Interactions of Recombinant and Wild-Type Baculoviruses with Classical Insecticides and Pyrethroid-Resistant Tobacco Budworm (Lepidoptera: Noctuidae)

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ABSTRACT In tests with neonate *Heliothis virescens* (F.), we characterized interactions of all combinations of a recombinant *Autographa californica* (Speyer) nuclear polyhedrosis virus (AcAaIT) that expresses an insect-selective neurotoxin (AaIT) and wild-type AcNPV when combined with low concentrations of several conventional insecticides. All combinations of the recombinant virus AcAaIT and insecticides showed a positive interaction (decrease in the median lethal time (LT_{50}) compared with the LT_{50} for either component alone). A type II pyrethroid (cypermethrin, which modifies currents of sodium channels) and a carbamate (methomyl, an inhibitor of acetylcholinesterase) were synergistic in combination with AcAaIT. Other insecticides also showed a positive interaction when tested in combination with the recombinant virus, but joint activity was slightly antagonistic (i.e., less than predicted activity when combined) with wild-type AcNPV. We also characterized the effectiveness of AcAaIT against pyrethroid-resistant *H. virescens* larvae. Our results show that a resistant strain of *H. virescens* is more sensitive to the recombinant virus compared with a susceptible strain. Results of these studies should be useful in planning of future field trials to increase the effectiveness of nuclear polyhedrosis viruses and to manage resistance to pyrethroids and other insecticides.

KEY WORDS *Heliothis virescens*, baculovirus, insecticide, pyrethroid, recombinant microorganism, nuclear polyhedrosis virus

SYNTHETIC ORGANIC CHEMICALS are important components of modern agriculture (Mellor and Adams 1984, Croft 1990). However, their use is increasingly criticized because of problems associated with environmental contamination and toxicity to nontarget organisms. These problems are compounded by the increasing costs of discovery, development, and registration of new insecticides. In addition, an increasing number of insect species have become resistant to the available insecticides.

Consequently, use of alternative control agents (including insect pathogens) is being explored. However, many of these pathogens lack the inherent properties of synthetic organic insecticides. Recombinant DNA technology may provide a tool to engineer microbials to yield increased insecticidal properties (Miller et al. 1983, Keeley and Hayes 1987). Recently, the pathogenic virus *Autographa californica* (Speyer) nuclear polyhedrosis virus (AcNPV) (Baculoviridae) has been genetically modified to increase its speed of kill. The introduction of toxins selective to insects (Maeda et al. 1991; McCutchen et al. 1991; Stewart et al. 1991; Tomalski and Miller 1991, 1992), the enzyme juvenile hormone esterase derived from insects (Hammock et al. 1990, Bonning and Hammock 1994), and a maize mitochondrial protein, URF13 (Korth and Levings 1993), has resulted in ≈20–30% reduction in time to death.

Although recombinant NPVs are becoming more competitive with synthetic insecticides, these modified viruses will encounter other problems before they are used for insect control (Hammock et al. 1993, McCutchen and Hammock 1994). Besides formulation, registration, and production problems, recombinant NPVs must effectively compete with classical insecticides to gain a share of the commercial market. Currently, biological agents account for <1% of total insecticide sales; *Bacillus thuringiensis* Berliner accounts for most of this percentage (Jutsum 1988).

Interactions Between Classical Insecticides and AcNPV. One strategy for use of natural or recombinant NPVs may be the simultaneous application of low rates of conventional insecticides. Although several investigators have documented a positive interaction between classical insecticides and microorganisms, a few insecticides have been shown

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Table 1. Insecticides combined with wild-type and recombinant baculoviruses

Compound	Type	Mode of action	Source
Allethrin	Pyrethroid type I	Sodium channel agonist	Roussel Uclaf, Agrovetennaire, Paris
Cypermethrin	Pyrethroid type II	Sodium channel agonist	ICI Americas (Zeneca), Goldsboro, NC
DDT	Chlorinated hydrocarbon	Sodium channel agonist	Synthesis by Hammock, UC Davis
Endosulfan	Cyclodiene	GABA channel agonist	FMC, Ag Chemical Group, Middleport, NY
Methomyl	Carbamate	AChE inhibitor	DuPont, Wilmington, DE
Profenofos	Organophosphate	AChE inhibitor	Ciba, Greensboro, NC

GABA, γ-aminobutyric acid; AChE, acetylcholinesterase.

to improve the activity of wild-type NPVs (Ignoffo and Montoya 1966, Benz 1971).

The 1st objective of our study was to identify combinations of insecticides and NPVs that hold promise for field trials by demonstrating potentiation. To achieve this objective, we characterized the interaction of a representative recombinant NPV (AcAaIT) expressing an insect-selective neurotoxin (AaIT), and wild-type AcNPV with representative compounds from different classes of synthetic organic insecticides.

Efficacy of a Recombinant NPV in Pyrethroid-Resistant Larvae. Another concern associated with the use of classical insecticides is increased tolerance and resistance of pest populations to the compound. For example, pyrethroids are the most commonly used insecticides for control of *Heliothis virescens* (F.), tobacco budworm, and *Helicoverpa zea* (Boddie), cotton bollworm, 2 of the most economically destructive pests in North America. Resistance of *H. virescens* to pyrethroids has been a widespread problem in cotton in the southeastern United States for more than a decade. Control failures and monitoring studies in the late 1970s and early 1980s revealed that resistance levels to pyrethroids (primarily permethrin and fenvalerate) were increasing rapidly (Harding et al. 1977, Plapp 1981, Martinez-Carillo and Reynolds 1983). The increased occurrence of pyrethroid resistance in the tobacco budworm has raised interest in the search for strategies to prolong the use of pyrethroids (e.g., Luttrell and Roush 1987, Campanhola and Plapp 1988, McCutchen et al. 1989, Plapp et al. 1990).

Therefore, our 2nd objective was to investigate the effectiveness of the recombinant virus, AcAaIT, against tobacco budworm resistant to pyrethroids. Both pyrethroids and AaIT bind to receptors in the sodium channel of the insect nerve and modify the influx of sodium through the channel, resulting in paralysis of muscle tissue. Because AaIT has a similar mode of action to that of the pyrethroids, we investigated cross-resistance to assess whether resistant *H. virescens* larvae were more or less tolerant to AcAaIT than the susceptible strain.

Materials and Methods

Interactions Between Classical Insecticides and AcNPV. A susceptible strain (Stoneville) of tobacco budworm was provided by the Southern Field Crop Insect Management Laboratory, USDA-ARS, Stoneville, MS; this strain has been reared for >5 yr without exposure to insecticides. Laboratory colonies of tobacco budworm were maintained on artificial diet as described by Vanderzant et al. (1962).

Six synthetic organic insecticides were tested alone and in combination with wild-type or recombinant NPVs (Table 1). All chemicals were supplied as technical grade materials. In addition, phenobarbital (maleate salt, Sigma, St. Louis, MO) was tested as an antidote to the neurotoxic effects induced by AcAaIT. For this study, phenobarbital was dissolved in double-distilled water to produce a 10% solution. Second-instar *H. virescens* that had been infected with a $>LC_{99}$ (unpublished data) of AcAaIT 24 h earlier were placed on a 2-mm^3 cube of diet saturated with 5 μl of the phenobarbital solution. Polyhedron occlusion bodies (POBs) of AcAaIT, AcJHE.KK (another recombinant NPV that expresses a modified form of juvenile hormone esterase; Bonning and Hammock 1994), and wild-type AcNPV were prepared from cell culture as described by O'Reilly et al. (1992).

Neonate tobacco budworm were exposed to chemicals films on the inner surfaces of 20-ml glass liquid scintillation vials as described in Plapp and Campanhola (1986) and Campanhola and Plapp (1989). Concentration-response curves were then estimated for each insecticide. Interactions of the NPVs and each insecticide were determined. Initially, larvae infected with NPVs were exposed to 2,000 POBs ($>LC_{99}$) using the modified droplet feeding method (Hughes et al. 1986). Three NPVs were used: wild-type AcNPV, AcAaIT, and AcJHE.KK. AcJHE.KK was used as a control for the recombinant NPV AcAaIT to demonstrate that synthetic insecticides do not indiscriminately synergize recombinant NPVs. Larvae were transferred immediately to vials treated with the LC_{10}–LC_{20} of the insecticide (except for controls). The LC_{10}–

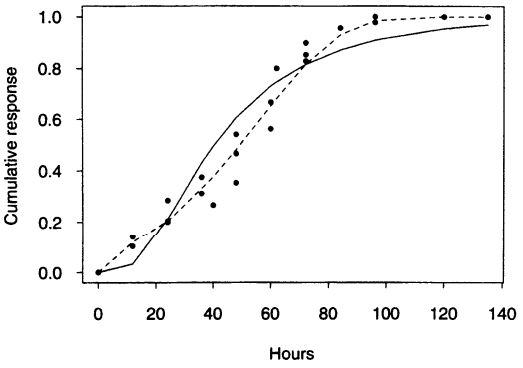

Fig. 1. Probit curve (solid curve) and a nonparametric smooth curve (dashed lines) fitted to cumulative mortalities over time. Probit analysis is not appropriate for bioassays over time because the probit curve often gives a bad fit, as illustrated here.

LC_{20} for each insecticide was estimated from the percentage mortalities of larvae that were exposed to the insecticide only at 24 h after initial exposure. Five neonate larvae were placed in each vial with ≈500 mg of artificial diet (Campanhola and Plapp 1989). All treatments were replicated 3 times using at least 25–30 larvae per treatment. NPV only and insecticide only controls were run concurrently with each replicate of NPV/insecticide combinations.

During the synergism and antidotal bioassays, insects were kept in an incubator maintained at 25 ± 1°C and a photoperiod of 14:10 (L:D) h. Larvae that moved when probed with a small brush were considered alive. Mortality was recorded at 8- to 12-h intervals.

Efficacy of a Recombinant NPV in Pyrethroid-Resistant Larvae. A strain of tobacco budworm resistant to pyrethroids (PEG) was obtained from Zeneca (Richmond, CA). The resistant strain was prepared from a mixture of 10 different populations collected from cotton fields in different states. Selection for resistance has been continuous since 1983 by exposure to permethrin and cypermethrin. The presence of target-site resistance has been identified in the PEG strain (Campanhola and Plapp 1989, McCaffery et al. 1989). We obtained eggs from Zeneca, and a population of neonate larvae (3 replicates of at least 25–30 insects per replicate) were tested for resistance with a discriminating concentration (0.5 μg cypermethrin per vial) as described in McCutchen et al. (1989). In addition, for each replicate a sample of the population (10 insects per replicate) was reared to 3rd instar and exposed to vials containing a concentration of 75 μg cypermethrin per vial (Campanhola and Plapp 1989) to confirm that resistance levels were ≥50 times that of the Stoneville strain.

Resistant and susceptible neonate larvae were tested with wild-type AcNPV and AcAaIT by using the same conditions and parameters described previously by McCutchen et al. (1991). Treatments were replicated 10 times using 24 larvae per treatment for each replicate.

Statistical Methods. Probit analyses with standard statistical programs (e.g., POLO-PC [LeOra 1987]) or PROBIT procedure in SAS (SAS Institute 1988) are not appropriate for bioassays over time for several reasons. First, observations on the same group of larvae at different times are not independent. Second, the probit curve often gives a bad fit when used to model concentration-responses over time or responses to mixtures of compounds over time (Fig. 1). This problem is demonstrated by a plot of the observed responses, the estimated log-probit curve, and an estimated nonparametric smooth curve for insects exposed to a mixture of insecticide and NPV (Fig. 1).

Therefore, we used an alternative procedure. We let the expected probability of an insect in the jth replicate dying by time t_{ij}, be characterized by

$$p_{ij} = 1 - \exp[-\exp(\alpha + \beta\tau_{ij})], \quad (1)$$

where α and β are unknown parameters and τ_{ij} is a transformation of the time point t_{ij}. We calculated estimates of the parameters α and β by maximizing the conditional likelihood function

$$\prod_{j=1}^{I}\prod_{i=1}^{I_j}\left[\frac{p_{(i+1)j} - p_{ij}}{1 - p_{ij}}\right]^{d_{ij}}\left[1 - \frac{p_{(i+1)j} - p_{ij}}{1 - p_{ij}}\right]^{s_{ij}-d_{ij}}, \quad (2)$$

where s_{ij} is the number of insects in replicate j that are still alive at time t_{ij}, and d_{ij} is the number of insects that have died in time interval $(t_{ij}, t_{(i+1)j}]$.

All calculations were done with the S-PLUS statistical package (Statistical Sciences 1993). Estimation was done in 2 steps. First, we evaluated the transformations τ_{ij} by fitting the cumulative mortalities over time with the **loess** smoothing routine within the generalized additive fitting function **gam( )** (Hastie 1992). Next, we used the transformed values τ_{ij} and the likelihood function in equation 2 to calculate estimates of parameters and appropriate standard errors. We used the general minimizing routine **ms( )** to minimize minus the log-likelihood. Estimates of LT_{50} and plots of the estimated mortality probabilities were used to study the effects of mixtures of small amounts of conventional insecticides and virus on the speed of kill. LT_{50}s for different treatments were considered significantly different when the 95% CI did not overlap.

We tested 2 hypotheses regarding the mode of action of the mixtures. We tested the *independent action* model of 2 compounds in a mixture by comparison of estimated probabilities of response of the observed mixture with expected probabilities of response. The expected probability of response for a given time and assuming independence was estimated by the equation

$$\hat{p}_{12} = \hat{p}_1 + \hat{p}_2 - \hat{p}_1\hat{p}_2, \quad (3)$$

Table 2. Lethal concentrations of classical insecticides used in combination with wild-type or recombinant NPVs against larvae of tobacco budworm

Compound	*n*	μg insecticide/vial	% mortality at 24 h
Allethrin	121	0.05	14.0
Cypermethrin	110	0.008	11.8
DDT	80	0.02	18.8
Endosulfan	80	0.002	17.5
Methomyl	125	0.02	20.8
Profenofos	150	0.06	16.3

Lethal concentrations of insecticides (LC_{10}-LC_{20}) were chosen based on results from Campanhola and Plapp (1989).

where $\hat{p}_1$ and $\hat{p}_2$ are estimated probabilities of mortality for a given time for insects exposed to the single compounds, respectively. The model for independent action assumes that the probabilities of effects of 2 compounds are *additive* (equation 3). Biologically, additive means that the insect dies when the amount of at least 1 of the compounds exceeds the threshold of tolerance of the insect.

The 2nd hypothesis, *similar action additive* interaction, assumes that both compounds act on the same site (or have the same mode of action), and the compounds are additive at the dose level. The insect dies when the sum of the doses at the site of action exceeds the threshold of mortality of the insect. In bioassays in which only one concentration of each compound is used, this model assumes that the insect dies when the sum of the doses at the site of action exceeds insect tolerance level by time t. Although doses at the site of action at a given time are not measured, values proportional to these amounts can be estimated as follows: let $D_1(t)$ and $D_2(t)$ be the dose of the single compound at the site of action at time (t); and

$$p_1 = 1 - \exp[-\exp[\alpha + \beta \log(D_1[t])] \quad (4)$$

$$p_2 = 1 - \exp[-\exp[\alpha + \beta \log(D_2[t])] \quad (5)$$

be the expected cumulative mortality probabilities for single compounds. Then the expected cumulative mortality probability for the mixture, assuming similar action additive model, is

$$p_{12} = 1 - \exp[-\exp(\alpha + \beta \log[D_1(t) + D_2(t)])]. \quad (6)$$

We tested the similar action additive model by first estimating α, β, $D_1(t)$, $D_2(t)$ in equations 4 and 5 with data from the single compound bioassays. Next, we substituted these estimates into equation 6 to calculate expected cumulative response probabilities of mixtures, assuming the similar action model.

Thus, we define a combination to be synergistic when the probabilities estimated from the observed mortalities are significantly greater ($P < 0.05$) than those calculated assuming the independent or additive models. We further define an interaction to be antagonistic ($P < 0.05$) when the probabilities are less than those calculated from the independent or additive models. For all hypotheses, curves were considered significantly different ($P < 0.05$) when the expected curve was outside the 95% CI of observed responses.

Results

Interactions Between Classical Insecticides and AcNPV. When we examined the interaction of low rates (Table 2) of several insecticides in combination with wild-type AcNPV or the recombinant NPVs, AcAaIT and AcJHE.KK (>LC_{99}) in neonate *H. virescens*, plots of the observed mortalities for bioassays with single compounds indicated that the 2 recombinant NPVs and the wild-type NPV produced no significant mortality in the first 50 h after infection (Fig. 2). Mortality resulting from conventional insecticides, however, occurred much earlier. Almost all the insects that died by the end of the experiment from the insecticides did so in the first 40–60 h.

Many researchers use a comparison of LT_{50}s (the time required to kill 50% of the sample population) to evaluate the efficacy of baculoviruses (McCutchen et al. 1991, O'Reilly and Miller 1991, Tomalski and Miller 1991, Cory et al. 1994). Estimated LT_{50}s for insects treated with the mixture of NPV and a conventional insecticide were shorter than those obtained with the NPV alone (Fig. 3). However, the decrease in LT_{50} was significant ($P < 0.05$) only in bioassays in which conventional insecticides were added to AcAaIT (i.e., the LT_{50} for all combinations of AcAaIT and an insecticide were significantly shorter than those with the virus alone).

Decreases in LT_{50}s for combinations of wild-type NPV (Fig. 3) or the recombinant AcJHE.KK (data not shown) with an insecticide were not significantly different (i.e., $P > 0.05$) from the LT_{50}s with the virus alone (Fig. 3) for all but one of the insecticides (methomyl). Only methomyl significantly decreased ($P < 0.05$) the median time to death when added to the wild-type NPV.

Lethal Times of Mixtures of Conventional Insecticides and Recombinant NPVs. Although median times to death by the virus expressing the neurotoxin AaIT in combination with any of the insecticides tested were shorter than the corresponding time for the virus alone, only the type II pyrethroid (cypermethrin) and the carbamate (methomyl) interacted synergistically with the virus. Fig. 4 shows plots of observed responses (and 95% confidence regions for the estimated response probabilities) for insects treated with mixtures of conventional insecticides and the recombinant AcAaIT. These plots also indicate the expected response probabilities assuming the independent action model and the similar action additive model, suggesting synergism between AcAaIT and both cypermethrin and methomyl. With AcAaIT, cypermethrin and metho-

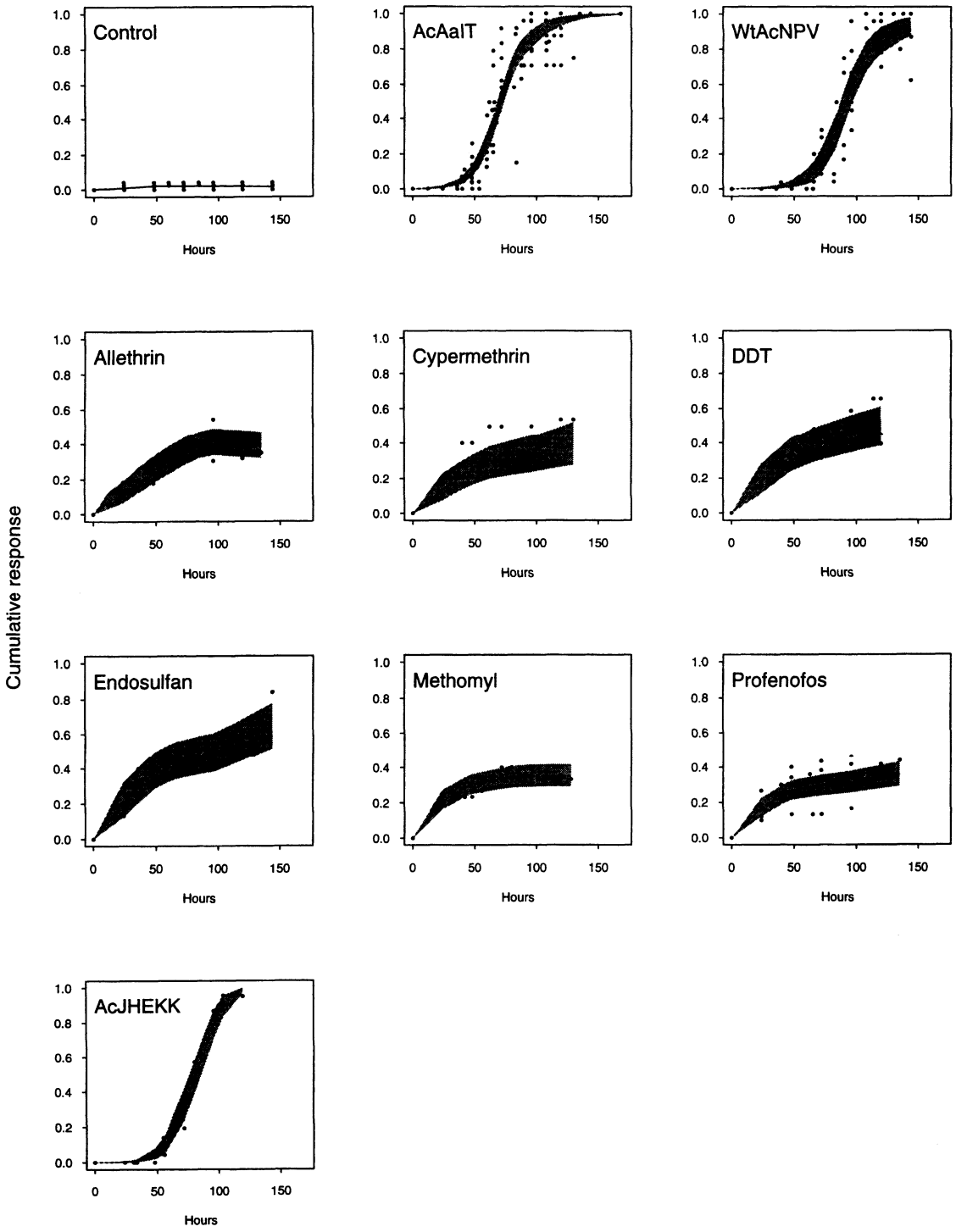

Fig. 2. Observed cumulative mortalities over time for the bioassays with single preparations. Dashed lines through the points are the fitted curves. Shaded areas are approximate point-wise 95% CL of the fitted curve. The control was treated with acetone (solvent for insecticides) only. AcAaIT is the recombinant form of *A. californica* nuclear polyhedrosis virus (AcNPV) that expresses a scorpion toxin. Wt AcMNPV is the wild-type of AcNPV; AcJHE.KK is the recombinant form of AcNPV that expresses a modified form of juvenile hormone esterase.

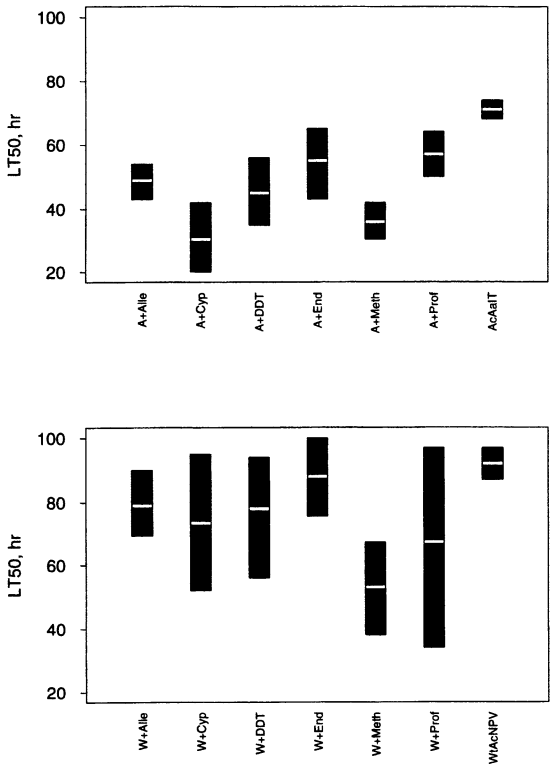

Fig. 3. Upper panel shows estimates (and 95% CL) for 50% LTs of insects treated with the mixture of AcAaIT and synthetic organic insecticides; estimates for wild-type NPV (WtAcNPV) with conventional insecticides are shown in the lower panel. A is NPV (AcAaIT); W is NPV (Wt AcNPV); Alle is allethrin; Cyp is cypermethrin; End is endosulfan; Meth is methomyl; Prof is profenofos. The LT_{50} for all combinations of AcAaIT and an insecticide were significantly shorter ($P < 0.05$) than those with the virus alone. Decreases in LT_{50}s for combinations of wild-type NPV and an insecticide were not significantly different (i.e., $P > 0.05$) from the LT_{50}s with the virus alone, except for methomyl ($P < 0.05$).

myl had an LT_{50} of 30 h after infection (a reduction of 58% compared with the virus alone).

Although interactions of allethrin, DDT, endosulfan, or profenofos with AcAaIT were not synergistic by our definitions (Fig. 4), these mixtures significantly reduced their respective median times to death compared with the corresponding values for the virus alone (Fig. 3) and the insecticide alone (Fig. 2). The rates of mortality for these combinations were consistent with either the independent or the similar action additive models. The LT_{50}s of profenofos and DDT were reduced by 37%, whereas LT_{50}s of allethrin and endosulfan were reduced 31% compared with the virus alone.

With the exception of methomyl, responses observed when insects were treated with the wild-type NPV plus conventional insecticides suggest a slight antagonism between the 2 agents. Estimated response curves for the combinations of insecticides and wild-type NPV were marginally higher than the expected responses assuming the hypotheses of independent or similar action additive (Fig. 4). Although estimates of LT_{50}s from bioassays with the wild-type NPV alone were not significantly different from those of the mixtures of wild-type NPV and conventional insecticides (Fig. 4), the total percentage mortalities at 120 h were significantly lower than those expected assuming models of independent or similar additive action (see Fig. 4 and Table 3).

The combination of some recombinant viruses with insecticides will not enhance activity, as shown by the combination of cypermethrin with the recombinant virus AcJHE.KK. We found no evidence of synergism or antagonism when cypermethrin was combined with AcJHE.KK (Fig. 5).

Activity of AcAaIT with Phenobarbital. Because phenobarbital is an anticonvulsant (Katzung 1995), we hypothesized that alleviation of symptoms of neurotoxicity may occur in larvae infected with AcAaIT, thus increasing the lethal times. However, we observed no significant difference in LT_{50}. The LT_{50} for AcAaIT ($n = 79$) and for the AcAaIT/phenobarbital combination were 94.3 h (90.5–98.1, 95% CL) and 94.5 h (90.7–98.3 95% CL), respectively. In summary, we found no significant differences in the mortality curves for these 2 treatments.

Efficacy of a Recombinant NPV in Pyrethroid-Resistant Larvae. In comparing the lethal times of larvae infected with wild-type AcNPV or AcAaIT against pyrethroid-susceptible and -resistant (PEG) larvae of *H. virescens*, no significant differences in the LT_{50} or LT_{90} for the 2 strains treated with the wild-type virus were found (Fig. 6; Table 4). However, when the recombinant virus was compared for effectiveness against susceptible and pyrethroid-resistant larvae, the results were surprising. Analysis of these data indicated a significant difference both at the LT_{50} and LT_{90} between the 2 strains treated with AcAaIT. The resistant strain died from infection by AcAaIT significantly faster. The LT_{50} and LT_{90} for the PEG strain were faster by 11 and 25%, respectively, compared with the susceptible Stoneville strain (Table 4).

Discussion

Interactions Between Classical Insecticides and AcNPV. Evaluation of the interactions between the baculovirus and conventional insecticides based on examination of the effect of our treatment combinations on the entire survival curve for each agent and each combination (Fig. 2) suggest that decreases in lethal times resulting from a combination of an NPV and an insecticide might occur assuming several different models. For example, decreases in median times to death would be expected if the insecticide and the NPV acted independently. Assuming the model for independent action, the insecticides might cause most deaths in the first 50 h

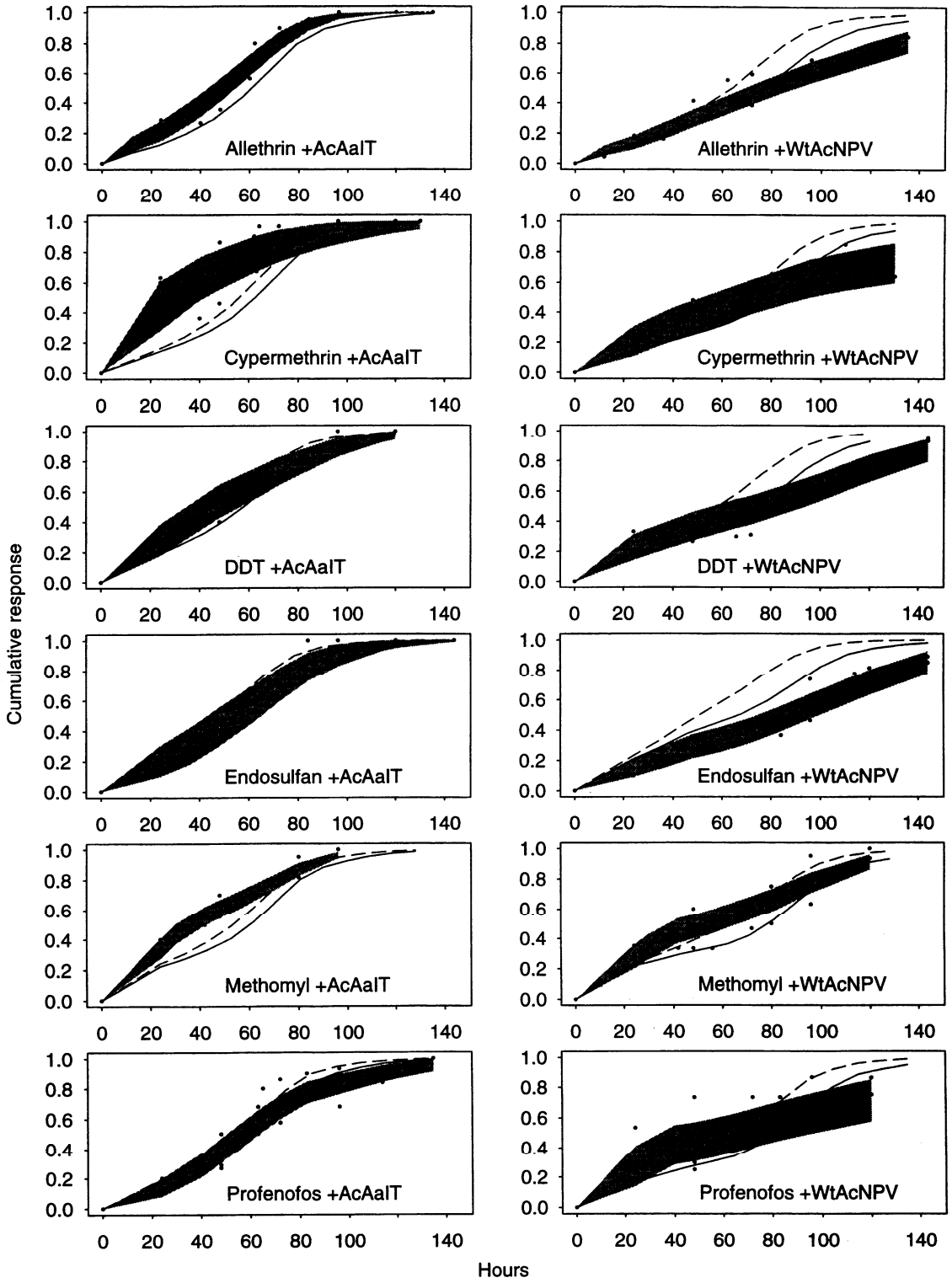

Fig. 4. Observed responses and 95% confidence regions (gray bands) of the estimated response curve (dotted lines) for insects treated with mixtures of conventional insecticides and NPV. Solid curves are expected response probabilities assuming independent action model. Dashed curves are the expected response curves assuming similar action additive model.

Table 3. Estimated percentage of mortality at 120 h postinfection of neonate tobacco budworm to wild-type AcNPV with low concentrations of classical insecticides

WT AcPNV +	% mortality at 120 h postinfection		
	Observations (95% CL)	Independent model	Additive model
Allethrin	74 (66–81)	91	98
Cypermethrin	70 (57–83)	91	97
DDT	76 (66–84)	93	99
Endosulfan	72 (64–80)	94	99
Methomyl	92 (86–96)	91	97
Profenofos	72 (57–85)	90	97

(i.e., during the period when the NPV levels at the site of action are not lethal). Thus, 50% mortality would occur more quickly in bioassays with insecticides and NPV, rather than with the NPV alone. Alternatively, shorter lethal times might also occur assuming the interactive model. Both models would produce shorter lethal times although the models do not assume synergistic or antagonistic interactions between the 2 agents.

Another model that could predict decreased lethal times involves synergism between insecticide and NPV. Synergism might have a number of biological explanations based on site of action: the same target site/tissue (e.g., insect nerve, same ionic channel); the same target site/tissue, but a different location (e.g., different ionic channels in an insect nerve; the same target site/tissue, same location in the tissue, but different binding site (e.g., insect nerve, same sodium channel, but different binding sites on the sodium channel); or other physiological interactions (e.g., NPV infection might facilitate absorption or distribution of insecticide, suppress detoxication mechanisms, infection of nerve cells results in increased sensitivity of nervous tissue to insecticides).

Despite the fact that AcJHE.KK kills insects at a rate comparable with that of AcAaIT, lack of increased speed of kill by AcJHE.KK combined with conventional insecticides suggests that enhanced speed of kill will not necessarily occur when other recombinant baculoviruses are combined with conventional insecticides. AcJHE.KK expresses a modified version of juvenile hormone esterase, which is an insect-derived enzyme important in the regulatory development of many lepidopteran insects. The modified JHE is lethal to several lepidopteran species (Bonning and Hammock 1994). Although the mode of action of JHE.KK is not yet fully understood, it probably does not interfere with the insect nervous system. Thus, classical insecticides may not indiscriminately synergize any recombinant baculovirus.

Our results do suggest, however, that the best predictions of positive (=synergistic) interaction among recombinant biological and synthetic pesticides might be based on the potential for interaction between the pesticide and the recombinant product at a receptor (in this case, an ion channel). More subtle interactions may occur at a tissue or physiological level. AaIT and cypermethrin both bind at the insect sodium channel. In support of this interpretation, Herrmann et al. (1995) showed that different scorpion peptide toxins that bind to different sites on the sodium channel can interact

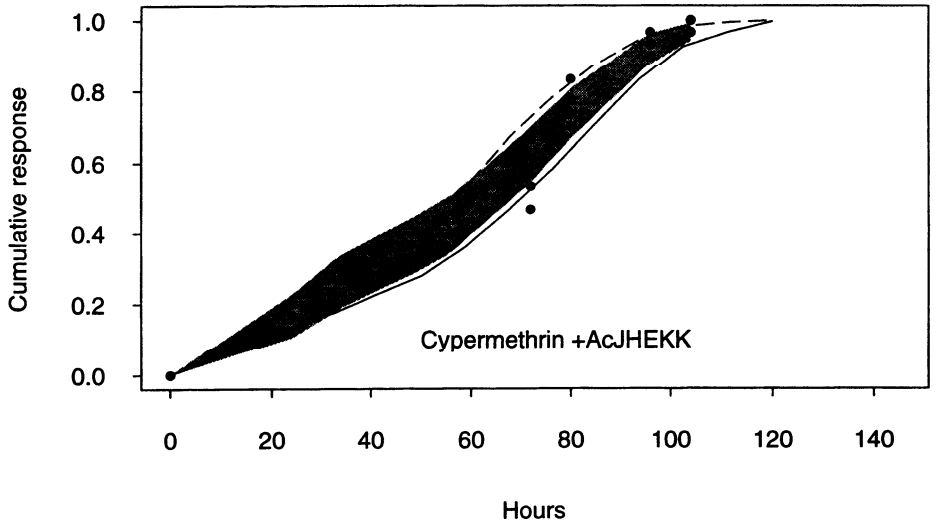

Fig. 5. Observed responses and 95% confidence regions (gray bands) of the estimated response curve (dotted lines) for insects treated with a mixture of cypermethrin and AcJHE.KK. Solid curves are expected response probabilities assuming independent action model. Dashed curves are the expected response curves assuming similar action additive model.

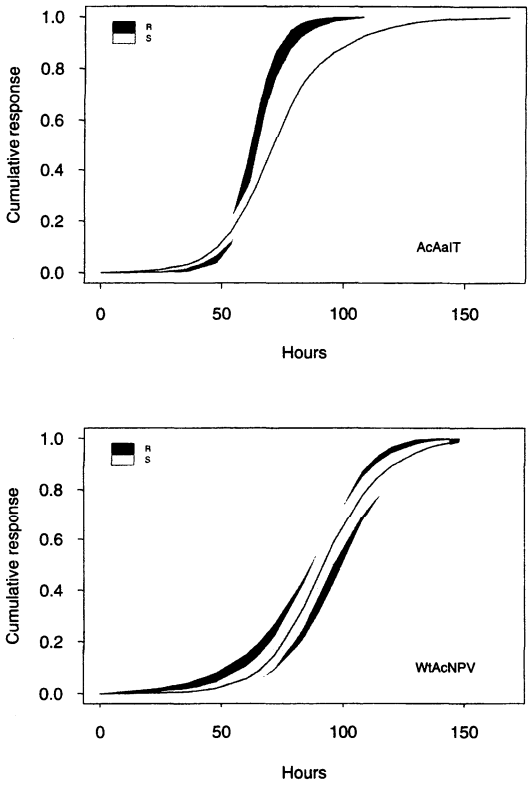

Fig. 6. Estimated cumulative mortality probabilities (and 95% confidence regions) for resistant and susceptible strains treated with AcAaIT (upper panel) and wild-type AcNPV (lower panel).

synergistically, whereas toxins that displace each other from the same binding do not.

Another type of synergistic interaction is illustrated by the carbamate and organophosphate insecticides that act as acetylcholinesterase inhibitors. Accumulation of acetylcholine produces a series of neurological responses that result in paralysis. In the case of synergism between methomyl and AaIT, methomyl probably produces a generalized hyperexcitability of the nervous system, reducing the amount of AaIT needed to cause paralysis. Thus, the materials act at different sites, but a synergistic interaction may be predictable based upon the known pharmacology of each agent.

Although we reported on temporal synergism between a recombinant organism and classical insecticides, synergism may also occur between conventional insecticides and other baculoviruses expressing neurotoxins; other microbials (especially those expressing toxins such as certain fungi and *B. thuringiensis*) and with transgenic plants, especially those expressing *B. thuringiensis* toxins. In many cases, transgenic organisms will be used in pest management programs with synthetic pesticides and other biological agents. We suggest that, as researchers develop these agents for pest control, they test them with a series of commonly used classical pesticides and other pest management agents for potential synergistic interactions.

Table 4. Time-response of pyrethroid-resistant and -susceptible neonate larvae of *H. virescens*

Treatment[a]	*n*	LT_{50} (h) (95% CL)	LT_{90}, h (95% CL)
S AcNPV	306	92 (87–96)	121 (113–132)
PEG AcNPV	168	94 (87–99)	118 (111–128)
S AcAaIT	411	71 (68–74)	103 (95–112)
PEG AcAaIT	240	63 (62–65)	77 (74–84)

PEG, pyrethroid resistant strain; S, pyrethroid susceptible strain.

[a] Neonate larvae of PEG and S strains of *H. virescens* were infected with an $>LC_{99}$ of wild-type AcNPV, or AcAaIT. Infected larvae were monitored for mortality at 8- to 12-h intervals.

Efficacy of a Recombinant NPV in Pyrethroid-Resistant Larvae. These results indicate that the recombinant virus AcAaIT may be more effective against pyrethroid-resistant larvae than pyrethroid-susceptible larvae in field situations, and might be useful combatting pyrethroid resistance. A possible explanation for our results may include a fitness deficit associated with the pyrethroid-resistant strain of *H. virescens*. Campanhola et al. (1991) indicated that this resistant strain has several biological characteristics including slower developmental rates, reduced fecundity and lowered mating efficiency. However, our data obtained with larvae of the PEG strain infected with wild-type AcNPV indicate that the biological constraints identified by Campanhola et al. (1991) are not major factors in time-response. In another study, *H. virescens* populations with an increased tolerance to an organophosphate or pyrethroid insecticide did not show increased susceptibility to infection by NPV (Ignoffo and Roush 1986). Possibly, the pyrethroid-resistant larvae are more sensitive to the neurotoxin AaIT, indicating negatively correlated cross-resistance. Only 1 study to date has confirmed the presence of negative correlation of cross-resistance in insects (Yamamoto et al. 1993).

Pyrethroids now dominate the insecticide market and have accounted for a larger percentage of the total market for insecticides over several years both in the United States and worldwide. However, sales are declining caused in part by the wide-scale presence of pyrethroid resistance. AcAaIT or other recombinant and nonrecombinant baculoviruses with selectivity for resistant insects might provide a means to deter the onset of insecticide resistance. Such viruses might be used to drive resistant populations toward susceptibility.

Slow mortality and resultant crop damage is often cited as a major limitation to the commercial success of viral insecticides. Twenty-four- to 48-h mortality is a common target for conventional in-

secticides, but recombinant baculoviruses designed for enhanced speed of kill have yet to reach this target (Maeda et al. 1991; McCutchen et al. 1991; Stewart et al. 1991; Tomalski and Miller 1991, 1992; Korth and Levings 1993; Bonning and Hammock 1994). Given the emphasis on developing agents that kill target pests more quickly, we concentrated on studying speed of kill expressed as lethal times. Analogous approaches might also emphasize factors likely to be of major economic importance such as lethal doses or concentrations.

In summary, our data indicate that conventional insecticides, especially those that act on the insect nervous system (e.g., pyrethroids and ACHE inhibitors), may provide the greatest chance of synergizing the recombinant viruses in the field. For the pyrethroids, our data suggest that a simultaneous exposure of AaIT and Type II pyrethroids result in a synergistic interaction at the insect sodium channel. Based on these results, we recommend that varying rates of insecticides, in combination with AcAaIT and other recombinant baculoviruses that express insect neurotoxins, be tested for efficacy in the field. Recently, several studies have documented that the specificity of recombinant NPVs has not been affected (Heinz et al. 1995, McNitt et al. 1995, McCutchen et al. 1996). We used an approximate LC_{10}–LC_{20} (24 h) of the pesticide to minimize symptoms and mortality resulting directly from the conventional pesticide. In practice, various factors would influence the rates used, including the cost and availability of the biological and synthetic chemical pesticide, environmental concerns, level of resistant populations in the field, and other pest management considerations. The combination of low rates of insecticides and recombinant viruses may also be useful in resistance management strategies.

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