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Authors
Hoover, Kelli
Yee, Julie L.
Schultz, Christine M.
et al.

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EFFECTS OF PLANT IDENTITY AND CHEMICAL CONSTITUENTS ON THE EFFICACY OF A BACULOVIRUS AGAINST *Heliothis virescens*

KELLI HOOVER,[1-3] JULIE L. YEE,[4] CHRISTINE M. SCHULTZ,[1] DAVID M. ROCKE,[5] BRUCE D. HAMMOCK,[1,2,*] and SEAN S. DUFFEY[1]

[1]*Department of Entomology*
[2]*Department of Environmental Toxicology*
[4]*Division of Statistics*
[5]*Center for Statistics in Science and Technology*
University of California
Davis, California 94616

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Abstract—Baculoviruses are arthropod-specific, dsDNA viruses primarily used to control lepidopteran pests. A limitation of the use of baculoviruses for pest control is that their efficacy is modifiable by host-plant chemicals. The levels of phenolic substrates and two foliar oxidative enzymes, peroxidase (POD) and polyphenol oxidase (PTO), were significant predictors of disease caused by a baculovirus in *Heliothis virescens* fed on either cotton or lettuce; POD was the more influential of the two enzymes. The higher the plant phenolase activity, the lower the percent mortality and the slower the insects died from viral infection. Whether a particular class of phenolic substrates was correlated with enhanced or attenuated baculoviral disease depended upon context, i.e., admixture. Diminution of viral efficacy by plant oxidative activity may compromise the compatibility of baculoviruses with other components of an integrated pest management system such as host plant resistance.

Key Words—Baculovirus, host-plant resistance, peroxidase, polyphenol oxidase, *Heliothis virescens*, tritrophic interactions, cotton, lettuce, phenolics.

*To whom correspondence should be addressed.
[3]Current address: Department of Plant and Microbial Biology, 251 Koshland Hall, University of California, Berkeley, California 94720.

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INTRODUCTION

There is increasing interest in the use of microbial control agents such as the nuclear polyhedrosis viruses (NPVs, a subgroup of the Baculoviridae) for the control of several important agricultural pests, particularly some in the family Noctuidae (e.g., *Heliothis*, *Helicoverpa*, and *Spodoptera* spp.). This renewed interest is due in part to the safety of baculoviruses to nontarget organisms (Heinz et al., 1995; McCutchen et al., 1996) and the ability of researchers to engineer baculoviruses to express gene products, resulting in improved speed of kill (Bonning and Hammock, 1992, 1996; Miller, 1995). A limitation of the use of baculoviruses as biopesticides is that their efficacy, as well as that of a number of entomopathogens, is unpredicatably modified by the host plant (see Duffey et al., 1995, for review; Farrar et al., 1996). Previous studies have shown that when insects were fed foliage from different plant species (Keating and Yendol, 1987; Keating et al., 1990; Forschler et al., 1992) or plants of disparate quality (Ebihara, 1966; Hayashiya et al., 1968; Sosa-Gomez et al., 1991; Hunter and Schultz, 1993) treated with baculoviruses, susceptibility to lethal infection[6] varied as much as 50-fold. In some cases, strong correlations (the sign dependent on the chemical) have been shown between foliar levels of certain phytochemicals and viral efficacy (Uchida et al., 1984; Felton et al., 1987; Keating et al., 1988, 1989; Felton and Duffey, 1990; Young et al., 1995).

Most investigations of the impact of host plant on baculoviral disease in insects have been phenomenological. Although it is well established that plant type and plant chemistry often have a profound influence on the course and severity of baculoviral disease (reviewed by Duffey et al., 1995), identification of the causal agents and elucidation of their mechanisms of action have been ill-defined or neglected (see Hayashiya et al., 1976; Keating et al., 1988, 1990; Felton and Duffey, 1990; Watanabe et al., 1990 for notable exceptions; and review by Duffey et al., 1995), primarily because of the experimental difficulty of establishing causality and mechanism. Several groups are making notable progress in overcoming some of these difficulties by the study of the impact of phenolics upon baculoviral disease in larval hosts (Felton et al., 1987; Felton and Duffey, 1990; Keating et al., 1990; Hunter and Schultz, 1993; Young et al., 1995). In a few cases the mechanism of inhibition of viral disease is being unraveled (Hayashiya et al., 1976; Uchida et al., 1984; Felton and Duffey, 1990; Keating et al., 1990; Watanabe et al., 1990). Several critical concepts emerge from these studies that bear on the above difficulties:

[6]We use the term "lethal infection" rather than simply "infection" because we have no evidence that plant compounds prevent binding and/or assimilation of the nucleocapsids after release from the polyhedral occlusion body. Infection may still occur in the presence of inhibitory plant compounds, but the infected midgut cells may be damaged and sloughed as a result of oxidative stress before the virus can spread beyond the midgut, effectively eliminating the infection from the insect.

1. Multiplicity: viral disease is simultaneously influenced by more than one phytochemical, each exerting its effect simultaneously. Thus, the influence of phytochemicals upon viral disease is multivariate; hence, there are causes and effects, not a cause and an effect.

2. Interactivity: a chemical does not influence viral disease independently of other phytochemicals. It interacts with other dietary components, often in nonlinear fashions; hence, context (what other chemicals are in admixture) is important.

3. Multifunctionality: a chemical often exhibits multiple mechanisms of action in its interactions with other chemicals and/or with baculoviruses, the degree and number being dependent upon context.

Our study was designed to reinforce the importance of the above concepts in understanding the impact of plant phenolics in crop plants upon the progression and severity of baculoviral disease in noctuid larvae. Phenolics do not act in isolation when ingested by the insect; they are modified by pH, redox conditions, oxygen availability, and/or other dietary chemicals (Appel and Schultz, 1992; McEvily et al., 1992; Appel, 1993, 1994; Duffey and Stout, 1996; Johnson and Felton, 1996). The plant oxidative enzymes polyphenol oxidase (PPO) and peroxidase (POD) act upon a variety of phenolics, particularly *o*-dihydroxyphenolics (catecholic phenolics), to produce highly reactive quinones (Pierpoint, 1983) and free radicals (Butt, 1981; Butt and Lamb, 1981; Robinson, 1991). Reactive products from phenolic oxidation can bind to proteins, damage membranes, and are implicated as defensive responses of plants against herbivores (Felton et al., 1989, 1992; Appel and Schultz, 1992; Bi et al., 1994; Summers and Felton, 1994; Duffey and Stout, 1996; Stout and Duffey, 1996). More recent reports implicate the oxidation of phenolics by oxidative enzymes as important processes capable of inhibiting baculoviral disease (Felton and Duffey, 1990).

In addition to catecholic phenolics, polymeric phenolics (tannins) have been implicated as factors capable of inhibiting mortality of susceptible hosts treated with baculoviruses (Keating et al., 1988, 1989, 1990; Young et al., 1995). Tannins in oak and cotton foliage were negatively correlated with larval mortality caused by baculoviruses of *Lymantria dispar* (Keating et al., 1988, 1990) and *H. zea* (Forschler et al., 1992), respectively. These reports might lead one to conclude that tannins are the causal agents of inactivation of baculoviruses on cotton. However, this conclusion is not fully supported by the evidence. Condensed tannins are not sufficiently correlated with decreased mortality by baculovirus in vivo (Keating et al., 1990), although negative relationships between condensed tannins and viral disease have been demonstrated by diet incorporation (Keating et al., 1989; Young et al., 1995). Finally, treatment of *H. zea* larvae on cotton cultivars that are isogenic except for tannin content (a high tannin line vs. a low tannin line) did not differ in susceptibility to bacu-

loviral infection (G. W. Felton and S. Y. Young, personal communication). Thus, poor performance of baculoviruses as pest control agents on cotton requires further exploration.

Herein, we test the effects of POD, PPO, and various classes of phenolics on the virulence of baculoviruses. These results suggest that oxidative processes catalyzed by peroxidase occurring in the insect midgut are, at least in part, responsible for the observed attenuation of viral disease on certain host plants. In this study, we also demonstrate an important paradigm concerning viral efficacy—that phytochemicals influence viral disease in a manner that is: (1) multiplex, (2) interactive, and (3) multifunctional, each of which is dependent on chemical context.

METHODS AND MATERIALS

To evaluate our hypothesis that plant phenolase activity is a major factor responsible for inhibiting baculoviral disease, we identified and quantified several plant chemical factors (e.g., phenolics, protein, and the oxidative enzymes PPO and POD), which we correlated by multiple regression with viral efficacy as modified by four different host plants of *H. virescens* with a permissive virus, *Autographa californica* multiple nucleocapsid nuclear polyhedrosis virus (AcMNPV). Specifically, we performed two sets of bioassays in which we examined two different response variables (percent mortality and rate of mortality) as functions of level of plant protein, phenolics, and oxidative enzyme activities. In bioassay 1, percent mortality (modeled by logistic regression) of third-instar *H. virescens* dosed with wild-type AcMNPV (WT AcMNPV) at a range of viral doses was determined. In bioassay 2, the rate of mortality (modeled by fitting survivorship curves) of neonate larvae of *H. virescens* infected with an LD_{99} was determined. Having determined from bioassay 1 above that host plant has only a minor influence on percent mortality at the LD_{99}, we chose to investigate whether host plant exerts any influence at this high viral dose on rate of mortality. Thus, larvae were dosed at an LD_{99} with either WT AcMNPV or a recombinant virus derived from AcMNPV, termed AcAaIT, which expresses an insect-selective neurotoxin derived from the scorpion *Androctonus australis* (McCutchen et al., 1991). AcAaIT kills larvae of *H. virescens* approximately 30% faster than wild-type virus (McCutchen et al., 1991; Hoover et al., 1995).

For these two sets of bioassays, the following methods were used:

Plants. Two cultivars of cotton (*Gossypium hirsutum*, cv. Acala SJ2 and Delta pine 51) and lettuce [*Latuca sativa* L., cv. Valmaine (romaine) and Salinas (iceberg)] were grown in a greenhouse under natural conditions to the four to five-leaf stage. These plants were chosen because these two species differ in

phenolic, oxidative enzyme, and/or protein levels. To obtain an array of phenolic and enzyme levels within a plant species for our regressions, which evaluated the influence of phytochemicals on rate of mortality (bioassay 2), we did not attempt to regulate photoperiod or temperature (except for the maximum temperature) in the greenhouse. Thus, we did not use artificial lighting; the photoperiod averaged 10L:14D in winter and 16L:8D in summer. The minimum/maximum temperatures averaged 21/27° in winter and 24/30°C in summer. Bioassay 1 above was performed in the summer months.

Insects. Tobacco budworm eggs were obtained from the United States Department of Agriculture Agricultural Research Station (Stoneville, Mississippi). Neonate larvae reared to third instar were placed individually on 8 cm^3 cubes of semisynthetic diet (BioServ, Inc.) in 24-well tissue culture plates (Fisher, St. Louis, Missouri) and maintained at 26 ± 1°C and 16L:8D.

Viruses. WT AcMNPV (C6 clone) (Ayers et al., 1994) and the recombinant AcAaIT were amplified in larvae of *H. virescens* and were extracted, partially purified, and stored until use as described in Hoover et al. (1995).

Foliar Chemical Assays. The protein content was determined by extraction of foliage in 0.5 N NaOH followed by the Bradford assay (Bradford, 1976), with ribulose, 1, 5-diphosphate carboxylase-oxygenase as a standard and the addition of 3% polyvinylpyrrolidone to the assay mixtures to minimize interference from phenolics (Jones et al., 1989). PPO and POD activities were determined colorimetrically with caffeic acid and guaiacol/H_2O_2, respectively, as substrates. Activities were measured as the increase in OD_{470} per minute per gram (Ryan et al., 1982; Felton et al., 1989). Catecholic phenolic content was determined colorimetrically with a 0.5% diphenylborinic acid-ethanolamine complex. Chlorogenic acid and rutin were used as standards at OD_{390} and OD_{440}, respectively (Broadway et al., 1986). Total phenolics were measured with the Folin-Ciocalteau reagent with chlorogenic acid as a standard (Singleton and Rossi, 1965). We calculated noncatecholic phenolics as the difference between total phenolics and catecholic phenolics. Total gossypol content was measured by the aniline method (Hedin et al., 1992) and reported as gossypol equivalents with gossypol acetate as a standard. Because this method also measures other aromatic terpenoid aldehydes (except benzaldehyde), HPLC was used to determine the terpenoid aldehyde content of the two cotton cultivars (courtesy of R. D. Stipanovic, USDA, ARS; Stipanovic et al., 1988). Condensed tannins were determined by the butanol/HCl assay (Lane and Schuster, 1981) using purified condensed tannins extracted from Acala cotton as a standard (Hagerman and Butler, 1980).

Percent Mortality of Third-Instar H. virescens *Dosed with AcMNPV.* Leaf disks, 0.5 cm diameter, were cut from foliage of each plant (12–15 plants of each cultivar per replicate) and placed on agar in 24-well tissue culture plates. Treatments consisted of plant cultivar and viral dose. Four to five leaf disks

from each of the 12 plants of the same cultivar were distributed among all viral treatments (four viral doses per plant type). Viral inoculum suspended in double-distilled H_2O was applied to each leaf disk at 3000, 300, 100, or 30 polyhedra/larva representing the approximate LD_{99}, LD_{75}, LD_{50}, and LD_{25}, respectively. Viral inoculum was allowed to dry at ambient temperature. LDs were determined by dosing a group of insects on artificial diet as controls concurrently with each replicate.

Within 6 hr after molting, third instars were removed from diet and starved overnight to void gut contents. Starved larvae were transferred individually to a leaf disk. Insects that consumed their entire virally tainted leaf disk or diet cube were transferred individually to excess artificial diet in 35-ml cups and maintained until death or pupation at 26 ± 1°C and 16L:8D. Mortality was scored at eight to nine days after infection. There were 35–48 larvae per dose per treatment, and the experiment was replicated three times. Foliar chemical content was determined on the same day that leaf disks were prepared.

Data were analyzed by logistic regression to determine if the probability of an insect dying could be predicted from chemical content (Statistica, StatSoft; Kalbfleisch and Prentice, 1980; Collett, 1994). The model estimates the unknown parameter coefficients as follows: $\log (p/1 - p) = \beta X$, where $\beta X = \beta_0 + \beta_1 x_1 + \beta_2 x_2 \ldots$. Thus, the probability of dying $(p) = \exp \{(\beta X)/[1 + \exp (BX)]\}$. Parameter coefficients β with a positive sign indicate a variable that increases the probability of an insect dying; negative coefficients indicate a decrease in the probability of dying.

We used two separate models to determine if the probability of an insect dying could be predicted from phytochemical levels (independent variables). For the first model, we analyzed pooled data with plant type as a categorical variable. For the second model, we analyzed each plant species separately (i.e., the two cotton cultivars were analyzed separately from the two lettuce cultivars). Analysis of the pooled data permitted us to ask if plant type is a critical variable, i.e., can viral performance be predicted by simply knowing plant type, or does prediction of viral efficacy require knowledge of plant chemistry among plant types? Analysis of the two plant species separately allowed us to ask if the same phytochemical variables influence viral efficacy in the same way in the two different plant species. Parameter coefficients for plant type were also used to calculate odds ratios as described in Armitage and Berry (1994). Each independent variable was regressed separately in combination with viral dose, followed by backward stepwise logistic regression to determine the most predictive model. In addition, we subjected the arcsin-transformed percentage mortalities for each plant cultivar at each viral dose to two-way ANOVA to further test whether viral performance can be predicted by simply knowing plant type (Steel and Torrie, 1980).

Rate of Mortality of Neonates Infected with WT AcMNPV or Recombinant AcAaIT. Time–response bioassays were conducted to determine the influence of host plant on the rate of mortality of neonate *H. virescens* treated with baculoviruses. True leaves were removed from plants and placed individually in 100- × 15-cm Petri dishes on top of a 9-cm piece of Whatman No. 1 filter paper moistened with 1 ml of water. Leaves were exchanged for fresh ones every 48 hr. Two leaves were used from each plant per treatment group. Neonate larvae of *H. virescens* were droplet fed a viral suspension of 2000 polyhedra/ μl (LD_{99}) of either AcAaIT or WT AcMNPV (Hughes et al., 1986). The viral formulation contained polyhedra in double distilled H_2O with 5% blue food dye (v/v) and 6% maltose (w/v). Controls consisted of larvae fed the same formulation without virus. Two hours after dosing, larvae that had consumed the viral suspension (or H_2O) were transferred individually to a leaf, and the Petri dish was sealed with parafilm. Larvae were maintained at 26 $\pm$ 1°C and 16L:8D, and mortality was scored every 4–8 hr, depending upon the mortality rate. Thirty-five larvae were infected per bioassay for each treatment. Bioassays were replicated two to three times during each of the four seasons of the year for a total of nine replicates. Each replicate also contained a group of insects fed on artificial diet instead of foliage as a control to determine if monthly differences in rate of mortality were the result of seasonal variability in physiological susceptibility of the insects.

Survival data were analyzed by Cox's proportional hazards model (S-plus; Kalbfleisch and Prentice, 1980; Collett, 1994) to determine if the survival curves could be predicted from levels of phytochemicals (independent variables). In addition, plant type and month were entered as categorical variables. The model calculates the baseline hazard function $\lambda(t) = \lambda_0(t) \exp(\beta X)$ where $\lambda_0(t)$ is the baseline hazard function representing the differential probability of dying at time (t), given survival to time (t). The βs are unknown parameters to be estimated by the model and Xs are the levels for each independent variable. By definition $\exp(\beta X) = \exp(\beta_0 + \beta_1 x_1 + \beta_2 x_2 \ldots)$ and is the amount by which $\lambda_0(t)$ is multiplied. Thus, an estimated parameter coefficient with a positive sign indicates a variable correlated with increased speed of kill; a coefficient with a negative sign indicates a variable correlated with decreased speed of kill. The best model was chosen by comparing the likelihood ratio chi-square (LRCS) values for each model.

We entered each variable in the model separately, followed by backward stepwise multiple regression to determine the most predictive model. We fitted two types of models. For the same reasons as described in the methods for the first set of percent mortality bioassays, we first examined the influence of phytochemicals on the survival curves using pooled data; we then evaluated models separately for each plant species. Because survival times were known only up

to an interval of time, the survival times of the insects were estimated as the midpoints of the intervals in which they died. The Kaplan-Meier product limit estimator was used to estimate the LT_{50}s for each treatment (S-plus; Kalbfleisch and Prentice, 1980; Collett, 1994).

RESULTS

Percent Mortality of Third-Instar H. virescens *Dosed with AcMNPV*

Combined Cotton and Lettuce Model. There was a dramatic influence of host plant on lethal infections in *H. virescens* treated with baculovirus, with the greatest impact occurring at lower doses (Figure 1). Except at the highest viral dose, mortality appeared highest on iceberg lettuce, lower on romaine lettuce, and lowest equally on the two cultivars of cotton; however, these differences were not significant at the 5% level using ANOVA to compare mean percent mortalities ($F = 1.62$, $df = 3, 9$, $P = 0.2030$). The odds of dying (odds ratios)

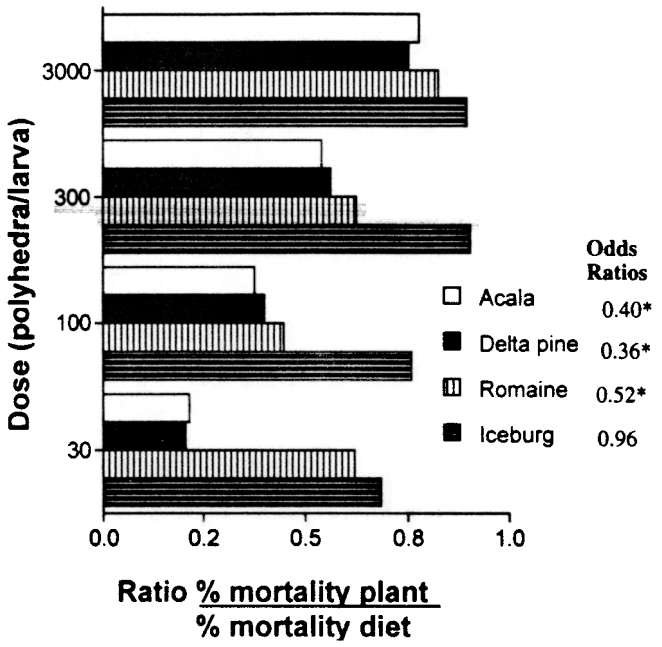

FIG. 1. Influence of host plant on mortality of third-instar *H. virescens* treated with AcMNPV. Bars represent the ratio of mean percent mortality for insects dosed on one of four host plants to mean percent mortality for insects dosed on artificial diet (control). Means are percentage mortality of three replicates (N = 12–15 plants/replicate). Odds ratios significantly less than 1 at the 5% level of significance (followed by asterisks) indicate treatments that protected the insect from dying compared to being dosed on artificial diet.

when insects were dosed on cotton or romaine lettuce were, however, significantly lower than the odds of dying on artificial diet. The odds ratios calculated for each plant type permitted us to determine whether any of the host plants protected the insect from lethal infection compared to the artificial diet control (Figure 1). The two cultivars of cotton were equally most protective, followed by romaine; iceberg lettuce was not significantly different from diet as a substrate for infection.

The lack of significant differences in mean percent mortality among host plants did not mean that viral efficacy was not influenced by host plant. On the contrary, larval mortality varied considerably within plant type from replicate to replicate as a function of plant phenolase activity, making it difficult to detect significant differences among treatments with ANOVA (a linear model which assumes a normal distribution). PPO activity was not detectable in cotton despite use of a diversity of substrates (chlorogenic acid, rutin, (±)-catechin, quercetin, and 2, 3-dihydroxybenzoic acid). Phytochemical levels within plant type varied considerably with photoperiod and temperature (experiments were replicated in late winter and early spring, data not shown), although within each replicate, cotton had significantly higher POD activity and phenolic content (catecholic and total phenolics) than lettuce (Table 1). Romaine lettuce contained slightly higher levels of all chemicals assayed than iceberg lettuce. The best relationship between mortality and an individual phytochemical variable was with POD (simple effect). With the exception of gossypol, only POD was useful for predicting mortality when other phytochemical variables were excluded from the model (Figure 2, simple effect). Total phenolics ($t = 1.54$, $P = 0.1270$), condensed tannins ($t = 0.80$, $P = 0.4237$), protein ($t = -0.21$, $P = 0.8367$), and plant type ($t = 1.61$, $P = 0.1109$) were not useful predictors for mortality either by themselves or in combination with other phytochemical variables.

When the best combination of phytochemical variables was examined, both POD and PPO had significantly negative effects on mortality (Table 2). Thus, the higher the level of these oxidative enzymes, the less likely the insect was to die from infection. In contrast, the higher the levels of catecholic phenolics and viral dose, the higher the probability of an insect dying. Finally, POD and PPO interacted in their effect on mortality ($\beta = +0.31$, Table 2). Thus, for a given level of PPO, the higher the level of POD, the less impact PPO had on decreasing the probability of an insect dying (a positive value for the interaction term added to the negative values of the individual terms). Similarly, the higher the PPO activity, the less impact POD had on protecting the insect from disease. However, POD was more influential in decreasing infectivity of the virus than PPO ($\beta = -1.19$ for POD vs. $\beta = -1.05$ for PPO; Table 2).

Separate Cotton and Lettuce Models. When data for the two cotton cultivars were analyzed separately from lettuce to permit examination of the influence of plant chemical content on mortality within a host plant species, the two models

TABLE 1. MEAN PHYTOCHEMICAL CONTENT OF FOUR HOST PLANTS USED TO TEST LETHAL INFECTIVITY OF ACMNPV TO *H. virescens*

Plant	POD (Δ Abs/g/min)	PPO (Δ Abs/g/min)	*o*-Dihydroxy phenolics (μmol/g)	Total phenolics (μmol/g)	Condensed tannins (μmol/g) (dry wt)	Gossypol (% gossypol equivalents) (dry wt)[a]	Protein (% Rubisco equivalents)
Acala cotton	170 ± 48	ND	17 ± 5.7	60 ± 22	26 ± 8.4	3.1 ± 0.80	2.7 ± 0.22
Delta pine cotton	184 ± 26	ND	14 ± 5.7	32 ± 14	23 ± 7.1	3.6 ± 0.49	3.0 ± 0.38
Romaine lettuce	15 ± 2.5	14 ± 1.4	4.0 ± 1.2	4.9 ± 0.81	NA	NA	1.5 ± 0.27
Iceberg lettuce	12 ± 1.6	11 ± 2.5	3.8 ± 0.90	4.3 ± 1.0	NA	NA	1.3 ± 0.29

Cotton cultivar	Hemigossypol	Gossypol	Heliocide H4	Heliocide H1	Heliocide H3	Heliocide H2
Acala SJ-2	0.49 ± 0.03	0.13 ± 0.01		0.71 ± 0.02	0.39 ± 0.01	1.1 ± 0.04
Delta Pine 15	1.0 ± 0.03	0.40 ± 0.02		0.72 ± 0.02	0.33 ± 0.01	0.90 ± 0.02

[a] Assay for gossypol also detects other aromatic terpenoid aldehydes. The concentrations of specific terpenoids (in μg/mg leaf tissue) are shown above. Concentrations were determined by HPLC using the appropriate standards (Stipanovic et al., 1988). NA = not applicable. ND = not detectable.

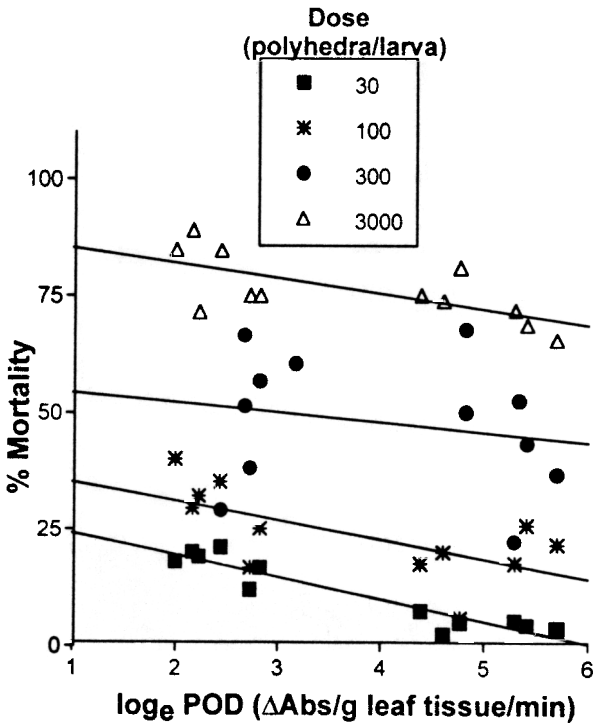

FIG. 2. Effect of peroxidase (POD) level on percent mortality of third-instar *H. virescens* dosed on one of four different host plants at 4 different viral doses. Data were analyzed for cotton and lettuce cultivars combined by logistic regression. The simplest model that fit the data consisted of viral dose ($\beta = +0.05$, $t = 19.54$, $P < 0.0001$) and $\log_e$ POD ($\beta = -0.10$, $t = -2.35$, $P = 0.0208$); Model chi-square = 525, $df = 2$, $P < 0.0001$; see Table 2 for best full model.

TABLE 2. COMBINED MODEL DEPICTING INFLUENCE OF PHYTOCHEMICAL CONTENT ON MORTALITY OF *H. virescens* TREATED WITH AcMNPV ON ALL PLANTS[a]

Variable	Parameter coefficient	*t* statistic	*df*	*P*
Catechols	+0.06	+4.65	88	<0.0001
PPO	−1.05	−4.82	88	<0.0001
POD	−1.19	−5.87	88	<0.0001
Viral Dose	+0.76	+19.74	88	<0.0001
PPO * POD	+0.31	+3.47	88	0.0008

[a] Model chi square = 555, $df = 5$, $P < 0.0001$; model equation: probability of dying (p) = exp (βX)/[1 + exp (βX)], where βX = [−0.74 + 0.06(Catecholic phenolics) − 1.19($\log_e$ POD) − 1.05(sqrtPPO) + 0.76($\log_e$ Viral dose) + 0.31($\log_e$ POD) * sqrtPPO]. Plant type was not significant ($t = 1.41$, $df = 88$, $P = 0.1618$).

were not the same (Table 3, simple model). Mortality on cotton and lettuce varied with POD and PPO activities, respectively. On cotton, the higher the POD activity, the lower the mortality. On lettuce, the higher the PPO activity, the lower the mortality.

The impact of condensed tannins on larval mortality depended upon whether it was regressed separately (with viral dose) or in combination with other phytochemicals (Table 3). By itself, the higher the condensed tannin content of cotton foliage, the lower the mortality (simple effect, negative coefficient). In combination with POD, however, tannins increased the probability of an insect dying from disease (full model, positive coefficient). POD and viral dose interacted in their effect on mortality (Table 3). Given that dose has a positive coefficient and POD a negative one, the higher the dose, the less negative was the slope of POD's impact on mortality. At higher viral doses, POD had less ability to inhibit viral disease. At lower doses, the influence of POD on inhibiting viral disease was much greater (more negative slope).

When insects were treated with baculovirus on lettuce, PPO, but not POD, was predictive of a lower probability of dying (Table 3, full model). In addition, insects dosed on lettuce were less likely to die from viral infection the higher the noncatecholic phenolic level of foliage (negative coefficient), but the probability of dying was increased by higher catecholic phenolic content of foliage (positive coefficient). Phytochemical effects in lettuce did not change whether the impact of the variables on mortality were considered separately or together.

Rate of Mortality of Neonates Infected with WT AcMNPV or Recombinant AcAaIT

Combined Cotton and Lettuce Model. A simplified examination of monthly survival times is depicted as mean LT_{50}s for each virus among the four plant types (Figure 3). LT_{50}s for insects fed on plants and artificial diet controls were slower in the summer months than the winter months. Mean foliar catecholic phenolic content for the four plant types followed a similar trend; catecholic phenolic levels were higher in the summer months and lower in the winter months (Figure 3). Although LT_{50}s for insects fed on plants parallel catecholic phenolic levels, the LT_{50}s on plants also paralleled monthly variability in LT_{50}s for insects fed on control diet. Thus, it is likely that seasonal differences in physiological susceptibility to viral infection also played a role in influencing lethal times. However, choosing one time point (the LT_{50}) in a survival curve (Figure 3) did not accurately reflect the statistical significance of month as a predictor of the entire survival curve, which is the probability of surviving up to time (t) for diet controls. Furthermore, month by itself was not the only significant predictor of the survival curves.

We examined the influence of host-plant chemical content as an indepen-

TABLE 3. SEPARATE MODELS DEPICTING INFLUENCE OF PHYTOCHEMICAL CONTENT ON MORTALITY OF *H. virescens* TREATED WITH AcMNPV ON COTTON OR LETTUCE[a]

Cotton				Lettuce			
Variable	Parameter coeff.	*t* statistic[b]	*P*	Variable	Parameter coeff.	*t* statistic[b]	*P*
Each variable regressed separately							
POD	−0.13	−0.69	0.4960	PPO	−0.06	−3.08	0.0035
Protein	−0.31	−2.46	0.0179	Protein	−0.01	−0.06	0.9527
Catechols	+0.01	+1.33	0.1912	Catechols	+0.07	+1.75	0.0870
Total phenolics	+0.06	+2.17	0.0354	Total phenolics	−0.18	−2.38	0.0214
Noncatechols[c]	+0.05	+2.16	0.0344	Noncatechols[c]	−0.32	−2.38	0.0214
Tannins	−0.01	−2.18	0.0346	POD	+0.11	0.44	0.6638
Gossypol	+0.03	+0.40	0.6946				
			Best full models				
Model[d]							
POD	−1.80	−2.46	0.0180	PPO	−0.05	−2.53	0.0150
Tannins	+0.02	+3.05	0.0039	Catechols	+0.98	+2.23	0.0309
Viral dose	+0.09	+5.92	<0.0001	Noncatechols[c]	−1.27	−2.21	0.0326
POD * dose	+1.13	+2.90	0.0058	Viral Dose	+0.05	+13.40	<0.0001
Model 2[e]							
Viral Dose	+0.05	+13.89	<0.0001				
POD	−0.75	−2.52	0.0156				
Catechols	+0.03	+2.75	0.0086				

[a] Data for cotton and lettuce were analyzed separately by logistic regression. Regressions were first run using viral dose + individual chemical variables (viral dose invariably had a P value of <0.0001 in combination with each chemical variable), followed by stepwise regression to determine the best full model for each plant species. For each model, the probability of dying (p) = $\exp(\beta X)/[1 + \exp(\beta X)]$. Cotton model 1: βX = [−1.80($\log_e$POD) + 0.02(Tannin) + 0.09(viral dose) + 1.13($\log_e$POD *Viral dose)]; chi-square = 288, $df = 4$, $P < 0.0001$; model 2: βX = [0.05(viral dose) − 0.75($\log_e$POD) + 0.03(catechols)]; chi-square = 282, $df = 3$, $P < 0.0001$. Lettuce model: βX = [−0.05(PPO) + 0.98(Catechols) − 1.27(Noncatechols)]; chi-square = 263, $df = 4$, $P < 0.0001$.

[b] $df = 45$ for all variables regressed separately with viral dose.

[c] Noncatechols = total phenolics − catecholic phenolics.

[d] $df = 41$.

[e] $df = 42$.

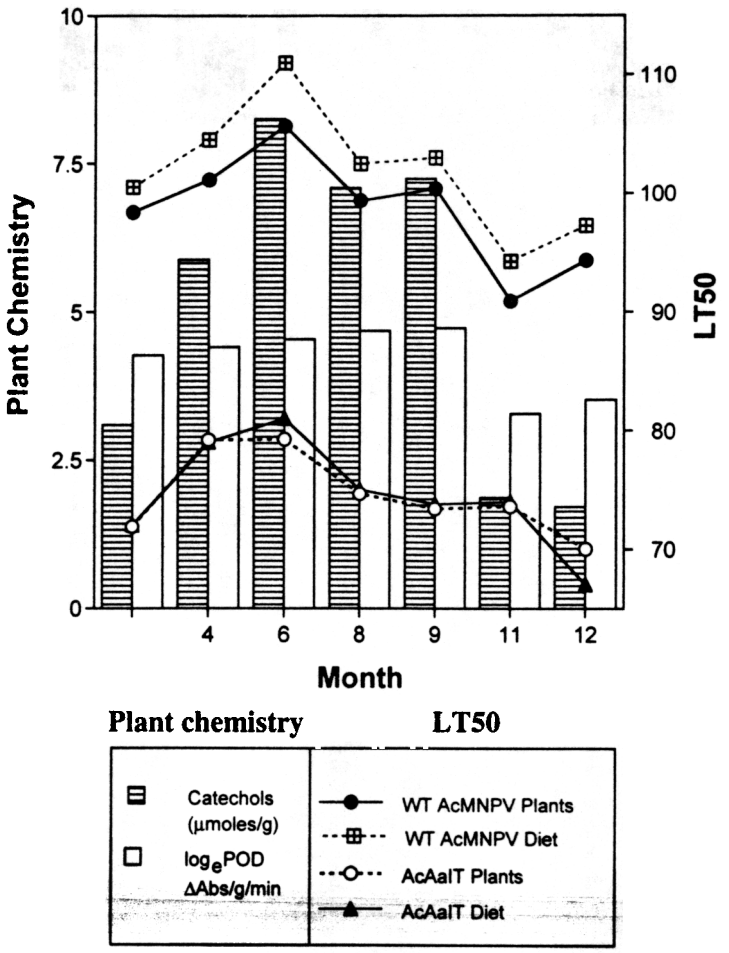

FIG. 3. Monthly variability in mean LT_{50} of *H. virescens* infected with wild-type AcMNPV or AcAaIT and fed on one of four host plants or artificial diet (controls) are shown as line graphs (right *Y* axis). Mean POD (solid bars) and catecholic phenolic levels (striped bars) of foliage are shown for each month (left *Y* axis). Month was a significant predictor of the survival curves for diet with WT AcMNPV (LRCS = 20.3, $P = 0.0024$), but the sign of the coefficients was opposite those for plants with one exception (Nov.). Despite the usefulness of month as a predictor of the survival curves of insects infected with AcAaIT and fed on plants (Table 7), there were no significant differences in monthly LT_{50}s for control insects (fed on diet) infected with AcAaIT (LRCS = 6.07, $P = 0.4160$).

dent variable on the survival times of neonate larvae of *H. virescens*, the dependent variable, infected with the WT AcMNPV or the recombinant baculovirus AcAaIT by fitting Cox's proportional hazards model. For both viruses, we first examined the relationship of each independent variable to the survival curve by entering each variable into the model separately. Then we fit the model using the best combination of variables. This procedure demonstrated some significant

TABLE 4. INFLUENCE OF EACH VARIABLE ON RATE OF MORTALITY OF *H. virescens* INFECTED WITH WILD-TYPE AcMNPV (IN ORDER OF RELATIVE IMPORTANCE) AND FED ON COTTON OR LETTUCE[a]

Variable	*z* statistic	Likelihood ratio (chi-square)	*df*	*P*
Month		70.2	6	<0.0001
Catechols	−4.78	24.6		<0.0001
POD	−4.75	24.0		<0.0001
Gossypol	−4.74	22.8		<0.0001
Plant type		19.2	3	0.0002[b]
Condensed tannins	−4.16	18.7	1	<0.0001
Protein	−3.99	16.2		<0.0001
Total phenolics	−3.70	14.9		0.0001
PPO	+3.20	10.0		0.0015

[a] Pooled survival data were analyzed for cotton and lettuce combined by Cox's proportional hazards model (N = 784).
[b] Insects fed on romaine lettuce died significantly faster than on the other plant types.

commonalities. For both viruses, rate of mortality varied with the levels of each phytochemical variable (Tables 4 and 5). For all chemical variables examined (except PPO), the higher the level, the slower the rate of mortality. In contrast, the higher the activity of PPO, the faster the insects died.

TABLE 5. INFLUENCE OF EACH VARIABLE ON RATE OF MORTALITY OF *H. virescens* INFECTED WITH AcAaIT (IN ORDER OF RELATIVE IMPORTANCE) AND FED ON COTTON OR LETTUCE[a]

Variable	*z* statistic	Likelihood ratio (chi-square)	*df*	*P*
Month		94.5	6	<0.0001
Condensed tannins	−4.68	23.6		<0.0001
Catechols	−4.32	20.0		<0.0001
Total phenolics	−4.00	17.6		<0.0001
PPO	+3.43	11.6		0.0006
Protein	−2.99	9.02		0.0027
POD	−2.92	8.81		0.0030
Gossypol	−2.72	7.46	1	0.0063
Plant type		7.02	3	0.0712

[a] Pooled survival data were analyzed for cotton and lettuce cultivars combined by Cox's proportional hazards model (N = 763).

When we examined the best combination of variables for predicting the survival curves, rate of mortality varied seasonally for both viruses (Tables 6 and 7). Although month was also a useful predictor of the survival curves for insects infected with WT AcMNPV that fed on diet, when the entire survival curve was modeled, the sign of the statistically significant coefficients for insects that fed on diet were opposite those for insects that fed on plants in the same months (with one exception, November; Table 6). Thus, month was not sufficient to explain the variability in the survival curves by itself. For the wild-type virus, in addition to month, rate of mortality varied with POD levels; plant type was not significant (Table 6). The higher the POD activity, the longer it took infected insects to die. Despite the fact that higher catecholic phenolic levels were strongly correlated with slower lethal times as a simple effect (Table 4), catechols dropped out of the final model (Table 6). This may be explained by the fact that catecholic phenolic content varied in parallel to month (Figure 3), and thus its presence in the model was probably redundant (i.e., multicollinearity).

For the recombinant virus AcAaIT, the best fit of the data required a more complex model (Table 7). POD, catecholic phenolics, condensed tannins, month, and plant type were predictive of rate of mortality. The higher the POD or phenolic level, the slower the insects died. Insects that fed on lettuce died

TABLE 6. BEST COMBINED MODEL FOR PREDICTING RATE OF MORTALITY OF *H. virescens* INFECTED WITH WILD-TYPE AcMNPV AMONG ALL PLANTS[a]

	Plants			Diet controls		
Variable	Parameter coeff.	*z* statistic	*P*	Parameter coeff.	*z* statistic	*P*
POD	−0.0007	−3.93	**<0.0001**			
Month (Feb. = 0)						
April	−0.2245	−3.25	**0.0012**	+2.27	+2.92	
June	−0.1202	−3.07	**0.0021**	+2.23	+2.74	
Aug.	+0.0116	+0.44	0.6600	+2.98	+3.74	
Sept.	+0.0235	+1.14	0.2600	1.35	+1.07	
Nov.	+0.1248	+6.05	**<0.0001**	1.99	+2.45	
Dec.	+0.0444	+2.99	**0.0028**	1.47	+1.24	

Pooled survival data were analyzed for cotton and lettuce cultivars by Cox's proportional hazards model (Model: $\lambda = \exp(\beta X)$, where $\beta X = \exp[\beta_{month}(\text{Month}) - 0.0007(\text{POD})]$; LRCS = 87.1, $df = 7$, $P < 0.0001$, $N = 784$). *P* values for variables with coefficients that were significant at the 5% level are shown in bold. Despite a highly significant correlation between catecholic phenolics and survival times as a simple effect (chi-square = 24.6, $df = 1$, $P < 0.0001$; Table 4), this variable was not needed in the final model when month entered (catechols z = −1.01, $P = 0.3100$).

TABLE 7. BEST COMBINED MODEL FOR PREDICTING RATE OF MORTALITY OF *H. virescens* INFECTED WITH ACAAIT AMONG ALL PLANTS

Variable	Parameter coefficient	*z* statistic	*P*
POD	−0.0008	−1.99	**0.0470**
Catechols	−0.0413	−3.21	**0.0013**
Condensed tannins	−0.0066	−2.59	**0.0097**
Month (February = 0)			
April	−0.4379	−5.93	**<0.0001**
June	−0.0924	−2.16	**0.0031**
August	−0.0662	−2.02	**0.0440**
September	+0.1001	+4.48	**<0.0001**
November	−0.0063	−0.28	0.7800
December	+0.0504	+2.2	**0.0260**
Plant (Acala cotton = 0)			
Delta pine cotton	−0.0175	−0.27	0.7900
Romaine lettuce	+0.2197	+3.42	**0.0006**
Iceberg lettuce	+0.0868	+2.21	**0.0270**

[a] Pooled survival data were analyzed for cotton and lettuce cultivars combined by Cox's proportional hazards model $\{\lambda = \exp(\beta X)$, where $\beta X = \exp[\beta_{\text{month}}(\text{Month}) - 0.0008(\text{POD}) - 0.0413(\text{Catechols}) - 0.0065(\text{Tannins}) + \beta_{\text{plant type}}(\text{Plant type})]$; LCRS = 126, $df = 12$, $P < 0.0001$, $N = 763]$. *P* values for variables with coefficients that were significant at the 5% level are shown in bold. Romaine and iceberg lettuce required different intercepts compared with cotton, i.e., rate of mortality was significantly faster when insects were fed on lettuce than cotton.

significantly faster than insects that fed on cotton. Mortality was again slower in the summer months and faster in the fall and winter months (Table 7; Figure 3). Although month was a significant predictor of the survival curves for insects that fed on plants (Table 7), it was not significant for insects that fed on control diet despite the parallel relationship in LT_{50}s for insects that fed on diet or plants depicted in Figure 3. Because lettuce does not contain condensed tannins, it could be argued that inclusion of this variable in the model introduces bias. If tannins are removed from the model, month, plant type, POD ($\beta = -0.01$, $z = -2.47$, $P = 0.0140$), and catechol content ($\beta = -0.037$, $z = -2.90$, $P = 0.0037$) provided a useful, biologically meaningful model without specifying plant type (LRCS = 111, $df = 8$, $P < 0.0001$). Clearly, inclusion of POD level is very important because predicting larval mortality using a model containing only month and plant type had a lower likelihood ratio chi-square (LRCS) value than one that includes POD (LRCS for month + plant type + POD = 110, $df = 10$ vs. LRCS for month + plant type = 103, $df = 9$).

Separate Cotton and Lettuce Models. Because POD activities were always markedly higher in cotton than lettuce (Table 1), one might argue that bias is

introduced in the model because the data in essence represent two points of clustered data, which define a line. Thus, we analyzed cotton cultivars separately from lettuce and obtained models very similar to each other and to the combined model described above.

For insects infected with WT AcMNPV, the higher the POD and catecholic phenolic levels, the slower the insects died for both plant species (Table 8), also, the higher the gossypol content of cotton foliage, the slower the insects died. The full models for cotton and lettuce analyzed separately are similar to those for all plant types combined. On both cotton (Figure 4A) and lettuce (Figure 4B), rate of mortality varied with POD level and month (Table 9). For cotton, gossypol and catecholic phenolics were also needed in the model, although only gossypol had a statistically significant z statistic at the 5% level (Table 9). POD was not significant in the absence of catecholic phenolics. Thus, in agreement with the model using all plant types combined (Table 6), the higher the POD activity of each plant species, the slower the insects died (Figures 4A and 4B).

Relationships between individual variables and rate of mortality of insects infected with AcAaIT that fed on cotton (Table 10) were also similar to those obtained using data for all plants combined. Again the higher the POD, catecholic phenolics, gossypol, and condensed tannin levels of cotton foliage, the slower the insects died (Table 10). For lettuce, however, only POD was significantly correlated (negative coefficient) with rate of mortality by itself (Table 10). The most robust full models required inclusion of month for both plant species (Table 11). However, the inclusion of other variables in addition to month was not required to explain the variability in survival times for insects

TABLE 8. INFLUENCE OF EACH VARIABLE ON RATE OF MORTALITY OF *H. virescens* INFECTED WITH WILD-TYPE AcMNPV AND FED ON COTTON OR LETTUCE[a]

	Cotton		Lettuce	
Variable	z statistic	P	z statistic	P
Month		**<0.0001**		<0.0001
Catechols	−2.86	**0.0043**	−1.46	**0.0150**
POD	−3.19	**0.0140**	−5.85	**<0.0001**
Total phenolics	−1.55	0.1200	−0.43	0.0670
Gossypol	−2.93	**0.0034**		NA
Condensed tannins	−1.91	0.0570		NA
PPO		NA	+0.26	0.7960

[a]Survival data for the two cultivars each of cotton and lettuce were analyzed separately by Cox's proportional hazards model. P values for variables with coefficient that were significant at the 5% level are shown in bold. NA = not applicable.

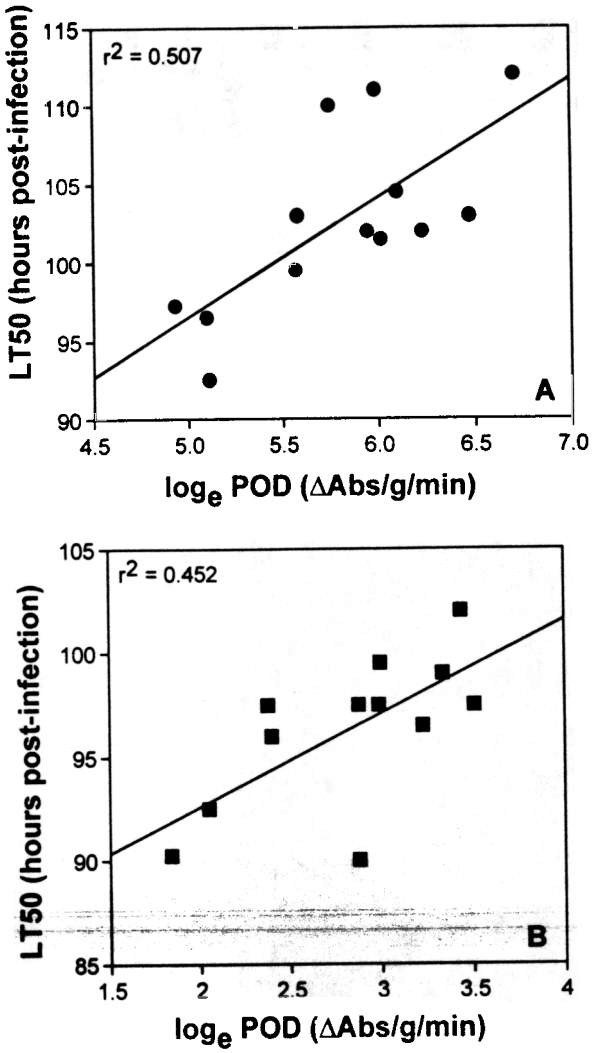

FIG. 4. Influence of peroxidase (POD) level on the LT_{50} of insects infected with WT AcMPV and fed on cotton (A) or lettuce (B) foliage. LT_{50}s were determined by the Kaplan-Meier estimator.

fed on cotton (Table 11). For lettuce, there were two equally useful models. In separate models PPO and POD were each negatively correlated with rate of mortality (Table 11). If PPO was in the model, it was also necessary to include noncatecholic phenolics (negative coefficient) and catecholic phenolics (positive coefficient) to describe the survival curves, although their z statistics were not significant in the final model. Similarly, if POD was in the model, it was also necessary to include catecholic phenolics (negative coefficient) to help explain some of the variability despite the fact that the z statistic for catechols was not significant at the 5% level in the final model.

TABLE 9. BEST MODELS FOR PREDICTING RATE OF MORTALITY OF *H. virescens* INFECTED WITH WILD-TYPE AcMNPV FED ON COTTON OR LETTUCE[a]

	Cotton			Lettuce		
Variable	Parameter coeff.	*z* statistic	*P*	Parameter coeff.	*z* statistic	*P*
Month			**<0.0001**			**<0.0001**
POD	−0.3420	−3.19	**0.0014**	−0.259	−1.98	**0.0470**
Catechols	−0.0255	−1.05	0.3000			
Gossypol	−0.2223	−2.06	**0.0400**			

[a] Survival data for cotton and lettuce were analyzed separately by Cox's proportional hazards model [$\lambda = \exp(\beta X)$]. Cotton model: $\beta X = \exp[\beta_{month}(\text{Month}) - 0.342(\log_e \text{POD}) - 0.0255(\text{Catechols}) - 0.2223(\text{Gossypol})]$; LRCS = 32.8, $df = 9$, $P = 0.0001$, $N = 380$; Lettuce model: $\beta X = \exp[\beta_{month}(\text{Month}) - 0.259(\log_e \text{POD})]$; LRCS = 53.5, $df = 7$, $P < 0.0001$, $N = 404$, *P* values for variables with coefficients that were significant at the 5% level are shown in bold.

TABLE 10. INFLUENCE OF EACH VARIABLE ON RATE OF MORTALITY OF *H. virescens* INFECTED WITH AcAaIT AND FED ON COTTON OR LETTUCE[a]

	Cotton		Lettuce	
Variable	*z* statistic	*P*	*z* statistic	*P*
Month		**<0.0001**		**<0.0001**
Catechols	−3.89	**<0.0001**	−0.71	0.4800
POD	−3.56	**0.0004**	−3.48	**0.0005**
Total phenolics	−3.27	**0.0011**	−0.58	0.5600
Gossypol	−1.99	**0.0470**		NA
Condensed tannins	−4.63	**<0.0001**		NA
PPO		NA	+0.86	0.3920

[a] Survival data for cotton and lettuce were analyzed separately by Cox's proportional hazards model. *P* values with coefficients that were significant at the 5% level are shown in bold. NA = not applicable.

DISCUSSION

Plant phenolase activity influenced the progression and severity of baculoviral disease in noctuid larvae in a nonlinear, highly context-dependent fashion. Furthermore, in the plants examined, POD had a greater and more consistent role than PPO in inhibiting baculoviral disease (to aid the reader models are

TABLE 1. BEST MODELS FOR PREDICTING RATE OF MORTALITY OF *H. virescens* INFECTED WITH AcAaIT AND FED ON COTTON OR LETTUCE[a]

Variable	Cotton			Lettuce		
	Parameter coeff.	*z* statistic	*P*	Parameter coeff.	*z* statistic	*P*
Month			**<0.0001**			**<0.0001**
PPO				−0.0597	−2.05	**0.0410**
Catechols (model 1)				+0.0875	+1.30	0.1900
Catechols (model 2)				−0.0495	−1.46	0.1400
Noncatechols[b]				−0.0374	−1.58	0.1100
POD				−0.0423	−2.14	**0.0320**

[a] Survival data for cotton and lettuce were analyzed separately by Cox's proportional hazards model. *P* values for variables with coefficients that are significant at the 5% level are shown in bold. For cotton, only month was significant (LRCS = 78.5, $df = 6$, $P < 0.0001$, $N = 363$). For lettuce there were two equally useful models: $\lambda = \exp(\beta X)$ where βX for model 1 = $\exp[\beta_{month}(\text{Month}) - 0.0597(\text{PPO}) + 0.0875(\text{Catechols}) - 0.0374(\text{Noncatechols})]$; LRCS = 60.3, $df = 9$, $P < 0.0001$, $N = 400$. βX for model 2 = $\exp[\beta_{month}(\text{Month}) - 0.0423(\log_e \text{POD}) - 0.0495(\text{Catechols})]$; LRCS = 58.6, $df = 8$, $P < 0.0001$, $N = 400$.

[b] Noncatechols = total phenolics − catecholic phenolics.

presented as schematics in Figures 5 and 6). In addition to the importance of plant phenolase activity as a modulator of baculoviral disease, several important concepts were reinforced concerning the means by which host plants influence disease—because the influence of phytochemicals on viral disease depends on chemical context, the impacts of phytochemicals are multiplex, interactive, and multifunctional.

Multiplicity. The influence of host plant on viral efficacy (measured as the probability of dying or time to death) is multivariate. Predicting the impact of phytochemicals on disease required regression of multiple independent variables, not single variables. For example, larval mortality on lettuce could be predicted from PPO, catecholic phenolics, or noncatecholic phenolic levels (and viral dose). (Figure 5). However, analysis of the influence of these individual phytochemicals on baculoviral disease explained only a part of the story. When these phytochemical variables were analyzed together (as a full model), we obtained a better fit of the data (chi-square for individual variables 16.5, 12.8, 5.5, and 248, respectively vs. chi-square = 273 for full model; Table 3, Figure 5). In addition, consideration of the impact of multiple phytochemicals on viral disease allowed us to construct rational hypothetical mechanisms for the inhibition of viral disease, the veracity of which can be subjected to further investigation (see discussion below on biological interpretation of the models).

Interactivity. A second major concept to be gleaned from this study is that

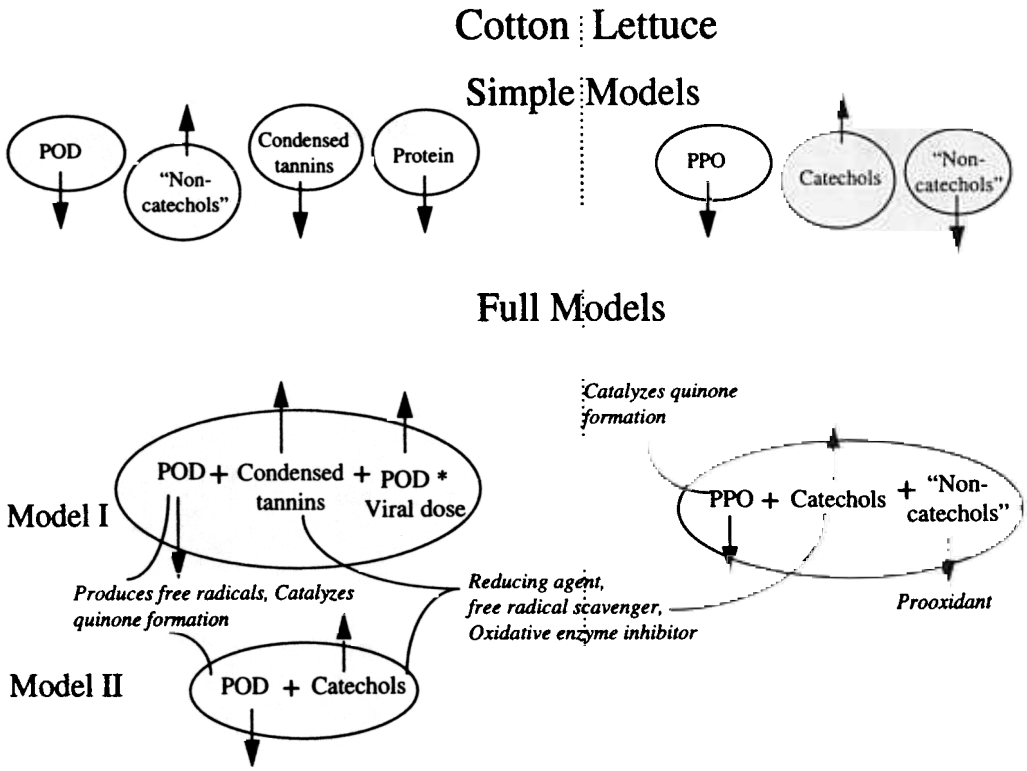

FIG. 5. Schematic representation of the impact of phytochemicals on lethal infection caused by the wild-type baculovirus AcMNPV in *H. virescens*. Arrows pointing upward or downward increase or decrease, respectively, the probability of an insect dying from viral infection. Note that some variables have different effects whether they are considered separately (simple regression, separate circles) or in combination with other phytochemicals (full models, multiple variables within same circle).

the impact of a given phytochemical on viral disease depended upon its interaction with other phytochemicals. Most illustrative of this concept was the influence of different classes of phenolics on viral disease. There were occasions where the impact of a given phytochemical variable changed (sign of the β coefficient) when it was considered in isolation versus in combination with other phytochemicals, i.e., in context. For example, condensed tannins were correlated with decreased larval mortality as a simple effect. In contrast, tannins were correlated with increased larval mortality in the context of POD activity.

As a simple effect, our finding that inhibition of lethal infection by increased tannin content of cotton foliage is in agreement with other studies that have shown a relationship between hydrolyzable and/or condensed tannins and a decrease in lethal infections caused by the *Lymantria dispar* NPV (LdMNPV) or *Helicoverpa zea* NPV (HzSNPV) (Keating et al., 1988, 1989; Schultz and Keating, 1991; Young et al., 1995). However, these studies either incorporated

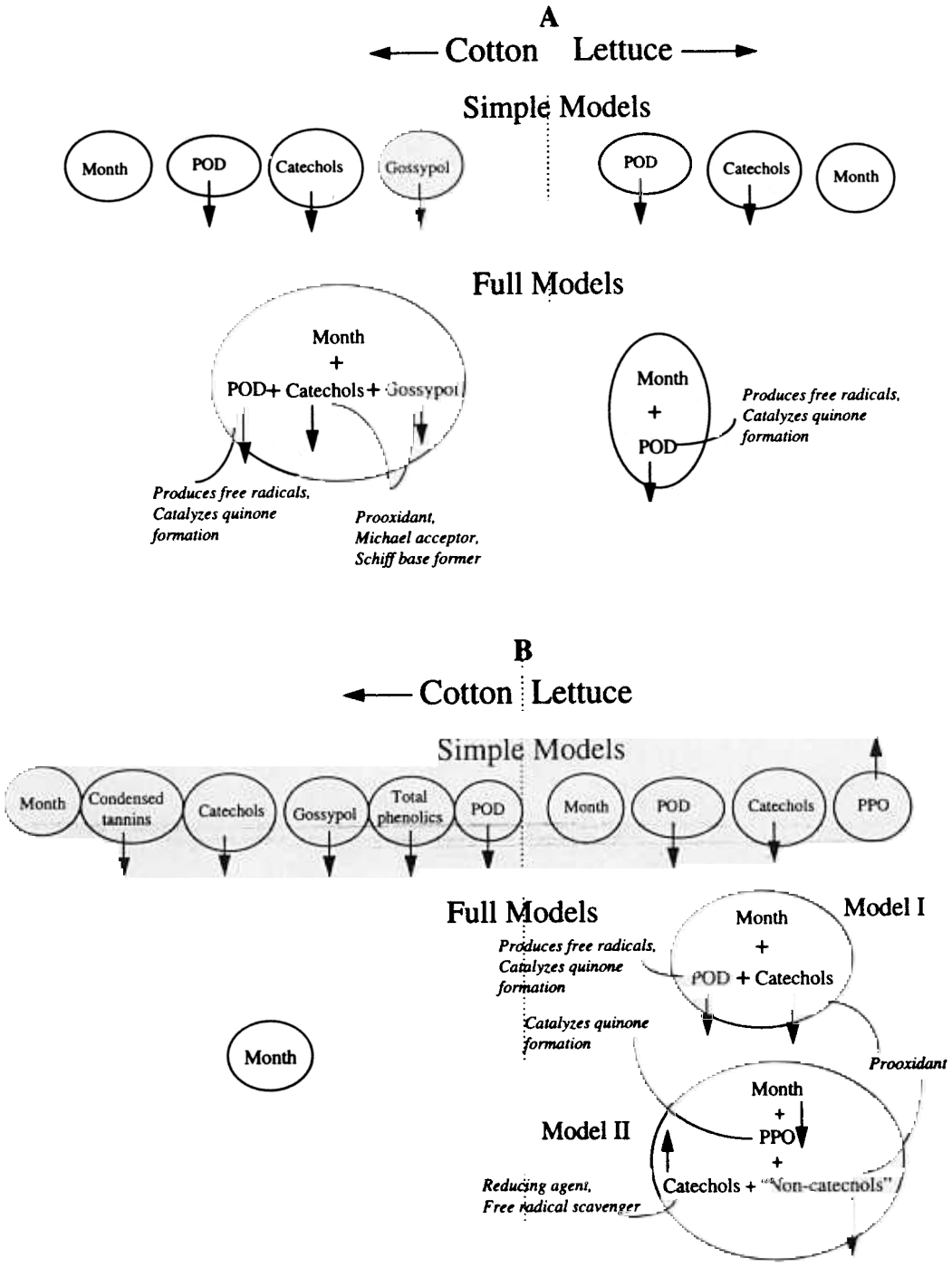

FIG. 6. Schematic representation of the impact of phytochemicals on rate of mortality of neonate larvae of *H. virescens* infected with either (A) the WT AcMNPV or (B) the recombinant AcAaIT. Arrows pointing upward or downward increase or decrease, respectively, the speed of kill. Note that some variables have different effects whether they are considered separately (simple regression, separate circles) or in combination with other phytochemicals (full models, multiple variables within same circle).

different concentrations of tannin into artificial diet or considered the impact of tannins in foliage in the absence of other phytochemicals, such as their possible interaction with plant oxidative enzymes and/or other types of phenolic compounds in foliage. Given that condensed tannins in our study were correlated with an opposite effect on viral disease when considered in isolation versus in the context of admixture, we suggest that estimating the impact of a phytochemical on viral disease out of context may lead to misleading conclusions.

A second example of interactions influencing viral disease was the impact of the host plant in the context of viral dose. Insects treated with a baculovirus were more likely to die from viral infection when fed on cotton than when fed on lettuce, with the greatest impact of the host plant on lethal infection at lower viral doses. Furthermore, POD interacted with viral dose such that the higher the viral dose, the less influence POD had in decreasing larval mortality on cotton. Differential impacts of phytochemicals on larval susceptibility to viral infection at low but not high viral doses was also reported by Felton et al. (1987). Furthermore, the absence of significant host plant effects on percent mortality at the highest viral dose (LD_{99}) suggests that when viral inoculum builds to levels capable of initiating an epizootic in natural environments, host plant chemistry probably has little if any effect on whether the insect will ultimately die from viral infection but may still influence the rate of mortality. The viral dose for these experiments represented the LD_{99}.

Multifunctionality. A final concept that can be inferred from our results is that the same phytochemical class can function by more than one mechanism in its impact on viral activity; the particular mechanism assumed is dependent upon context (admixture). This concept is well exemplified by the impact of catecholic phenolics on percent or rate of mortality. Catecholic phenolics increased the probablity of an insect dying from viral infection in both cotton and lettuce. In contrast, catecholic phenolics were correlated with slower lethal times, with one exception—insects infected with the recombinant virus AcAaIT died faster the higher the catecholic phenolic content of lettuce. In another instance, noncatecholic phenolics appeared to enhance viral disease (correlated with increased mortality) on cotton, whereas this variable appeared to attenuate disease on lettuce. Thus, a given phytochemical may enhance or attenuate viral disease depending upon context.

Biological Interpretation of the Models. How plant phenolase activity inhibits the ability of baculoviruses to produce lethal infections in larval hosts is not known, but we now have sufficient information to begin exploration of some potential mechanisms. In the plants examined, POD had a greater and more consistent role than PPO in exerting a negative influence on viral efficacy. Since both enzymes convert a variety of phenolics to highly reactive quinone end products, why is POD more influential?

There are several possible explanations for the more influential role of POD

in diminishing viral disease. Firstly, POD oxidizes a much broader range of substrates than does PPO, including intact proteins (Singleton, 1987; Robinson, 1991), thereby increasing the diversity of reaction products that may negatively affect the baculovirus. Secondly, the midgut of heliothines is nearly anaerobic (K. S. Johnson, personal communication), which may limit the available O_2 for PPO-mediated reactions. Reactions catalyzed by PPO are O_2 dependent, whereas many reactions (including phenolic oxidation) catalyzed by POD are O_2 independent (but H_2O_2 dependent) (Singleton, 1987; Robinson, 1991). Thirdly, POD may be more stable in the midgut of noctuid larvae than PPO; thus, POD activity may be further enhanced by the production of H_2O_2 by salivary glucose oxidase in oral secretions of heliothines (G. W. Felton, personal communication). In addition to the above, we believe that a critical process leading to the attenuation of viral disease by POD is the generation of free radicals.

POD oxidizes phenolics to quinones by a one-electron transfer mechanism employing H_2O_2 as a cosubstrate. The mechanism of oxidation produces a highly reactive semiquinone free radical intermediate, which can then initiate further propagation of free radicals (Butt, 1981; Butt and Lamb, 1981; Ahmad, 1995; Bi et al., 1997). We propose this intermediate is the most significant negative factor leading to inhibition of baculoviral disease. In contrast, PPO directly produces the quinone using molecular oxygen as a cosubstrate (Mayer, 1987), which, if our hypothesis is correct, assumes that the quinone is much less detrimental to the virus because it is less effective at secondarily producing free radicals by redox cycling. It is also possible that redox cycling initiated by POD generates active oxygen species (AOS) that are detrimental to the virus.

Because the polyhedral occlusion body (OB) of the baculovirus is primarily composed of protein (Vlak and Rohrmann, 1985; Whitt and Manning, 1987, 1988) with oxidizable and alkylatable amino acid residues (e.g., tyrosine, lysine, cysteine, methionine, and histidine), the OB is subject to covalent binding by reactive end products such as quinones formed by phenolic oxidation catalyzed by phenolases (Pierpoint, 1983; Felton et al., 1992). Although it has been shown that covalent binding can occur between oxidized phenolics, such as chlorogenoquinone formed by PPO activity and the OB in vitro (Felton and Duffey, 1990), it is not known whether this process has any effect on the ability of baculoviruses to infect their hosts on intact foliage.

Viral disease may also be attenuated via processes initiated by POD in foliage, such as oxidation of phenolics and other reaction products that produce free radicals that attack proteins and lipids (Summers and Felton, 1994). These processes may generate oxidative stress in the insect midgut leading to damage of the infected gut cells. Damaged midgut cells containing virus may be sloughed before the virus can spread beyond the midgut, effectively eliminating the infection from the insect.

Interactions between plant phenolases and phenolic substrates support the

notion that plant constituents influence viral disease in a manner that is multiplex, interactive, and multifunctional, each depending upon context. For example, the influence of different classes of phenolics was highly dependent upon context. Thus, the distinction between catecholic and noncatecholic phenolics (i.e., monohydroxyphenolics) is both chemically and biologically relevant in our experimental system. The two enzymes, PPO and POD, show a marked preference for oxidizing catecholic phenolics (Felton et al., 1989). For example, PPO readily oxidizes *o*-dihydroxyphenolics, such as chlorogenic acid, caffeic acid, and (±)-catechin, but has no activity on the monohydroxyphenolics, ferulic, *p*-coumaric, or *p*-hydroxybenzoic acids (Butt, 1981; Singleton, 1987). In addition, we observed 13-fold and 7-fold increases in the PPO and POD activities, respectively, of several plants during oxidation of catecholic phenolics in mixture, including chlorogenic acid plus rutin or catechin, compared to during oxidation of each phenolic species in isolation (Hoover et al., unpublished). In contrast, PODs from several plants have potent abilities to oxidize monohydroxyphenolics (Robinson, 1991), although PODs from cotton and tomato were more active on *o*-dihydroxyphenolics both singly and in mixture than on monohydroxyphenolics (Hoover et al., unpublished). Hence, the ratios and absolute amounts of catecholic phenolics and monohydroxyphenolics are important elements determining oxidative damage to viruses.

In general, phenolics, particularly some of the catecholic phenolics, have a variety of reactivities that, in a basic environment and/or in the presence of PPO or POD, include antioxidant activities such as radical and electrophile scavenging and reducing power [e.g., chlorogenic, caffeic, and ferulic acids (Huang and Ferraro, 1992) and tannins (Huang et al., 1992)], prooxidative activity (initiation of chain reactions generating AOS, organic free radicals, and/or quinones, e.g., quercetin), and chelation (Ho et al., 1992; Huang et al., 1992; Ahmad, 1995). The degree and proportion to which they exhibit these reactivities appear to be dependent upon context, identity, and dose. For example, under aerobic conditions and in the presence of trace metals, quercetin can produce superoxide and other AOS (Ho et al., 1992; Huang et al., 1992; Ahmad, 1995). However, in another context quercetin and other flavanoids such as rutin can also scavenge superoxide anions (Robak and Gryglewski, 1988). The reactivity of phenolic species may also be dose-dependent in a manner similar to that of ascorbate. Ascorbate can function as a prooxidant or antioxidant, depending on its concentration (Englard and Seifter, 1986; Frei et al., 1989; Felton, 1995; Felton and Summers, 1995). Thus, it is possible that catecholic phenolics in lettuce and condensed tannins in cotton are serving as reducing agents and/or free radical scavengers, thus protecting the virus from inactivation by oxidative activity. Furthermore, because tannins and other phenolics are known to act as competitive inhibitors of PPO and POD (Golan-Goldhirsh and Whitaker, 1984; McEvily et al., 1992), it is possible that these phytochemicals can also

enhance viral disease in combination with oxidative enzymes by inhibiting oxidative enzyme activity, thereby protecting the virus from inactivation. In contrast, noncatecholic phenolics were correlated with decreased mortality on lettuce, suggesting that a variety of such phenolic compounds in lettuce may serve as substrates for oxidative enzymes with subsequent negative impact on viral activity.

Despite the problem of multicollinearity in some models, we believe inclusion of phenolics in these models is necessary to obtain a more complete understanding of the impact of host plant on viral efficacy. Not only is multicollinearity common in regression models (Neter et al., 1990), but it was expected, given the conditions of our study involving growing plants at different times of year under natural conditions (Karban, 1987; Bryant et al., 1988; Chaves et al., 1997). Furthermore, we could not eliminate multicollinearity by excluding month from some models because it was sometimes required to explain the variability in the remaining phytochemicals to discern the importance of phenolics in influencing viral disease. More importantly, inclusion of phenolics in our models makes sense biologically. It is possible that increasing amounts of catecholic phenolics beyond a minimum level may not be necessary to inactivate (or enhance) viral efficacy. This is because enzymes are only required in catalytic amounts and there is an excess of phenolic substrates in these plants. It is also possible that increasing catecholic phenolic levels beyond a minimum concentration is inconsequential for the virus. Moreover, high phenolic levels can even interfere with enzyme activity, a phenomenon often referred to as autoinhibition (Golan-Goldhirsh and Whitaker, 1984). Assuming redox reactions that generate free radicals are involved as a potential mechanism of viral inactivation in these systems, and because these reactions are propagative, small amounts of phenolics may be all that is required in the presence of high enzymatic activity for viral inactivation to occur.

The mechanism(s) whereby plant phenolase activity influences speed of kill after infection by baculoviruses is also unknown. We suspect that under the conditions of this study, we may be witnessing a postinfectional impact on speed of kill of the larval host mediated through the physiological status of the insect in response to interactions between the insect and phytochemicals, not a direct effect of phytochemicals on the virus. Phenolase activity may retard the rate of mortality by affecting the growth rates of infected insects. In a previous study, we found that slower growth rates of *H. virescens* infected with AcMNPV were strongly correlated with slower speeds of kill (Hoover et al., 1996). Thus, it is possible that oxidative enzymes and phenolic compounds normally correlated with antinutritive effects in noctuid larvae (Felton et al., 1992; Duffey and Stout, 1996) have the inadvertent effect of slowing lethal times of infected insects.

In addition to host plant effects, seasonal differences in physiological susceptibility to viral infection likely contributed to the monthly differences in

survival times of insects treated with baculovirus. Month was a significant predictor of the survival curves of insects infected with WT AcMNPV and fed on artificial diet, although month was not a significant predictor of the survival curves for AcAaIT on diet. A variety of biotic and abiotic factors have been shown to influence susceptibility of insects to entomopathogens including differences in genetic susceptibility to viruses among conspecific strains of insects (Watanabe, 1987).

Research is currently underway in our laboratory to determine the mechanisms of the differential ability of POD and PPO to inhibit baculoviral disease, to determine the interaction of these enzymes with various classes of phenolic compounds in this process, and to distinguish free radical from enzymatic effects on viral activity. By manipulating phytochemicals in admixture we hope to establish a better understanding of the mechanism(s) whereby phytochemicals influence viral efficacy among different host plants. The antiviral effects of these phytochemicals may have profound consequences for the compatibility of baculoviruses with host plants expressing these plant defensive compounds at high levels. Knowing the mechanism(s) whereby phytochemicals inhibit baculoviral disease will facilitate a more rational exploration of formulation chemistry that may mitigate these impacts.

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REFERENCES

AHMAD, S. 1995. Oxidative Stress and Antioxidant Defenses in Biology. Chapman & Hall, New York.

APPEL, H. M. 1993. Phenolics in ecological interactions: The importance of oxidation. *J. Chem. Ecol.* 19:1521-1551.

APPEL, H. M. 1994. The chewing herbivore gut lumen: Physicochemical conditions and their impact on plant nutrients, allelochemicals, and insect pathogens, pp. 209-223, *in* E. A. Bernays (ed.). Insect-Plant Interactions. CRC Press, Boca Raton, Florida.

APPEL, H. M., and SCHULTZ, J. C. 1992. Activity of phenolics in insects: The role of oxidation, pp. 609-620, *in* R. W. Hemingway and P. E. Laks (eds.). Plant Polyphenols. Plenum Press, New York.

ARMITAGE, P., and BERRY, G. 1994. Statistical Methods in Medical Research, 3rd ed. Blackwell Scientific Publications, Oxford, 620 pp.

AYERS, M. D., HOWARD, S. C., KUZIO, J., LOPEZFERBER, M., and POSSEE, R. D. 1994. The complete DNA sequence of *Autographa californica* nuclear polyhedrosis virus. *Virology* 202:586–605.

BI, J. L., FELTON, G. W., and MUELLER, A. J. 1994. Induced resistance in soybean to *Helicoverpa zea:* Role of plant protein quality. *J. Chem. Ecol.* 20(1):183–198.

BI, J. L., MURPHY, J. B., and FELTON, G. W. 1997. Antinutritive and oxidative components as mechanisms of induced resistance in cotton to *Helicoverpa zea. J. Chem. Ecol.* 23:95–115.

BONNING, B. C., and HAMMOCK, B. D. 1992. Development and potential of genetically engineered viral insecticides. *Biotech. Gen. Eng. News* 10:455–489.

BONNING, B. C., and HAMMOCK, B. D. 1996. Development of recombinant baculoviruses for insect control. *Annu. Rev. Entomol.* 41:191–210.

BRADFORD, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.

BROADWAY, R., DUFFEY, S. S., PEARCE, G., and RYAN, C. A. 1986. Plant proteinase inhibitors: A defense against herbivorous insects. *Entomol. Exp. Appl.* 41:33–38.

BRYANT, J. P., TUOMI, J., and NIEMALA, P. 1988. Environmental constraint of constitutive and long-term inducible defenses in woody plants, pp. 367–389, *in* K. Spencer (ed.). Chemical Mediation of Coevolution. Pergammon Press, New York.

BUTT, V. S. 1981. Direct oxidases and related enzymes, pp. 81–123, *in* P. K. Stumpf and E. E. Conn (eds.). The Biochemistry of Plants: A Comprehensive Treatise. Academic Press, New York.

BUTT, V. S., and LAMB, J. C. 1981. Oxygenases and the metabolism of plant products, pp. 627–655, *in* P. K. Stumpf and E. E. Conn. (eds.). The Biochemistry of Plants. A Comprehensive Treatise, Secondary Plant Products. Academic Press, New York.

CHAVES, N., ESCUDERO, J. C., and GUTIERREZ-MERINO, C. 1997. Role of ecological variables in the seasonal variation of flavonoid content of *Cistus ladanifer* exudate. *J. Chem. Ecol.* 23:579–603.

COLLETT, D. 1994. Modelling Survival Data in Medical Research. Chapman and Hall, London.

DUFFEY, S. S., and STOUT, M. J. 1996. Antinutritive and toxic components of plant defense against insects. *Arch. Insect Biochem. Physiol.* 32:3–37.

DUFFEY, S. S., HOOVER, K., BONNING, B. C., and HAMMOCK, B. D. 1995. The impact of host-plant on the efficacy of baculoviruses, pp. 137–275, *in* M. Roe and R. Kuhr (eds.). Reviews in Pesticide Toxicology. CTI Toxicology Communications, Raleigh, North Carolina.

EBIHARA, T. 1966. Effect of mulberry leaf quality on resistance of silkworm to a cytoplasmic polyhedrosis virus. *Rep. Ibaraki Seric. Exp. Stn.* 1:61–68.

ENGLARD, S., and SEIFTER, S. 1986. The biochemical functions of ascorbic acid *Annu. Rev. Nutr.* 6:364–406.

FARRAR, R. R., JR., MARTIN, P. A. W., and RIDGWAY, R. L. 1996. Host plant effects on activity of *Bacillus thuringiensis* against gypsy moth (Lepidoptera: Lymantriidae) larvae. *Environ. Entomol.* 25:1215–1223.

FELTON, G. W. 1995. Oxidative stress of vertebrates and invertebrates, pp. 356–434, *in* S. Ahmad (ed.). Oxidative Stress and Antioxidant Defenses in Biology. Chapman & Hall, New York.

FELTON, G. W., and DUFFEY, S. S. 1990. Inactivation of a baculovirus by quinones formed in insect-damaged plant tissue. *J. Chem. Ecol.* 16:1211–1236.

FELTON, G. W., and SUMMERS, C. B. 1995. Antioxidant systems in insects. *Arch. Insect Biochem. Physiol.* 29:187–197.

FELTON, G. W., DUFFEY, S. S., VAIL, P. V., KAYA, H. K., and MANNING, J. 1987. Interaction of nuclear polyhedrosis virus with catechols: Potential incompatibility for host-plant resistance against noctuid larvae. *J. Chem. Ecol.* 13:947–957.

FELTON, G. W., DONATO, K. K., DEL VECCHIO, R. J., and DUFFEY, S. S. 1989. Activation of plant polyphenol oxidases by insect feeding reduces the nutritive quality of foliage for noctuid herbivores. *J. Chem. Ecol.* 15:2667–2694.

FELTON, G. W., DONATO, K. K., BROADWAY, R. M., and DUFFEY, S. S. 1992. Impact of oxidized plant phenolics on the nutritional quality of dietary protein to a noctuid herbivore. *J. Insect Physiol.* 38:277–285.

FORSCHLER, B. T., YOUNG, S. Y., and FELTON, G. W. 1992. Diet and the susceptibility of *Helicoverpa zea* (Noctuidae: Lepidoptera) to a nuclear polyhedrosis virus. *Environ. Entomol.* 21:1220–1223.

FREI, B., ENGLAND, L., and AMES, B. N. 1989. Ascorbate is an outstanding antioxidant in human blood plasma. *Proc. Natl. Acad. Sci. U.S.A.* 86:6377–6381.

GOLAN-GOLDHIRSH, A., and WHITAKER, J. R. 1984. Relation between structure of polyphenol oxidase and prevention of browning, pp. 437–456, *in* M. Friedman. (ed.). Nutritional and Toxicological Aspects of Food Safety. Plenum Press, New York.

HAGERMAN, A. E., and BUTLER, L. G. 1980. Condensed tannin purification and characterization of tannin associated proteins. *J. Agric. Food Chem.* 28:947–952.

HAYASHIYA, K., NISHIDA, J., and MATSUBARA, F. 1968. Inactivation of nuclear polyhedrosis virus in the digestive juice of silkworm larvae, *Bombyx mori* L. I. Comparison of anti-viral activities in the digestive juice of larvae reared on natural and artificial diets. *Jpn. J. Entomol. Zool.* 12:189–193.

HAYASHIYA, K., NISHIDA, J., and UCHIDA, Y. 1976. The mechanism of the formation of the red fluorescent protein in the digestive juice of the silkworm larvae. The formation of chlorophyllidae-a. *Jpn. J. Appl. Entomol. Zool.* 20:37–43.

HEDIN, P. A., JENKINS, J. N., and PARROTT, W. L. 1992. Evaluation of flavonoids in *Gossypium arboreum* (L.) cottons as potential source of resistance to tobacco budworm. *J. Chem. Ecol.* 18(2):105–114.

HEINZ, K. M., MCCUTCHEN, B. F., HERRMANN, R., PARRELLA, M. P., and HAMMOCK, B. D. 1995. Direct effects of recombinant nuclear polyhedrosis viruses on selected nontarget organisms. *J. Econ. Entomol.* 88:259–264.

HO, C.-T., LEE, C. Y., and HUANG, M.-T. 1992. Phenolic Compounds in Food and Their Effects on Health I: Analysis, Occurrence, & Chemistry. American Chemical Society, Washington, DC, 338 pp.

HOOVER, K., SCHULTZ, C. M., LANE, S. S., BONNING, B. C., MCCUTCHEN, B. F., DUFFEY, S. S., and HAMMOCK, B. D. 1995. Reduction in damage to cotton plants by a recombinant baculovirus that knocks moribund larvae of *Heliothis virescens* off the plant. *Biol. Control* 5:419–426.

HOOVER, K., BONNING, B. C., LANE, S. S., SCHULTZ, C. M., HAMMOCK, B. D., and DUFFEY, S. S. 1996. Effects of diet age and streptomycin on virulence of *Autographa californica* M nucleopolyhedrovirus against the tobacco budworm. *J. Invert. Pathol.* 69:46–50.

HUANG, M.-T., and FERRARO, T. 1992. Phenolic compounds in food and cancer prevention, pp. 8–34, *in* M.-T. Huang, C.-T. Ho and C. Y. Lee (eds.). Phenolic Compounds in Food and Their Effects on Health II: Antioxidants and Cancer Prevention. ACS Symposium Series 507. American Chemical Society, Washington, DC.

HUANG, M.-T., HO, C.-T., and LEE, C. Y. 1992. Phenolic Compounds in Food and Their Effects on Health II: Antioxidants and Cancer Prevention. American Chemical Society, Washington, DC, 402 pp.

HUGHES, P. R., VAN BEEK, N. A. M., and WOOD, H. A. 1986. A modified droplet feeding method for rapid assay of *Bacillus thuringiensis* and baculoviruses in noctuid larvae. *J. Invertebr. Pathol.* 48:187–192.

HUNTER, M. D., and SCHULTZ, J. C. 1993. Induced plant defenses breached? Phytochemical induction protects an herbivore from disease. *Oecologia* 94:195–203.

JOHNSON, K. S., and FELTON, G. W. 1996. Physiological and dietary influences on midgut redox conditions in generalist lepidopteran larvae. *J. Insect Physiol.* 42:191–198.

JONES, C. G., HARE, J. D., and COMPTON, S. J. 1989. Measuring plant protein with the Bradford assay: Evaluation and standard method. *J. Chem. Ecol.* 15:979–992.

KALBFLEISCH, J. D., and PRENTICE, R. L. 1980. The Statistical Analysis of Failure Time Data. Wiley, New York.

KARBAN, R. 1987. Environmental conditions affecting the strength of induced resistance against mites in cotton. *Oecologia* 73:414–419.

KEATING, S. T., and YENDOL, W. G. 1987. Influence of selected host plants on gypsy moth (Lepidoptera: Lymantriidae) larval mortality caused by a baculovirus. *Environ. Entomol.* 16:459–462.

KEATING, S. T., YENDOL, W. G., and SCHULTZ, J. C. 1988. Relationship between susceptibility of gypsy moth larvae (Lepidoptera: Lymantriidae) to a baculovirus and host-plant constituents. *Environ. Entomol.* 17:942–958.

KEATING, S. T., MCCARTHY, W. J., and YENDOL, W. G. 1989. Gypsy moth (*Lymantria dispar*) larval susceptibility to a baculovirus affected by selected nutrients, hydrogen ions (pH), and plant allelochemicals in artificial diets. *J. Invertebr. Pathol.* 54:165–174.

KEATING, S. T., HUNTER, M. D., and SCHULTZ, J. C. 1990. Leaf phenolic inhibition of gypsy moth nuclear polyhedrosis virus: Role of polyhedral inclusion body aggregation. *J. Chem. Ecol.* 16:1445–1457.

LANE, H. C., and SCHUSTER, M. F. 1981. Condensed tannins of cotton leaves. *Phytochemistry* 20:425–427.

MAYER, A. M. 1987. Polyphenol oxidases in plants-recent progress. *Phytochemistry* 26:11–20.

MCCUTCHEN, B. F., CHOUDARY, P. V., CRENSHAW, R., MADDOX, D., KAMITA, S. G., PALEKAR, N., VOLRATH, S., HAMMOCK, B. D., and MAEDA, S. 1991. Development of a recombinant baculovirus expressing an insect-selective neurotoxin: potential for pest control. *Bio/Technology* 9:848–852.

MCCUTCHEN, B. F., HERRMANN, R., HEINZ, K. M., PARRELLA, M. P., and HAMMOCK, B. D. 1996. The effects of recombinant baculoviruses on a non-target insect: An endoparasitoid of *Heliothis virescens. Biol. Control* 6:45–50.

MCEVILY, A. J., IYENGAR, R., and GROSS, A. T. 1992. Inhibition of polyphenol oxidase by phenolic compounds, pp. 318–325, *in* C.-T. Ho, C. Y. Lee, and M.-T. Huang (eds.). Phenolic Compounds in Food and Their Effects on Health I: Analysis, Occurrence, and Chemistry. ACS Symposium Series 506. American Chemical Society, Washington, D.C.

MILLER, L. K. 1995. 1994 Founders lecture: Genetically engineered insect virus pesticides: Present and future. *J. Invertebr. Pathol.* 65:211–216.

NETER, J., WASSERMAN, W., and KUTNER, M. H. 1990. Applied Linear Statistical Models: Regression, Analysis of Variance, and Experimental Designs, 3rd ed. Richard D. Irwin, Homewood, Illinois.

PIERPOINT, W. S. 1983. Reactions of phenolic compounds with proteins, and their relevance to the production of leaf protein, pp. 235–267, *in* L. Telek and H. D. Graham (eds.). Leaf Protein Concentrates. Avi Publ. Westport, Connecticut.

ROBAK, J., and GRYGLEWSKI, R. J. 1988. Flavanoids are scavengers of superoxide anions. *J. Biochem. Pharmacol.* 37:837–841.

ROBINSON, D. S. 1991. Peroxidases and catalases in foods, pp. 1–47, *in* D. S. Robinson and N. A. M. Eskin (eds.). Oxidative Enzymes in Foods. Elsevier Applied Science, London.

RYAN, J. D., GREGORY, P., and TINGEY, W. M. 1982. Phenolic oxidase activities in glandular trichomes of *Solanum berthaultii. Phytochemistry* 21(8):1185–1187.

SCHULTZ, J. C., and KEATING, S. T. 1991. Host-plant-mediated interactions between the gypsy moth and a baculovirus, pp. 489–530, *in* Microbial Mediation of Plant-Herbivore Interactions. John Wiley & Sons, New York.

SINGLETON, V. L. 1987. Oxygen with phenols and related reactions in musts, wines, and model systems: Observations and practical implications. *Am. J. Enol. Vittic.* 38:69–77.

SINGLETON, V. L., and ROSSI, J. A., JR. 1965. Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16:144–158.

SOSA-GOMEZ, D. R., ALVES, S. B., and MARCHINI, L. C. 1991. Variation in the susceptibility of *Bombyx mori* L. to nuclear polyhedrosis virus when reared on different mulberry genotypes. *J. Appl. Entomol.* 111:318–320.

STEEL, R. G. D., and TORRIE, J. H. 1980. Principles and Procedures of Statistics: A Biometrical Approach, 2nd ed. McGraw-Hill, New York, 633 pp.

STIPANOVIC, R. D., ALTMAN, D. W., BEGIN, D. L., and BENEDICT, G. A. 1988. Terpenoid aldehydes in upland cottons: Analysis by aniline and HPLC methods. *J. Agric. Food Chem.* 36:509–515.

STOUT, M. J., and DUFFEY, S. S. 1996. Characterization of induced resistance in tomato plants. *Entomol. Exp. Appl.* 79:273–283.

SUMMERS, C. B., and FELTON, G. W. 1994. Prooxidant effects of phenolic acids on the generalist herbivore *Helicoverpa zea* (Lepidoptera: Noctuidae): potential mode of action for phenolic compounds in plant antiherbivore chemistry. *Insect Biochem. Mol. Biol.* 24:943–953.

UCHIDA, Y., KAWAMOTO, F., HIMENO, M., and HAYASHIYA, K. 1984. A virus-inactivating protein isolated from the digestive juice of the silkworm, *Bombyx mori*. *J. Invertebr. Pathol.* 43:182–189.

VLAK, J. M., and ROHRMANN, G. F. 1985. The nature of polyhedrin, pp. 489–542, *in* K. Maramorosch and K. E. Sherman (eds.). Viral Insecticides for Biological Control. Academic Press, Orlando.

WATANABE, H. 1987. The host population, pp. 71–112, *in* J. R. Fuxa and Y. Tanada (eds.). Epizootiology of Insect Diseases. John Wiley & Sons, New York.

WATANABE, H., NAGATA, M., and WANG, Y. 1990. The effect of protein content of an artificial diet on protease and antiviral activities of the gut juice of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae). *Appl. Entomol. Zool.* 25:150–152.

WHITT, M. A., and MANNING, J. S. 1987. Role of chelating agents, monovalent anion and cation in the dissociation of *Autographa californica* nuclear polyhedrosis virus occlusion body. *J. Invertebr. Pathol.* 49:61–69.

WHITT, M. A., and MANNING, J. S. 1988. A phosphorylated 34-kDa protein and a subpopulation of polyhedrin are thiol linked to the carbohydrate layer surrounding a baculovirus occlusion body. *Virology* 163:33–42.

YOUNG, S. Y., YANG, J. G., and FELTON, G. W. 1995. Inhibitory effects of dietary tannins on the infectivity of a nuclear polyhedrosis virus to *Helicoverpa zea* (Noctuidae: Lepidoptera). *Biol. Control* 5:145–150.