THE ROYAL SOCIETY

Received 2 April 2003
Accepted 25 June 2003
Published online 3 September 2003

Rapid evolution and the cost of resistance to Bacillus thuringiensis in greenhouse populations of cabbage loopers, Trichoplusia ni

Alida F. Janmaat* **and Judith Myers**

Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia, V6T 1Z4, Canada

The microbial insecticide *Bacillus thuringiensis* (*Bt*) has become the mainstay of non-chemical control of Lepidopteran pests, either as sprays or through the incorporation of *Bt* toxins into transgenic crops. Given the wide use of *Bt*, it is striking that currently only one pest species, *Plutella xylostella*, has been reported to have developed significant resistance to *Bt* outside the laboratory. By contrast, we report here the frequent and rapid development of resistance to *B. thuringiensis kurstaki* (Dipel, Abbott) in populations of cabbage loopers, *Trichoplusia ni*, in commercial greenhouses. Resistance to *Bt* appears to be costly and there is a rapid decline of resistance in populations collected from greenhouses and maintained in the laboratory without selection. Management of pests resistant to *Bt* in vegetable greenhouses will require sporadic use of *Bt*-based sprays or alternatively use of sprays containing other *Bt* toxins.

Keywords: *Bacillus thuringiensis*; resistance; *Trichoplusia ni*; fitness costs

1. INTRODUCTION

Bacillus thuringiensis (*Bt*) has been used successfully for over 30 years to control insect pests, but surprisingly only one species of target pest, the diamondback moth, *Plutella xylostella*, has been shown to have evolved resistance to this microbial control in the field (Ferre & van Rie 2002). We report here the second occurrence, to our knowledge, of *Bt* resistance in an agricultural situation: the resistance of cabbage loopers, *Trichoplusia ni*, in vegetable greenhouses in British Columbia, Canada. In addition, we show that this resistance is rapidly lost when selection ceases, which implies that high costs are associated with this resistance.

The mode of action of *Bt* is based on the production of protein crystals that are toxic to particular insect groups. Owing to its specificity and limited environmental impact, *Bt* has become the primary alternative to chemical insecticides for control of moth pests of forests and agriculture. The use of transgenic plants engineered to produce *Bt* endotoxins is on the rise globally. However, the continued use of both *Bt* sprays and *Bt* transgenic crops depends on preventing the evolution of resistance in target pest populations (Ferre & van Rie 2002). Resistance to *Bt* in field situations has been predicted from the results of laboratory experiments involving over 16 pest species in which resistance to *Bt* has been selected (Tabashnik 1994). As an example, the genetic potential for evolution of *Bt* resistance in *T. ni* has been demonstrated by the successful selection of laboratory populations for resistance (Estada & Ferre 1994). However, the predicted evolution of resistance has thus far not been borne out outside the laboratory (Tabashnik 1994; Ferre & van Rie 2002).

The current lack of *Bt* resistance in the field may be a result of an inherent instability of resistance in the absence of *Bt* exposure. Newly arisen resistance traits are often assumed to be associated with a fitness cost (Coustau *et al.* 2000). This assumption arises from the observation that resistance genes are rarely fixed in populations, and the maintenance of genetic polymorphisms is thought to be a result of counterbalanced selection pressures (Coustau *et al.* 2000). Resistance to *Bt* has been reported to decline in the absence of selection in a number of laboratory colonies (McGaughey & Beeman 1988; Tabashnik *et al.* 1991; Hama *et al.* 1992; Sayyed & Wright 2001) and this decline has been attributed to fitness costs, such as a lower growth rate (Liu *et al.* 1999), survival (Groeters *et al.* 1994), or fecundity and mating success (Groeters *et al.* 1993) in resistant than in susceptible individuals in the absence of *Bt*. However, estimates of overall intrinsic growth rates of field-derived resistant *P. xylostella* populations have not been found to differ from those of susceptible populations (Sayyed & Wright 2001). Furthermore, no differences in survival or larval weight were found between resistant and susceptible forms of *Heliothis virescens* in the absence of *Bt* (Gould & Anderson 1991). Despite the uncertainty of fitness costs associated with resistance, many proposed resistance-management strategies rely on their presence (Tabashnik *et al.* 1994; Ferre & van Rie 2002). It is, therefore, imperative to identify and assess fitness costs in order to develop appropriate resistance-management strategies.

Commercial greenhouse vegetable growers in British Columbia, Canada, rely heavily on *Bt* for the control of cabbage loopers, *T. ni*, because it is compatible with other control agents. These greenhouse *T. ni* populations most probably originate from immigrants from field populations, which enter through ceiling vents during the summer months. *Trichoplusia ni* moths can then cycle continuously throughout the growing season with multiple overlapping generations per year. Resistance has been detected in field *P. xylostella* populations with multiple generations per year in regions such as Hawaii, Malaysia, the Philippines, Florida and Thailand (Tabashnik 1994).

*Author for correspondence (janmaat@zoology.ubc.ca).

The relative containment of *T. ni* populations in greenhouses may be highly conducive to resistance development. In another example of contained populations, *Plodia interpunctella* collected from *Bt*-treated grain bins were modestly more resistant (1.2-fold) to *Bt* than populations from untreated bins (McGaughey 1985).

The use of *Bt*-based sprays against *T. ni* provides an ideal environment for the evolution of resistance, and selection for resistance can be intense following multiple sprays of the high concentrations of *Bt* used to control severe pest outbreaks. Following reports of poor *Bt* efficacy in commercial greenhouses, we surveyed *Bt* resistance in *T. ni* populations to ascertain whether resistance was indeed evolving. In addition, life-history characteristics of *T. ni* were measured to determine whether selection for resistance to *Bt* was associated with a fitness cost.

2. MATERIAL AND METHODS

(a) Trichoplusia ni *collection*

We surveyed the *Bt* resistance of cabbage loopers in commercial vegetable greenhouses in the lower mainland of British Columbia between Vancouver and Abbotsford, 100 km to the east. We sampled greenhouses ranging in size from 7000 m^2 to 81 000 m^2, with an average growing area of 44 800 m^2, that were reported to have *T. ni* infestations. Sample dates and collections are listed in table 1. In 2001, three broccoli fields situated more than 1 km from commercial greenhouses and treated with no more than one *Bt* application were also sampled for *T. ni* and assayed for comparison (table 1).

All *T. ni* larvae seen during the greenhouse visit were collected, placed in 473 ml paper cups with collected leaves and returned to the laboratory for rearing using a method modified from Ignoffo (1963). The larvae were reared individually in 30 ml cups containing 2 ml of a wheat-germ-based artificial diet in a controlled-temperature room at 26 °C with a photoperiod of 16 L : 8 D. The number of larvae collected per greenhouse depended on the level of infestation and the final number of parents depended on the proportion of collected larvae that pupated successfully in the laboratory (table 1). Collected larvae were weighed at pupation (only in 2001 and 2002) and placed in a cage for emergence and mating. The cages were supplied with paper towelling for oviposition and a 10% sucrose solution contained in 30 ml plastic cups with cotton wicks. Once the first eggs were laid, egg sheets were harvested every 2 days until fewer than 10 adults remained in the cage. Egg sheets were maintained at 4 °C until use for a maximum of 12 days in 2000, whereas in 2001 and 2002, eggs were stored for no more than 7 days owing to a lower observed fertility of eggs stored for longer than 7 days in 2000. In the majority of collections, development time to pupation of collected larvae differed by less than seven days. To account for any differences in resistance arising from the parental life-stage at collection, multiple egg sheets from throughout the egg-laying period were assayed per population.

T. ni larvae from a laboratory colony that had been maintained for more than 10 years without exposure to *Bt* were used to initiate a new laboratory colony to serve as an unselected reference group for the greenhouse populations. The laboratory colony was susceptible to *Bt* and was assayed 10 times over the 3 years, with LC_{50} values ranging from 0.9 to 5.5 kInternational Units ml^{-1} diet with a mean of 2.2 ± 0.4 (s.e.m.) kIU ml^{-1} diet. Throughout this paper, different greenhouses are designated by crop (T, tomato; P, pepper; C, cucumber; B, broccoli fields), greenhouse number and year.

(b) *Bioassays*

Five-day-old first-generation larvae obtained from parents collected as larvae from greenhouses were used for bioassays. *Bt*-concentration mortality assays were performed by incorporating *Bt* (Dipel, Abbott laboratories) into freshly made artificial diet. Dipel was serially diluted in distilled water and mixed at a 1 : 10 ratio (*Bt* solution : diet) with diet that had cooled to 50 °C. Diet plus *Bt* (2 ml) was dispensed into 30 ml plastic cups and one larva was placed into each cup. Each population was assayed at five different doses ranging from 2.5 to 320 kIU ml^{-1} diet and, if possible, assays were repeated a minimum of twice (table 1).

The number of concentrations tested for each population varied depending on the number of larvae available. A minimum of three concentrations was tested in any one replicate of the assay per population, with 10 or more larvae per concentration. The numbers of larvae tested from each population are presented in table 1. Larval mortality was observed 3 days following the experimental set-up. Mortality was assessed visually and any suspect larvae were prodded gently with a toothpick to ensure a correct evaluation. Five-day-old larvae in the control treatment were weighed at the time of the experimental set-up.

Five resistant greenhouse populations were maintained in the laboratory in the absence of *Bt* exposure on artificial diet. A total of 200 larvae were maintained per generation. For each generation, eggs were chosen from the peak of the egg-laying period when the most adult moths were present. Populations were re-assayed for *Bt* resistance after three and either seven or eight generations of laboratory rearing. The rate of decrease of resistance (R) was calculated as described in Tabashnik (1994) with respect to the LC_{50} of the first-generation larvae and the LC_{50} of subsequent generations, where the inverse of R is the number of generations required for a 10-fold change in LC_{50}.

(c) *Statistical analysis*

Probit analysis was performed using GENSTAT 5 (1998) to calculate the LC_{50} and 95% fiducial limits for each greenhouse population. The probit-analysis procedure in GENSTAT involves methods outlined by Finney (1971). The average mortality in the control groups was less than 5% after 3 days and assays with greater than 20% control mortality at this assessment were not included in the analysis. LC_{50} ratios are reported for 3 days of feeding unless otherwise stated and are calculated with respect to the mean LC_{50} of the reference laboratory colony ($LC_{50} = 2.2$ kIU ml^{-1} diet). The 3-day assessment was reported preferentially since mortality caused by other factors became apparent after 6 days of feeding.

Mean parental pupal weights and mean first-generation 5-day larval weights were regressed against log-transformed population LC_{50} data (JMPIN 4.0). Populations with high mortality in the control group (greater than 40%) at day 6 following the experimental set-up were discarded from the pupal- and larval-weight analyses owing to the potential sublethal effects of disease such as nuclearpolyhedrovirus infections on growth and pupal size. Larvae that hatched from eggs stored for longer than 10 days (in 2000) were also discarded from the larval- and pupal-weight analyses, as prolonged storage at 4 °C decreases larval growth rates (Milks 2002). Pupal weights of populations treated with chemical insecticides in the greenhouse were also not included owing to potential sublethal effects. Pupal weights and larval weights were compared between collection dates of the same

Table 1. Summary of the *Trichoplusia ni* greenhouse populations surveyed for *Bt* resistance.
(The number of collected larvae pupating in the laboratory (parental number), the number of first-generation larvae assayed and the number of assays per greenhouse collection are listed. Greenhouse collections (and broccoli fields (B)) are represented by greenhouse crop (C, cucumber; P, pepper; T, tomato), year and date of collection.)

year	greenhouse	sampling date	no. of parents	total assayed	no. of assays	resistance ratio[a]	probit slope (± s.e.)
2000	C1[b]	30 August	76	589	7	14	0.86 ± 0.01
	C2	15 August	94	292	3	26	0.55 ± 0.11
	C3	30 August	69	243	4	23	0.74 ± 0.15
	C4	10 August	68	181	2	12	0.75 ± 0.16
	P1	23 June	36	413	4	47	0.69 ± 0.09
	P2	29 June	31	364	3	16	1.10 ± 0.18
	P3	19 June	16	218	2	15	0.56 ± 0.11
	P4	16 August	133	403	4	25	0.56 ± 0.09
	T1	24 August	97	245	4	55	1.13 ± 0.38
2001	P4a[b]	1 August	43	364	3	4	1.00 ± 0.13
	P4b[b]	29 August	67	415	3	3	0.84 ± 0.09
	P5[b]	17 July	74	334	4	24	0.85 ± 0.19
	P6	8 May	17	371	2	22	0.97 ± 0.12
	C4a[b]	20 July	127	240	2	3	0.72 ± 0.08
	C4b	31 August	139	596	5	39	1.53 ± 0.37
	C5a	17 July	190	136	2	25	0.55 ± 0.26
	C5b[c]	5 September	91	278	2	5	0.82 ± 0.09
	T1a[b]	28 June	51	311	2	3	0.79 ± 0.10
	T1b[b]	23 August	168	320	3	5	0.64 ± 0.12
	T2a	11 September	223	234	2	44	0.65 ± 0.11
	T2b	9 October	113	390	2	90	0.48 ± 0.15
	T2c	23 November	90	343	2	113	0.62 ± 0.11
	B1[b]	8 August	76	392	2	4	0.73 ± 0.08
	B2[b]	28 August	138	392	3	1	0.99 ± 0.11
	B3[b]	29 August	82	370	3	1	1.63 ± 0.31
2002	T2a	10 July	120	328	2	47	0.97 ± 0.18
	T2b	18 September	157	285	2	160	0.25 ± 0.09
	T3a[b]	18 June	55	258	2	13	0.47 ± 0.09
	T3b	28 August	171	360	2	28	0.50 ± 0.09
	T3c	26 September	164	294	2	31	0.84 ± 0.17
	T4a[b]	30 July	50	257	2	2	0.41 ± 0.08
	T4b[b]	11 September	48	146	2	4	0.42 ± 0.15
	P4	10 June	15	438	1	18	0.49 ± 0.06
	P7[b]	2 September	45	198	2	4	0.53 ± 0.12
	C4	11 September	278	350	1	50	0.51 ± 0.08

[a] Ratios were calculated relative to an LC_{50} of 2.2 kIU ml^{-1} diet for the reference population.
[b] No *Bt* sprays prior to larval collection of *T. ni* within the year indicated.
[c] Treated with a chemical insecticide one week prior to collection.

greenhouse population using *t*-tests (JMPIN 4.0). Mean LC_{50} data of field and untreated greenhouse populations were compared using multiple-comparison procedures (Student's *t*-tests in JMPIN 4.0). LC_{50} results are reported as mean ± 95% fiducial limits unless otherwise indicated.

3. RESULTS

(a) *Surveys for* Bt *resistance*

In each of the survey years, populations of *T. ni* were found that were significantly more resistant to *Bt* than the reference laboratory colony (figure 1). All sampled greenhouse populations that had been treated with *Bt* displayed elevated levels of resistance. Among the collections, the resistance ratio (greenhouse LC_{50}/laboratory colony LC_{50}) varied by more than 100-fold (table 1). In the greenhouses that harboured the two most resistant populations in 2000, the three most resistant populations in 2001 and the two most resistant populations in 2002, the growers reported poor *Bt* efficacy.

The field populations of *T. ni* sampled in 2001 had a mean LC_{50} of 4.7 ± 1.6 kIU ml^{-1} diet, which did not significantly differ from that in the reference colony (figure 1). Over the three survey years, a total of eight greenhouse populations were not treated with *Bt* prior to the initial collection in the growing season. Collections of *T. ni* larvae from the same greenhouse in separate growing seasons were treated as separate populations. This assumption seems reasonable, since at the end of each year greenhouse crops are removed and the structures are cleaned and fumigated to eradicate any insects. Five of the untreated populations surveyed had resistance levels similar to those

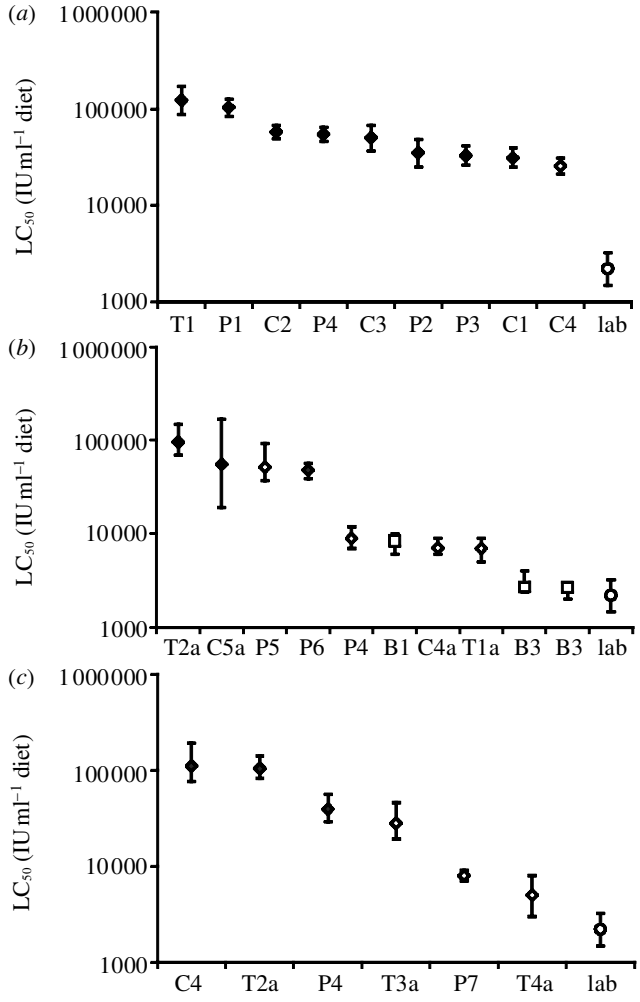

Figure 1. LC_{50} data and 95% fiducial limits of *Trichoplusia ni* populations collected from greenhouses in (*a*) 2000, (*b*) 2001 and (*c*) 2002. Greenhouse populations treated with *Bt* are shown as filled diamonds, and as open diamonds when untreated; field populations are shown as open squares, and the reference laboratory colony as open circles.

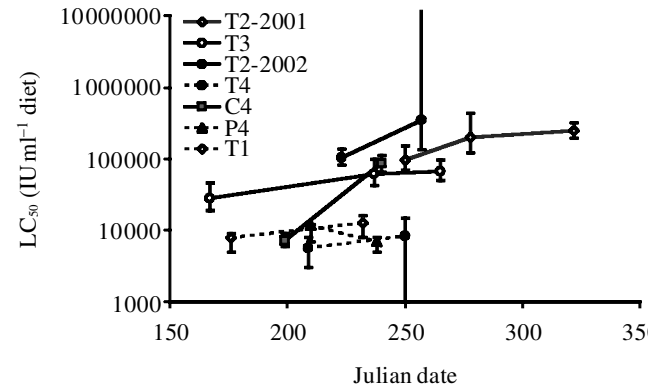

Figure 2. Changes in LC_{50} over time in greenhouse *Trichoplusia ni* populations in 2001 and 2002. Populations with solid lines were treated with *Bt* and populations with dashed lines were not. LC_{50} values with 95% fiducial limits were calculated using Probit analysis (GENSTAT 5).

of the sampled field populations, with a mean LC_{50} of 7.4 ± 1.2 kIU ml^{-1} diet, but they had significantly higher resistances than the reference laboratory colony. The remaining three untreated populations had resistance levels that were higher than those of the field populations, with a mean LC_{50} of 37 ± 7.5 kIU ml^{-1} diet. The most resistant untreated population in 2001 (P5) was physically located between two treated populations (P6 and C5a) with similar resistance levels, suggesting that resistant moths immigrated into P5.

Four of the treated greenhouse populations (T2-2001, T2-2002, T3-2002 and C5-2001) were sampled repeatedly within each growing season. (As mentioned earlier, larval collections from the same greenhouse in different years were treated as separate populations.) Three of these populations were frequently treated with *Bt* throughout the growing season and this was reflected in significant increases in the LC_{50} values from the first to the last collections (figure 2). The fourth population had become uncontrollable with *Bt* and was treated with a chemical insecticide. Larvae were collected a week after the chemical application and the LC_{50} was five times lower than that of the initial collection (table 1). Four out of the eight untreated greenhouse populations were also sampled multiple times within the growing season. Three of these remained untreated and displayed no change in LC_{50} between collection dates (P4-2001, T1-2001, T4-2001) (figure 2). Following the initial collection, the remaining untreated *T. ni* population (C4-2001) increased beyond economic threshold levels, which necessitated *Bt* treatment. After several *Bt* applications, the population was resampled and the LC_{50} had significantly increased relative to that in the initial collection.

Three greenhouse populations were sampled towards the end of 2000 following *Bt* applications and again in 2001 prior to any *Bt* treatments. The change in LC_{50} between years was examined to determine whether *Bt* resistance had been carried over. The LC_{50} values changed from 54 to 12, 25 to 7 and 122 to 7 kIU ml^{-1} diet in the three greenhouse populations (P4, C4 and T1, respectively). In all populations, resistance declined to levels equivalent to those of the field populations sampled in 2001. Growers experienced poor economic conditions at the beginning of 2001 owing to high petrol prices and many delayed the planting of their crops by up to four weeks, which may have affected the survivorship of *T. ni* between years.

To determine whether the assayed LC_{50} was related to grower management practices, the total amount of *Bt* applied prior to the first larval collection in each growing season was regressed against the population LC_{50} at the time of the first collection. The quantity of IU per hectare was calculated for each *Bt* application by converting the amount applied to IU for the two *Bt* formulations used by the growers (16×10^9 IU kg^{-1} for Dipel and 10.6×10^9 IU l^{-1} for Foray 48B, Abbott laboratories) from grower-reported application rates in kg ha^{-1} or l ha^{-1} for Dipel or Foray, respectively. The total amount of *Bt* applied prior to the larval collection per greenhouse was significantly related to the *T. ni* resistance level identified in the *Bt* dose–response assays ($r^2 = 0.78$, d.f. = 1, $p = 0.0086$) (figure 3). Only initial collections per greenhouse per growing season were included in the analysis to avoid pseudoreplication. No significant differences were found in the relationship between the amount of *Bt* applied and the assayed LC_{50} in the different years

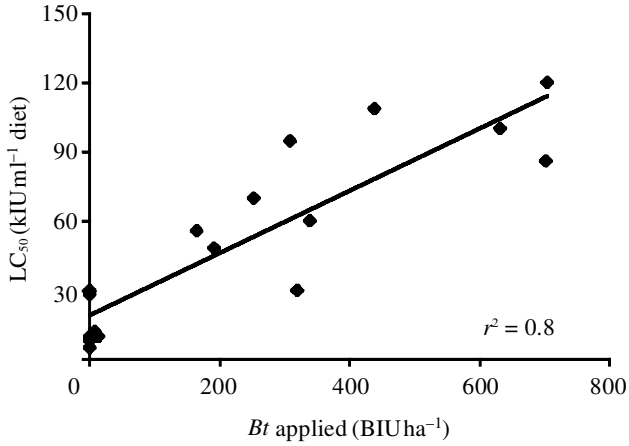

Figure 3. Relationship between the *Bt* dose–response assay LC_{50} values of the first-generation offspring of greenhouse-collected *Trichoplusia ni* and the total amount of *Bt* applied in the greenhouse. Only the results of the collections conducted between May and September and the first collection per greenhouse per year are included to avoid pseudoreplication. Populations with high mortality in the control treatment group were not included in the analysis.

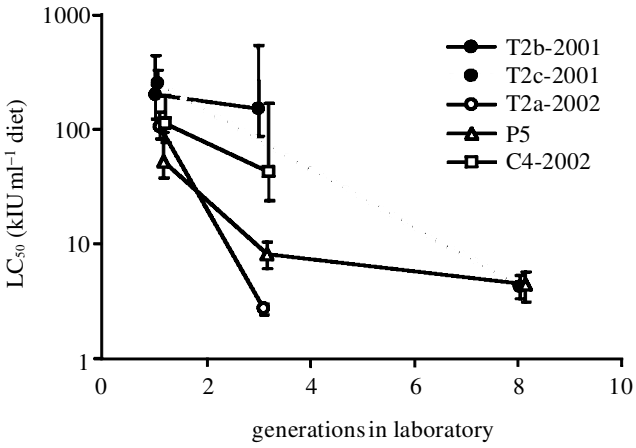

Figure 4. Decline in resistance of greenhouse *Trichoplusia ni* populations reared in the laboratory in the absence of *Bt* exposure. LC_{50} values and 95% fiducial limits are shown.

(d.f. = 2, $p = 0.66$) and therefore year was not included in the overall analysis.

(b) *Stability of* Bt *resistance*

In the absence of *Bt* exposure in the laboratory, resistance declined in all five established colonies (figure 4). The two most resistant of these colonies were initiated from separate collections in 2001 from the same greenhouse. Resistance declined rapidly in one colony, from 248 to 4.2 kIU ml^{-1} diet (T2c-2001) after seven generations, at a rate of decline of -0.25, which indicates that four generations were required for a 10-fold decrease in LC_{50}. In the other colony, established from the second collection from T2 in 2001 (T2b-2001), resistance decreased less than twofold, from 199 to 147 kIU ml^{-1} diet, in two generations ($R = -0.05$) and subsequently died out prior to eight generations. A third line established from the same greenhouse (T2a-2002) in 2002 showed an LC_{50} after the first laboratory generation of 104 kIU ml^{-1} diet, which declined to 3 kIU ml^{-1} diet after two generations—a rate of decline of -0.80, or a 10-fold decrease in LC_{50} in less than two generations.

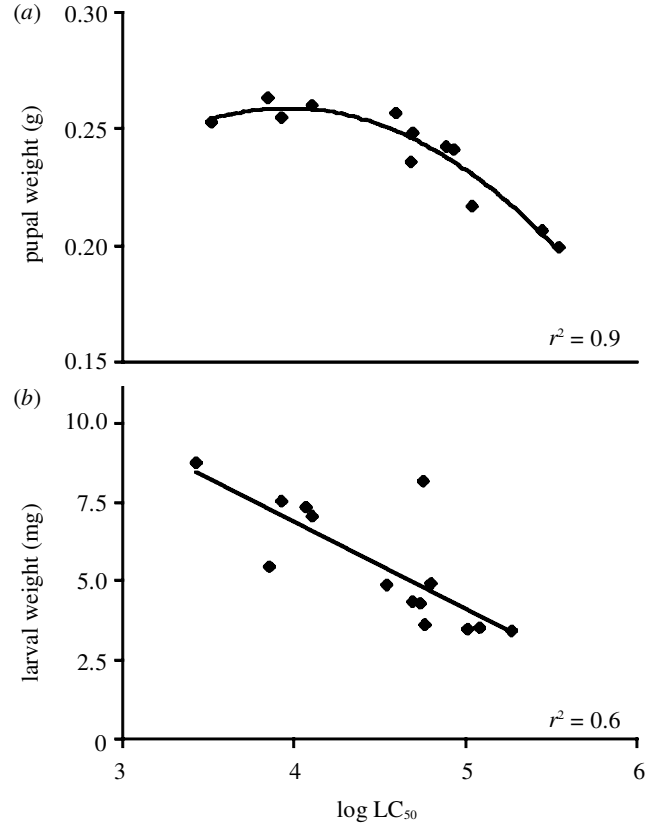

Figure 5. Relationships between (*a*) pupal weights of collected larvae and (*b*) 5-day larval weights of offspring and the population LC_{50}. Only the results of the last collection per greenhouse per year are included to avoid pseudoreplication. Only the results of the last collection per greenhouse per year are included to avoid pseudoreplication. Populations with high mortality in the control treatment group and larvae of eggs stored for more than seven days at 4 °C were not included in the analysis. Values of r^2 were calculated for a second-order polynomial equation ($y = 0.394 - 0.031x - 0.024(x - 4.602)^2$, $F = 46.7$, $p = 0.0001$) for pupal weights and a first-order polynomial equation ($y = 17.8 - 2.75x$, $F = 18.57$, $p = 0.001$) for 5-day larval weights, using JMPIN 4.0.

The remaining two of the five colonies were established from two separate resistant greenhouse populations. One colony (C4-2002) was resistant at an LC_{50} of 110 kIU ml^{-1} diet, which declined to 42 kIU ml^{-1} diet after two generations at a rate of decline of -0.21 (10-fold decrease in five generations). The final colony (P5) was moderately resistant at establishment, with an LC_{50} of 50 kIU ml^{-1} diet, which declined to 8 kIU ml^{-1} diet after two generations ($R = -0.40$) and to 4.3 kIU ml^{-1} diet after seven generations ($R = -0.15$).

(c) *Pupal and larval weights*

Parental pupal weights were significantly correlated with log-transformed population LC_{50} values ($r^2 = 0.91$) (figure 5*a*). The mean pupal weight (± s.e.m.) of the most resistant population was 199 ± 4 mg, over 20% lower than the mean pupal weight of the untreated populations (256 ± 2 mg). Pupal weights were observed to decrease by

17% between collections for two greenhouse populations that changed in resistance from 96 to 248 kIU ml^{-1} diet (T2-2001) and from 104 to 352 kIU ml^{-1} diet (T2-2002) ($t = 12.9$, d.f. = 380, $p < 0.0001$ and $t = 7.9$, d.f. = 275, $p < 0.0001$, respectively). For three untreated greenhouse populations that exhibited no change in LC_{50} over time, pupal weights were stable, with a 0–5% change between collections.

Weights of first-generation larvae at 5 days were also negatively related to population resistance levels ($r^2 = 0.61$, slope = -0.0027, $p = 0.001$) (figure 5*b*). The mean larval weight of the most resistant population ($LC_{50} > 100\,000$ IU ml^{-1} diet) was 3.45 ± 0.05 mg. Moderately resistant ($30\,000 < LC_{50} < 65\,000$ IU ml^{-1} diet) larvae had a mean weight of 5.0 ± 0.6 mg, compared with a mean larval weight of non-resistant colonies ($LC_{50} < 12\,000$ IU ml^{-1} diet) of 7.2 ± 0.8 mg.

4. DISCUSSION

Resistance to *Bt* clearly develops in cabbage loopers in commercial vegetable greenhouses in response to grower spray regimes. The rate of resistance development was similar between years and resistance alleles are apparently widespread in surrounding field populations and in the founding populations in greenhouses. Thus far, most cases of *Bt* resistance in Lepidoptera appear to be primarily associated with an autosomal recessive allele with an average estimated frequency of 0.001 to 0.0015 in field populations (reviewed by Ferre & van Rie 2002). One might predict relatively high frequencies of resistance alleles among founding populations of *T. ni* in greenhouses, since resistance develops rapidly in these populations and they are likely to have been initiated by a small number of immigrants.

Resistance-allele frequencies are often estimated through the use of a diagnostic dose that causes 99% mortality of a susceptible reference population (ffrench-Constant & Roush 1990). As the variation in the susceptibility of different laboratory strains and unexposed field populations is considerable (Robertson *et al.* 1995; Gonzalez-Cabrera *et al.* 2001), it is often difficult to decide on the appropriate diagnostic dose (Marcon *et al.* 1999). In the present study, a dose of 40 kIU ml^{-1} diet was sufficient to kill 99% of the reference laboratory colony and no survivors were found after a dose of 80 kIU ml^{-1} diet. Given that reports of poor *Bt* efficacy correspond to populations with LC_{50} values of 48 kIU ml^{-1} diet or greater, a 48 kIU ml^{-1} diet or more may be a suitable diagnostic dose. Combining the assay results of the three sampled field populations, four larvae out of 100 (4%) and eight larvae out of 160 (5%) survived at 80 and 40 kIU ml^{-1} diet, respectively. Therefore, in the most simplistic case, if the resistant trait was the result of a single recessive allele then the allele frequency may be estimated as 0.20 in the wild population when using a discriminating dose of 80 kIU ml^{-1} diet. Since foliar *Bt* applications contain a variety of *Bt* toxins, it seems likely that resistance may be a result of the action of more than one gene and the assumption may not be valid. However, high allele frequencies in the invading *T. ni* populations would further explain the rapid increase in *Bt* resistance in response to selection pressure and therefore more work is needed to address this issue. High frequencies of resistance alleles are not unheard of. In field populations of *Pectinophora gossypiella* in Arizona, frequencies were estimated to be as high as 0.18 (Tabashnik *et al.* 2000).

Various physiological mechanisms associated with the steps in the mode of action of *Bt* toxin proteins could be associated with resistance (Taylor & Feyereisen 1996; Ferre & van Rie 2002). These include solubilization, proteolytic processing, passage through the peritrophic membrane, receptor binding, membrane insertion, pore formation and osmotic lysis of midgut cells. Owing to this complexity in the mode of action of *Bt*, a variety of associated fitness costs are possible. The major mechanism observed in field-derived resistant *P. xylostella* populations is an alteration in the binding of *Bt* toxins to the gut receptor molecules (Ferre & van Rie 2002). Altered target sites could induce deleterious effects owing to the disruption of pre-existing pathways (Uyenoyama 1990).

The presence of resistance-associated fitness costs should result in counterselection in the absence of *Bt* and a subsequent decline in resistance. This supposition was borne out, as resistance declined rapidly in three field-derived resistant colonies, whereas two other highly resistant colonies exhibited limited decreases in resistance. The initial high resistance levels and the slow decline of resistance in these colonies suggest that they may have had a high frequency of resistance alleles and therefore few or no susceptible individuals. Both of these colonies subsequently died after four generations of laboratory rearing owing to poor fecundity and reduced growth, which may have been caused by negative pleiotropic effects. Interestingly, the mean stability of resistance in *T. ni* populations after three generations was similar to that reported for *P. xylostella* (Tabashnik 1994; Sayyed & Wright 2001), the only species known to have evolved resistance in the field.

Two indicators of reduced fitness of resistant *T. ni* are slower larval growth and smaller pupal size. The mean pupal weight of the most resistant strain was 20% lower than the mean pupal weight of untreated populations and larval weights were 49% smaller. Since pupal weights in *T. ni* are proportional to fecundity (Milks *et al.* 1998), any decrease in pupal weight would confer a negative fitness effect. Furthermore, a decrease in growth rate will increase the period in which larvae are vulnerable to predators, diseases and the weather, further affecting fitness (Gould *et al.* 1991). Therefore, the decreases in pupal weight and larval growth rates observed with increasing resistance demonstrate that *Bt* resistance in *T. ni* populations is associated with severe deleterious pleiotropic effects and this provides an explanation for the lack of resistance stability in the resistant colonies.

It is possible that sublethal effects or maternal effects resulting from prior exposure to *Bt* are responsible for the observed negative relationship between the growth rates of offspring and the LC_{50} values of the parental greenhouse population. It is known that *Bt* is a feeding inhibitor that can reduce growth rates and pupal weights (Salama & Sharaby 1988). However, sublethal effects were not observed to affect pupal weights of the spruce budworm, *Choristoneura fumiferana* (Ramachandran *et al.* 1993) and were absent or in fact opposite in resistant individuals feeding on *Bt* or *Bt* transgenic crops (Gould *et al.* 1995; Ramachandran *et al.* 1998). Few investigators have tested

potential maternal effects resulting from *Bt* exposure. However, in one study, strains of pink bollworm, *P. gossypiella*, with different levels of resistance showed no maternal effects on development time or larval weight (Carriere *et al.* 2001). In a preliminary study, we compared the growth characteristics of a colony initiated from a resistant population in 2001 that had reverted to susceptibility with those of a hybrid of the susceptible colony and its sister colony that had been selected to maintain resistance. Parents of the hybrids were grown for one generation without exposure to *Bt* to reduce any potential sublethal effects. Maternal effects were reduced by examining the hybrid offspring of matings between reverted susceptible females and resistant males. Mean ($\pm$ s.e.m.) hybrid pupal weights (217 ± 6.7 g) were significantly less than those from the susceptible colony (250 ± 5.7 g; $t = 3.6$, d.f. = 43, $p < 0.001$), which supports the concept of the existence of a resistance-correlated fitness cost rather than sublethal effects. By contrast, resistant *P. xylostella* derived from Malaysian field collections have been reported to exhibit increased growth rates and greater pupal weights than unselected subpopulations (Sayyed & Wright 2001).

Despite the uncertainty surrounding resistance-associated fitness costs, many proposed resistance-management strategies, particularly those for *Bt* transgenic crops, rely on their presence. Using insecticidal rotation (Ferre & van Rie 2002) or temporal refuges (periods with no *Bt* exposure) (Tabashnik *et al.* 1994) as a management strategy requires that resistance declines when selection ceases. Given that resistance to *Bt* rapidly declined in several of the resistant populations studied here and that a decrease in resistance was correlated with the use of an insecticidal spray, the use of insecticidal rotation may be an important tool in managing *Bt* resistance in greenhouse *T. ni* populations.

Populations of *T. ni* in greenhouses are likely to be initiated each year from small numbers of individuals either having survived over winter in the greenhouse or having immigrated from field populations. The presence of resistance genes in either wild or overwintered populations will allow the rapid development of resistance to be a continuing occurrence. Deleterious fitness costs associated with *Bt* resistance in *T. ni* populations would be predicted to cause the resistance of moths in both greenhouses and field populations to decline in the absence of *Bt* sprays. However, the estimate of relatively high frequencies of resistance alleles in wild populations does not support this prediction. Either the frequency of resistance alleles is overestimated or fitness costs are not as deleterious in the wild as in the laboratory. Continued selection for *Bt* resistance in greenhouses may lead to selection of resistance alleles with minimal pleiotropic effects or of modifier genes that could ameliorate fitness costs and thus stabilize resistance in the absence of *Bt* applications (Roush & McKenzie 1987). These possibilities put at risk the long-term viability of both foliar *Bt* applications and *Bt* transgenic crops.

We thank B. E. Tabashnik and Y. Carriere for discussion of the survey results. We gratefully acknowledge the technical assistance of M. Milks, J. Ware, J. Passmore, I. Hartasanchez, J. Manson, V. Cervantes, G. Weingartner and T. Sackett. We thank each greenhouse grower and the BC Hothouse Growers' Association for allowing us to survey *T. ni* populations. We also thank the anonymous referees for their thorough and helpful review of this manuscript. This research was supported by funding from the British Columbia Vegetable Greenhouse Growers' Research Council, British Columbia Investment Agriculture, a Science Council of British Columbia graduate scholarship and the NSERC Biocontrol Network.

REFERENCES

Carriere, Y., Ellers-Kirk, C., Liu, Y., Sims, M. A., Dennehy, T. J. & Tabashnik, B. E. 2001 Fitness costs and maternal effects associated with resistance to transgenic cotton in the pink bollworm (Lepidoptera: Gelechiidae). *J. Econ. Entomol.* **94**, 1571–1576.

Coustau, C., Chevillon, C. & ffrench-Constant, R. 2000 Resistance to xenobiotics and parasites: can we count the cost? *Trends Ecol. Evol.* **15**, 378–383.

Estada, U. & Ferre, J. 1994 Binding of insecticidal crystal proteins of *Bacillus thuringiensis* to the midgut brush border of the Cabbage Looper, *Trichoplusia ni* (Hubner) (Lepidoptera: Noctuidae) and the selection for resistance to one of the crystal proteins. *Appl. Environ. Microbiol.* **60**, 3840–3846.

Ferre, J. & van Rie, J. 2002 Biochemistry and genetics of insect resistance to *Bacillus thuringiensis*. *A. Rev. Entomol.* **47**, 501–533.

ffrench-Constant, R. H. & Roush, R. T. 1990 Resistance detection and documentation: the relative role of pesticidal and biochemical assays. In *Pesticide resistance in arthropods* (ed. R. T. Roush & B. E. Tabashnik), pp. 4–38. NY: Routledge, Chapman and Hall Inc.

Finney, D. J. 1971 *Probit analysis*, 3rd edn. Cambridge University Press.

Genstat 5 Release 4.1 1998 Rothamsted Experimental Station, Lawes Agricultural Trust: Harpenden, UK.

Gonzalez-Cabrera, J., Herrero, S. & Ferre, J. 2001 High genetic variability for resistance to *Bacillus thuringiensis* toxins in a single population of diamondback moth. *Appl. Environ. Microbiol.* **67**, 5043–5048.

Gould, F. & Anderson, A. 1991 Effects of *Bacillus thuringiensis* and HD-73 Delta-endotoxin on growth, behavior, and fitness of susceptible and toxin-adapted strains of *Heliothis virescens* (Lepidoptera: Noctuidae). *Environ. Entomol.* **20**, 30–38.

Gould, F., Kennedy, G. G. & Johnson, M. T. 1991 Effects of natural enemies on the rate of herbivore adaptation to resistant host plants. *Entomol. Exp. Appl.* **58**, 1–14.

Gould, F., Anderson, A., Reynolds, A., Bumgarner, L. & Moar, W. J. 1995 Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. *J. Econ. Entomol.* **88**, 1545–1559.

Groeters, F. R., Tabashnik, B. E., Finson, N. & Johnson, M. W. 1993 Resistance to *Bacillus thuringiensis* affects mating success of the diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* **86**, 1035–1039.

Groeters, F. R., Tabashnik, B. E., Finson, N. & Johnson, M. W. 1994 Fitness costs of resistance to *Bacillus thuringiensis* in the diamondback moth (*Plutella xylostella*). *Evolution* **48**, 197–201.

Hama, H., Suzuki, K. & Tanaka, H. 1992 Inheritance and stability of resistance to *Bacillus thuringiensis* formulations of the diamondback moth *Plutella xylostella* (Linnaeus) (Lepidoptera: Yponomeutidae). *Appl. Entomol. Zool.* **27**, 355–362.

Ignoffo, C. M. 1963 A successful technique for mass-rearing cabbage loopers on a semisynthetic diet. *Ann. Entomol. Soc. Am.* **50**, 178–182.

JMPIN v. 4.0.3 2000 SAS Institute, Inc. Cary, NC, USA. (Distributed by Duxbury Press.)

Liu, Y., Tabashnik, B. E., Dennehy, T. J., Patin, A. L. & Bartlett, A. C. 1999 Development time and resistance to *Bt* crops. *Nature* **400**, 519.

McGaughey, W. H. 1985 Insect resistance to the biological insecticide *Bacillus thuringiensis*. *Science* **229**, 193–195.

McGaughey, W. H. & Beeman, R. W. 1988 Resistance to *Bacillus thuringiensis* in colonies of Indian meal moth and almond moth (Lepidoptera: Pyralidae). *J. Econ. Entomol.* **81**, 28–33.

Marcon, P. C. R. G., Young, L. J., Steffey, K. L. & Siegfried, B. D. 1999 Baseline susceptibility of European corn borer (Lepidoptera: Crambidae) to *Bacillus thuringiensis* toxins. *J. Econ. Entomol.* **92**, 279–285.

Milks, M. L. 2002 Effect of cold storage of eggs on the response of cabbage looper (Lepidoptera: Noctuidae) larvae to a nucleopolyhedrovirus. *Can. Entomol.* **134**, 789–792.

Milks, M. L., Burnstyn, I. & Myers, J. 1998 Influence of larval age on the lethal and sublethal effects of the nucleopolyhedrovirus of *Trichoplusia ni* in the cabbage looper. *Biol. Control* **12**, 119–126.

Ramachandran, R., Raffa, K. F., Miller, M. J., Ellis, D. D. & McCown, B. H. 1993 Behavioral responses and sublethal effects of spruce budworm (Lepidoptera: Tortricidae) and Fall Webworm (Lepidoptera: Arctiidae) larvae to *Bacillus thuringiensis* CryIA(a) toxin in diet. *Environ. Entomol.* **22**, 197–211.

Ramachandran, S., Buntin, G. D., All, J. N., Tabashnik, B. E. & Raymer, P. L. 1998 Survival, development, and oviposition of resistant diamondback moth (Lepidoptera: Plutellidae) on transgenic canola producing a *Bacillus thuringiensis* toxin. *J. Econ. Entomol.* **91**, 1239–1244.

Robertson, J. L., Preisler, H. K., Ng, S. S., Hickle, L. A. & Gelernter, W. D. 1995 Natural variation: a complicating factor in bioassays with chemical and microbial pesticides. *J. Econ. Entomol.* **88**, 1–10.

Roush, R. T. & McKenzie, J. A. 1987 Ecological genetics of insecticide and acaricide resistance. *A. Rev. Entomol.* **32**, 361–380.

Salama, H. S. & Sharaby, A. 1988 Effects of exposure to sublethal doses of *Bacillus thuringiensis* (Berl.) on the development of the greasy cutworm, *Agrotis ypsilon* (Hufn.). *J. Appl. Entomol.* **106**, 396–401.

Sayyed, A. H. & Wright, D. J. 2001 Fitness costs and stability of resistance to *Bacillus thuringiensis* in a field population of the diamondback moth *Plutella xylostella* L. *Ecol. Entomol.* **26**, 502–508.

Tabashnik, B. E. 1994 Evolution of resistance to *Bacillus thuringiensis*. *A. Rev. Entomol.* **39**, 47–49.

Tabashnik, B. E., Finson, N. & Johnson, M. W. 1991 Managing resistance to *Bacillus thuringiensis*: lessons from the diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* **84**, 49–55.

Tabashnik, B. E., Finson, N., Groeters, F. R., Moar, W. J., Johnson, M. W. & Luo, K. 1994 Reversal of resistance to *Bacillus thuringiensis* in *Plutella xylostella*. *Proc. Natl Acad. Sci. USA* **91**, 4120–4124.

Tabashnik, B. E., Patin, A. L., Dennehy, T. J., Liu, Y., Carriere, Y., Sims, M. A. & Antilla, L. 2000 Frequency of resistance to *Bacillus thuringiensis* in field populations of pink bollworm. *Proc. Natl Acad. Sci. USA* **97**, 12 980–12 984.

Taylor, M. & Feyereisen, R. 1996 Molecular biology and evolution of resistance to toxicants. *Mol. Biol. Evol.* **13**, 719–734.

Uyenoyama, M. K. 1990 Pleiotropy and the evolution of genetic systems conferring resistance and pesticides. In *Pesticide resistance in arthropods* (ed. R. T. Roush & B. E. Tabashnik), pp. 207–221. NY: Routledge, Chapman and Hall Inc.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.