Effects of crop type on *Bacillus thuringiensis* toxicity and residual activity against *Trichoplusia ni* in greenhouses

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**Abstract:** We assessed the efficacy and persistence of a *Bacillus thuringiensis kurstaki* (*Btk*) formulation (Dipel) against *Trichoplusia ni* (Hubner) (Lep., Noctuidae), the cabbage looper, on three greenhouse vegetable crops (tomato, bell pepper and cucumber). First, *T. ni* larvae were fed leaf discs treated with *Btk* to assess how *Btk* toxicity varies with host plant. Secondly, *T. ni* larvae were fed leaf discs harvested from plants that had been sprayed with *Btk* 1, 5 and 9 days previously to assess the residual activity of *Btk* toxicity in greenhouse environments. Mortality of *T. ni* larvae fed tomato leaf discs was significantly higher than *T. ni* fed cucumber or pepper leaf discs. The toxicity of *Btk* had declined by less than 50% after 9 days, which suggests that *Btk* persistence is lengthy in greenhouse environments. No crop effects on the residual activity of *Btk* were found. These results demonstrate that the greenhouse environment and the crop should be considered when using *Btk* for insect management on greenhouse crops.

**Key words:** *Bacillus thuringiensis*, *Trichoplusia ni*, greenhouse, host plants, resistance, tri-trophic interactions

1 Introduction

The greenhouse vegetable industry in British Columbia, Canada is currently one of the fastest growing agriculture sectors in the province (BCMAFF 2003). The industry relies heavily on biological controls, such as *Bacillus thuringiensis kurstaki* (*Btk*), to manage insect pests (Murphy et al. 2002). One significant pest in the industry is *Trichoplusia ni* (Hubner) (Lep., Noctuidae), the cabbage looper, a noctuid moth indigenous to North America. In the past, growers used *Btk* products almost entirely to control *T. ni* populations. This heavy reliance on *Btk* provided the ideal conditions for the evolution of genetic resistance to *Btk* in greenhouse *T. ni* populations, which was detected in 2000 (Janmaat and Myers 2003). This finding prompted us to examine factors that affect *Btk* efficacy in greenhouses that may ultimately contribute to resistance evolution.

It is widely known that resistance to an insecticide will develop most rapidly in a pest population under situations where the selective pressure, in this case mortality, is high (Hedrick 2000). Accordingly, strategies to manage *Btk* resistance focus on concentrations of the selective agent, and often fail to consider the potential for tri-trophic interactions, those between *Btk*, the host plant and the pest, on resistance evolution. There are numerous studies on the effects of the host plant on insect pathogens, such as *Btk* (see Cory and Hoover 2006), and these results can be dramatic. In a study of the forest tent caterpillar, the estimated *Btk* LC₅₀ was 100-fold greater on quaking aspen than on sugar maple (Kouassi et al. 2001). Similar effects are even observed within a host plant species, such that different cultivars of a plant species with high pest resistance traits can interact positively with *Btk* efficacy (Meade and Hare 1993; Schuler and van Emden 2000; Giustolin et al. 2001a). In situations where *Btk* toxicity is increased by host plant resistance, selection for resistance to *Btk* in the pest population may be amplified leading to more rapid resistance evolution.

Meade and Hare (1994) proposed that the susceptibility of insect herbivores to entomopathogens, such as *Btk*, generally increases as host plant suitability decreases. For example, neonate *Spodoptera exigua* exhibited lower mortality due to *Btk* on a more suitable *Apium graveolens* var. *rapaceum* cultivar than on a less suitable cultivar (Meade and Hare 1993), and a decrease in the quality of dietary protein enhanced the toxicity of the *Btk* toxin Cry1Ac to *Manduca sexta* (Neal 1996). In the present system, *T. ni* is a pest on the primary crops grown in vegetable greenhouses in British Columbia (beefsteak and vine tomatoes, bell peppers and long English cucumbers), and the crops vary in suitability for *T. ni* growth. *T. ni* larval development is slowest on pepper leaves, intermediate
on tomato leaves, and fastest on cucumber leaves (Jannaat and Myers 2005). Therefore, if Btk efficacy is proportional to host plant suitability as suggested by Meade and Hare (1994), then Btk toxicity towards T. ni should vary between crops grown in commercial greenhouses.

A second factor affecting the rate of resistance evolution is the persistence of the selective agent, such that the longer the persistence the more rapidly resistance evolves in the pest population (Denholm et al. 1983; Taylor et al. 1983). Btk is known to decay within days following an application under field conditions, because of degradation by sunlight and rain (Leong et al. 1980; Behle et al. 1997). In a greenhouse, both exposure to UV and rain is reduced, and it is therefore likely that the persistence of Btk will be considerably longer than when applied in a field. Any increase in persistence would lengthen the exposure period of the pest population to Btk, thereby enhancing resistance evolution. Furthermore, plant–pathogen interactions may also affect the persistence of Btk on greenhouse crops. Many plant compounds are known to exhibit antibacterial properties that can decrease Btk persistence on leaf material (Ludlum et al. 1991). For example, survivorship of Manduca sexta larvae exposed to Btk increased with increasing levels of nicotine, an allelochemical in tobacco plants, and this effect was attributed to bactericidal effects of nicotine (Krischik et al. 1988). Therefore, knowledge of the residual activity of Btk in greenhouses and between greenhouse crops is needed to develop Btk use practices that are sustainable in these environments.

The primary objective of the present study was to determine: (1) if Btk toxicity differs between greenhouse vegetable crops, (2) the persistence of Btk in greenhouses, and (3) if the crop affects the persistence of Btk.

2 Materials and Methods

Trichoplusia ni larvae were fed Btk-treated leaf discs of three greenhouse vegetable crops to examine effects of host plant on Btk toxicity. Leaves treated with Btk were harvested at three different times following a Btk application to assess the persistence of Btk toxicity towards T. ni under greenhouse conditions. The assay was replicated five times.

Larvae of T. ni utilized in the experiment were obtained from a laboratory colony maintained for >10 years at University of British Columbia (UBC) and were reared on a wheat germ-based diet (methods modified from Ignoffo 1963), prior to being transferred to Btk-treated leaf discs.

Plants utilized in the experiment were grown in a glass-venlo greenhouse at UBC for 2 months prior to each assay. Three different crop plants were grown for the experiment: pepper (variety 444; Enza Zaden, Enkhuizen, The Netherlands), tomato (Rapsodie; Syngenta Seeds Canada, Arva, ON, Canada) and cucumber (Ventura; RijkZwaan, De Lier, the Netherlands). The varieties chosen were the most widely grown in BC at the time of the experiment. Plants were grown from seed and transplanted as seedlings to pots containing a sterilized peat-based soil-less media (UBC, Horticulture) and were supported by bamboo stakes. Pots were placed on benches and flood-irrigated with a re-circulated nutrient solution.

2.1 Btk persistence assay

One day prior to each Btk application, overlapping leaves were pruned from each plant to prevent light blockage, and to allow for high UV exposure. All plants were subjected to leaf removal. The remaining fully expanded leaves located within the top third of each plant were then marked with a flagging tape, and Btk was applied to these leaves the following day.

Plants were treated with six different Btk concentrations (DiPel WP, Valent Biosciences, containing 16 MIU/g, Libertyville, IL, USA) mixed in distilled water or a distilled water control (concentrations: 0, 0.625, 1.25, 2.5, 5.0, 10.0 and 20.0 kIU/ml H2O). DiPel was thoroughly mixed in distilled water using a vortex and automated stirrer and then placed into a hand-held spray container which was manually agitated prior to use for 30 s. Leaves marked the previous day were sprayed to drip, on both the upper and lower sides, with the Btk solution and then shaken gently for 10 s to remove any excess droplets. Sprayed plants were labelled and kept in the greenhouse for a period of 9 days. The assay was repeated five times, and each time new plants were placed haphazardly in the centre of a middle bench in the greenhouse, thereby varying plant position between replicates. During each replicate, two plants of each crop type were treated with each Btk concentration.

One leaf was harvested from each of the treated plants 1, 5 and 9 days after each Btk spray. Leaves were harvested according to leaf age, with the oldest leaf being collected on day 1. Leaf discs (2 cm diameter) were cut from the treated leaves and were placed into depressions in a 1% agar layer contained in six-well tissue culture dishes. Individual T. ni larvae, 6 days old, were transferred to individual leaf discs and were placed at 26°C, 16 : 8 h (light : dark). Larval mortality was assessed after 2 days. Twelve to 18 larvae were assayed per plant–treatment combination at each time. For days 1, 5 and 9 following the Btk application, the total number of larvae assayed were: pepper n = 419, 436 and 342; tomato n = 421, 430 and 342; cucumber n = 375, 312 and 210, respectively.

2.2 Statistical analysis

Percent mortality was analysed using a general linear model (GLM) in JMP 5.0.1 (1989–2003 SAS Institute Inc). The Btk concentration (natural logarithm transformed), plant and post-spray period (DPS) and their interactions were included in the model as main effects. Replicate date was included as a blocking factor. Residuals from the full model were examined for departures from normality using a Shapiro–Wilk W-test, and no departures from normality were noted (W = 0.98, P = 0.60). Further mortality comparisons were made between each plant treatment group and between days after initial spray using Student’s t multiple comparisons on least-square means produced from the full GLM.

3 Results

3.1 Control mortality

After 2 days of feeding on the leaf discs, mortality was low in the control treatment group over all sampling dates and was not related to the plant type or date of assay (plant: F = 0.93, d.f. = 2, P = 0.41; date of assay: F = 0.48, d.f. = 4, P = 0.75). Mortality was
2.9 ± 1.7%, 3.5 ± 2.1%, and 5.1 ± 1.8% in the cucumber, pepper, and tomato treatment groups, respectively.

3.2 Btk toxicity

All main effects (Btk concentration, plant, and post-spray interval) had a significant effect on larval mortality, whereas no significant interactions between main effects were found (table 1). Percent mortality increased with Btk concentration as expected, demonstrating that the Btk application was successful (fig. 1). Larval mortality was higher for larvae fed on Btk-treated tomato leaf discs, than those fed on Btk-treated cucumber or pepper leaf discs. Mean larval mortality was 35.6 ± 2% in the tomato treatment group relative to 18.0 ± 2.0% and 17.4 ± 2.0% for the cucumber and pepper treatment groups, respectively. This increase in mortality was evident across all three post-spray intervals (fig. 2).

Larval mortality declined from 1 day post-spray to 9 days post-Btk application (table 1; fig. 2). Mean mortality at day 1 was 24.2 ± 1.8%, which declined to 16.9 ± 2.8% 9 days following Btk treatment. Mean mortality at day 5 was 27.5 ± 2.4% which did not appear to differ from the day 1 treatment group. In the analysis of least-square mean values (Student’s t comparisons) derived from the full model however, mortality declined significantly between 1 and 5 days post-spray. Least-square mean values for day 1 and day 5 were 27.2 ± 1.8% and 18.1 ± 2.4%, respectively. This discrepancy between the actual mean values and the least-square mean values is probably due to the significant date effect. In the last post-spray interval, mortality in the lowest Btk concentration (0.625 kIU/ml H2O) remained eightfold higher than that of the control treatment which demonstrated that even a minimal Btk concentration remained active for an extended period (2.8 ± 4.7% and 16.7 ± 6.9% for the control and 0.625 Btk concentration, respectively).

Table 1. A general linear model analysis of the effects of Btk concentration, crop, days post-spray and their interactions on percent mortality of 6-day-old Trichoplusia ni larvae

<table>
<thead>
<tr>
<th>Factor</th>
<th>d.f.</th>
<th>F-ratio</th>
<th>Prob. &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Btk concentration</td>
<td>6</td>
<td>22.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Crop</td>
<td>2</td>
<td>30.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Days post-spray (DPS)</td>
<td>2</td>
<td>13.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Date</td>
<td>4</td>
<td>10.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Crop × DPS</td>
<td>4</td>
<td>1.08</td>
<td>0.36</td>
</tr>
<tr>
<td>Crop × Btk conc.</td>
<td>12</td>
<td>1.47</td>
<td>0.15</td>
</tr>
<tr>
<td>DPS × Btk conc.</td>
<td>12</td>
<td>0.82</td>
<td>0.63</td>
</tr>
<tr>
<td>Crop × DPS × Btk conc.</td>
<td>24</td>
<td>0.49</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Fig. 1. The percentage mortality of 6-day-old Trichoplusia ni larvae after 2 days of feeding on leaf discs treated with Btk 1 day previously for the three different plants. Lines shown represent mean predicted values obtained from the general linear model with plant, Btk concentration, and date included as factors

Fig. 2. The percentage mortality (mean ± SE) of Trichoplusia ni larvae after 2 days of feeding at the highest Btk concentration (20 kIU/ml H2O) 1, 5 and 9 days after Btk was applied for the three different host plants

4 Discussion

Plant–pathogen interactions were found to affect the efficacy of Btk towards T. ni, and therefore the selection pressure for resistance, among greenhouse crops (table 2). These plant–pathogen interactions did not extend to Btk persistence, yet Btk remained toxic for a lengthy period following application under greenhouse conditions. These results demonstrate that the greenhouse environment and the crop should be considered when using Btk for insect management.

4.1 Crop effects

Previous research has shown that pepper is the least suitable of the three greenhouse crops tested for T. ni growth (Janmaat and Myers 2005). We expected therefore that Btk toxicity would be highest for larvae on pepper leaves. In contrast, larval mortality was highest on tomato leaf discs and equivalent on cucumber and pepper leaves, the best and worst host plant for larval growth, respectively. This response was consistent over all post-spray intervals and thus host plant suitability, as suggested by Meade and Hare (1994), was not the primary factor affecting Btk toxicity in the present study.

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It is likely that factors unique to tomato leaves, such as secondary defense compounds are the cause of the enhanced Btk toxicity towards T. ni. Previous studies have shown Btk toxicity to be higher on tomato relative to other host plants for a variety of lepidopteran species (Giustolin et al. 2001b; Ali et al. 2004; Asano et al. 2004). Tomato leaves are known to contain an assortment of constitutive defense compounds that include the glycoalkaloid, α-tomatine, and the catecholic phenols, chlorogenic acid and rutin (Kennedy 2003). Each compound is known to negatively affect the growth of insect herbivores (Elliger et al. 1981; Isman and Duffy 1982a,b; Bloem et al. 1989), and may interact with Btk toxicity. Interestingly, chlorogenic acid in combination with peroxidase, an inducible defensive compound in tomato, increased Bt toxicity towards H. zea (Ludlum et al. 1991). Therefore, it is probable that synergistic interactions between tomato phytochemicals and Btk toxins enhanced mortality on tomato leaves in the present study.

### 4.2 Persistence

The Btk formulation is known to rapidly degrade within days following application under field conditions because of sunlight degradation and rain (Leong et al. 1980; Behle et al. 1997), and, therefore, Btk persistence has been of little concern in the management of Btk resistance. In a recent study, the half-life of Btk spores was estimated to be < 24 h on field maize (Haddad et al. 2005). In the present study however, Btk toxicity declined by 50% after 9 days under greenhouse conditions. The lack of precipitation in greenhouses almost certainly plays a major role in the lengthy persistence of Btk; however, reduced sunlight degradation may also be a factor. In a second study, in which Btk applied to a field crop was protected from precipitation, the half-life of insecticidal activity due to sunlight degradation alone was estimated to be 4.3 days for DiPel 2X applied to field-grown cabbage (Behle et al. 1997). In the same study, complete shading of cabbage plants treated with Btk formulations with black plastic provided protection from sunlight degradation for 7 days (Behle et al. 1997), which was similar to persistence in the present study. We attempted to ensure that UV exposure was maximal by pruning leaves to prevent shading and by using no measures to reduce the plant’s sun exposure (e.g. whitewashing of the greenhouse walls or shade-cloth barriers). The longer persistence of activity of Btk, therefore, probably indicates reduced sunlight degradation of Btk in greenhouses.

The absence of a plant by post-spray interval interaction demonstrates that the crop type did not affect the rate at which Btk is degraded. This finding is consistent with another study in which no significant differences in field persistence of Btk endospores were found between beans, broccoli or cabbage (Leong et al. 1980). Therefore, plant–pathogen interactions appear to be more likely to modulate Btk efficacy, than its persistence.

### 4.3 Implications

Knowledge of the differences in Btk toxicity between crops can be used by growers to tailor spray application rates to optimize efficacy and to manage Btk resistance. Btk efficacy, and therefore the selection pressure for Btk resistance, is significantly higher on tomato than on the other crops. Other host plant effects that can potentially influence the development of Btk resistance are outlined in table 2, and were obtained from two other studies on the same study system (Janmaat and Myers 2005, 2007). Thus, it is not surprising that of the greenhouses surveyed, tomato greenhouses harboured T. ni populations with the highest Btk resistance levels (Janmaat and Myers 2003). As a result, to help mitigate the evolution of resistance, tomato growers in particular should reduce Btk application rates, and utilize measures other than Btk, such as natural enemies, to control T. ni.

In addition, knowledge of the lengthened persistence of Btk in greenhouses can help growers to adjust the interval between Btk applications. It is common practice by greenhouse growers to apply Btk at short intervals (within 7 days) to manage T. ni outbreaks. These short intervals are chosen because of the overlapping lifestages of T. ni in greenhouse populations. Applying Btk at such intervals will however lead to a large buildup of Btk on the greenhouse crop because of the extended persistence of Btk in greenhouses and will increase efficacy. In the long term, however, these conditions will select for Btk resistance of the treated pest populations. It is probable that these results are not unique to Btk and the persistence of other insecticidal products in greenhouse environments should be considered in order to develop sustainable management practices.

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**Table 2. Impacts of plant species on factors influencing the selection for resistance to Bt in cabbage loopers, Trichoplusia ni based on the results of this study and Janmaat and Myers (2005, 2007)**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Growth</th>
<th>Cost of resistance</th>
<th>Bt mortality</th>
<th>Bt persistence</th>
<th>Genetic expression</th>
<th>Selection for resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td>Intermediate</td>
<td>Intermediate</td>
<td>Higher</td>
<td>Same</td>
<td>NA</td>
<td>High</td>
</tr>
<tr>
<td>Cucumber</td>
<td>Fastest</td>
<td>Lowest</td>
<td>Higher</td>
<td>Same</td>
<td>Partially recessive</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Pepper</td>
<td>Slowest</td>
<td>Highest</td>
<td>Lower</td>
<td>Same</td>
<td>Recessive</td>
<td>Lowest</td>
</tr>
</tbody>
</table>

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