

Impact of Pesticide Resistance on Toxicity and Tolerance of Hostplant Phytochemicals in *Amyelois Transitella* (Lepidoptera: Pyralidae)

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Abstract

For some polyphagous insects, adaptation to phytochemically novel plants can enhance resistance to certain pesticides, but whether pesticide resistance expands tolerance to phytochemicals has not been examined. *Amyelois transitella* Walker (navel orangeworm) is an important polyphagous pest of nut and fruit tree crops in California. Bifenthrin resistance, partially attributable to enhanced cytochrome P450 (P450)-mediated detoxification, has been reported in an almond-infesting population exposed to intense pesticide selection. We compared the toxicity of bifenthrin and three phytochemicals—chlorogenic acid, and the furanocoumarins xanthotoxin and bergapten—to three strains of *A. transitella*: pyrethroid-resistant R347 (maintained in the laboratory for ~10 generations), fig-derived FIG (in the laboratory for ~25 generations), and CPQ—a laboratory strain derived from almonds ~40 years ago). Whereas both *Ficus carica* (fig) and *Prunus dulcis* (almond) contain chlorogenic acid, furanocoumarins occur only in figs. Both R347 and FIG exhibited 2-fold greater resistance to the three phytochemicals compared with CPQ; surprisingly, bifenthrin resistance was highest in FIG. Piperonyl butoxide, a P450 synergist, increased toxicity of all three phytochemicals only in CPQ, implicating alternate tolerance mechanisms in R347 and FIG. To test the ability of the strains to utilize novel hostplants directly, we compared survival on diets containing seeds of *Wisteria sinensis* and *Prosopis pallida*, two non-host Fabaceae species; survival of FIG was highest and survival of R347 was lowest. Our results suggest that, while P450-mediated pesticide resistance enhances tolerance of certain phytochemicals in this species, it is only one of multiple biochemical adaptations associated with acquiring novel hostplants.

Key words: bergapten, chlorogenic acid, resistance, synergist, xanthotoxin

Pesticide resistance in herbivorous insects has been associated with pre-existing detoxification mechanisms that evolved in response to hostplant phytochemicals; specific detoxification enzymes, particularly in polyphagous species, may metabolize both phytochemicals and insecticides (Li et al. 2007). Consequently, adaptation that accompanies host shifts to phytochemically novel hostplants may at the same time confer enhanced resistance to pesticides. Bass et al. (2013) demonstrated that a population of the polyphagous aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae) recently adapted to tobacco (*Nicotiana tabacum* L.) exhibited both enhanced resistance to the tobacco alkaloid nicotine and cross-resistance to synthetic neonicotinoid insecticides. A shared mechanism, overexpression of the cytochrome P450 monooxygenase *CYP6CY3* caused by gene amplification, facilitated enhanced metabolism of both natural and synthetic substrates. Similarly, Dermauw et al. (2013) tested the

hypothesis that mechanisms mediating hostplant phytochemical resistance can preadapt a herbivore for pesticide resistance, by propagating a pesticide-susceptible strain of the highly polyphagous two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) for five generations on a chemically challenging hostplant, tomato (*Solanum lycopersicum* L.). After hostplant adaptation, pesticide resistance in this strain increased and transcriptional profiles of tomato-adapted mites, particularly for cytochrome P450 and other detoxification genes, were similar to profiles of pesticide-resistant strains.

Whether selection in the opposite direction—i.e., for pesticide resistance—may facilitate hostplant shifts in a polyphagous species by conferring enhanced ability to metabolize phytochemicals is an open question with important economic implications. Many, if not most, of the 580+ pest species resistant to at least one pesticide (Sparks

and Nauen 2015) are polyphagous. If mechanisms mediating resistance to synthetic pesticides simultaneously expand the range of phytochemicals that a pest species can tolerate, then acquisition of pesticide resistance may increase the likelihood of hostplant shifts, resulting in expanded economic problems.

In the Central Valley of California, the navel orangeworm *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) is a New World species that is a major pest of many Old World orchard crops, including almonds (*Prunus dulcis* (Mill.) D.A. Webb, Rosaceae), pistachios [*Pistacia vera* (L.), Anacardiaceae], figs [*Ficus carica* (L.), Moraceae], and pomegranates [*Punica granatum* (L.), Lythraceae], and a minor pest on several other tree crops. This pyralid is also reported from several native species in the family Fabaceae and a few genera in other families (Sapindaceae, Rubiaceae, Asparagaceae, Arecaceae, and Juglandaceae) (Heinrich 1956, Heppner 2003). In the range of navel orangeworm in the southern USA, honeylocust (*Gleditsia triacanthos*, L.) is a major larval host (Neunzig 1979); the fact that the largest proportion of native hostplants recorded for this species, approximately one-third, as well as the major hosts for several of its close relatives, are species in the family Fabaceae suggests that the native ancestral hosts may be in this family (Heppner 2003, Niu et al. 2011).

In general, navel orangeworms preferentially infest fruits and nuts that have reached an advanced stage of maturity or that have been damaged by fungal infection, sun exposure, or infestation by other insects. The ability of the navel orangeworm to utilize hostplants that collectively produce a broad range of phytochemicals is due in part to cytochrome P450-mediated detoxification (Niu et al. 2011). Management of this pest, particularly in almond and pistachio, has historically relied on both cultural and chemical control, but as commodity prices have increased so has pesticide use; in particular, application of bifenthrin, a pyrethroid (IRAC Group 3A), increased almost 15-fold in almonds from 2007 to 2010 (California Department of Pesticide Regulation 2010, Demkovich et al. 2015b). Bifenthrin resistance was first identified in Kern County, California and Demkovich et al. (2015b) demonstrated that individuals from a colony derived from this field-collected resistant population (designated R347) exhibited up to ten-fold greater tolerance to bifenthrin. Synergism bioassays with the cytochrome P450 inhibitor piperonyl butoxide (PBO) and the esterase inhibitor S,S,S-tributyl phosphorothioate implicated both enzyme systems in resistance to the pyrethroids bifenthrin and β -cyfluthrin. In addition to mediating pesticide resistance, P450s contribute to the ability of *A. transitella* to detoxify both phytochemicals and mycotoxins (Lee and Campbell 2000, Niu et al. 2009, 2011, 2012).

As a highly polyphagous economic pest in which pesticide resistance has developed, *A. transitella* is an ideal insect to test the hypothesis that changes in detoxification systems associated with insecticide resistance can confer enhanced resistance to novel phytochemicals. In this study, we compared the toxicity of phytochemicals found in almonds and figs to three strains of navel orangeworm—a bifenthrin-resistant population from almond orchards in Kern County CA (R347) and maintained in the laboratory for ca. 10 generations, a population collected from fig orchards in Madera County CA (FIG) and maintained in the laboratory for ca. 25 generations, and a colony derived originally from almonds and maintained in the laboratory (free from insecticide selection) for 40+ years (CPQ). Toxicity of three phytochemicals, two furanocoumarins and a phenolic acid, was compared among strains. Bergapten is a linear furanocoumarin found in figs, at concentrations reaching 2.68 mg/100 g dry weight in peel and 4.58 mg/100 g dry weight in pulp (Oliveira et al. 2015; see also Qin et al. 2015, Veberic and Mikuli-Petkovsek 2015); although

present in *Citrus* hosts, it has not to our knowledge been reported from almonds. Xanthotoxin, an isomer of bergapten, is not known to occur in any of the hostplants reported for *A. transitella* in the Central Valley. Chlorogenic acid, an ester of caffeic acid and (-)-quinic acid, is a major phytochemical constituent of almonds but is found in far lower concentrations in figs. In almond hulls, chlorogenic acid occurs at concentrations up to 3.77 μ g/g (Garrido et al. 2008), whereas in fig fruits it is found in concentrations of 1.71 mg per 100 g fresh weight (0.06 μ g/g) (Veberic et al. 2008). In addition, we determined the LC₅₀ for bifenthrin in the FIG and CPQ strains, for comparison with the resistance levels in the R347 line.

In this study, we conducted three experiments. First, we tested the hypothesis that the pesticide-resistant R347 strain, with its elevated levels of P450 activity, should display increased tolerance to phytochemicals, independent of prior association with a particular phytochemical—i.e., tolerance should be greater not only for chlorogenic acid, regularly encountered in host almonds, but also for the less frequently encountered furanocoumarins. By contrast, the FIG strain should, by virtue of exposure through its hostplant, display the greatest capacity to tolerate furanocoumarins. Second, we compared the ability of the three strains to utilize novel hosts directly. The ecological status of the FIG strain is difficult to infer; concerns about navel orangeworm infestations reflects at least in part economic complaints related to phytosanitary rejection in figs for the fresh market rather than extensive crop loss, so the size of populations infesting figs and the degree to which they are specialized are unknown. Moreover, because the acreage in California dedicated to fig production is tiny relative to that of nut crops and is concentrated primarily in a single county, the high dispersal capacity of *A. transitella* (Sappington and Burks 2014) suggests that the fig population might not be genetically distinct, although differences in phenology and body size are suggestive of some degree of differentiation (J.P.S., unpublished data). Accordingly, to determine the degree of specialization for *F. carica* in the FIG strain, we also tested all three strains for the ability to utilize leguminous species that are either not native to the range of navel orangeworm (*Wisteria sinensis* (Sims) DC *Prosopis pallida* (Humb. & Bonpl. ex Willd.) H.B.K.) or found within the range of navel orangeworm but never reported as a hostplant (mesquite, *Prosopis pallida*). In this way, we could differentiate between diet breadth contraction due to selection for increased efficiency on fig or diet breadth expansion due to enhanced ability to utilize chemically novel hostplants. Finally, we specifically evaluated the contributions of cytochrome P450 monooxygenases to detoxification of these phytochemicals by conducting comparison diet incorporation assays using the synergist PBO, a methylenedioxyphenyl compound that, among other effects on metabolism, is known to inhibit P450-mediated detoxification in this species (Demkovich et al. 2015a).

Materials and Methods

Chemicals

Xanthotoxin and chlorogenic acid were purchased from Sigma-Aldrich (St Louis, MO); bergapten was purchased from Fisher Chemicals (Hampton, NH), bifenthrin was purchased from Chem Service Inc. (West Chester, PA), and PBO was purchased from Tokyo Kasei Kogyo (Tokyo, Japan).

Insects

Three strains of navel orangeworm were used for bioassays. CPQ, originally derived from almond orchards in California and

maintained at the USDA-ARS laboratory in Parlier, California, was kept in a colony at University of Illinois at Urbana-Champaign, occasionally supplemented from individuals shipped from Parlier. FIG originated in a fig orchard in Madera County, California, and has been maintained at the USDA-ARS laboratory; like CPQ, this strain was kept in a colony at UIUC and occasionally supplemented by individuals shipped from Parlier. The bifenthrin-resistant R347, derived from a population in Kern County, California, was provided by Bradley Higbee, Wonderful Orchards (Shafer, CA). These strains were kept in continuous culture in an insectary at the University of Illinois at Urbana-Champaign at $28 \pm 4^\circ\text{C}$ and a photoperiod of 16:8 (L:D) h cycle. Larvae were mass-reared until pupation in 500-ml plastic containers containing 200 g of a wheat bran diet (Finney and Brinkmann 1967). Adults were transferred to clean 900 ml (32-oz) jars with paper towels placed inside the jar and covering the top, which served as an oviposition surface. For bioassays, paper towels with eggs were placed into a clean 0.94-liter Ziploc (SC Johnson, Racine WI) sandwich bag and hatching neonates were collected after 24 h.

Novel Hostplant Material

Raw Mesquite Powder (SKU 1914) originating from plants grown in Peru was purchased from Sunfood Superfoods (El Cajon, CA), to evaluate performance on mesquite (*P. pallida*). Seeds of *W. sinensis* were obtained from a private residence in St Louis, MO; seed pods were air-dried in the laboratory and seeds were freshly ground in a commercial coffee grinder on the day of diet preparation.

LC₅₀ Bioassays

Xanthotoxin, bergapten, chlorogenic acid, and bifenthrin were incorporated separately into a semi-defined artificial diet (Waldbauer et al. 1984) at a range of concentrations. Xanthotoxin was added at six concentrations to bracket the LC₅₀: 2.5, 5, 7.5, 10, 12.5, and 15 mg/g, because Niu et al. (2012) reported that a 2 mg/g concentration of xanthotoxin caused at least 20% mortality to first instars at 48 h. Bergapten and chlorogenic acid were added to the diet at concentrations of 5, 10, 15, and 20 mg/g, to provide a broad range for calculating an accurate LC₅₀. Although chlorogenic acid could be added directly to the diet, furanocoumarins are prone to crystallize in liquids and accordingly they were first combined with the dry ingredients of the artificial diet (Alpha-Cel, Wesson's salt mixture, and sucrose), ground with a mortar and pestle, and then added to the liquid components of the diet. Bifenthrin was added to the diet at concentrations of 200 ng/g, 500 ng/g, 1 µg/g, 1.5 µg/g, 2 µg/g, 5 µg/g, and 10 µg/g for the FIG strain and 100 ng/g, 200 ng/g, 500 ng/g, 1 µg/g, and 2 µg/g for the CPQ strain.

For each series of bioassays, 20 newly hatched larvae from each strain (collected between 12 and 24 h after hatching) were used for testing. Four larvae were gently transferred by paintbrush into each 30 ml (1-oz) plastic cup SOLO (Dart Corporation, Lake Forest, IL containing 5 g of standard artificial diet (control diet), or standard diet to which were added varying amounts of test chemicals (*vide supra*). Mortality was recorded at 48 h. The larvae that did not move when touched with a soft brush were scored as dead and all bioassays were repeated three times.

Novel Hostplant Bioassay

Artificial diet was prepared as described, with adjustments made to compensate for the reduced water content of the plant material incorporated. For each diet, 60 g pre-ground mesquite or wisteria powder was mixed with 30 ml of a 1:1 mixture of glycerol:distilled

water, along with an additional 9 ml distilled water to improve the consistency of the diet. For each hostplant, the diet was divided roughly equally into 30 1-oz. (28 g) plastic SOLO cups and pressed into a uniform surface. Two individual first instars were placed into each cup, using a fine paintbrush. A total of 60 larvae from each of three colonies of navel orangeworm were placed on both diets (10 cups/diet/lab strain), for a total of 120 individuals in 60 cups.

Larvae were reared in a photoperiod of 16:8 (L:D) h cycle at room temperature and were checked daily for the first four weeks and weekly thereafter until 107 days and mortality was scored as a lack of response to gentle contact stimulus with a fine paintbrush. When cannibalism was observed, it was noted as the cause of mortality. When pupation did occur, the pupa was removed from its loose silk cocoon, sexed, weighed, and placed it in an empty cup to record the time to eclosion.

Synergist Incorporation Assays

PBO was added to a semi-defined artificial diet at 200 µg/g, a concentration previously determined to be nonlethal for first instars (Niu et al. 2012). The LC₅₀ concentrations for the three phytochemicals for each strain were mixed into the standard insect diet in the presence or absence of PBO (200 µg/g). Controls for each bioassay consisted of the standard artificial diet and the standard artificial diet with the addition of PBO. For each series of bioassays, 20 newly emerged larvae from each strain were placed on the diets. Mortality was recorded at 48 h as described. All bioassays were repeated three times.

Statistical Analysis

The Probit Analysis function in SPSS version 22 software (SPSS Inc., Chicago, IL) was used to calculate the median lethal concentration (LC₅₀) at 48 h in the rangefinder studies for xanthotoxin, bergapten, chlorogenic acid and bifenthrin. Differences in mortality on the two leguminous novel hostplants were determined by G-test with Bonferroni's Correction for multiple comparisons ($P=0.025$). Analyses were conducted independently at each timepoint for the different strains on diet containing each hostplant. For the synergism assays, Tukey's HSD Multiple Comparisons test in Graphpad Prism 6 (Graphpad Software Inc., La Jolla, CA) was used to determine differences among treatments. Analysis of variance was run using JMP (v10., SAS Institute, Cary, NC) and Relative Risk was calculated by Chi Square (Kelsey et al. 1986).

Results

LC₅₀ Assays

The FIG and R347 strains displayed comparable levels of tolerance to all three phytochemicals, which were 2.6× greater than CPQ tolerance to these phytochemicals (Table 1). LC₅₀ values in the R347 strain displayed the greatest variability when larvae consumed bergapten and chlorogenic acid, with a ratio of 4.1 and 2.9 between the upper and lower 95% confidence limits, respectively. These ratios suggest a heterogeneous tolerance in this strain, with some individuals that are highly tolerant and others that are very susceptible. The lower variability in FIG and CPQ indicates that most individuals responded similarly. LC₅₀ values in all three strains were least variable when larvae ingested xanthotoxin. Exposure to bifenthrin led to differences among all strains. CPQ was the most susceptible, followed by R347 (4.95-fold more tolerant than CPQ) and then FIG (18.95-fold more tolerant than CPQ). The FIG strain was 3.8-fold more tolerant to bifenthrin than the R347 strain.

Table 1. LC₅₀ values (mg or µg of active ingredient per gram of diet) for first instars determined by probit analysis for three navel orangeworm strains after 48 h on diets containing phytochemicals (xanthotoxin, bergapten, chlorogenic acid, and bifenthrin)

Chemical	Strain	LC ₅₀	95% confidence limit lower	95% confidence limit upper
Xanthotoxin	CPQa	4.52 mg/g	2.90	5.85
	FIG	9.39 mg/g	8.18	10.97
	R347	11.56 mg/g	9.77	14.59
Bergapten	CPQa	6.48 mg/g	3.11	8.79
	FIG	15.21 mg/g	11.36	27.14
	R347	16.93 mg/g	11.90	49.15
Chlorogenic acid	CPQa	7.44 mg/g	5.67	9.08
	FIG	16.75 mg/g	13.22	25.80
	R347	19.57 mg/g	14.43	42.41
Bifenthrin	CPQa	0.38 µg/g	0.31	0.46
	R347bc	1.88 µg/g	1.36	2.46
	FIGd	7.2 µg/g	5.35	11.08

^aCPQ strain differs from R347 and FIG strains.

^bR347 strain differs from CPQ and FIG strains.

^cLC₅₀ value from Demkovich et al. 2015b.

^dFIG strain differs from R347 and CPQ strains.

Hostplant Utilization Assays

Differences in survival across the three strains tested on the two legumes were apparent at 48 h on mesquite (Table 2). At that time-point, CPQ experienced higher mortality than the other strains, and at 21 d both R347 and CPQ experienced mortality twice as high as that in FIG. At 107 d, mortality was similar for all three strains (65–90%). In contrast, on wisteria, there was no mortality in any strain at 48 h; differences among strains became apparent at 14 d, at which point R347 displayed mortality twice as high as in the other two strains. This trend, with 2-fold greater mortality in R347, continued to 107 d, at which point the experiment was terminated.

Individuals from all three strains tested were able to survive to pupation on wisteria. In contrast, although individuals of all three strains succeeded in surviving to pupation on mesquite, cumulative survival was significantly reduced relative to survival on wisteria (Table 2). There were too few surviving to compare pupal weights across all lines but the pupal weights obtained were consistent with the overall survival pattern. To illustrate, across all of the individuals that successfully pupated, the 20 heaviest pupae were obtained on wisteria, with two females exceeding 50 mg in weight and several males exceeding 40 mg in weight, and the two smallest pupae were males reared on mesquite (10.9 and 13.5 mg).

Synergist Incorporation Assays

The three strains differed in their response to the phytochemicals and synergist. Overall, the addition of PBO significantly increased mortality ($P < 0.0001$) but the strains differed in their response to the presence of this synergist (Table 3). There were two significant two-way interactions, of Strain*Phytochemical and Strain*PBO. The first interaction reflects the greater susceptibility of CPQ and R347 to xanthotoxin and chlorogenic acid compared with FIG and the second interaction reflects the greater response of CPQ to the addition of PBO, especially compared with FIG. Table 4 summarizes

Table 2. Mortality of laboratory (CPQ), pyrethroid-resistant (R347), and fig (FIG) strains of navel orangeworm on two legume species not recorded as hostplants

Hostplant	Mortality					
	2 d	7 d	14 d	21 d	107 d	
<i>Wisteria</i>	FIG	0 ^A	0 ^A	0.20 ^A	0.20 ^A	0.35 ^A
	CPQ	0 ^A	0.05 ^A	0.30 ^A	0.35 ^A	0.40 ^A
	R347	0 ^A	0.05 ^A	0.65 ^B	0.80 ^B	0.80 ^B
<i>Mesquite</i>	FIG	0.10 ^A	0.20 ^A	0.20 ^A	0.35 ^A	0.65 ^A
	R347	0.25 ^A	0.30 ^A	0.50 ^{AB}	0.70 ^B	0.90 ^A
	CPQ	0.65 ^B	0.75 ^B	0.75 ^B	0.80 ^B	0.85 ^A

Significant differences designated by different letters after applying Bonferroni's Correction ($P = 0.025$) to the G-Test results. Analyses were conducted independently at each time for the different strains on diet containing each hostplant.

the results for each phytochemical; the controls are not included in the table because mortality on all control diets was negligible (Control mortality, 5/600 or 0.8%; PBO mortality, 11/1,200 or 0.9%). There is considerable variability in the dataset, with a coefficient of variation >100% for all three strains. For xanthotoxin, addition of PBO did not significantly increase mortality for the FIG and R347 strains but did increase mortality for the CPQ strain by 52%. For bergapten, addition of PBO did not increase mortality in the FIG strain but did increase mortality in the R347 strain by 46.5% and mortality in the CPQ strain by 82.5%. For chlorogenic acid, addition of PBO did not significantly increase mortality for the FIG or R347 strain but did increase mortality for the CPQ strain by 61.9%. When the responses to phytochemicals are pooled for each strain, addition of PBO increased mortality in FIG by 1.35× compared with the phytochemicals alone (Chi Square 8.4, DF=1; $0.005 > P > 0.001$); increased mortality in R347 by 1.31× compared with phytochemicals alone (Chi Square 7.9, DF=1; $0.05 > P > 0.001$); and increased mortality in CPQ by 1.64× compared with phytochemicals alone (Chi Square 32.4, DF=1; $P < 0.0001$).

Discussion

According to our hypothesis, the insecticide-resistant R347 strain was expected to be more tolerant of phytochemicals than the CPQ strain because its resistance to bifenthrin is associated with enhanced cytochrome P450 and carboxylesterase detoxifying activity (Demkovich et al. 2015b). Although our LC₅₀ assays determined that the R347 strain displayed 2-fold greater resistance to all three phytochemicals relative to the CPQ strain, the LC₅₀ of bergapten in the FIG strain was similar to that exhibited by the R347 strain; these values, 15.21 and 16.93 mg/g for FIG and R347, respectively, were more than twice the LC₅₀ value for bergapten in the CPQ strain. Although these findings alone are consistent with the interpretation that, while *A. transitella* is broadly polyphagous as a species, hostplant-associated populations may be more specifically adapted to the phytochemistry of their hostplants, the fact that the FIG strain exhibits an elevated tolerance for bifenthrin exceeding even that of the field-selected bifenthrin-resistant strain (LC₅₀ of 7.2 vs 1.88 µg/g) indicates that the "FIG" strain may well be a strain that is pre-adapted for acquiring novel hostplants by virtue of enhanced

Table 3. Three-way analysis of variance of strain, phytochemical, and synergist (PBO) nested within phytochemical

Source	DF	Sum of squares	Mean square	F ratio	Probability > F
Model	11	0.9038	0.0822	23.5133	
Error	48	0.1677	0.0035		
C. Total	59	1.0715			<0.001

Source	DF	Sum of squares	F ratio	Probability > F	
Strain	2	2	0.0790	11.3109	<0.0001
Chemical	2	2	0.0070	0.9945	0.3774
PBO	1	1	0.6962	199.2355	<0.0001
Strain*Phytochemical	4	4	0.0659	4.7169	0.0027 ^a
Strain*PBO	2	2	0.0382	5.4643	0.0073 ^a

^aSignificant two-way interaction.

Table 4. Mean mortality (\pm SD) for each of three navel orangeworm strains by chemical. An asterisk* indicates that addition of PBO significantly increased mortality ($P \leq 0.05$)

Chemical	Strain	Mortality \pm SD
Xanthotoxin	FIG	0.43 \pm 0.50
	FIG + PBO	0.59 \pm 0.50
	R347	0.55 \pm 0.50
	R347 + PBO	0.64 \pm 0.48
	CPQ	0.50 \pm 0.50
	CPQ + PBO*	0.76 \pm 0.43
Bergapten	FIG	0.42 \pm 0.50
	FIG + PBO	0.53 \pm 0.50
	R347	0.43 \pm 0.50
	R347 + PBO*	0.63 \pm 0.49
	CPQ	0.40 \pm 0.49
	CPQ + PBO*	0.73 \pm 0.45
Chlorogenic acid	FIG	0.47 \pm 0.50
	FIG + PBO	0.63 \pm 0.49
	R347	0.42 \pm 0.50
	R347 + PBO	0.58 \pm 0.50
	CPQ	0.42 \pm 0.50
	CPQ + PBO*	0.68 \pm 0.47

resistance mechanisms. Bifenthrin is not registered for use in figs; the only insecticide currently registered for figs is the anthranilic diamide Altacor (AI chlorantraniliprole), which has been used in the orchard of origin since 2012 (J.P.S., personal observation). From a management perspective, these findings suggest that fig-infesting navel orangeworm can also become established in orchards where pyrethroids (IRAC group 3A) are the dominant insecticide used.

That both the R347 and FIG strains exhibited similar tolerance for xanthotoxin, an isomer of bergapten, despite the lack of long-term exposure to this phytochemical suggests that the detoxification mechanisms for these two furanocoumarins in these two strains is independent of the position of the methoxy group. The equivalent toxicity of these isomers to both strains contrasts with differential P450-mediated metabolism and toxicity of these two compounds in at least two lepidopterans specialized on furanocoumarin-containing hostplants, the parsnip webworm *Depressaria pastinacella* Duponchel (Lepidoptera: Oecophoridae) (Berenbaum and Zangerl 1992) and the black swallowtail caterpillar *Papilio polyxenes* (F.) (Lepidoptera:

Papilionidae) (Berenbaum and Zangerl 1993). For both of these species, bergapten is more toxic than xanthotoxin.

Although our hypothesis was that bifenthrin resistance would enable R347 to exhibit the highest survivorship on two novel fabaceous plant species, counter to our expectations, overall survival at all timepoints was highest for the FIG strain on both plant species (Table 2). Although we were unable to carry out a statistical comparison of pupal weights due to the small number of survivors in some treatments, particularly on mesquite, the pupal weights we were able to obtain are consistent with suitability of Chinese wisteria as a hostplant for this species. Why there are no records of utilization of *W. sinensis* as a host by navel orangeworm despite the fact that the plant was introduced into California more than a century ago may be due to the plant's limited distribution in the state (where, in contrast with the southeastern USA, it does not appear to be invasive) or to the status of the plant as an ornamental, rather than a crop of economic importance. Additionally, Chinese wisteria pods exhibit a ballistic seed dispersal mechanism, and navel orangeworms tend to infest intact fruits, as opposed to individual seeds.

The ability of the FIG strain to survive on plants generally less suitable for the almond-derived CPQ strain or the R347 strain indicates that this "strain", despite deriving ~20 generations ago from *F. carica*, is not narrowly specialized. Rather, this strain appears to be well-equipped for metabolizing both natural toxins and pyrethroid insecticides. The poor performance of R347 on both wisteria and mesquite was unexpected; evidently, its enhanced tolerance of bifenthrin, attributable in part to elevated P450-mediated metabolism, does not confer general tolerance of defense chemicals in novel hostplants. Demkovich et al. (2015b) recorded lower pupal weights in individuals of R347 reared on wheat bran-based artificial diet compared with individuals of CPQ across six generations; the low pupal weights characteristic of this strain may be reflective of a cost of pesticide resistance.

The effects of PBO augmentation, to test for synergism of P450-mediated metabolism, varied across strains and treatments (Table 4). Ingestion of PBO increased the susceptibility of the CPQ strain to chlorogenic acid, implicating P450-mediated metabolism of this compound, although FIG and R347, which were unaffected by PBO, may have other mechanisms for metabolizing this compound. The effects of PBO differed by strain to an even greater extent for the furanocoumarins. In the FIG strain, susceptibility to xanthotoxin and bergapten was unaffected by PBO, which may indicate the presence of detoxification mechanisms other than P450s in furanocoumarin metabolism in general. In contrast, while PBO did not increase the susceptibility of the R347 strain to xanthotoxin, it did increase its susceptibility to bergapten. In the polyphagous

caterpillar *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), xanthotoxin, chlorogenic acid and the pyrethroid insecticide α -cypermethrin all induce and are detoxified at least in part by the same cytochrome P450 enzyme, CYP6B8 (Li et al. 2004); such does not appear to be the case for *A. transitella*.

When data were pooled by phytochemical and strain differences were evaluated, ingestion of PBO had the greatest impact on the CPQ strain, increasing mortality 1.64-fold, while the FIG and R347 strains responded similarly (mortality increased 1.35- and 1.31-fold, respectively). These results are consistent with the hypothesis that detoxification in CPQ is primarily by cytochrome P450s and that other systems may play a role in detoxification for the FIG and R347 strains. Interpreting the ecological significance of the toxicological responses of CPQ laboratory strain in comparison with the FIG and R347 strains more recently derived from field populations, however, must be done cautiously, because multiple generations of maintenance on an artificial diet may have led to inadvertent selection for losses (or other alterations) of phytochemical detoxification capacity. That said, the enhanced ability of R347 larvae to tolerate phytochemicals not known to be present in almonds, the hostplant from which the strain originated, coupled with a reduced ability to survive on novel potential hostplants suggests that phytochemical detoxification, even for a polyphagous species, is only one of multiple behavioral and physiological adaptations associated with acquisition of new hostplant species (Henniges-Janssen et al. 2014).

In view of the diversity of Old World agricultural plants comprising the current host range of the native *A. transitella*, hostplant shifts have undoubtedly occurred with some frequency in the recent past. Elevated capacity for P450-mediated detoxification associated with pesticide resistance may help facilitate (and increase the rate of) such shifts in the future, in the context of the hyperdiverse agricultural landscape of California. Such may have been the case for the FIG strain, which our rearing experiment suggests may be an opportunistic variant pre-adapted for colonizing new hostplant species. In the past 3 years, navel orangeworm infestation of both pomegranates and mandarin oranges (*Citrus reticulata* Blanco) has increased substantially, coinciding with the increase in pyrethroid resistance and the acreage expansion of almonds, citrus and pistachios (J.P.S., personal observation). Our finding that the FIG strain was as capable as the pesticide-resistant R347 strain of tolerating bifenthrin, possibly due to enhancement of alternate detoxification pathways, raises the possibility that navel orangeworm strains developing on hosts with challenging phytochemistry may be preadapted for rapidly developing resistance to structurally novel insecticides. Such capacity, if it is widespread, has implications for the design of integrated pest management programs for this polyphagous pest.

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