Romário de Carvalho et al. (2014) found temperature to play an important role in the foraging behavior of *Trichogramma pretiosum* Riley and Pak and vanHeiningen (1985) found field temperature to influence behavior in *Trichogramma* spp. with *Trichogramma pretiosum* performing best at 24 °C - 27 °C. At 18 °C or 21 °C, parasitism did occur, but at lower rates than parasitism occurring at higher temperatures. In the PEI field during 2014, mean daily temperatures were above 18 °C up to 7 September. After this point, the average daily temperature dropped to between 12 °C and 15 °C with only 2 days reaching an average temperature of 18 °C. This could have influenced the hatching rate and subsequent foraging behavior of *Trichogramma* during the latter part of this study. During the study done by Lundgren et al. (2002), the mean daily temperature was approximately 21 °C, and similarly in Romania, Stoleru et al. (2012) had

temperatures above 20 °C for the duration of the mid-season crop but this dropped toward the end of their study. As our study utilized a late season cabbage variety, further study could utilize short season varieties. These may benefit from using *Trichogramma* as exposure time would be reduced and daily temperatures would be more suitable for *Trichogramma* foraging throughout the growing period.

There was some concern that the architecture and spacing of the cabbage plants would negatively influence *Trichogramma* distribution within the plot. Cabbages were planted with a spacing of 30 cm in early July. By early August, when *Trichogramma* was released, the plants were considerably larger and had leaves well into the gap between plants. Although the canopy was not dense, it is expected that *Trichogramma* would have been able to move between plants within a plot. As other studies using *Trichogramma* in cabbage (Stoleru et al. 2012) reported success, it is more likely that length of season and temperature contributed to the results obtained in our study.

Economically, the use of *Trichogramma* alone is a moderately expensive management option (Table 4). Dipel 2X is the least expensive option costing \$163.85/ha when used three times during the season. Addition of *Trichogramma*, as used in this study (three applications of Dipel 2X and five sets of cards), increases the cost to \$551.35/ha, with no significant increase in marketable cabbage heads. These options are less expensive than using Entrust, a spinosad product, on its own (\$612.85/ha). Another option used by growers is to alternate Entrust and Dipel 2X with two applications each, costing \$517.81/ha. Clearly Dipel 2X is the least expensive option, however, there is definitely room to improve upon the percentage of marketable heads at harvest. Should growers wish to use *Trichogramma* for control of *Pieris rapae*, further study of *Trichogramma* on PEI, incorporating shorter season varieties, is required before its use becomes economically feasible. A short season variety, e.g., one that needs only 40-60 days to reach maturity, would mean fewer cards to be deployed and, if planted early in the season, would ensure that hatching *Trichogramma* are exposed to adequate temperatures for foraging. Reducing the number of sets of cards from 5 to 2 or 3 would make use of *Trichogramma* either alone or in combination with Dipel 2X more cost effective method for control of *Pieris rapae*.

This study found the best treatment for control of *Pieris rapae* in organic cabbage to be *Bt+Trichogramma*, however these results must be taken with caution due to the lack of randomization in the plot layout. Further study using a randomized plot design could confirm the

**Table 4:** Economic comparison of control methods for *Pieris rapae* in organic cabbage production on PEI.

Product	Unit cost	Application rate	# of applications required or allowed	Cost/ha
Entrust	\$561.20/1L	364 mL/ha	3	\$612.85
Dipel 2X DF	\$50.20/500 g	544 g/ha	3	\$163.85
<i>Trichogramma</i>	\$1.55/card	50 cards/ha	5	\$387.50
<i>Trichogramma</i> + Dipel			3 Dipel + 5x cards	\$551.35
Entrust + Dipel			2 Entrust + 2 Dipel	\$517.81

results obtained here. Plot layout may have influenced oviposition, and further study is warranted to confirm the results obtained from this study. This study combined *Bt* sprays (Dipel 2X) with *Trichogramma* release, as growers were interested in knowing if combining the two treatments would yield an additive effect and increase percentage of marketable heads at harvest. Results from this study show that a combination of *Bt*+*Trichogramma* will lead to a significant increase in marketable cabbage heads over the controls and *Trichogramma* alone, however, the increase is not large enough to offset the cost of both products. Until further trials have confirmed the potential of *Trichogramma* for control of *Pieris rapae*, organic cabbage growers on PEI should continue to use Dipel 2X.

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