Refuges in reverse: The spread of *Bt* resistance to unselected greenhouse populations of cabbage looper *Trichoplusia ni*

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Citation

***Agricultural and Forest Entomology* (2008), 10, 119–127**

Keywords

Trichoplusia ni, dispersal, resistance management, genetically modified crops, transgenic crops, *Bacillus thuringiensis,* structured populations, greenhouse crops

Abstract 1 The dispersal of susceptible insects between refuges and *Bt* treated fields is the key to resistance management of *Bt* crops. Here we describe the opposite situation; the movement of *Bt* resistant *Trichoplusia ni* moths from over-wintered, greenhouse populations in British Columbia (BC) exposed to high *Bt* use to neighbouring greenhouses where *Bt* sprays have not been used.

2 The spread of *Bt* resistance to non-selected populations of *T. ni*, and the resulting increase in resistance, indicates a surprising level of dispersal of resistant moths among greenhouses even in the face of fitness costs.

3 Field populations of *T. ni* in BC are seasonal migrants from regions of California where *Bt* cotton is grown. In 2006, field populations surveyed along the migration path from California through Oregon were highly susceptible to *Bt* insecticides and thus, showed no indication of selection for resistance among these source populations.

4 The arrival of the immigrant moths provides a potential source of susceptible individuals to dilute the levels of resistance in greenhouse populations in BC later in the summer, but this has not occurred. Thus field populations in BC do not appear to serve as refuges to combat *Bt* resistance in greenhouse populations.

Introduction

The high dose refuge strategy has become the primary method to delay resistance evolution in major insect pests of transgenic crops expressing *Bacillus thuringiensis* (*Bt*) proteins (Gould, 1998). This approach depends on the persistence of susceptible moths in untreated refuges to act as mates for resistant moths arising from selection by the high doses of *Bt* toxin in the genetically modified plants. Many theoretical considerations of moth movement and spatial structure have provided insights into the potential role of refuges in preventing resistance adaptation (Caprio & Tabashnik, 1992; Peck *et al.,* 1999; Ives & Andow, 2002; Cerda & Wright, 2004; Sisterson *et al.,* 2005). Far less information has been gathered on the actual patterns of movement of resistant moths, and thus the potential spread of *Bt* resistance from selected to non-selected populations. Here, we demonstrate a situation in which cabbage looper *Trichoplusia ni* (Hübner), selected for resistance through high use of *Bt* sprays in some vegetable greenhouses, colonize untreated greenhouses. This leads to elevated levels of resistance in unsprayed moth populations in neighbouring greenhouses. Immigration from susceptible field populations does not apparently counteract this flow of resistance among greenhouse populations and thus, these do not serve as refuges to the greenhouse populations.

Trichoplusia ni is a sub-tropical insect that over-winters in the southern USA (Mitchell & Chalfant, 1984) and migrates northwards each summer as far as British Columbia (BC), Canada. It is a pest on many crop species and is frequently controlled using *Bt* based microbial agents on field crops in western North America. In addition, in

southern California (CA) the first generation transgenic cotton, Bollgard, expressing the single toxin protein gene *Cry1Ac* and the latest variety, Bollgard II, expressing two toxin genes, *Cry1Ac* and *Cry2Ab* or *Cry1Ac* and *Cry1F,* could serve as a host for *T. ni* populations (Li *et al.,* 2007). In BC, *T. ni* are only able to survive in greenhouses if the cleanup at the end of the growing season is not complete, and new field populations are re-established each year from the over-wintering regions of southern CA (Cervantes, 2005). *Trichoplusia ni* have become resistant in vegetable greenhouses in BC following extensive use of *Bt* sprays. This is particularly the case for moth populations that have successfully over-wintered in greenhouses (Janmaat & Myers, 2003).

We have investigated the spatial and temporal patterns of *Bt* resistance in *T. ni* greenhouse and field populations in BC, and regional patterns of *Bt* resistance in field populations collected from CA, Oregon (OR), and BC. The aims of our surveys were to determine (1) if seasonal migrants from the southern USA to Canada were preadapted to *Bt* resistance thus facilitating the development of resistance in greenhouse populations, (2) if resistant populations of *T. ni* that have successfully over-wintered in greenhouses in BC served as a source of resistance genes to field and "unselected" greenhouse populations, and (3) if moths from susceptible field populations disperse into greenhouses later in the summer and reduce the levels of *Bt* resistance.

Materials and methods

Greenhouse and field collections

To examine local patterns of *Bt* resistance, *T. ni* larvae were collected from commercial vegetable greenhouses including those growing tomato, cucumber and pepper crops and cruciferous field crops throughout the lower mainland of BC between March 2005 and

September 2006. All commercial vegetable greenhouses surveyed used integrated pest management practices for the control of insect pests. Foliar applications for the control of Lepidopteran pests in greenhouses included *Bacillus thuringiensis* subsp. *kurstaki* (Berliner) *Btk* (Dipel and Foray; Valent Biosciences, Libertyville, IL) in all crops, tebufenozide (Confirm; Dow AgroSciences, Calgary, Alberta, Canada) in pepper and tomato crops, and spinosad (Success; Dow AgroSciences) in pepper and cucumber crops. The majority of cruciferous field crops sampled in BC used organic farming practices and limited foliar applications to *Btk* (Dipel) and spinosad (Entrust; Dow AgroSciences). Table 1 provides a summary of management practices and *Bt* applications for all greenhouse and field locations surveyed.

Several observers collected larvae by visually searching plants. Eggs and larvae were removed from the plants and placed in paper or plastic cups with leaves in groups of 30 or less. The growth stage of collected larvae ranged from first to fifth instar, with third instar larvae representing the median life stage. Containers were then put in an insulated cooler with ice packs for transport back to the laboratory for sorting.

Larvae were collected from four greenhouses and six fields in 2005 and nine greenhouses and three fields in 2006. Three of the greenhouses and one of the fields were sampled in both 2005 and 2006. Low infestations in some greenhouses and crop rotations in the majority of fields inhibited the resurveying of all greenhouses and fields sampled in both 2005 and 2006. The number of collections from each field and greenhouse varied between one and three per year depending on the abundance and timing of the infestation at each location. Multiple collections were performed

throughout the growing season in three of the greenhouses and one of the field sites in 2005 and five of the greenhouses and one of the field sites surveyed in 2006 (Table 2).

To examine patterns of *Bt* resistance on a larger geographical scale, larvae were collected from a broccoli field in Santa Maria, CA, a cabbage field and mixed cruciferous field in Oxnard, CA, and one broccoli field in Albany, OR during June and July 2006. One collection was performed at each of these sites. All cruciferous fields surveyed in CA and OR used conventional farming practices and no *Bt* sprays had been applied prior to our collections during the 2006 growing season. Management details for these fields are listed in Table 1 and collection dates for each site and GPS coordinates are provided in Table 2.

Greenhouse and field collected larvae were reared in the laboratory using methods modified from Ignoffo (1963). Larvae were transferred as groups of five to 30-ml plastic cups or groups of 15 to 175-ml polystyrene cups containing artificial wheat-germ-based diet and were reared at a temperature of 26˚C with a 16:8 (L:D) photoperiod until pupation. Pupae were removed from cups and placed in a 0.6% bleach solution for five minutes to prevent viral contamination. When *T. ni* was the only noctuid species present in greenhouses, pupae were counted and placed directly into a mating cage for emergence and mating. In several greenhouses and all fields however, other similar noctuid larvae were present, such as *Autographa californica* (Speyer). Pupae from these greenhouses and fields were placed into individual 30-ml plastic cups and upon adult emergence identified to confirm the species. *Trichoplusia ni* moths were counted and put into cages for mating.

Mating cages were housed in a temperature controlled chamber (Conviron, Winnipeg, Canada) at 24˚C with 16:8 (L:D) photoperiod. Moths were supplied with a 10% sucrose solution and paper towelling was placed around the perimeter of the cage. Moths laid eggs on these paper towels which were changed every two to three days and were stored at 4˚C for a maximum of seven days prior to use in bioassays.

Bioassay procedures

Progeny hatching from eggs laid throughout the laying period were used in all assays to ensure that the results were not biased by differences in development time of resistant and susceptible individuals (Janmaat & Myers, 2003). Susceptibility of larvae to *Btk* was assayed using Dipel (Abbott laboratories, Montréal, Canada). Dipel is composed of a mixture of five bacterial proteins including *Cry1Aa, Cry1Ab, Cry1Ac, Cry2Aa,* and *Cry2Ab* and a bacterial spore. *Bt* solutions, ranging in concentration from 0.625 to 160 KIU/ml diet, were prepared by serial dilutions with distilled water and mixed with the artificial diet, cooled to below 50˚C, in a 1:10 ratio (*Bt* solution : artificial diet). Two ml of *Bt* treated or control diet were dispensed into 30-ml plastic cups and allowed to cool to room temperature. Five, five-day-old larvae were placed in each cup and mortality was assessed by gently probing the larvae for movement three days following dosing. A minimum of 20 larvae was tested per *Bt* concentration, and when possible, assays were replicated twice for each population. The number of parents and progeny tested from each population is listed in Table 2.

Data analysis

Bioassays with greater than 20% mortality in the control treatment were not included in analyses. All analyses were performed separately for 2005 and 2006 data. At sites where

multiple collections were performed, linear regression analysis between proportion dead and log-concentration were used to test for parallelism among collection dates (SAS 9.1, 2003). The average proportion dead in each treatment was used for all comparisons since there were no significant interactions between log-concentration and collection date or differences between collection dates at all locations. For the regional analysis, the average proportion dead for the two fields in Oxnard, CA and two fields in Abbotsford, BC were used to test for differences among regions as there was no evidence of interactions (log-concentration and site) or differences between sites. We obtained 50% lethal concentration (LC_{50}) values and fiducial limits for each location using probit procedures in GENSTAT 5 (1997). Methods for calculating fiducial limits in GENSTAT 5 follow procedures outlined by Finney (1971). Abbott's formula (Abbott, 1925) was used to correct for control mortality when the average proportion dead in the control treatment group was greater than 10% for a sampling location.

For the following analyses all locations sampled were classified as being field or greenhouse. Concentration was represented using a scale from 1 to 6, with 1 representing the control treatment and 6 a concentration of 10 KIU/ml diet, respectively. Concentrations ranging from 20 to 160 KIU/ml diet were excluded from these analyses because only a few populations with high levels of resistance were tested at those doses causing several parameters not to be estimated when included in the model. Because of the unequal number of observations, a Generalized Linear Model (GLM) procedure in SAS 9.1 was used to test site (field or greenhouse) and concentration as main effects and their interactions for local differences in resistance among greenhouse and field sites in the lower mainland of BC. We tested for regional differences in moth resistance to *Bt*

between CA, OR, and BC field populations using a PROC GLM in SAS 9.1 with concentration and region as main effects and their interactions.

We used analysis of covariance (ANCOVA) with the covariate *Bt* concentration and main effect "location" to compare *Bt* resistance among populations from all locations in the lower mainland of BC. The covariate concentration was transformed to ln-concentration+100 to ensure a linear relationship between concentration and mortality. Bonferroni multiple comparison procedures were used to adjust for the number of meaningful comparisons. The geographic distances between greenhouses surveyed in 2006 were estimated using spherical distance measures based on the latitude and longitude coordinates for each greenhouse in PASSAGE 1.1 (Rosenberg, 2001).

Assumptions of normality and homogeneity of variances were met for all analyses conducted. Reported means and standard errors of the means are based on least square means to adjust for the unequal number of observations and control for the effect of the covariate concentration in covariance analyses.

Results

Local patterns of *Bt* resistance

Greenhouse populations of *T. ni* were more resistant to *Bt* than were field populations in both 2005 and 2006. This is most clearly seen by their significantly lower mortality when exposed to *Bt* for all doses tested in 2005 ($F_{1,8} = 9.77$, $P = 0.01$; Fig. 1). Among greenhouse populations resistance levels varied significantly however, ($F_{3,23} = 12.43$, $P < 0.0001$), one population (G1), a greenhouse in which *Bt* was used extensively, had a much higher resistance level than the other three in 2005 (G1 vs. G2 $t_{23} = 3.17$, $P = 0.0042$; G1 vs. G3 $t_{23} = 4.14$, $P = 0.0004$; G1 vs. G4 $t_{23} = 5.97$, $P < 0.0001$). No

significant differences occurred in resistance levels among local field populations in 2005 ($F_{5,29} = 1.79$, $P = 0.15$).

Higher levels of resistance for BC greenhouse populations compared to field populations of *T. ni* are also shown by the LC_{50} values. In 2005 these ranged from 1.29 to 67.5 KIU/ml diet for greenhouse populations and from 0.124 to 1.71 KIU/ml diet for field populations (Fig. 2). No significant interaction occurred between site (greenhouse or field) and dose in 2005 ($F_{5,33} = 1.51$, $P = 0.21$).

In 2006, LC_{50} values for BC *T. ni* greenhouse populations were slightly lower than in 2005 and ranged from 0.72 to 9.66 KIU/ml diet. For BC field populations LC_{50} values ranged from 1.43 to 2.24 KIU/ml diet (Fig. 2). In this year a significant interaction existed between site (greenhouse or field) and dose ($F_{5,50} = 3.30$, $P = 0.01$). Resistance levels were higher in greenhouse populations when compared to field populations for four of the doses tested (Dose = 1.25 KIU/ml diet $t_{50} = 4.30$, $P < 0.0001$; Dose = 2.5 KIU/ml diet $t_{50} = 3.38$, $P = 0.001$; Dose = 5.0 KIU/ml diet $t_{50} = 3.01$, $P = 0.004$; Dose = 10 KIU/ml diet $t_{50} = 3.14$, $P = 0.003$; Fig. 1). Similar to the results from 2005, resistance levels varied significantly among greenhouse populations ($F_{8,51} = 6.83$, $P < 0.0001$), while field populations showed no significant variation in resistance ($F_{2,14} = 1.33$, $P = 0.30$).

Spatial patterns of *Bt* resistance

The survey of nine greenhouse populations in 2006 allowed for a comparison of spatial patterns of *Bt* resistance among populations. Spatial patterns of resistance indicate that moths moved between greenhouses in close proximity to one another (Fig. 3 and 4). One greenhouse population (G11) had persisted through the winter cleanup in 2005 into the

2006 growing season. This population was exposed to *Bt* applications during both the 2005 and 2006 growing seasons and likely served as the original source of moths for the greenhouses surveyed that were located 3 to 5 km away that did not have populations that persisted through the winter cleanup (G1, G5, and G6). Attempts to quantify resistance levels in this population however were unsuccessful, as larvae from this greenhouse did not survive in the laboratory.

The greenhouse population with the highest level of resistance (G5) was likely colonized by moths from G11 and was subsequently exposed to nine *Bt* sprays during the 2006 growing season and thus strong selection. That moths had likely migrated from G5 and G11 to other greenhouses between 1 and 5 km away is indicated by the heightened levels of resistance and later first collection dates in the neighbouring greenhouse populations (G1 and G6) in which *T. ni* had not persisted through the 2005 winter cleanup and *Bt* sprays had not been used during 2006 (Fig. 3 and 4). The "unselected" neighbouring populations, G1 and G6 had similar levels of resistance to each other and to the selected population (G5) (G1 vs. G5 $t_{51} = 1.03$, $P = 0.31$; G1 vs. G6 $t_{51} = 0.29$, $P = 0.7698$; G5 vs. G6 $t_{51} = 1.52$, $P = 0.13$).

Two other "unselected" greenhouse populations located less than 4 km apart also had similarly high levels of resistance (G4 vs. G7 $t_{51} = 0.38$, $P = 0.71$). These greenhouses are located in close proximity to many other greenhouses that may have served as sources of resistant moths. The first sampling date for eight of the nine greenhouse populations surveyed in 2006 occurred prior to when moth larvae were found in field samples. This indicates that greenhouse populations persisting from the previous

year probably colonized other greenhouses before field populations of moths were present.

Temporal patterns of *Bt* resistance

Resistance levels in both greenhouse and field populations that were sampled multiple times during 2005 and 2006 did not change within growing seasons ($P > 0.10$ for all comparisons) and showed no trends towards increased susceptibility. Thus, even though the field populations were much more susceptible to *Bt* than greenhouse populations, the resistance of greenhouse populations was not apparently reduced through immigration of susceptible field moths.

Regional patterns of *Bt* resistance

We expected that if southern field populations of *T. ni* had been exposed to transgenic plants or *Bt* sprays, they may have had elevated levels of resistance. For *T. ni* from fields surveyed in CA and OR however, LC_{50} values were similar to BC field populations and ranged from 1.51 to 2.35 KIU/ml diet (Fig. 5). No significant interactions existed between region and dose ($F_{10,41} = 1.83$, $P = 0.1191$) and no significant difference in resistance levels occurred among CA, OR and BC field populations ($F_{2,4} = 0.39$, $P = 0.7007$).

Discussion

Our results strongly suggest that dispersal of resistant moths can lead to the spread and persistence of *Bt* resistant genes to greenhouse moth populations that are not treated with *Bt*. We observed heightened levels of resistance in two "unselected" greenhouse populations that were located only 5 km from two *Bt* treated greenhouse populations. Two other "unselected" greenhouse populations located 4 km apart showed elevated

levels of resistance that had likely spread from one or more of the many surrounding greenhouse populations. The maintenance of resistance in "unselected" populations is unexpected, considering that significant fitness costs are associated with resistance in *T. ni* populations (Janmaat & Myers, 2003). We discuss a number of key factors that could have contributed to the spread of *Bt* resistance among greenhouse populations including timing of colonization, dominance of resistance, dispersal, and selection pressures.

Greenhouse populations of *T. ni* that are not eliminated through the winter cleanup process can increase rapidly on the newly planted crops in the spring. If *Bt* had been used in the previous year, these populations tend to rapidly develop resistance when sprays are used to reduce the populations the next spring. For example, the population in greenhouse 1 (G1) persisted from the 2004 growing season and *Bt* resistance levels reached over 60 KIU/ml diet by March of 2005. Compared to greenhouse populations, selection for *Bt* resistance was much weaker in BC field populations because cold winter temperatures and short growing seasons help to keep population densities low. Despite longer growing seasons and warmer temperatures in CA and OR, there was likely no selection for *Bt* resistance at the field sites sampled, since these fields were farmed conventionally and were not treated with *Bt*.

Moths occurred earlier in most greenhouses than fields. This implies that persistent greenhouse populations were the source of resistant moths for greenhouses in the surrounding area. Consistent with our findings, a model developed by Ives & Andow (2002) to evaluate the effectiveness of the high dose refuge strategy found that if a purely susceptible population is not persistent, then resistance could spread rapidly. This occurs because no susceptible individuals are available for mating and thus, resistant individuals

mate with each other and the frequency of the resistant genes rapidly rises. By the time transitory field populations established in BC, the frequency of resistant genes was likely too high in greenhouse populations for susceptible individuals to have a significant impact on resistance levels. The lack of reduced resistance in greenhouse populations throughout the growing season supports this interpretation.

The high dose refuge strategy developed for *Bt* crops relies on the key assumption that resistance is functionally recessive (Tabashnik & Croft, 1982). Under this scenario, the mating of susceptible individuals (SS) from the refuge and resistant individuals (RR) from the *Bt* transgenic crop is expected to delay resistance by producing heterozygous (RS) offspring that are killed by feeding on transgenic plants (Ferré & Van Rie, 2002). When resistance is not functionally recessive, models predict that resistance will develop rapidly (Crowder *et al.,* 2005; Cerda *et al.,* 2006). Genetic determination of *Bt* resistance in *T. ni* varies with host plant (Janmaat & Myers, 2007). On pepper plants resistance was completely recessive or potentially underdominant, while on cucumber plants resistance of larvae showed incomplete dominance (Janmaat & Myers, 2007). The two cucumber greenhouses surveyed were the only greenhouses treated with *Bt* in 2006. Resistance may have been difficult to delay in these cucumber greenhouses, since the partial dominance of the resistant trait would have favoured the survival of heterozygous (RS) individuals. All other greenhouses surveyed in 2006 grew peppers and were not treated with *Bt*.

In contrast to our expectations of a rapid decline in resistance in "unselected" populations due to reduced fitness costs, resistance was able to persist in the offspring of several greenhouse populations that were not treated with *Bt*. Fitness costs such as

reduced pupal and larval weight, progeny size and number, have been identified in laboratory tests of greenhouse collected strains of *Bt* resistant *T. ni* (Janmaat & Myers, 2003; Janmaat & Myers, 2006). The majority of these populations showed a corresponding decrease in resistance over several generations in the laboratory (Janmaat & Myers, 2003).

Current resistance levels in BC greenhouse populations have declined considerably since Janmaat & Myers (2003) identified negative pleiotropic effects associated with resistance. Thus fitness costs that manifest at high levels of resistance may be weak or absent in strains that are only moderately resistant to *Bt*. Fitness costs may also be overstated in highly resistant strains if strong selection reduces the effective population size and increases deleterious mutations due to inbreeding (Carriere *et al.,* 2006). Modelling results for *Pectinophora gossypiella* in *Bt* cotton fields indicate that resistance can spread when weak to moderate fitness costs are combined with other parameters such as small refuges and incomplete resistance (Tabashnik *et al.,* 2005). Thus in the absence of field populations in the winter in BC that could serve as refuges for susceptible individuals, it is understandable that resistant genes can spread among greenhouse populations despite the possible presence of weak fitness costs.

Although *T. ni* have long seasonal migrations, local patterns of *Bt* resistance in selected and "unselected" greenhouse populations suggest dispersal distances in the range of 1 to 5 km between greenhouses. Similarly, mark-recapture estimates for other Lepidopteran insects indicate that a large fraction of moths only disperse very short distances (Mo *et al.,* 2003; Quereshi *et al.,* 2006; Bailey *et al.,* 2007). For example, greater than 90% of released *Diatraea grandiosella* and *Plutella xylostella* were

recaptured or expected to stay within 300 m of their release sites (Mo *et al.,* 2003; Quereshi *et al.,* 2006). Short-distance dispersal may increase the frequency of non-random matings and increase the rate of resistance evolution (Bailey *et al.,* 2007). In *Ostrinia nubilalis* predispersal matings have been found to be common, while matings between resident males and immigrant females occur infrequently (Dalecky *et al.,* 2006). Indeed, it is quite probable that resistant resident *T. ni* moths mate before dispersing to greenhouses in the surrounding area. With the large reproductive potential of *T. ni* females (up to 1000 eggs laid per female) (Mitchell & Chalfant, 1984) postcopulatory dispersal could easily contribute to the spread of resistance among greenhouse populations in BC.

Patterns of *Bt* resistance in BC greenhouse populations suggest that dispersal distances of *T. ni* moths are sufficiently large to allow matings between resistant individuals from greenhouses and susceptible individuals from fields. Populations of *T. ni* are transitory in BC and are not able to over-winter, and this long-range migration can link widely separated populations. As a secondary pest of *Bt* cotton (Ehler *et al.,* 1973), we predicted that the use of *Bt* cotton in southern CA might increase the frequency of resistant genes in southern CA *T. ni* populations that could then spread to populations in northern CA, OR, and BC. Contrary to our prediction, the results presented here indicate that resistance levels remain low and homogeneous in all the field populations that we surveyed in CA, OR, and BC. Consistent with these findings, feeding experiments indicate that no *T. ni* larvae were able to survive when fed *Bt* cotton for their entire development (Li *et al.,* 2006). In addition, simulation studies have indicated that resistance is unlikely to develop in *T. ni* populations inhabiting *Bt* cotton due to the

presence of spatial refuges and temporal refuges (Gutierrez *et al.,* 2006), created by a decline in larval susceptibility with developmental stage and toxin concentration with plant age (Li *et al.,* 2007). Variation in susceptibility to *Bt* among populations of *T. ni* has, however, recently been reported for populations in Bajio guanajuatense area of Mexico (Tamez-Guerra *et al.,* 2006). Furthermore, in Arizona, feeding experiments indicated that *T. ni* was less susceptible to *Bt* cotton than other Lepidopteran pests, including *P. gossypiella* and *Heliothis virescens* (Henneberry *et al.,* 2003). Thus the potential remains for increased resistance to occur in permanent southern populations.

Our study provides the first evidence that *Bt* resistance can spread from selected *T. ni* populations to "unselected" populations. Resistance likely develops in greenhouse populations because of strong selection, year-round persistence and the temporal elimination of susceptible field populations in the winter. Resistance then spreads to other neighbouring greenhouses through local dispersal of resistant moths, prior to the establishment of susceptible field populations. The rapid evolution of *Bt* resistance in vegetable greenhouses poses a serious threat to crop production in BC. Information gathered from studying resistance adaptation in BC populations can aid us in evaluating the effectiveness of the high dose refuge strategy in delaying resistance adaptation in *T. ni* feeding on *Bt* crops. Given the low environmental risk of *Bt* products and the dramatic rise in their use (Betz *et al.,* 2000), it is imperative that management strategies incorporate knowledge of the insect's biology and key factors that facilitate the development of *Bt* resistance.

Acknowledgements

We would like to thank T. Richardson, P. Duffels, J. Yam, I. Shikano, K. Scott, M. Fraser, and N. Vander Wal for their technical assistance, J. Cory, and M. Tseng for their valuable comments and T. Kozak for his statistical advice. We would like to acknowledge BC Hothouse Growers' Association and E.S. Crop Consult, and the Agricultural Extension Services in Oregon and California for helping us to identify suitable field and greenhouse sites to collect *T. ni* from. Financial support for this project was provided by the British Columbia Vegetable Greenhouse Growers' Research Council, British Columbia Investment Agriculture, NSERC Biocontrol Network, and NSERC Discovery grant program.

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Table 1 Summary of crops, management practices, and *Bt* applications used in greenhouses and fields, prior to sampling, in 2004, and during *T. ni* larval collections, in 2005 and 2006. Farming practices used included integrated pest management (IPM), organic, and conventional methods.

Year	Site	Crop	Farming Practices	*Bt* application
Local collections				
2004	G1	Tomato	IPM	Yes
	G2	Tomato	IPM	No
	G3	Pepper	IPM	Yes
	G4	Tomato	IPM	Yes
2005	G1	Pepper	IPM	Yes
	G2	Tomato	IPM	Yes
	G3	Pepper	IPM	Yes
	G4	Tomato	IPM	No[a]
	F1	Broccoli	Organic	No
	F2	Broccoli	Organic	Yes
	F3	Broccoli	Organic	Yes
	F4	Broccoli	Organic	Yes
	F5	Broccoli	Organic	Yes
	F6	Mixed crucifers	Conventional	Yes
2006	G1	Pepper	IPM	No[b]
	G3	Pepper	IPM	No
	G4	Pepper	IPM	No[a]
	G5	Cucumber	IPM	Yes
	G6	Pepper	IPM	No
	G7	Pepper	IPM	No[a]
	G8	Cucumber	IPM	Yes
	G9	Pepper	IPM	No[c]
	G10	Pepper	IPM	No
	G11	Cucumber	IPM	Yes
	F6	Mixed crucifers	Conventional	No
	F7	Broccoli	Organic	No
	F8	Rutabaga	Conventional	No
Regional collections				
2006	Abbotsford 1 BC	Mixed crucifers	Conventional	No
	Abbotsford 2 BC	Rutabaga	Conventional	No
	Delta BC	Broccoli	Organic	No
	Albany OR	Broccoli	Conventional	No
	Oxnard 1 CA	Mixed crucifers	Conventional	No
	Oxnard 2 CA	Cabbage	Conventional	No
	Santa Maria	Broccoli	Conventional	No

[a] *Bt* was used in these greenhouses subsequent to our *T. ni* collections.

[b] *Bt* was used in adjoining greenhouse for the control of cutworms. *T. ni* were not exposed to *Bt* and were collected four months after *Bt* application when no residue would have remained.

[c] *Bt* was used for the treatment of *T. ni* in an adjoining greenhouse compartment where cucumbers were grown.

Table 2 Summary of local and regional collections of *T. ni* performed in 2005 and 2006. Included is a list of greenhouse and field locations, sampling dates, number of pupae or moths caged (number of parents), number of offspring assayed, and number of assays performed. Greenhouse and field populations are represented by the letters G and F, respectively.

Local collections							
Year	Location	Sampling date	No. of parents	No. offspring assayed	No. of assays	Latitude (N)	Longitude (W)
2005	G1	March 18	41	224	2	49°02.800′	122°35.577′
	G1	May 2	188	573	2		
	G2	July 14	41	300	1	49°03.795′	123°06.573′
	G2	October 14	129	670	2		
	G3	April 26	164	300	1	49°02.615′	122°26.897′
	G4	June 10	50	591	2		
	G4	July 6	31	274	1	49°04.014′	123°03.097′
	G4	August 10	254	240	1		
	F1	July 12	11	629	2	49°03.007′	123°03.343′
	F2	August 2	9	276	1	49°07.886′	123°02.043′
	F2	August 25	63	660	2		
	F3	July 8	17	524	2	49°02.450′	123°03.858′
	F4	September 8	71	232	1	49°03.177′	123°05.683′
	F5	July 20	13	601	2	49°05.036′	123°08.288′
	F6	July 26	17	300	1	49°03.507′	122°05.667′
2006	G1	September 15	46	510	2	49°02.852′	122°35.591′
	G3	July 25	75	571	2	49°02.615′	122°26.897′
	G4	August 25	144	480	2	49°04.014′	123°03.097′
	G5	June 30	315	840	3	49°02.726′	122°38.291′
	G5	August 24	70	361	1		
	G5	September 30	60	265	1		
	G6	July 10	110	595	2	49°02.180′	122.38.284′
	G6	September 27	30	300	1		
	G7	July 12	21	360	1	49°04.944′	123°00.394′
	G7	August 21	246	661	2		
	G8	June 9	15	120	1	49°15.123′	122°41.421′
	G8	August 10	115	285	1		
	G9	June 9	35	300	1	49°05.867′	122°17.605′
	G9	August 11	129	300	1		
	G10	September 5	212	281	1	49°05.045′	123°08.290′
	G11	May 19	261	0	0	49°02.443′	122°38.366′
	G11	August 7	175	0	0		
	F6	August 23	23	360	1	49°03.507′	122°05.667′
	F7	August 8	19	300	1	49°06.723′	123°02.266′
	F7	September 19	35	210	1		
	F8	September 14	40	510	2	49°05.046′	122°05.805′
Regional collections							
2006	Abbotsford 1 BC	August 23	23	360	1	49°03.507′	122°05.667′
	Abbotsford 2 BC	September 14	40	510	2	49°05.046′	122°05.805′
	Delta BC	August 8	19	300	1	49°06.723′	123°02.266′
	Delta BC	September 19	35	210	1		
	Albany OR	July 27	28	695	2	44°43.865′	123°07.455′
	Oxnard 1 CA	June 29	158	780	2	34°12.561′	119°03.403′
	Oxnard 2 CA	June 29	11	421	2	34°19.803′	119°08.339′
	Santa Maria CA	June 27	130	992	2	34°53.550′	120°30.853′

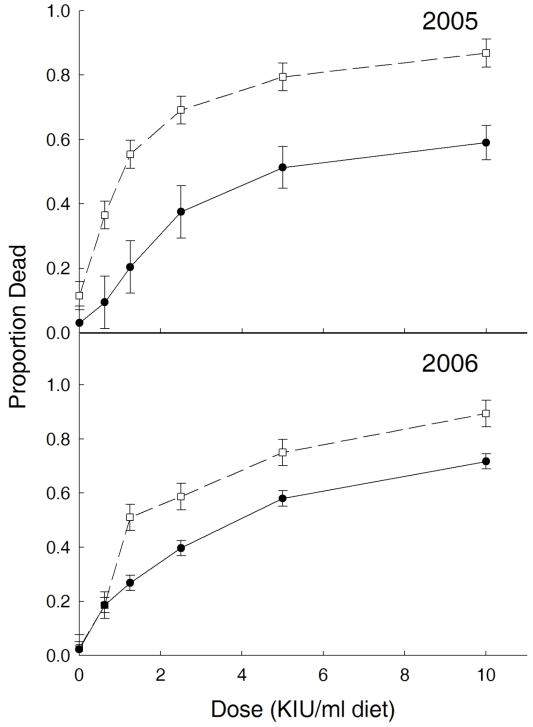
Figure 1 Mean proportion dead (± SE) for *T. ni* progeny assayed for *Bt* resistance from greenhouse and field populations surveyed in 2005 and 2006 in British Columbia. Doses ranged from 0 to 10 KIU/ml diet. Greenhouses and field collections were denoted with —●— and - -□- -, respectively. Greenhouse populations in 2005 had significantly lower mortality than field populations for all doses ($P = 0.01$). In 2006, greenhouse populations had significantly lower mortality for all doses greater than 1.25 KIU/ml diet ($P < 0.005$ for all comparisons).

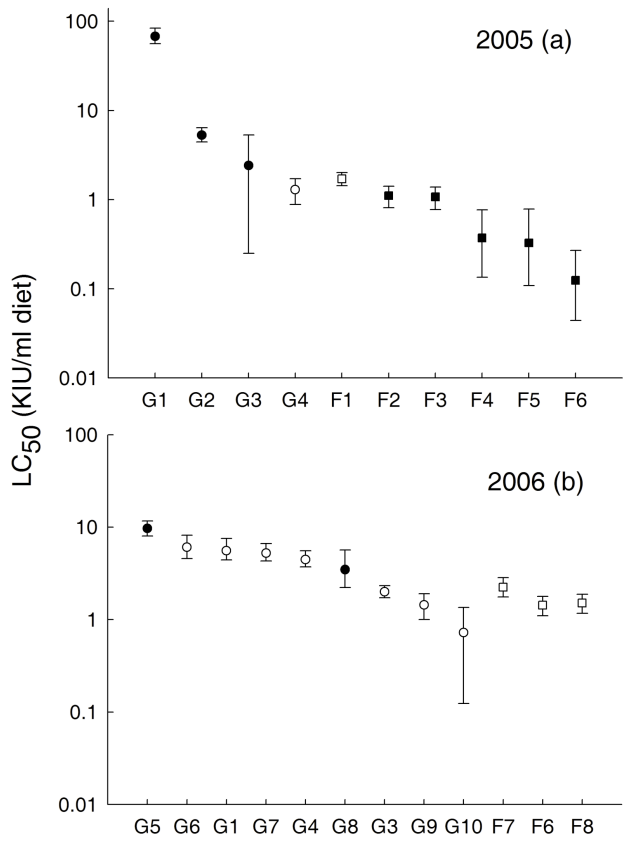
Figure 2 LC_{50} values and fiducial limits for *T. ni* collected from greenhouses and fields throughout the lower mainland of British Columbia in 2005 (a) and 2006 (b). Greenhouse populations that were treated with *Bt* are represented by ● and those untreated by ○ and fields treated with *Bt* are denoted by ■ and those untreated by □.

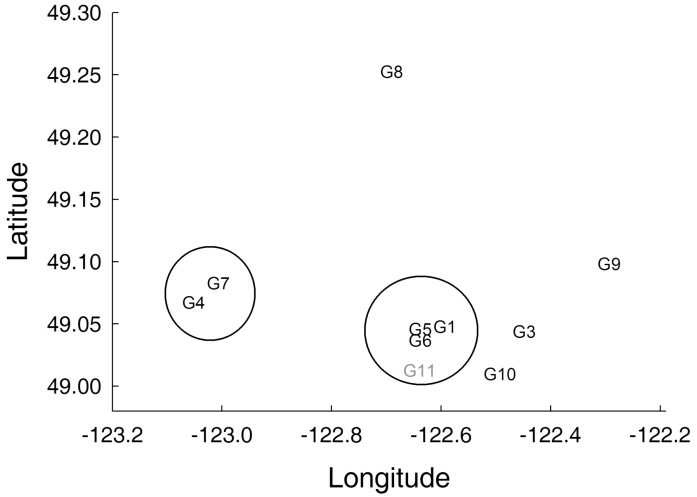
Figure 3 Locations of greenhouses surveyed in British Columbia in 2006. Each greenhouse is represented by latitude and longitude coordinates. Circles encompassing greenhouses G4 and G7, and G1, G5, G6 indicate that resistance levels do not differ significantly within these greenhouse groups ($P > 0.1$). G11 had a population that persisted through the winter and had been exposed to frequent *Bt* sprays. This population likely served as the source of resistant individuals for greenhouses G1, G5, and G6. Attempts were made to test this population for *Bt* resistance, however collected individuals did not survive in the laboratory.

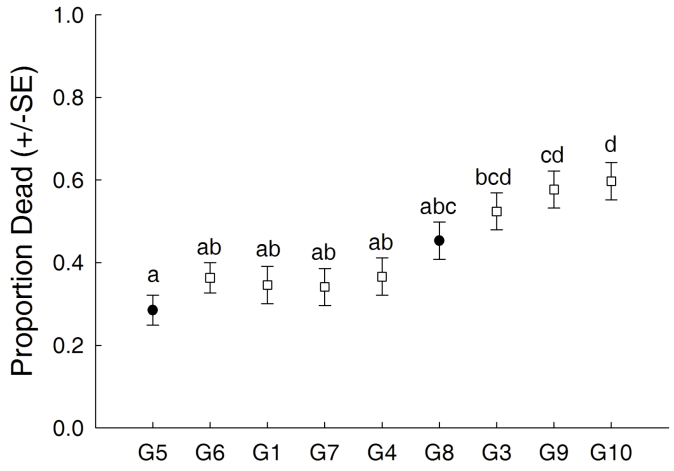
Figure 4 Mean proportion dead (± SE) for the nine greenhouse *T. ni* populations that were assayed for *Bt* resistance in 2006 in British Columbia. Greenhouse populations with significantly different levels of mortality ($P < 0.001$) are represented by different letters (a, b, c, d). Greenhouses treated with *Bt* are denoted by ● and those that were not treated by □.

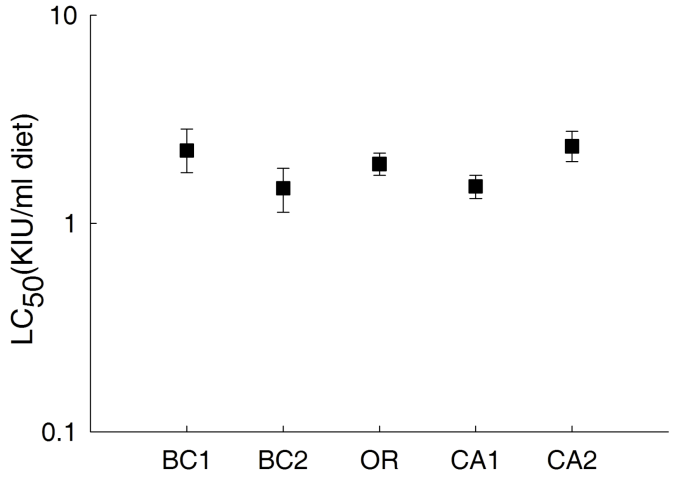
Figure 5 LC_{50} and fiducial limits for *T. ni* field populations collected from California (CA), Oregon (OR), and British Columbia (BC) in 2006. The locations surveyed include: BC1=Delta, BC2=Abbotsford, OR=Albany, CA1=Santa Maria, and CA2=Oxnard.


2005
2006
Proportion Dead
Dose (KIU/ml diet)
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10


2005 (a)
2006 (b)
LC50 (KIU/ml diet)
100
10
1
0.1
0.01
G1 G2 G3 G4 F1 F2 F3 F4 F5 F6
G5 G6 G1 G7 G4 G8 G3 G9 G10 F7 F6 F8


Latitude
Longitude
49.30
49.25
49.20
49.15
49.10
49.05
49.00
-123.2
-123.0
-122.8
-122.6
-122.4
-122.2
G8
G9
G7
G4
G5
G1
G6
G3
G11
G10


Proportion Dead (+/-SE)
1.0
0.8
0.6
0.4
0.2
0.0
a
ab
ab
ab
ab
abc
bcd
cd
d
G5
G6
G1
G7
G4
G8
G3
G9
G10


LC50(KIU/ml diet)
10
1
0.1
BC1
BC2
OR
CA1
CA2