Cabbage Looper Resistance to a Nucleopolyhedrovirus Confers Cross-Resistance to Two Granuloviruses

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ABSTRACT Previously, we showed that cabbage loopers (*Trichoplusia ni* Hübner) can evolve >20X resistance to the single (S) nucleocapsid nucleopolyhedrovirus (NPV) of *Trichoplusia ni* (TnSNPV). In this study, we investigate one potential cost that resistant cabbage loopers may incur, increased susceptibility to other mortality agents. Contrary to expectation, no such cost was observed with any of the six mortality agents tested. In fact, the LD_{50} of selected larvae was always greater than that of control caterpillars for each agent tested. However, the differences were never significant for permethrin or *Bacillus thuringiensis* subsp *kurstaki* (Berliner). The differences in the LD_{50} of control and selected *T. ni* for the wild-type multiple (M) nucleocapsid NPV of *Autographa californica* Speyer (AcMNPV, clone *C6*) and the recombinant AcMNPV (AcMNPV-AaIT) were small (≈2X) and significant in only one of three generations. Surprisingly, the highest level of cross-resistance was to the granuloviruses of *Pieris rapae L.* (4–5X; significant in two of three generations) and *T. ni* (20–30X; significant in three of three generations). This suggests that the infection pathway of TnSNPV may be more similar to TnGV than to that of AcMNPV.

KEY WORDS *Trichoplusia ni*, nucleopolyhedrovirus, TnSNPV, cost of resistance, cross-resistance, TnGV

INSECT RESISTANCE TO CHEMICALS and pathogens often entails costs (Roush and Tabashnik 1990). Traditionally, these possible costs have been measured by comparing the fitness (e.g., developmental time to adulthood and/or reproductive success) of susceptible and resistant insects reared in the absence of the mortality agent. While many studies have been successful in documenting resistance costs using this approach (e.g., Groeters et al. 1994, Fuxa and Richter 1998), several others have failed (e.g., McGaughey and Beeman 1988, Glenn et al. 1994). Furthermore, this approach may overlook more subtle costs that are not directly related to fitness. Most notably, resistance to one mortality agent may increase the susceptibility of insects to other ones. Sheppard and Joyce (1998) showed that horn flies (*Hematobia irritans* L.) that were resistant to pyrethroids were more susceptible to chlorfenapyr (for other examples see Drummond et al. 1995; Pimprale et al. 1997).

Previously, Milks and Myers (2000) conducted two experiments in which they selected cabbage loopers (*Trichoplusia ni* Hübner) for resistance to the single (S) nucleocapsid nucleopolyhedrovirus of *T. ni* (TnSNPV), and concurrently selected the virus for increased virulence. The cabbage loopers of experiment 1 evolved 4.4× resistance to the virus after 15 generations of selection with TnSNPV whereas those of experiment 2 developed 22× resistance after 26 generations of selection. However, the virus did not evolve greater virulence in either experiment. Furthermore, resistance to TnSNPV did not appear to incur any costs when investigated using the traditional protocol. Cabbage loopers from the control and selected lines developed at the same rate and had the same reproductive success (egg production and percent hatch) when reared in the absence of TnSNPV (Milks and Myers 2002).

The purpose of the current study was to determine the effect of resistance to TnSNPV on the susceptibility of *T. ni* to four other baculoviruses. The wild-type multiple (M) nucleocapsid NPV of *Autographa californica* Speyer (AcMNPV, clone *C6*) was chosen because it is the archetype NPV. The recombinant AcMNPV (AcMNPV-AaIT) expressing the neurotoxin of the scorpion (*Androctonus australis* Hector) under the control of P10 promoter (McCutchen et al. 1991) was of particular interest because it kills *T. ni* ≈30% faster than wild type AcMNPV, and shows a great deal of potential for being developed as a bioinsecticide against cabbage loopers (Cory et al. 1994). The granuloviruses of *T. ni* (TnGV) and of *Pieris rapae* L. (PrGV) were chosen to determine if resistance to TnSNPV affected the susceptibility of cabbage loopers to viruses belonging to the other Baculoviridae genus.

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Table 1. Slope (SE), LD_{50} (95% fiducial limits), goodness of fit (chi-square/degrees of freedom), and resistance ratio for various mortality agents for two lines of *Trichoplusia ni*, one control and one selected for resistance to the single nucleocapsid nucleopolyhedrovirus of *T. ni*.

Mortality agents[a]	Generation	Control line					Selected line					
		n	Slope	LD_{50} (95% FL)	χ^2/df	*P*	n	Slope	LD_{50} (95% FL)	χ^2/df	*P*	RR[b]
TnSNPV[c]	20	120	0.9 (0.3)	927 (424–2,284)	3/2	0.22	120	0.9 (0.3)	29,622 (15,472–154,451)	3/2	0.23	34*
	25	120	1.3 (0.3)	5,736 (3,150–8,958)	2.3/2	0.32	119	1.2 (0.4)	122,674 (66,408–909,526)	1.3/3	0.73	21*
	27	120	1.8 (0.3)	5,059 (3,309–7,065)	1.7/2	0.43	120	1.8 (0.7)	109,248 (71,528–339,338)	7.3/3	0.06	22*
B. thuringiensis	25	118	2.2 (0.7)	127 (87–168)	12/3	<0.01	120	2.4 (0.9)	183 (137–231)	15/3	<0.01	1.4
	27	125	3.3 (0.7)	175 (131–216)	5.2/3	0.15	119	2.9 (0.8)	239 (206–268)	4.3/3	0.23	1.4
Permethrin	27	120	3.7 (1.0)	2.8 (1.8–3.9)	2.1/2	0.35	120	3.3 (0.9)	3.2 (2–4.6)	2.1/2	0.35	1.4
wt-AcMNPV	20	120	3.0 (0.7)	447 (305–567)	0.1/2	0.97	120	1.7 (0.3)	596 (351–853)	0.1/2	0.99	1.3
	25	119	2.1 (0.4)	586 (413–782)	0.3/3	0.96	119	1.5 (0.3)	1,466 (1,015–2,393)	5.1/3	0.16	2.5*
	27	120	2.4 (0.6)	705 (525–925)	7.3/3	0.06	119	2.6 (0.6)	1,060 (813–1,390)	6.7/3	0.08	1.5
AcMNPV-AaIT	20	117	1.8 (0.4)	413 (295–685)	2.1/3	0.55	120	1.4 (0.3)	713 (426–2,263)	1.6/3	0.66	1.7
	25	120	1.6 (0.4)	367 (230–987)	4.5/3	0.21	119	1.6 (1.4)	1,084 (482–62,426)	3.0/3	0.38	3.0
	27	119	2.3 (0.5)	486 (318–640)	2.4/2	0.29	117	2.7 (0.8)	781 (698–1,011)	5.9/2	0.06	1.6*
PrGV ($\times 10^7$)	20	144	1.4 (0.3)	13 (6.3–33)	4.9/4	0.30	143	1.3 (0.3)	52 (23–160)	3.2/4	0.52	4.0
	25	120	1.5 (0.2)	62 (37–110)	5.0/3	0.17	120	1.9 (0.7)	280 (170–510)	12/3	<0.01	4.5*
	27	119	1.3 (0.3)	58 (26–103)	0.4/1	0.84	120	2.3 (0.5)	317 (194–519)	0.1/1	0.93	5.5*
TnGV ($\times 10^8$)	20	144	1.1 (0.2)	4 (2–7.4)	8.7/4	0.07	144	0.9 (0.1)	75 (36–215)	1.3/4	0.87	19*
	25	120	1.6 (0.3)	14 (9–22)	3/3	0.40	120	0.6 (0.2)	687 (256–2,364)	5.6/3	0.13	49*
	27	142	1.7 (0.3)	11 (7–17)	1.1/4	0.90	142	0.9 (0.2)	249 (120–880)	6/4	0.20	23*

[a] *B. thuringiensis* doses: 38,000, 3,800, 380, 270 or 40 international units/larva; Permethrin concentrations: 12, 6, 3 or 1.5% (v/v) permetrin; AcMNPV doses: G20: 7500, 1875, 750 or 375 OBs per larva; G25 and 27: 3750, 1875, 940, 470 or 235 OBs/larva; AcMNPV-AaIT doses: G20 and 25: 750, 375, 190, 95 or 50 OBs per larva; G27: 7500, 3750, 750 or 375 OBs/larva; PrGV doses: G20: 1×10^9, 1×10^8, 1×10^7, 5×10^6, 2×10^6 or 1×10^6 granules/arva; G25: 1×10^9, 1×10^8, 1×10^7, 5×10^6 or 2×10^6 granules/larva; G27: 1×10^9, 1×10^8 or 1×10^7 granules/larva; TnGV doses: G20 and 27: 5×10^{10}, 1×10^{10}, 5×10^9, 1×10^9, 5×10^8 or 1×10^8 granules/larva; G25: 5×10^{10}, 1×10^{10}, 5×10^9, 1×10^9 or 5×10^8 granules/larva.

[b] Resistance ratio = LD_{50} of selected line/LD_{50} of control line.

[c] From Milks and Myers (2000).

* Significant difference between the LD_{50} of control and selected lines based on overlap of 95% fiducial limits.

Two mortality agents commonly used against *T. ni* in the wild were also tested, the microbial insecticide *Bacillus thuringiensis* Berliner subspecies *kurstaki* and the synthetic pyrethroid permethrin. *Bacillus thuringiensis* causes the epithelial cell lining of the midgut to rupture while permethrin attacks the nervous system of insects. Finally, we chose to conduct this study with the cabbage loopers of experiment 2 of Milks and Myers (2000) because those insects had evolved greater resistance to TnSNPV and thus may be more likely to show increased susceptibility to the mortality agents tested.

Materials and Methods

Source of Mortality Agents. Samples of AcMNPV (clone C6) and of AcMNPV-AaIT were obtained respectively from Drs. D. A. Theilmann (Agriculture and Agri-Food Canada) and B. D. Hammock (University of California at Davis). Once received, the viruses were passed once in our *T. ni*. The occlusion bodies (OBs) were purified from the cadavers as described in Hostetter et al. (1990), resuspended in sterile distilled water and quantified using a hemocytometer. A PrGV solution produced in *P. rapae* was quantified by and obtained from Dr. R. P. Jaques (Agriculture and Agri-Food Canada). A TnGV solution produced in *T. ni* was quantified by and obtained from Dr. R. R. Granados (Boyce-Thompson Institute, Cornell University). *B. thuringiensis* subspecies *kurstaki* crystals were obtained from Safer's Ltd (Scarborough, Ontario) and the insecticide permethrin from Horticulture Laboratories, Inc (Mississauga, Ontario). All mortality agents were serially diluted in distilled water.

Bioassays. At generations 20, 25, and 27 (=offspring of the last generation of selection), neonates of the control and selected lines were individually placed in 25-ml plastic cups containing high wheat germ diet (Jaques 1967), and reared at 26 ± 1°C with a 16:8 (L:D) photoperiod. At 168 h of age (fifth instar), caterpillars were transferred to a second cup containing only a plug of diet that had been inoculated with one of three to six doses (see Table 1) of TnSNPV, AcMNPV, AcMNPV-AaIT, PrGV, TnGV or *B. thuringiensis* (generations 25 and 27 only) or with distilled water as control. Larvae that consumed the entire plug within 24 h were returned to their original cup while those failing to do so were discarded (typically ≈3%). For permethrin, caterpillars (generation 27 only) were individually immersed for 2 s using forceps in a permethrin solution (one of four concentrations: see Table 1) or distilled water, and then returned to their cup. In each bioassay, the doses were replicated three times and there were 8–10 larvae per replicate (depending on availability). The cups were subsequently checked daily until adult emergence. The LD_{50} of control and selected lines were calculated using PROC PROBIT (SAS Institute 1990) and were judged to be significantly different on the basis of overlap of their 95% fiducial limits. The data were pooled across replicates (SAS Institute 1990) and were not corrected for control mortality because few (≤5%) of the insects that were mock infected died. Finally, all assays

were conducted with 168-h-old larvae because this is the stage at which caterpillars had been selected for resistance to TnSNPV (Milks and Myers 2000).

Results and Discussion

Resistance to TnSNPV did not adversely affect the susceptibility of *T. ni* to any of the mortality agents tested. If anything, it may have conferred cross-resistance to some of them. The LD_{50} of selected larvae was always greater than that of control caterpillars for each agent tested (Table 1). However, the differences were never significant for permethrin or *B. thuringiensis,* and were significant in only one of three generations for wt-AcMNPV and AcMNPV-AaIT (Table 1). Hence, whatever cross-resistance to these mortality agents exists is likely to be marginal and of questionable biological significance. Surprisingly, the greatest cross-resistance was to the granuloviruses, in particular the granulovirus of *T. ni* (Table 1). Selected caterpillars were 4–5× more resistant to PrGV (significant in two of three generations), and at least 20× more resistant to TnGV (significant in three of three generations) than control larvae (Table 1).

Fuxa and Richter (1990) also examined the effect of resistance to a nucleopolyhedrovirus on the susceptibility of caterpillars to other mortality agents and obtained similar findings to those of our study. They showed that resistance (3×) to the nucleopolyhedrovirus of *Spodoptera frugiperda (*SfMNPV) did not affect the susceptibility of *S. frugiperda* larvae to *B. thuringiensis* or to the chemical methyl parathion. Resistance to SfMNPV did confer weak (≈2×) but statistically significant cross-resistance to AcMNPV and to the granulovirus of *S. frugiperda* (SfGV) (Fuxa and Richter 1990). However, the assays were conducted only once with each virus

Why did ≈20× resistance to TnSNPV translate into at best very small (≤2×: average across generations 20, 25, and 27) cross-resistance to AcMNPV when *S. frugiperda* that evolved 3X resistance to SfMNPV showed about the same level (2×) of cross-resistance to AcMNPV? Sequencing of NPVs (Ayres et al. 1994, Ahrens et al. 1997, Ijkel et al. 1999, Gomi et al. 1999, Kuzio et al. 1999, Chen et al. 2001, Hyink et al. 2002) suggest that there can be considerable genetic variation among viruses, and perhaps important differences in their routes of infection. For example, the GP64 protein that occurs on the surface of AcMNPV budded viruses and which is responsible for the spread of the infection throughout the body of the caterpillar is absent from the NPV of *Lymantria dispar* (Kuzio et al. 1999). Hence, the lower cross-resistance to AcMNPV observed in this study may reflect greater genetic difference between AcMNPV and TnSNPV than between AcMNPV and SfMNPV. Conversely, the high level of cross-resistance to TnGV suggests that TnSNPV and TnGV may have similar routes of infection and perhaps be closely related either via convergent evolution or a common ancestry.

In this study and those of Milks and Myers (2000) and Milks et al. (2003), we have established that cabbage loopers can evolve resistance to TnSNPV and that resistance does not adversely affect the developmental time, reproductive success or the susceptibility of *T. ni* to six mortality agents. The next step should be to investigate the genetic and physiological mechanisms of cabbage looper resistance to TnSNPV. Resistance to SfMNPV is controlled by ≥1 gene lacking dominance in *S. frugiperda* (Reichelderfer and Benton 1974), to a single dominant gene in *Phthorimaea operculella* Zeller resistant to *P. operculella* GV (Briese 1982), and is polygenic *in Bombyx mori* L. resistant to *B. mori* NPV (Aratake 1973). A number of physiological mechanisms have been suggested as possible factors determining resistance to NPVs. Fuxa and Richter (1990) showed that the mortality of susceptible and resistant *S. frugiperda* to SfMNPV did not differ when virions were injected into the hemocoel and concluded that resistance was associated with the midgut. Resistant individuals may be able to slough off the primary target cells of the virus in the midgut before the virus spreads to other tissues thus increasing their chance of survival (Keddie et al., 1989; Washburn et al. 1995, 1998; Hoover et al. 2000). However, recent findings suggest that the hemocoel may also play a role in resistance to TnSNPV. Washburn et al. (1996) showed that infected cells were encapsulated by hemocytes and destroyed (see also Begon et al. 1993). Determining the mechanism of resistance to TnSNPV may provide an explanation for the difference in cross-resistance to wt-AcMNPV and TnGV and perhaps also provide insight into strategies for engineering recombinant NPVs for increased insecticidal activity.

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