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

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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 *Bacillus 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. Resistance management in vegetable greenhouses will require sporadic use of *Bt*-based sprays or alternative use of sprays with other *Bt* toxins.●●3●●

Keywords: ●●4●●

1. INTRODUCTION

Bacillus thuringiensis (*Bt*) has been used successfully for over 30 years for the control of 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. We report here the second occurrence 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 *Bt* resistance evolution 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 decreased growth rate (Liu *et al.* 1999), survival (Groeters *et al.* 1994), fecundity and mating success (Groeters *et al.* 1993) of resistant versus 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 as regards the development of 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 summer months. *Trichoplusia ni* moths can then cycle continuously throughout the growing season with multiple overlapping generations per year. Similarly, resistance has been detected in field *P. xylostella* populations that undergo multiple generations per year in regions such as Hawaii, Malaysia, the Philippines, Florida and Thailand (Tabashnik 1994). The relative containment of *T. ni* populations in greenhouses

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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 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 if 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 enumerated in table 1. In 2001, three broccoli fields situated more than 1 km from commercial greenhouses and treated with one or fewer *Bt* applications 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 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 less 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 lower observed fertility of eggs stored longer than 7 days in 2000. In the majority of collections, larval stages differed by less than 7 days in development time to pupation. 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.

Larval *T. ni* 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 diet and a mean of 2.2 ± 0.4 ●●5●●kIU ml^{-1} diet. In the results, different greenhouses are designated by crop (T, tomato; P, pepper; C, cucumber), greenhouse number and year. Broccoli fields are indicated by the letter B.

(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 diluted serially 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 two times (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 repeat of the assay per population, with 10 or more larvae per dose. The numbers of larvae tested per 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. Two hundred larvae were maintained per generation. For each generation, eggs were chosen from the peak of the egg-laying period when the largest number of adult moths were present. Populations were re-assayed for *Bt* resistance following 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 treatment 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 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 owing to 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●●6●● 4.0). Populations with high mortality in the control treatment group (greater than 40%) at day 6 following the experimental set-up were discarded from the pupal and larval weight analyses owing to potential sublethal effects of disease such as nuclearpolyhedrovirus infections on growth and pupal size. In 2001 and 2002, larvae that hatched from eggs stored for longer than 10 days 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 greenhouse populations, using *t*-tests (JMPIN 4.0). Mean LC_{50} data of field and untreated greenhouse

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	res. ratio[a]	slope
2000	C1[b]	8/30	76	589	7	14	0.86 ± 0.01
	C2	8/15	94	292	3	26	0.55 ± 0.11
	C3	8/30	69	243	4	23	0.74 ± 0.15
	C4	8/10	68	181	2	12	0.75 ± 0.16
	P1	6/23	36	413	4	47	0.69 ± 0.09
	P2	6/29	31	364	3	16	1.10 ± 0.18
	P3	6/19	16	218	2	15	0.56 ± 0.11
	P4	8/16	133	403	4	25	0.56 ± 0.09
	T1	8/24	97	245	4	55	1.13 ± 0.38
2001	P4[b]	8/1	43	364	3	4	1.00 ± 0.13
	P4[b]	8/29	67	415	3	3	0.84 ± 0.09
	P5[b]	7/17	74	334	4	24	0.85 ± 0.19
	P6	5/8	17	371	2	22	0.97 ± 0.12
	C4a[b]	7/20	127	240	2	3	0.72 ± 0.08
	C4b	8/31	139	596	5	39	1.53 ± 0.37
	C5a	7/17	190	136	2	25	0.55 ± 0.26
	C5b[c]	9/5	91	278	2	5	0.82 ± 0.09
	T1a[b]	6/28	51	311	2	3	0.79 ± 0.10
	T1b[b]	8/23	168	320	3	5	0.64 ± 0.12
	T2a	9/11	223	234	2	44	0.65 ± 0.11
	T2b	10/9	113	390	2	90	0.48 ± 0.15
	T2c	11/23	90	343	2	113	0.62 ± 0.11
	B1[b]	8/8	76	392	2	4	0.73 ± 0.08
	B2[b]	8/28	138	392	3	1	0.99 ± 0.11
	B3[b]	8/29	82	370	3	1	1.63 ± 0.31
2002	T2a	7/10	120	328	2	47	0.97 ± 0.18
	T2b	9/18	157	285	2	160	0.25 ± 0.09
	T3a	6/18	55	258	2	13	0.47 ± 0.9
	T3b	8/28	171	360	2	28	0.50 ± 0.09
	T3c	9/26	164	294	2	31	0.84 ± 0.17
	T4a[b]	7/30	50	257	2	2	0.41 ± 0.08
	T4b[b]	9/11	48	146	2	4	0.42 ± 0.15
	P4	6/10	15	438	1	18	0.49 ± 0.06
	P7[b]	9/2	45	198	2	4	0.53 ± 0.12
	C4	9/11	278	350	1	50	0.51 ± 0.08

[a] Ratios were calculated with 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. ●●16●●

populations were compared using multiple comparison procedures (Student's *t*-tests in JMPIN 4.0).●●7●●

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 greenhouses that harboured the two most resistant populations in 2000, the three most resistant treated 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, table 1). Over the three survey years, eight greenhouse populations had not been 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 structures are cleaned and fumigated to eradicate any insects. Five of the untreated populations surveyed had resistance levels similar to those of the sampled field populations, with a mean

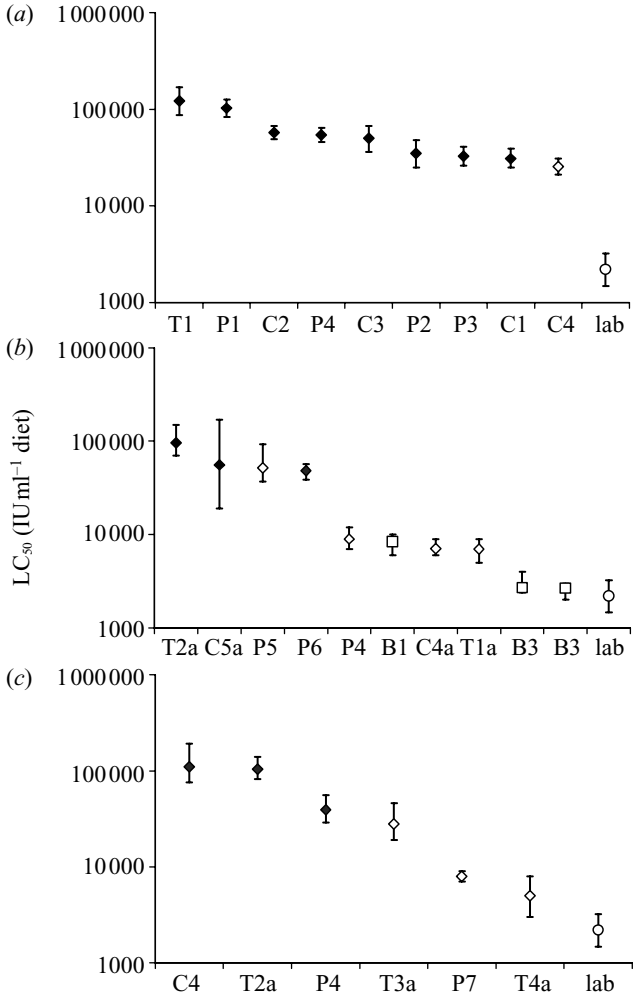

Figure 1. LC_{50} data and 95% fiducial limits of *Trichoplusia ni* populations collected from greenhouses in (*a*) 2000, (*b*) 2001 and (*c*) 2002. Greenhouse populations treated with *Bt* are shown as filled diamonds, and open diamonds when untreated, field populations as open squares, and the reference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

LC_{50} of 7.4 ± 1.2 kIU ml^{-1} diet, but they had significantly higher resistance 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 greenhouses (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* for the entire 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 following the chemical application and the LC_{50} was 10-fold lower than that of the initial collection (table 1). Four of the eight untreated greenhouse populations were also sampled multiple times within a growing season. Three of these remained untreated and displayed no change in LC_{50} between collection dates (P4-01, T1-01, T4-01) (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 versus that in the initial collection.

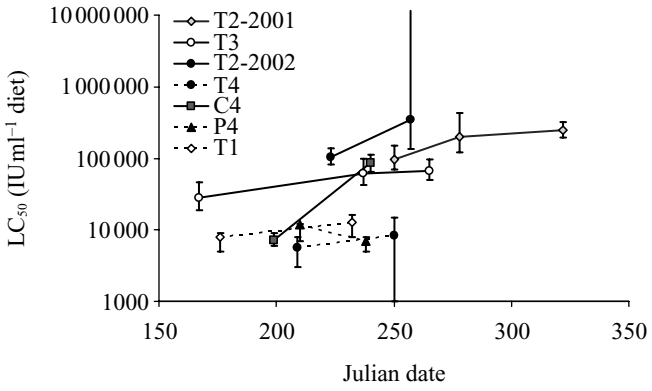

Figure 2. Changes in LC_{50} over time in greenhouse *Trichoplusia ni* populations in 2001–2002. Populations with solid lines were treated with *Bt* and populations with dashed lines were not. LC_{50} values with 95% fiducial limits were calculated using Probit analysis (GENSTAT 5).

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 if *Bt* resistance had been carried over. The LC_{50} values changed from 54 to 12, 25 to 7 and from 122 to 7 kIU ml^{-1} diet in the three greenhouse populations (P4, C4 and T1, respectively) (table 1). In all populations, resistance declined to levels equivalent to that of the field populations sampled in 2001. Growers experienced poor economic conditions at the beginning of 2001 owing to high petrol●●8●● 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 if 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 International Units per hectare was calculated for each *Bt* application by converting the amount applied to IU for both *Bt* formulations used by the growers (16×10^{9}●●9●● IU kg^{-1} Dipel and $10\,600 \times 10^{9}$●●9●● IU L^{-1} 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 pseudo-replication. No significant differences were found in the relationship between the amount of *Bt* applied and the assayed LC_{50} in the different years (d.f. = 2, $p = 0.66$) and therefore year was not included in the overall analysis.

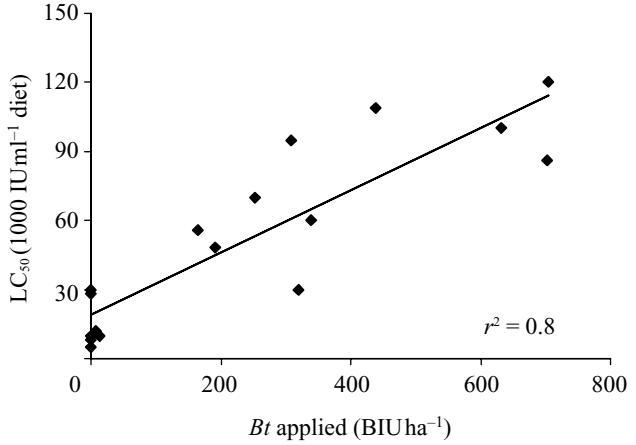

Figure 3. Relationship between the *Bt* dose-response assay LC_{50} values of the first generation offspring of greenhouse-collected *Trichoplusia ni* and the total amount of *Bt* applied in the greenhouse. Only the results of the collections conducted between May and September and the first collection per greenhouse per year are included to avoid pseudo-replication. BIU, billion International units.

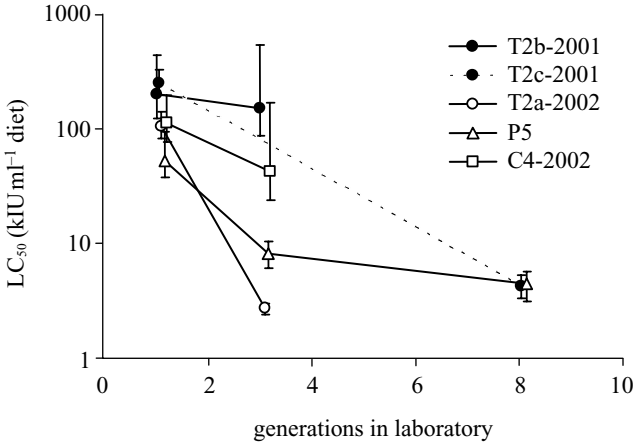

Figure 4. Decline in resistance of greenhouse *Trichoplusia ni* populations reared in the laboratory in the absence of *Bt* exposure. LC_{50} values and 95% fiducial limits are shown.

(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 (T2c-2001) from 248 to 4.2 kIU ml^{-1} diet after eight generations, at a rate of decline of -0.22, which indicates that less than five 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 two-fold, from 199 to 147 kIU ml^{-1} diet in three generations ($R = -0.04$) and it subsequently died out prior to eight generations. A third line (T2a-2002) established from the same greenhouse 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 three generations, at a rate of decline of -0.53, or a 10-fold decrease in LC_{50} in two generations.

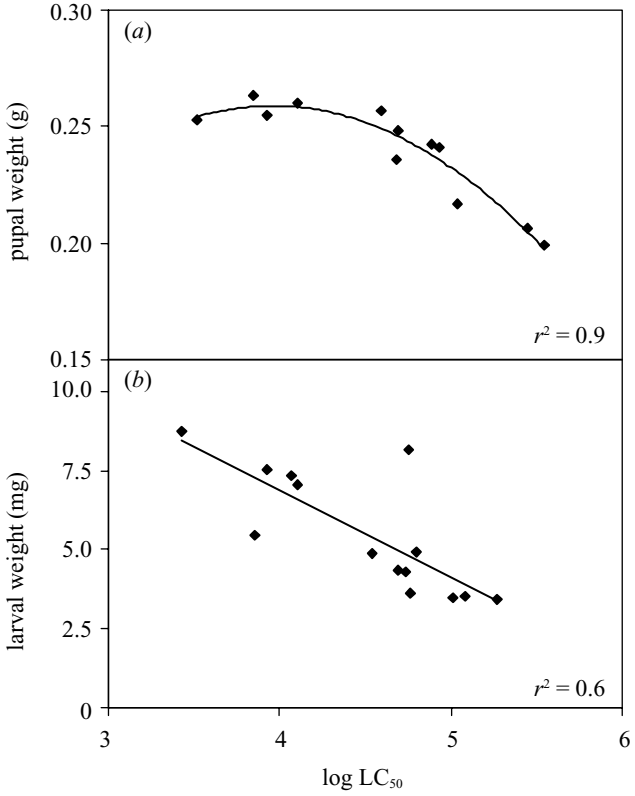

Figure 5. Relationship between pupal weights of collected larvae (*a*) and 5-day larval weights of offspring (*b*) versus the population LC_{50}. Only the results of the last collection per greenhouse per year are included to avoid pseudo-replication. Values of r^2 were calculated for a second order 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 JMP 4.●●15●●

The remaining two of the five colonies were established from two separate resistant greenhouse populations. One colony (C4b-2001)●●10●● was resistant at an LC_{50} of 86 kIU ml^{-1} diet, which declined to 63 kIU ml^{-1} diet after three generations at a rate of decline of -0.14 (10-fold decrease in seven generations). The final colony (P5) was moderately resistant at establishment, with an LC_{50} of 52 kIU ml^{-1} diet, which declined to 8 kIU ml^{-1} diet after three generations ($R = -0.27$) and to 4.3 kIU ml^{-1} diet after eight generations ($R = -0.13$).

(c) *Pupal and larval weights*

Parental pupal weights were significantly correlated to log-transformed population LC_{50} values ($r^2 = 0.91$) (figure 5). The mean pupal weight 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 (T2-01) and from 104 to 352 kIU ml^{-1} diet (T2-02) ($t = 12.9$, d.f. = 380, $p < 0.0001$ and $t = 7.9$, d.f. = 275, $p < 0.0001$). 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). 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) larval weights were a mean 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●●11●● 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●●12●● & 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 diet or more may be a suitable diagnostic dose. After 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 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 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 slow decline of resistance in these colonies suggests 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 that 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 weather, further affecting fitness (Gould *et al.* 1991). Therefore, the decrease 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 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 as regards pupal weights of 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, *Pectinophora gossypiella*, with different levels of resistance, showed no maternal effects on development time and 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 hybrid offspring of matings between reverted susceptible females and resistant males. 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 relative to 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 management strategies 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 modifier genes that could ameliorate fitness costs and thus could 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* crops.

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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. Economic 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. ●●12●●.

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 ●●12●●.

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

Genstat 5 Release 4.1 1998 Rothamsted Experimental Station, Lawes Agricultural Trust, 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. *Entomologia Experimentalis et Applicata* **58**, 1–14.

Gould, F., Anderson, A., Reynolds, A., Bumgarner, L. & Moar, W. J. 1995 Selection and genetics●●13●● analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. *J. Economic 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. Economic Entomol.* **86**, 1035–1039.

Groeters, F. R., Tabashnik, B. E., Finson, N. & Johnson, M. W. 1994 Fitness costs of resistance to *Bacillus thuringiesis* 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.

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. Economic 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. Economic 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. Entomologist* **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. Economic Entomol.* **91**, 1239–1244.

Robertson, J. L., Preisler, H. K., NG●●14●●, S. S., Hickle, L. A. & Gelernter, W. D. 1995 Natural variation: a complicating factor in bioassays with chemical and microbial pesticides. *J. Economic 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. *Ecological 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. Economic 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.

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