# Inheritance of Resistance to *Bacillus thuringiensis* Cry1Ac Toxin in a Greenhouse-Derived Strain of Cabbage Looper (Lepidoptera: Noctuidae)

WENDY C. KAIN,[1, 2] JIAN-ZHOU ZHAO,[1, 2], ALIDA F. JANMAAT,[3] JUDITH MYERS,[3] ANTHONY M. SHELTON,[1] AND PING WANG[1, 4]

J. Econ. Entomol. 97(6): 2073–2078 (2004)

**ABSTRACT** A population of cabbage looper, *Trichoplusia ni* (Hübner), collected from commercial greenhouses in the lower mainland of British Columbia, Canada, in 2001 showed a resistance level of 24-fold to Dipel, a product of *Bacillus thuringiensis* (Bt) subspecies *kurstaki*. This population was selected with Cry1Ac, the major Bt Cry toxin in Dipel, to obtain a homogenous population resistant to Cry1Ac. The resulting strain of *T. ni*, named GLEN-Cry1Ac, was highly resistant to Cry1Ac with a resistance ratio of ≈1000-fold. The larvae from the GLEN-Cry1Ac strain could survive on Cry1Ac-expressing transgenic broccoli plants that were highly insecticidal to *T. ni* and diamondback moth, *Plutella xylostella* (L.). The inheritance of Cry1Ac resistance in this *T. ni* strain was autosomal and incompletely recessive. The degree of dominance of the resistance was −0.402 and −0.395, respectively, for the neonates in reciprocal crosses between the GLEN-Cry1Ac and a laboratory strain of *T. ni*. Using $\chi^2$ goodness-of-fit test, we demonstrated that the inhibition of larval growth resulting from testing 12 toxin doses in the progeny of the backcross fit the predicted larval responses based on a monogenic inheritance model. Therefore, we conclude that the inheritance of the resistance to Cry1Ac in the *T. ni* larvae is monogenic.

**KEY WORDS** *Trichoplusia ni*, *Bacillus thuringiensis*, Cry1Ac, resistance, transgenic plants

*Bacillus thuringiensis* (Bt) has been widely used as a biological control agent for insect pest control. Since the commercial introduction of transgenic Bt plants in 1996, the acreage of Bt crops planted worldwide has been increasing steadily and reached 18 million ha in 2003 (James 2003). The widespread and prolonged application of Bt formulations and planting of Bt transgenic plants have created the threat of evolution of Bt resistance in field insect populations (Tabashnik 1994, Gould 1998, Ferre and van Rie 2002). Laboratory selection studies have demonstrated the high potential of resistance development to Bt in several insect species upon prolonged exposure to Bt (Ferre and van Rie 2002). In the field, Bt-resistant populations of diamondback moth, *Plutella xylostella* (L.), have been identified in various geographic regions where sprayable Bt formulations were applied (Ferre and van Rie 2002, Tabashnik et al. 2003). Very recently, Bt-resistant populations of the cabbage looper, *Trichoplusia ni* (Hübner), were found in commercial greenhouses where foliar sprays of Bt formulations were frequently applied (Janmaat and Myers 2003). For management of Bt resistance development in the field, it is important to understand the genetic basis of the resistance.

*T. ni* has a very diverse range of host plants, including >160 plant species in 36 families (Sutherland and Greene 1984), and is one of the most damaging lepidopteran pests on cabbage and other cole crops (Shelton et al. 1982, Liu 1999, Hines and Hutchison 2001). Control of *T. ni* is becoming increasingly difficult due to resistance to common synthetic insecticides and restrictions imposed on the applications of synthetic pesticides on fresh market vegetables (Liu et al. 2002). Bt formulations have been effective in the control of *T. ni* in both field and vegetable greenhouses (Liu 1999, Lundgren et al. 2002, Janmaat and Myers 2003). Similarly, transgenic Bt plants, including broccoli, Chinese cabbage, and cotton, are highly insecticidal to *T. ni* (Cao et al. 1999, Allen et al. 2000, Cho et al. 2001). However, the development of Bt resistance in *T. ni* populations (Janmaat and Myers 2003) alerts us to take necessary measures to monitor and manage resistance development in field *T. ni* populations.

The Bt-resistant *T. ni* populations identified from the commercial greenhouses by Janmaat and Myers (2003) showed various levels of resistance to Dipel, a product of *B. thuringiensis* subspecies *kurstaki* (Btk) containing multiple endotoxins and spores. It is known that different Cry toxins may interact with different insect midgut molecules as receptors (Ferre and van

[1] Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456.
[2] These authors contributed equally to this study.
[3] Department of Zoology, University of British Columbia, 6270 University Blvd., Vancouver, British Columbia, V6T 1Z4, Canada.
[4] Corresponding author, e-mail: pw15@cornell.edu.

0022-0493/04/2073–2078$04.00/0 

Rie 2002). Therefore, to understand the genetic basis of resistance to Dipel in field populations of *T. ni*, it is necessary to first study the resistance to different types of Bt toxins separately. To understand the genetic basis for the resistance of *T. ni* to the major Bt toxin in Dipel, Cry1Ac, we established a strain of *T. ni* highly resistant to Cry1Ac from a population collected from a commercial vegetable greenhouse where Bt resistance in *T. ni* has developed (Janmaat and Myers 2003) and studied the inheritance of the Cry1Ac-resistance in the *T. ni* strain.

## Materials and Methods

**Cry1Ac Protoxin.** Btk strain HD-73, received from the *Bacillus* Genetic Stock Center at The Ohio State University (Columbus, OH), was used for producing the Cry1Ac protoxin. The Bt culture was grown in glucose-Tris medium (Aronson et al. 1971), and the protoxin crystals were prepared by suspending the bacterial cells in one M NaCl, followed by two washes with deionized water by centrifugation. The protoxin crystals were solubilized in 50 mM sodium carbonate buffer (pH 9.5) containing 25 mM EDTA and 5% $\beta$-mercaptoethanol. The dissolved Cry1Ac protoxin in the supernatant was collected after removal of insoluble materials by centrifugation and precipitated by addition of 7% volume of 4 M sodium acetate (pH 4.5). The precipitated protoxin was then collected by centrifugation and resuspended in 50 mM sodium carbonate buffer (pH 9.5). To quantify the protoxin in a preparation, the proteins in the preparation were separated by SDS-PAGE analysis, and the quantity of the Cry1Ac protoxin in the SDS-PAGE gel was determined by comparing the Commassie staining intensity of the Cry1Ac protoxin band with a series of known quantities of bovine serum albumin on the same SDS-PAGE gel as standards.

**Insects.** A laboratory-susceptible strain of *T. ni*, which has been maintained on an artificial diet (Shorey and Hale 1965) in the laboratory for >20 yr without exposure to Bt toxins, was provided by Dr. Wendell Roelofs' laboratory at the New York State Agricultural Experiment Station (Cornell University, Geneva, NY). A Bt-resistant *T. ni* strain (GLEN) was collected in 2001 from commercial greenhouses in the lower mainland of British Columbia, Canada. When collected from the greenhouses, this strain showed a resistance of 24-fold to Dipel and was designated as P5 in the earlier report by Janmaat and Myers (2003). After two generations ($F_4$ and $F_5$) of selection on Dipel in Canada, the $F_6$ eggs were shipped to the New York State Agricultural Experiment Station for further selection by using the Bt protoxin Cry1Ac, and the resulting *T. ni* colony was designated GLEN-Cry1Ac. We also maintained a colony of the GLEN strain with no Bt selection, which is designated GLEN-Nonsel. Both GLEN-Cry1Ac and GLEN-Nonsel colonies were maintained on high wheat germ diet (Bell et al. 1981). The *T. ni* colonies were kept in environmental chambers at 27°C, 50% RH, and a photoperiod of 16:8 (L:D) h.

**Resistance Selections.** The GLEN-Cry1Ac colony was selected with Cry1Ac protoxin by using neonates with a diet overlay method (Zhao et al. 2002). Styrofoam cups (480 ml) were filled with ≈80 ml of diet (surface area of the diet was ≈50 $cm^2$). In each diet cup, 2 ml of Cry1Ac solution was applied to and distributed over the diet surface. After the solution was dried, 200–300 neonates were placed into each cup. Surviving larvae remained in these cups until pupation, unless the cups became too crowded, at which time some of the larvae (usually fourth or fifth instars) were transferred to new cups of untreated diet. The concentrations of Cry1Ac protoxin used for selections were 50 ($F_1$), 100 ($F_2$), 100 ($F_3$), 200 ($F_4$), 316 ($F_6$), 500 ($F_7$), and 500 mg([AI])/liter ($F_8$), and the survival rate for each selection ranged from 10 to 30%.

**Bioassays.** A diet overlay assay similar to the method described by Iracheta et al. (2000) and Zhao et al. (2002) was used to determine the susceptibility of neonates to Cry1Ac. Five to six concentrations of Cry1Ac plus a control (no Cry1Ac) and four or five cups (replications) for each concentration were included in each bioassay. An aliquot of 0.2 ml of Cry1Ac solution was applied to and evenly distributed over the diet surface (surface area ≈7 $cm^2$) of 30-ml plastic cups with 5 ml of high wheat germ diet. Ten neonates were transferred into each cup. Cups were covered with lids and held at 27°C, 50% RH, and a photoperiod of 16:8 (L:D) h for 4 d to determine mortality or growth inhibition. The larvae that reached second instar after 4 d could all survive and finish pupation, whereas, most of those that did not reach second instar after 4 d would eventually die before pupation. Inhibition of larval growth by Bt toxins has been shown to be a less variable criterion for lepidopteran larval response to Bt toxins (Sims et al., 1996; Wu et al., 2002). Therefore, we used growth inhibition (neonates not reaching second instar after 4 d) as the indicator for the susceptibility of *T. ni* neonates to the Bt protoxin Cry1Ac.

**Transgenic Plants Expressing Cry1Ac Toxin and Insect Tolerance Efficacy Tests.** Cytoplasmic male sterile broccoli, *Brassica oleracea* subspecies. *Italica*, was transformed with a full-length synthetic *cry1Ac* gene of *B. thuringiensis* (Metz et al. 1995). Progeny were produced by pollinating transformed plants with 'Green Comet' hybrid broccoli. The expression of the transgene *cry1Ac* in the transgenic plants was confirmed at both mRNA and protein levels by Northern blot analysis to detect the transcript of the transgene and by enzyme-linked immunosorbent assay (ELISA) to verify the protein production with antibodies specific to the Cry1Ac protein. The Cry1Ac protein expression level in the plants was >600 ng/g measured by ELISA. In addition, the toxicity of the expressed Cry1Ac protein in the plants was verified by screening the plants with *P. xylostella* neonates when plants were 4–5 wk old as described by Tang et al. (2001) and Zhao et al. (2002). Only those plants on which the neonates showed 0% survival were used as Bt plants in the tests. The transgenic and nontransgenic broccoli plants

**Table 1. Response of *T. ni* larvae to Cry1Ac protoxin on artificial diet**

| Colony | Slope (SE) | $IC_{50}$ (95% CL), mg (AI)/liter | $\chi^2$ (df)[a] | Resistance ratio |
|---|---|---|---|---|
| Susceptible strain | 2.36 (0.34) | 0.263 (0.199–0.335) | 0.99 (3)* | |
| GLEN-Cry1Ac | 1.84 (0.26) | 258 (191–345) | 1.89 (3)* | 981 ($F_9$) |
| | 2.25 (0.39) | 337 (256–429) | 0.90 (3)* | 1,281 ($F_{14}$) |
| GLEN-Nonsel | | <10[b] | | <38 ($F_5$) |
| | 1.78 (0.19) | 1.44 (1.13–1.83) | 1.95 (4)* | 5.5 ($F_{13}$) |

[a] $\chi^2$ (df) values marked by * indicate good fit of the data to the probit model ($P > 0.05$).
[b] The corrected mortality of $F_5$ neonates was 85% at 10 ppm (AI), a discriminating concentration of Cry1Ac that kills 100% of susceptible and 98% of heterozygous larvae.

grown in the greenhouse were used for the assays. Ten neonates were transferred into a 30-ml plastic cup with a Bt or non-Bt broccoli leaf disk (10–15 $cm^2$), and five cups were used for each colony. Growth inhibition was used for comparison between colonies. More than 90% of larvae that reached second instar could survive until pupation on leaves of Bt plants.

**Inheritance Tests.** For inheritance analysis, reciprocal crosses were made between the GLEN-Cry1Ac (RR) and the susceptible laboratory colony (SS). Because of the incompletely recessive inheritance of the Bt resistance trait in the RR colony, a backcross (BC) was made between the $F_1$ progeny from the cross of RR × SS and the GLEN-Cry1Ac strain, i.e., BC = $F_1$ of (RR × SS) × RR. The adults used for the reciprocal crosses and the backcross were from larvae that were not selected against Cry1Ac for the generation before the backcross. Following the method of Stone (1968), the degree of dominance for resistance was calculated using the reciprocal $F_1$ crosses and the pooled data. The single-concentration method (Liu and Tabashnik 1997) to estimate dominance ($h$) also was used for analysis of dominance. The $\chi^2$ goodness-of-fit test was performed to determine whether the observed response of the neonates from the backcross at each Cry1Ac testing concentration fit the predicted response based on the monogenic inheritance model (Tabashnik 1991, Zhao et al. 2000). Twelve Cry1Ac concentrations and 40 larvae for each concentration were used in the backcross bioassay.

**Statistical Analysis.** The POLO program (LeOra Software 1997) was used for probit analysis of the dose–response data (Russell et al. 1977). Mortality was corrected using Abbott's formula (Abbott 1925) for each probit analysis and for the Bt plants efficacy tests. Resistance ratio values for the GLEN-Cry1Ac and GLEN-Nonsel colonies were calculated by dividing the respective $IC_{50}$ (concentration for 50% inhibition of larval development from neonates to second instar larvae in 4 d) values for these colonies by the $IC_{50}$ value for the susceptible laboratory colony. SAS programs were used for analysis of variance (SAS Institute 1985) of the responses of *T. ni* larvae to Bt plants. Treatment means were compared and separated by Tukey's studentized range test (honestly significant difference [HSD]) at $P = 0.05$.

## Results

**Selection for Resistance to Cry1Ac.** The resistance level of the GLEN colony to Cry1Ac increased rapidly upon selection with Cry1Ac. After seven generations of selections, the resistance ratio to Cry1Ac reached a level of 981- to 1,281-fold in GLEN-Cry1Ac neonates in comparison with the laboratory-susceptible colony (Table 1). The resistance ratio to Cry1Ac of the neonates from the GLEN-Nonsel colony remained at a low level, 5.5-fold, after rearing without selection for 13 generations.

**Resistance of GLEN-Cry1Ac to Transgenic Bt Broccoli.** Similar to the response to Cry1Ac on artificial diet (Table 1), the resistance level of neonates from GLEN-Cry1Ac to transgenic Bt broccoli increased dramatically over three generations of selection with Cry1Ac on artificial diet. The corrected mortality of the GLEN-Cry1Ac neonates on Bt broccoli was 36% for the selected generation $F_3$ (Table 2). The mortality further decreased and stabilized at 7.5 to 15% after generation $F_6$. In contrast, the mortality of the neonates from the susceptible colony on the Bt broccoli was 100%. The neonates of the GLEN-Nonsel colony also showed a high mortality (86%) on the Bt broccoli after 13 generations of rearing without selection. However, 14% survival of the GLEN-Nonsel larvae on the Bt broccoli was significantly higher than 0% survival of the susceptible strain.

**Table 2. Response of *T. ni* larvae to Bt broccoli expressing Cry1Ac toxin**

| Colony | Mean % of growth inhibition (SEM), 4 d | | |
|---|---|---|---|
| | Non-Bt broccoli | Bt broccoli[a] | Corrected % of growth inhibition |
| Susceptible colony | 2.0 (2.0) | 100 (0)a | 100 |
| GLEN-Cry1Ac ($F_3$) | 0 | 36 (5.1)c | 36 |
| ($F_6$) | 2.0 (2.0) | 10 (4.5)d | 8.2 |
| ($F_9$) | 0 | 7.5 (2.5)d | 7.5 |
| ($F_{14}$) | 10 (4.1) | 15 (6.5)d | 5.9 |
| Susceptible × (GLEN-Cry1Ac $F_9$) | 2.0 (2.0) | 100 (0)a | 100 |
| GLEN-Nonsel ($F_{13}$) | 2.0 (2.0) | 86 (2.5)b | 86 |

[a] Means (±SEM) within the column followed by the same letter are not significantly different ($P > 0.05$, HSD).

**Table 3. Inheritance of Cry1Ac resistance in GLEN-Cry1Ac strain of *T. ni***

| Colony | *n* | Slope (SE) | $IC_{50}$ mg(AI)/liter | 95% CL | $\chi^2$ (df) | RR | D[a] |
|---|---|---|---|---|---|---|---|
| SS | 200 | 2.36 (0.34) | 0.263 | 0.199–0.335 | 0.99 (3) | | |
| GLEN-Cry1Ac ($F_9$) | 200 | 1.84 (0.26) | 258 | 191–345 | 1.89 (3) | 981 | |
| S(f) × R (RS1) | 200 | 1.86 (0.26) | 2.06 | 0.898–4.15 | 5.77 (3) | 7.8 | −0.402 |
| R(f) × S (RS2) | 200 | 1.75 (0.15) | 2.11 | 0.699–5.05 | 6.84 (3) | 8.0 | −0.395 |

[a] Degree of dominance (neonates, 4 d).

**Inheritance of the Cry1Ac Resistance.** The resistance ratios of the $F_1$ progeny from the reciprocal crosses between the resistant (GLEN-Cry1Ac) and the susceptible parental colonies were only 7.8 and 8.0, respectively, in contrast to the resistance ratio 981 in the resistant parent (Table 3). The degree of dominance (D) of the resistance based on pooled $F_1$ data were −0.402 and −0.395, respectively, for the neonates in the reciprocal crosses. Therefore, the inheritance of the resistance in GLEN-Cry1Ac seems to be incompletely recessive. Similarly, the $F_1$ progeny from the cross of the resistant and the susceptible colonies showed a mortality of 100% on the Bt transgenic broccoli, although its resistant parent only had a mortality of 7.5% on the Bt broccoli (Table 2). The estimated dominance ($h$) of resistance using the single-concentration method tended to be more recessive as the concentration increased. The resistance was partially dominant at concentrations of 0.2–2 ppm ($h$ = 0.95–0.54), partially recessive at concentrations of 5–10 ppm (h = 0.36–0.03), and recessive at concentrations of 20 ppm or above ($h$ = 0) that killed 100% SS and $F_1$ progeny but caused <5% mortality to RR neonates.

The $IC_{50}$ values of the $F_1$ progeny from the reciprocal crosses between the resistant and susceptible parents were virtually the same (Table 3) and so were the slopes of the Cry1Ac dose–mortality response lines between the $F_1$ progeny from the two crosses (Fig. 1). Therefore, the inheritance of the resistance is autosomal, without significant sex linkage or maternal effects.

The Cry1Ac dose–response line of the progeny from the backcross showed typical monogenic inheritance characteristics (Fig. 1). In the direct test of monogenic inheritance for Cry1Ac resistance, the observed responses resulting from all of the 12 Cry1Ac doses did not deviate from the predicted responses based on the monogenic inheritance model (Table 4), which strongly indicates that the inheritance of the resistance to Cry1Ac in the *T. ni* colony was monogenic.

## Discussion

In 2001, the GLEN strain of *T. ni* was collected from greenhouses where Dipel had been frequently used. When collected from the greenhouses, this GLEN strain showed a resistance level to Dipel of 24-fold (Janmaat and Myers 2003). The GLEN population was heterogeneous when collected from the greenhouses, which was clearly shown by the declining level of resistance in the GLEN-Nonsel colony after several generations without selection (Table 1). After laboratory selection of the colony with Cry1Ac, the colony (GLEN-Cry1Ac) became a homogenous population resistant to Cry1Ac and the resistance level was stable without further selection for 10 generations (data not shown). Dipel contains multiple Bt Cry toxins, including Cry1Aa, Cry1Ab, Cry1Ac, Cry2Aa, and Cry2Ab, and spores, and different Cry toxins are known to

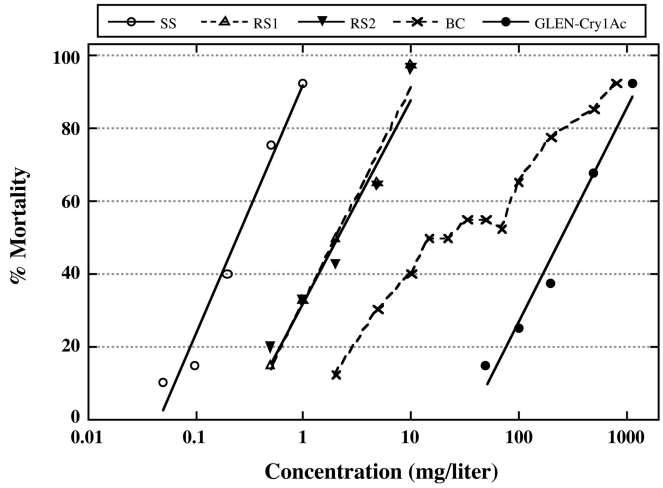


**Fig. 1.** Concentration–response curves for neonates from the resistant (GLEN-Cry1Ac) and susceptible (SS) colonies, progeny of the reciprocal crosses (RS1 and RS2), and progeny of the backcross (BC) to Cry1Ac.

**Table 4. $\chi^2$ test of monogenic inheritance for Cry1Ac resistance in the GLEN-Cry1Ac strain by using the expected and observed responses of the backcross progeny**

| Concn (mg[AI]/liter) | $n^a$ | Observed no. response | Expected no. response | $\chi^2$ (df = 1) | $P > \chi^{2b}$ |
|---|---|---|---|---|---|
| 2 | 40 | 5 | 9.8 | 3.11 | 0.078 |
| 5 | 40 | 12 | 15.2 | 1.09 | 0.296 |
| 10 | 40 | 16 | 18.0 | 0.41 | 0.523 |
| 15 | 40 | 20 | 19.1 | 0.08 | 0.774 |
| 22 | 40 | 20 | 19.9 | 0.00 | 0.974 |
| 33 | 40 | 22 | 20.7 | 0.16 | 0.688 |
| 50 | 40 | 22 | 21.8 | 0.00 | 0.945 |
| 70 | 40 | 21 | 22.9 | 0.38 | 0.540 |
| 100 | 40 | 26 | 24.5 | 0.25 | 0.618 |
| 200 | 40 | 31 | 28.4 | 0.84 | 0.361 |
| 500 | 40 | 34 | 34.0 | 0.00 | 0.992 |
| 800 | 40 | 37 | 36.3 | 0.13 | 0.716 |

[a] Number of larvae tested (4 d).
[b] Observed response did not significantly deviate from the model prediction in each concentration ($P > 0.05$).

interact with different midgut receptors in the insect hosts (Ferre and van Rie 2002). Therefore, to understand the genetic basis of *T. ni* resistance to Bt toxins, it is an appropriate approach to study the resistance to different Cry toxins separately. Among the Cry toxins in Dipel, Cry1Ac is a major toxin and highly active against *T. ni.* In this study, we established a strain of *T. ni*, GLEN-Cry1Ac, highly resistant to the Bt toxin Cry1Ac by selection of the *T. ni* colony GLEN with Cry1Ac. Larvae of GLEN-Cry1Ac became not only highly resistant to Cry1Ac on artificial diet but also to Bt transgenic broccoli expressing a high level of Cry1Ac.

The presence of multiple toxins without cross-resistance may significantly suppress the resistance development in an insect population (Zhao et al. 2003). Therefore, it is not surprising that after selection of the GLEN colony, which showed a moderate level of resistance to Dipel, with Cry1Ac without exposure to other Cry toxin proteins, the resistance level to Cry1Ac quickly increased to ≈1000-fold. Our bioassays using a diet overlay method reported in this article indicated that resistance in the *T. ni* strain GLEN-Cry1Ac was inherited as incompletely recessive and monogenic. Similarly, bioassays using Cry1Ac transgenic broccoli foliage also indicated that the inheritance of the resistance to Cry1Ac in GLEN-Cry1Ac larvae was recessive.

Our observations on the Bt resistance in the *T. ni* strain GLEN-Cry1Ac might resemble the "mode 1" resistance observed in other lepidopteran species (Tabashnik et al. 1998). Mode 1 resistance is characterized by a high level of resistance to one or more Cry1A toxins, recessive inheritance, reduced binding of one or more Cry1A toxins to the midgut brush-border membrane and little or no cross-resistance to Cry1C toxin (Tabashnik et al. 1998). The neonates from GLEN-Cry1Ac could not survive on the foliage of Bt transgenic broccoli with the *cry1C* gene or pyramided *cry1C* and *cry1Ac* genes (data not shown), suggesting that there was no cross-resistance to Cry1C in the larvae of GLEN-Cry1Ac. This observation is consistent with the mode 1 resistance. Previously, it was reported that a laboratory-selected Bt-resistant *T. ni* population produced 31-fold resistance to Cry1Ab (Estada and Ferre 1994). However, the resistance was limited to Cry1Ab and did not extend to Cry1Aa or Cry1Ac, even though Cry1Ac and Cry1Ab share the same binding site on the midgut brush-border membrane in *T. ni* (Iracheta et al. 2000). Apparently, that laboratory-selected *T. ni* strain is mechanistically different from the GLEN-Cry1Ac strain in the resistance.

Although several insect species have developed resistance to Bt formulations or toxins in laboratories (Ferre and van Rie 2002), only a few of these reported insect strains may survive on Bt transgenic plants (Tabashnik et al. 2003). Laboratory and greenhouse studies have shown that seven resistant laboratory strains of three lepidopterans, *P. xylostella*, the pink bollworm, *Pectinophora gossypiella* (Saunders), and the cotton bollworm, *Helicoverpa armigera* (Hübner), could complete their entire developmental cycle on Bt transgenic crops (Tabashnik et al. 2003). Of these species, only *P. xylostella* has been shown to have developed resistance in the field to a Bt spray, and with (Zhao et al. 2000) or without (Metz et al. 1995) further selection in the laboratory to a specific Bt toxin, to have developed sufficient resistance to be able to survive on a plant when that protein is expressed at a "high dose," according to U.S. Environmental Protection Agency standards (U.S. EPA 2000). The work presented herein indicates that *T. ni* is only the second species to have demonstrated this ability.

## Acknowledgments

We thank E. D. Earle for providing Bt transgenic broccoli plants and J. Zhang for providing Cry1Ac protoxin and technical assistance. This research was supported in part by the Cornell University Agricultural Experiment Station federal formula funds, Project No. NYG-621510 received from Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture.

## References Cited

**Abbott, W. S. 1925.** A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

**Allen, C. T., M. S. Kharboutli, C. Capps, L. D. Earnest, P. Dugger, and D. Richter. 2000.** Effectiveness of Bollgard II cotton varieties against foliage and fruit feeding caterpillars in Arkansas. Proc. Beltwide Cotton Conf. 2: 1093–1094. National Cotton Council, Memphis, TN.

**Aronson, A. I., N. Angelo, and S. C. Holt. 1971.** Regulation of extracellular protease production in *Bacillus cereus* T: characterization of mutants producing altered amounts of protease. J. Bacteriol. 106: 1016–1025.

**Bell, R. A., C. D. Owens, M. Shapiro, and J. R. Tardif. 1981.** Development of mass-rearing technology, pp. 599–633. *In* C. C. Doane and M. L. McManus [eds.], The gypsy moth: research toward integrated pest management. U.S. Dep. Agric., Washington, DC.

**Cao, J., J. D. Tang, N. Strizhov, A. M. Shelton, and E. D. Earle. 1999.** Transgenic broccoli with high levels of *Bacillus thuringiensis* Cry1C protein, control diamondback moth

larvae resistant to Cry1A or Cry1C. Mol. Breed. 5: 131–141.

**Cho, H. S., J. Cao, J. P. Ren, and E. D. Earle. 2001.** Control of lepidopteran insect pests in transgenic Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) transformed with a synthetic *Bacillus thuringiensis* cry1C gene. Plant Cell Rep. 20: 1–7.

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

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

**Gould, F. 1998.** Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu. Rev. Entomol. 43: 701–726.

**Hines, R. L., and W. D. Hutchison. 2001.** Evaluation of action thresholds and spinosad for lepidoteran pest management in Minnesota cabbage. J. Econ. Entomol. 94: 190–196.

**Iracheta, M. M., B. Pereyra-Alferez, L. Galan-Wong, and J. Ferre. 2000.** Screening for *Bacillus thuringiensis* crystal proteins active against the cabbage looper, *Trichoplusia ni.* J. Invertebr. Pathol. 76: 70–75.

**James, C. 2003.** Preview: global status of commercialized transgenic crops: 2003. ISAAA Briefs No. 30. International Service for the Acquisition of Agri-Biotech Applications, Ithaca, NY.

**Janmaat, A. F., and J. Myers. 2003.** Rapid evolution and the cost of resistance to *Bacillus thuringiensis* in greenhouse populations of cabbage looper, *Trichoplusia ni.* Proc. R. Soc. Lond. B. 270: 2263–2270.

**LeOra Software. 1997.** POLO-PC: probit and logit analysis. LeOra Software, Berkeley, CA.

**Liu, T.-X. 1999.** Effects of some new formulations of *Bacillus thuringiensis* for management of cabbage looper and diamondback moth on cabbage in South Texas. Southwest. Entomol. 24: 167–177.

**Liu, T.-X., A. N. Sparks, Jr., W. Chen, G.-M. Liang, and C. Brister. 2002.** Toxicity, persistence, and efficacy of indoxacarb on cabbage looper (Lepidoptera: Noctuidae) on cabbage. J. Econ. Entomol. 95: 360–367.

**Liu, Y.-B., and B. E. Tabashnik. 1997.** Inheritance of resistance to *Bacillus thuringiensis* toxin Cry1C in the diamondback moth. Appl. Environ. Microbiol. 63: 2218–2223.

**Lundgren, J. G., G. E. Heimpel, and S. A. Bomgren. 2002.** Comparison of *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) augmentation with organic and synthetic pesticides for control of cruciferous Lepidoptera. Environ. Entomol. 31: 1231–1239.

**Metz, T. D., R. T. Roush, J. D. Tang, A. M. Shelton, and E. D. Earle. 1995.** Transgenic broccoli expressing a *Bacillus thuringiensis* insecticidal crystal protein: implications for pest resistance management strategies. Mol. Breed. 1: 309–317.

**Russell, R. M., J. L. Robertson, and N. E. Savin. 1977.** POLO: a new computer program for probit analysis. Bull. Entomol. Soc. Am. 23: 209–213.

**SAS Institute. 1985.** SAS user's guide: statistics, 5th ed. SAS Institute, Cary, NC.

**Shelton, A. M., J. T. Andaloro, and J. Barnard. 1982.** Effects of cabbage looper, imported cabbage worm, and diamondback moth on fresh market and processing cabbage. J. Econ. Entomol. 75: 742–745.

**Shorey, H. H., and R. L. Hale. 1965.** Mass-rearing of the larvae of nine noctuid species on a simplified medium. J. Econ. Entomol. 58: 522–524.

**Sims, S. B, J. T. Greenplate, T. B. Stone, M. A. Caprio, and F. Gould. 1996.** Monitoring strategies for early detection of Lepidoptera resistance to *Bacillus thuringiensis* insecticidal protein, pp. 229–242. *In* T. M. Brown [ed.], Molecular genetics and evolution of pesticide resistance. ACS Symposium Series 645. American Chemical Society, Washington, DC.

**Stone, B. F. 1968.** A formula for determining degree of dominance in cases of monofactorial inheritance of resistance to chemicals. Bull. W.H.O. 38: 325–326.

**Sutherland, D. W., and G. L. Greene. 1984.** Chapter 1, cultivated and wild host plants, pp. 1–13. *In* Suppression and management of cabbage looper populations. USDA–ARS Technical Bulletin No. 1684. U.S. Dep. Agric. Washington, DC.

**Tabashnik, B. E. 1991.** Determining the mode of inheritance of pesticide resistance with backcross experiments. J. Econ. Entomol. 84: 703–712.

**Tabashnik, B. E. 1994.** Evolution of resistance to *Bacillus thuringiensis.* Annu. Rev. Entomol. 39: 47–79.

**Tabashnik, B. E., Y. B. Liu, T. Malvar, D. G. Heckel, L. Masson, and J. Ferré. 1998.** Insect resistance to *Bacillus thuringiensis:* uniform or diverse? Phil. Trans. R. Soc. Lond. B. 353: 1751–1756.

**Tabashnik, B. E., Y. Carrière, T. J. Dennehy, S. Morin, M. S. Sisterson, R. T. Roush, A. M. Shelton, and J.-Z. Zhao. 2003.** Insect resistance to transgenic *Bacillus thuringiensis* crops: lessons from the laboratory and field. J. Econ. Entomol. 96: 1031–1038.

**Tang, J. D., H. L. Collins, T. D. Metz, E. D. Earle, J.-Z. Zhao, R. T. Roush, and A. M. Shelton. 2001.** Greenhouse tests on resistance management of Bt transgenic plants using refuge strategies. J. Econ. Entomol. 94: 240–247.

**[EPA] U.S. Environmental Protection Agency. 2000.** SAP report no. 2000-07. (http://www.epa.gov/scipoly/sap/2000/october/octoberfinal.pdf).

**Wu, K. M., Y. Y. Guo, N. Lv, J. T. Greenplate, and R. Deaton. 2002.** Resistance monitoring of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis* insecticidal protein in China. J. Econ. Entomol. 95: 826–831.

**Zhao, J.-Z., H. L. Collins, J. D. Tang, J. Cao, E. D. Earle, R. T. Roush, S. Herrero, B. Escariche, J. Ferre, and A. M. Shelton. 2000.** Development and characterization of diamondback moth resistance to transgenic broccoli expressing high levels of Cry1C. Appl. Environ. Microbiol. 66: 3784–3789.

**Zhao J.-Z., Y. X. Li, H. L. Collins, and A. M. Shelton. 2002.** Examination of the $F_2$ screen for rare resistance alleles to *Bacillus thuringiensis* toxins in the diamondback moth. J. Econ. Entomol. 95: 14–21.

**Zhao, J.-Z, J. Cao, Y. X. Li, H. L. Collins, R. T. Roush, E. D. Earle, and A. M. Shelton. 2003.** Transgenic plants expressing two *Bacillus thuringiensis* toxins delay insect resistance evolution. Nat. Biotechnol. 21: 1493–1497.

*Received 28 April 2004; accepted 23 September 2004.*