Field testing the microbial tool (*Bacillus thuringiensis* subsp. *aizawai*) against caterpillar pests in cole crops
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Executive Summary

The management of caterpillar pests for organic cole crop production is reliant on *Bacillus thuringiensis* spp. *krustaki* (Btk) and spinosad-based insecticides. However, there are concerns around the organic formulations of these two active ingredients. Specifically, resistance is a concern with Btk and non-target effects on pollinators, and parasitoid wasps are a concern with spinosad. Another active ingredient that could be added to alleviate some of these concerns, is *Bacillus thuringiensis* spp. *aizawai* (Bta). Several formulations of Bta are registered in the US for caterpillar control and approved for organic production, including the product XenTari™. XenTari is not currently registered in Canada, however efficacy trials are being conducted on canola. The goal of this work was to collect additional data on minor use crops like head and stem cole crops. In this study we evaluated the efficacy of XenTari in field plots of kale at three different rates - 500g/ha, 750g/ha, and 1000 g/ha, against the organic industry standard Dipel (750g/ha) and a water only Control. Plots were sprayed three times and counted five times post-spray. Overall, the number of caterpillars observed in XenTari medium and high rate plots was significantly lower than the Control. Further, the total number of caterpillars recovered in post spray counts was similar between the XenTari-treatments and Dipel. Pest pressure, however, was low in the field plots and the trial should be repeated earlier in the field season (June or July) when caterpillar counts are higher. Results of this study will be shared with the registrant and will hopefully support a broad label in Canada.

Introduction and Objectives

Pest management for organic cole crop production in the Fraser Valley is a challenge because of the diversity of arthropod pests that feed below ground (cabbage root maggot - *Delia radicum*) and above ground (cabbage aphid - *Brevicoryne brassicae*, green peach aphid - *Myzus persicae*, crucifer flea beetle - *Phyllotreta crucifera*, western flower thrips - *Franklinella occidentalis*, imported cabbage worm - *Pieris rapae*, cabbage looper - *Trichoplusia ni*, and diamondback moth - *Plutella xylostella*). The caterpillar pests - imported cabbage worm, cabbage looper and diamondback - are problems for very young plants because they can cause enough defoliation to stunt growth (Delahaut and Newenhouse 1997). Caterpillars are also a problem for mature crops because the larvae or their droppings (frass) can contaminate the harvestable heads and foliage of cole crops (Delahaut and Newenhouse 1997).

Cultural controls, like crop rotation and timing of plantings, are of limited efficacy for caterpillars because adults can fly in from surrounding areas (Chaput 1990, Steffan and Whitaker 1996) and are active for most of the growing season in the Fraser Valley (May through to early October). Biological controls are available both commercially and naturally (Dosdall *et al*. 2012). There has been a substantial amount of work done on enhancing biological control by naturally occurring species (Steffan and Whitaker 1996, Landis *et al*. 2000). However, even in organic systems, with habitat for natural enemies, biological control may not be enough to prevent economic damage by caterpillars. Insecticides - approved for organic production via the Organic Materials Review Institute (OMRI) - are also available. For organic production, sprays are recommended when caterpillar infestations reach 30 to 50% on young plants (Fig. 1; Delahaut and Newenhouse 1997). A much lower threshold of 10% is used for both young plants (transplants or seed beds) and plants starting to mature (Fig. 1; Delahaut and Newenhouse 1997). Once caterpillars are established in a field, sprays may be needed on a weekly basis (Fig. 1). For control of caterpillar pests growers currently have two active ingredients - *Bacillus thuringiensis* spp. *krustaki* (Btk) and spinosad.

Bacillus thuringiensis spp. *krustaki* is a widely used insecticide for caterpillar control in both organic and conventional production. Resistance to Btk has been documented in diamondback moth (Tabashnik *et al.* 1997) and cabbage looper (Janmaat and Myers 2003). Locally, there is concern that diamondback populations are becoming less susceptible to Btk, however this has not been confirmed (E.S. Cropconsult Ltd. unpublished data). Spinosad is another active ingredient with the formulation Entrust™, (Dow Agrosciences Inc.) which is approved for organic production. Spinosad has a different mode of action than Btk and organic growers can use Entrust in rotation with Btk-products to delay resistance development. Additionally, spinosad has some efficacy on other pests of cole crops like beetles and thrips (Sparks *et al*. 2001). However, there are concerns about resistance to Entrust (Zhao *et al*. 2006) and the impact of spinosad based-products on pollinators (Biondi *et al*. 2012) and Hymenopteran natural enemies, like parasitoid wasps (Liu and Zhang 2012). An additional tool would be beneficial for organic brassica production, in order to avoid resistance and other concerns when using the currently used products and in order to maintain high production standards.

Bacillus thuringiensis spp. *aizawai* (Bta) is a candidate active ingredient for caterpillar control in organic cole crop production that is not currently registered in Canada. It is registered in the US and has been used widely in rotation with other products for caterpillar control in organic crops (Rowell and Bessin 2005). In particular, Bta has a different mode of action than Btk and therefore the two can be used in rotation. The formulation XenTari™(Valent Biosciences Corporation) is approved for organic agriculture in the US (Rowell and Bessin 2005). In Canada, trials in canola have been successfully completed, with good efficacy shown against diamondback moth (Anon. 2013).

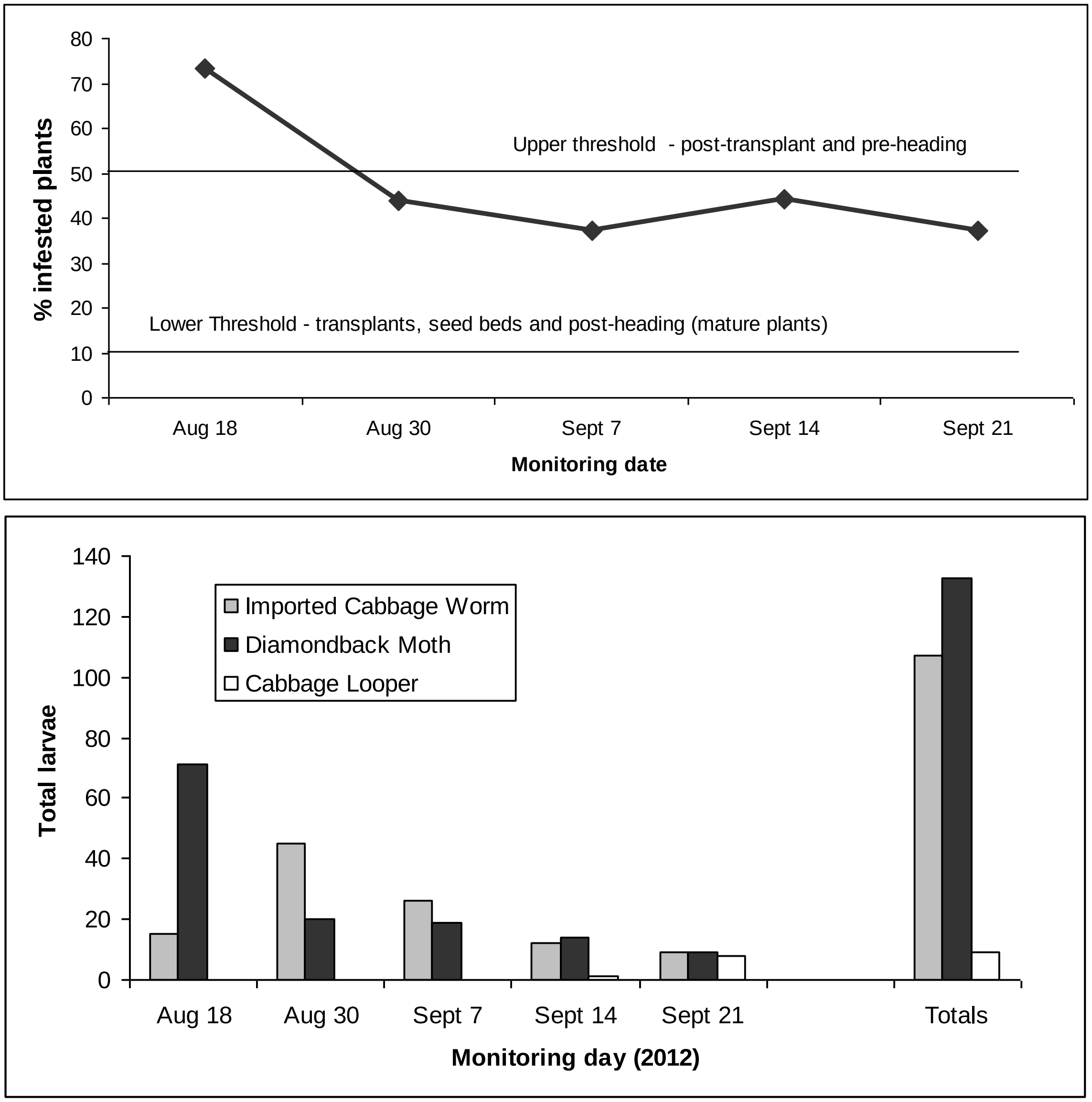

Figure 1. Caterpillar infestation levels (top) and counts (bottom) from crop monitoring of a 10-acre organic broccoli and cabbage planting in Chilliwack, BC, in 2012. Infestation levels and counts are based on observations of 60 plants/date. (E.S. Cropconsult Ltd. unpublished data).

Methods

Study location - The study was conducted at a mixed use (vegetable, berry and ornamental) farm in Chilliwack, BC, on piece of land that was in vegetable production earlier in the season. The site was selected as it met the Pest Management Regulatory Agency's research notification criteria for field testing an unregistered product. Specifically the site had a controlled access point, the surrounding plant material was not used for human or animal consumption, and the site could be secured. The area for the trial had not been previously sprayed with conventional insecticides or fungicides, however because of heavy weed pressure (Fig. 2) conventional herbicides were applied prior to planting.

Plot description and pest inoculation - The study was conducted between August 30 and October 11, 2013 and was repeated three times, with each of the three runs planted one week apart. We chose this time frame to mimic the timing of late season head crucifer plantings in the Fraser Valley. One month old kale (var. Winterbor) transplants were manually planted out into the field site on August 30, September 7 and September 14, 2013. We planted five plants/plot, that were spaced 20-cm apart. A 1-m gap was left between plots and all plots for each run were planted in either one or two rows (Fig. 2).

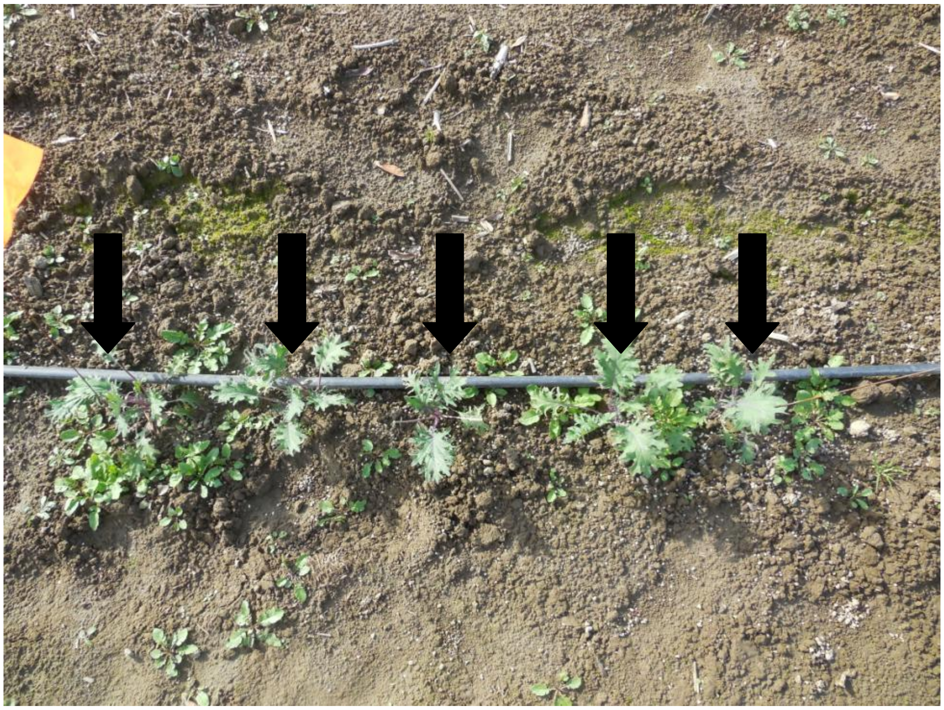

Figure 2. An individual plot for field testing the efficacy of XenTari for caterpillar control in cole crops. Plots were 1-m long, with 20-cm spacing between kale plants. Arrows drawn in to help separate kale plants from surrounding weeds. (Photo: R. Prasad)

After planting, transplants were watered with drip irrigation for three hours. The trial area was also treated with slug bait (iron phosphate) after transplant and then on an as needed basis for the trial duration. The trial area was adjacent to an over mature mixed brassica bed that the land owner left as a source of Lepidopteran pests for our trial. However to ensure that there was even pest pressure through out the trial area, diamondback larvae were collected from commercial rutabaga fields and transferred to our study area. Larvae were collected, and held in the fridge (8^{o}C) for one to two days prior to release into the trial area. A single second or third instar larva was transferred to the middle plant in each plot one day prior to the first spray and after the pre-treatment count. We chose to inoculate with diamondback larvae because this pest has, in previous seasons, been the most abundant, compared to the other caterpillar species in organic broccoli plantings in Chilliwack in late August and September (Fig. 1).

Experimental design and application of treatments - The study was designed as a randomized complete block experiment. Each of the three runs of the trial were treated as separate time blocks. There were five treatments (Table 1) each replicated six times for a total of 30 plots/run and a total of 90 plots for the entire study. Treatments were randomly assigned to plots just prior to doing the pre-treatment counts. Products were applied to plots with a single nozzle backsprayer hand-pumped to maintain full pressure and with a swath-width of 1-m for a total plot area of $1m^2$. Because of the low volume of solution required to treat each plot (Table 1) we mixed the spray solution for all six plots for each treatment and time application to ensure even coverage among plots. Treatments were applied in the late afternoon or early evening to minimize UV damage to the products (Griego and Spence 1978). Products were applied on both the upper and lower surfaces of leaves to ensure thorough coverage but to avoid run-off. No spreaders or stickers were used. Each run of the trial was treated three times and products were applied at 5 to 10 day intervals depending on weather conditions (Table 2). Plots were assessed five times - once prior to product application and pest inoculation, after each application, and one week after the final spray (Table 2).

Table 1. Treatment details (rate, amount of product and amount of water) for field testing efficacy of XenTari for caterpillar control on cole crops. Total plot area was $1m^2$

Treatment and Rate	Amount of product/plot	Amount of water/plot
Industry Standard Dipel 750 g/ha	0.075 g	50 mL
XenTari Low rate - 500g/ha	0.05 g	50 mL
XenTari Medium rate - 750 g/ha	0.075 g	50 mL
XenTari Medium rate - 1000 g/ha	0.10 g	50 mL
Water only Control	N/A	50 mL

Assessment and analysis - To assess the effect of product applications we counted the foliage on every plant in each plot. Both the top and underside of each leaf was checked and all of the larvae and pupae observed were counted. Both live and dead individuals were counted. Dead individuals were all larvae and these were removed once they were counted so as not to recount them. All live individuals were left unharmed. All Lepidoptera species encountered were counted - imported cabbage worm, diamondback larvae, cabbage loopers, and cutworms. We also recorded the number of parasitoid wasps and other natural enemies observed in plots. Any larvae or pupae encountered on the final count were collected and held for 2-weeks to observe emergence of natural enemies.

The effect of XenTari treatment on caterpillars was examined using two-way (Run X Treatment) repeated measures MANOVA. We also compared the effect of treatments on the total number of larvae observed on all post-treatment counts using one-way ANOVA. We did not analyze the data for Run 1 because a third of the plots could not be assessed for the final three counts because all plants had either died or were severely stunted. These plots were in an area of the trial that was inadvertently under watered during the first 10 days of the trials. We did not statistically analyze the effect of treatments on the number of dead larvae because the overall number of dead larvae observed was very low. Dead larvae were not observed in the majority of plots for Run 2 and 3; i.e. 17 and 23 out of 30 plots, respectively, without dead larvae. Finally, we did not analyze data collected from plots 2-weeks post the last treatment (i.e. Post Count 5 - Table 2), because there was a noticeable decline in plant quality across all plots due to slug feeding, resulting in uneven foliage and possibly confounding caterpillar counts. *Post-hoc* means comparisons were done using Tukey-Kramer HSD test. All data were analyzed using JMP-In Version 5.1 (SAS Institute, Chicago, IL).

Table 2. Schedule of spray and assessment activities for field evaluation of XenTari for caterpillar control in cole crops.

	Run 1	**Run 2**	**Run 3**
Activity			
Pretreatment Count	Sept 12	Sept 18	Sept 18
Pest Inoculation	Sept 13	Sept 18	Sept 18
Spray 1	Sept 14	Sept 19	Sept 19
Post Count 1	Sept 19	Sept 24	Sept 24
Spray 2	Sept 19	Sept 25	Sept 25
Post Count 2*	Sept 25	Oct 4	Oct 4
Spray 3	Sept 25	Oct 4	Oct 4
Post Count 3	Oct 4	Oct 7	Oct 7
Post Count 4	N/A	Oct 11	Oct 11
Post Count 5	N/A	Oct 18	Oct 18

*Post count 2 was delayed due to heavy rains from September 27 to October 2, 2013.

Results

Significantly more larvae were recovered from Run 2 than Run 3 ($F(1,50)=5.96$, $p=0.02$), however this difference did not impact treatment differences which were significant (Fig. 3; $F(4,50)=7.04$, $p=0.0001$) and consistent between the two runs ($F(4,50)=0.87$, $p=0.49$) and over the course of the trial (Time X Run: $F(3,48)=0.57$, $p=0.64$; Time X Treatment: $F(12, 127)=0.97$, $p=0.48$; Time X Treatment X Run: $F(12, 127)=1.81$, $p=0.05$). Following Spray 1, there was no significant difference between the treated plots and the water only Control. Following Spray 2 only the XenTari Medium treatment was significantly different from the Control. However, following the third spray we saw significantly fewer caterpillars in the XenTari Medium, High and Dipel treatments compared to the Control. This difference continued through to 1-week after the final application of products. Overall, there were significantly fewer caterpillars recovered from XenTari Medium, High and Dipel treated-plots than the Control over the course of the four post-treatment counts (Fig. 4; $F(4,55)=5.92$, $p=0.0005$). There was no difference between the XenTari treatments and Dipel (Fig. 4). For Run 2, the overall number of dead larvae observed was 16 and these were observed only in the treated plots and not in Control plots (Table 3). For Run 3, the overall number of dead larvae was 5 and these were also only found in treated plots (Table 3). Finally, the number of adult parasitoids observed during post-treatment counts was only 22 and no parasitoids emerged from the larvae and pupae collected at the end of the trial.

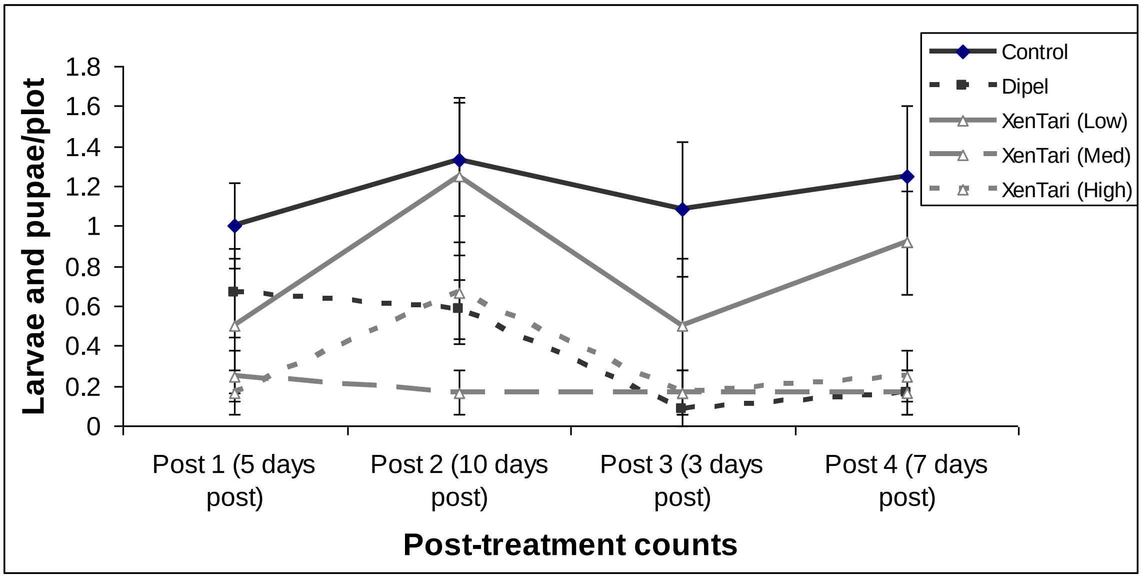

Figure 3. Effect of Bt-based insecticides, XenTari (at three rates) or Dipel, on the mean (± s.e.m.) number of Lepidoptera larvae and pupae observed in field plots following three sprays applications. Day counts accompanying X-axis labels indicate the number of days following each spray that counts were made, with the final count (Post 4) made 1 week after the third spray. For each point N=12.

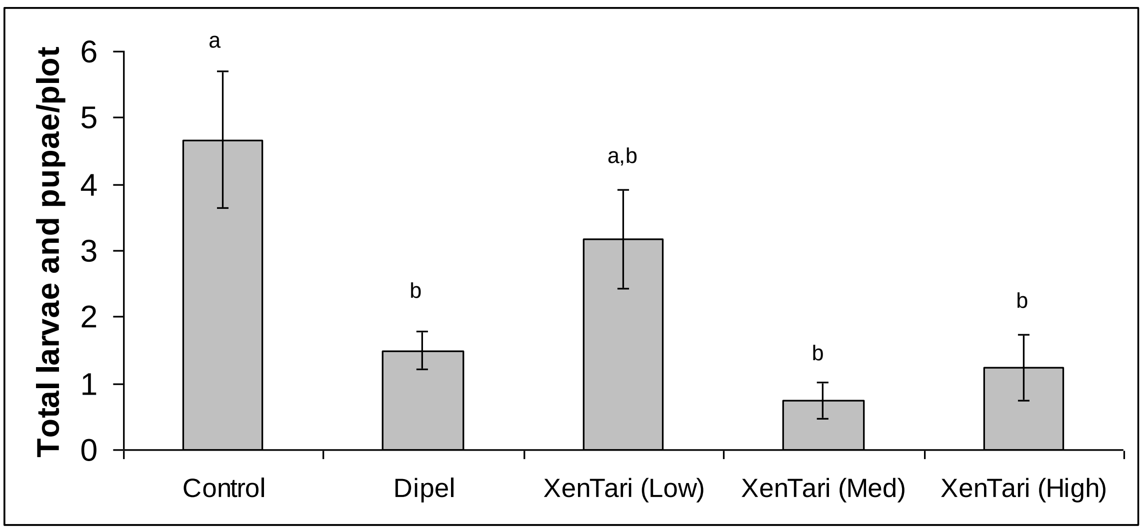

Figure 4. Effect of Bt-based insecticides XenTari (at three rates) and Dipel on the total (mean ± s.e.m.) number of Lepidoptera larvae and pupae observed in field plots over the course of four post-spray counts. Bars with the same letter are not significantly different from each other, based on Tukey-Kramer HSD test. For each treatment N=12.

Table 3. Total number of plots (out of 5) with dead larvae observed during post-treatment counts.

	Run 2	Run 3
Control	0/5	0/5
XenTari Low	2/5	2/5
XenTari Medium	3/5	0/5
XenTari High	5/5	1/5
Dipel	2/5	0/5

Discussion and Next Steps

The objective of this research was to collect local efficacy data on the product XenTari (Bta) for control of caterpillar pests in cole crops. Our results show that, like Btk-products, the efficacy of XenTari depends on a number of factors including conditions following application. When treatments were followed by rainfall within 24-hours we saw no efficacy compared to the untreated Control (i.e. Post 1 counts Fig. 1). However, when conditions were favorable we saw significant effects for both XenTari (Medium and High rates) and Dipel. The efficacy of the Medium and High rate at the low pest infestation levels observed in this trial needs to be confirmed at the higher levels that can sometimes occur in the field earlier in the field season (E.S. Cropconsult Ltd. unpublished data). In the canola work conducted in the prairies XenTari was tested under higher pest pressure and provided 57 to 63% reduction in diamondback counts (Anon. 2013). Our findings also confirm that repeat applications of XenTari are needed to maintain control as Bt-based products have no systemic or residual activity (Rowell and Bessin 2005) and therefore no impact on larvae hatching after product application.

Follow up research from this study should therefore focus primarily on repeating the trial in the early part of the field season (e.g. June or July) - when pest pressure is likely to be higher and the need for applications in closer intervals (i.e. less than 7 days apart) maybe needed. XenTari could be a good product for caterpillar control on cole crops because it will help to manage resistance against Btk and will not have the non-target impacts that spinosads have been found to have (Biondi *et al*. 2012). In this study natural enemy activity in field plots was too low to document any potential negative effects of XenTari, however to date there is not indication that this is a concern (Rowell and Bessin 2005). In the event that XenTari is registered, we recommend that there be a coordinated extension effort with regards to caterpillar control in cole crops - with the focus on training growers, consultants and agribusiness sales representatives on optimal product rotations and integration with biological controls.

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