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Accelerated Development and Toxin Tolerance of the Navel Orangeworm *Amyelois transitella* (Lepidoptera: Pyralidae) in the Presence of *Aspergillus flavus*

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Abstract

The navel orangeworm (*Amyelois transitella*) and the fungus *Aspergillus flavus* constitute a facultative mutualism and pest complex in tree nut and fruit orchards in California. The possibility exists that the broad detoxification capabilities of *A. flavus* benefit its insect associate by metabolizing toxicants, including hostplant phytochemicals and pesticides. We examined this hypothesis by conducting laboratory bioassays to assess growth rates and survivorship of pyrethroid-resistant (R347) and susceptible (CPQ) larval strains on potato dextrose agar diet containing almond meal with and without two furanocoumarins, xanthotoxin and bergapten, found in several hostplants, and with and without two insecticides, bifenthrin and spinetoram, used in almond and pistachio orchards. Additionally, fungi were incubated in liquid diets containing the test chemicals, and extracts of these diets were added to almond potato dextrose agar (PDA) diets and fed to larvae to evaluate the ability of the fungus to metabolize these chemicals. Larvae consuming furanocoumarin-containing diet experienced higher mortality than individuals on unamended diets, but adding *A. flavus* resulted in up to 61.7% greater survival. *Aspergillus flavus* in the diet increased development rate > two-fold when furanocoumarins were present, demonstrating fungal enhancement of diet quality. Adding extracts of liquid diets containing xanthotoxin and fungus decreased mortality compared to xanthotoxin alone. On diets containing bifenthrin and spinetoram, however, mortality increased. These results support the hypothesis that *A. flavus* enhances navel orangeworm performance and contributes to detoxification of xenobiotics. Among practical implications of our findings, this mutualistic association should be considered in designing chemical management strategies for these pests.

Keywords Navel orangeworm · Fungus · Pyrethroid · Spinosyn · Furanocoumarin · Detoxification

Introduction

The navel orangeworm *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) and the fungus *Aspergillus flavus* (Link) comprise a facultative mutualism constituting an important pest complex in tree nut and fruit orchards in California. The navel orangeworm is responsible for significant economic losses in almonds (*Prunus dulcis*), pistachios (*Pistacia vera*), and figs (*Ficus carica* var. *domestica*), among other orchard crops (Connell 2002). Damage results from internal feeding and contamination of the fruits with frass, webbing, and the conidia of *A. flavus* and other phytopathogens (Curtis and Barnes 1977; Palumbo et al. 2014; Haviland et al. 2017). *A. flavus* also infects diverse plant hosts and produces mycotoxins, including aflatoxin B1, that as potent carcinogens threaten human health and cause crop losses due to contamination (Amaike and Keller 2011; Campbell et al. 2003; Palumbo et al. 2014).

The navel orangeworm and *A. flavus* have both been the targets of widespread pest management programs. The most common method of navel orangeworm control has been the use of insecticides, and in recent years some populations have developed resistance to a commonly used class of insecticide, the pyrethroids (IRAC Group 3A in the Insecticide Resistance Action Committee's Mode of Action classification scheme), which increases the difficulty of managing this pest (Bagchi et al. 2016; Demkovich et al. 2015b). In spite of sanitation efforts (Haviland et al. 2017), toxigenic species of *Aspergillus*

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remain a serious threat to orchard crops in California. The atoxigenic strain "AF36" has been successfully used to replace toxigenic strains in cotton crops and is registered as a biocontrol agent (Cotty and Bayman 1993; Cotty 1994); efforts to replicate this success in orchard crops are underway, but aflatoxin contamination of nut crops remains an ongoing concern.

Management of these two pests is further complicated by their relationship with each other, which is a facultative mutualism. *Aspergillus* conidia often colonize plant hosts after hull damage by navel orangeworm larvae (Widstrom 1979), and *A. flavus* conidia and high aflatoxin levels are associated with navel orangeworm infestation (Doster and Michailides 1994; Luo et al. 2009; Phillips et al. 1976). Navel orangeworm moths and larvae transport the spores of *Aspergillus* species in orchards (Palumbo et al. 2014) and within the fruit or nut (Ampt et al. 2015), and both larvae and adults are attracted to the presence of the fungus, even preferentially ovipositing on substrates that it has infected (Ampt et al. 2015; Beck 2013; Bush et al. 2017). Finally, the navel orangeworm displays a high level of resistance to aflatoxins (and to *A. flavus* in general) relative to other insects (Niu et al. 2009). These behavioral and physiological attributes have resulted in a relatively strong mutualistic pest complex that may be more difficult to manage than the two species individually.

Little is known about how *A. flavus* affects the way that navel orangeworm cope with their multifarious environmental challenges, including the phytochemical defenses contained in their diverse hosts as well as the insecticides sprayed on these hosts. Among the phytochemical defenses encountered by navel orangeworm in some of their hostplants including figs and citrus, are furanocoumarins, which can bind irreversibly to pyrimidine bases in DNA and are toxic to a broad range of insects, including most generalist lepidopterans (Berenbaum 1991a, b). Although navel orangeworm have a limited capacity to detoxify some furanocoumarins (Niu et al. 2011), several of these phytochemicals when ingested can reduce survival and delay development (Bagchi et al. 2016). In addition to host chemicals, there are a number of common synthetic pesticides used by almond and pistachio growers to protect crops from navel orangeworm damage or other pests. Among these are insecticides belonging to chemical families such the pyrethroids (IRAC Group 3A), spinosyns (IRAC Group 5), diacyl hydrazines (IRAC Group 18), diamides (IRAC Group 28), and organophosphates (IRAC Group 1B) (Demkovich et al. 2015b, 2018). *Aspergillus flavus*, as a common associate of navel orangeworm larvae, would presumably encounter these same phytochemicals and insecticides when infecting a host fruit or nut.

The mutualistic association of *A. flavus* with the navel orangeworm is strengthened by the fact that larvae are attracted to the fungus and develop faster in its presence (Ampt et al. 2015), likely by providing nutritional benefits. Beyond any potential nutritional contribution, *A. flavus* may enhance navel orangeworm performance by providing detoxification services. Many fungi, particularly phytopathogens, detoxify phytochemicals, including furanocoumarins (Desjardins et al. 1989); this metabolic capacity has been documented in *Aspergillus* species (Myung et al. 2008). Moreover, multiple studies have documented the ability of fungi, including *Aspergillus* species, to metabolize synthetic insecticides (often via cytochrome P450-mediated detoxification), such as organophosphates (Alvarenga et al. 2014; Ramadevi et al. 2012) and cyclodienes (Mukherjee and Mittal 2005). Consequently, the possibility exists that *A. flavus* may provide benefits to navel orangeworm by detoxifying natural and synthetic toxins that larvae encounter while consuming their nut and fruit diet.

In this study, we conducted bioassays to determine whether navel orangeworm larvae experience lower mortality and more rapid development when natural and synthetic toxins are consumed in the presence of *A. flavus*. We reared navel orangeworm larvae on an almond potato dextrose agar (PDA) substrate mixed with either synthetic insecticides or host phytochemicals. The response variables measured were percentage mortality and time to pupation in the presence and absence of *A. flavus*. Both a pyrethroid-resistant strain (R347) and a susceptible strain (CPQ) were assayed in order to compare the effects of high levels of metabolic resistance to pesticides (as is found in the pyrethroid-resistant strain) to the effects of fungal presence. We included another set of assays utilizing a liquid minimal medium augmented with insecticides and phytochemicals in which *A. flavus* was grown in order to test whether the presence of *A. flavus* could benefit navel orangeworm through fungal metabolism of toxins.

Methods and Materials

Navel Orangeworm Rearing Navel orangeworm colonies were maintained at 28 ± 4 °C with a 16:8 (L:D) h photoperiod on wheat bran diet at the University of Illinois at Urbana-Champaign (Demkovich et al. 2015b; Finney and Brinkman 1967). Two different colonies were used in these experiments: a pyrethroid-resistant strain (R347) originally collected from almond orchards in 2016 and a susceptible strain (CPQ), originally recovered from almond orchards and maintained in a colony at the USDA-ARS in Parlier, California. First instar larvae were chosen for assays within 24 hr of hatching and placed on a semi-defined wheat germ diet (Waldbauer et al. 1984) in 1-oz. (30 ml) plastic soufflé cups (Solo Cup Company, Lake Forest, IL). In the development assays, larvae were grown to third instar, then transferred to the assay system, a Petri dish containing potato dextrose agar (PDA) with ~5% almond meal. In the assay testing for efficacy of fungal metabolism, the wheat germ diet was used for the assay itself.

Aspergillus flavus Colonies *Aspergillus flavus* colonies were isolated from wheat seeds (provided by T. Michailides, UC Davis) infected with an atoxigenic strain of *A. flavus*, "AF36," originally recovered from cotton and under investigation as a possible biocontrol agent in the form of the registered formulation AF36 Prevail (USEPA 2017). We isolated *A. flavus* and maintained continuously conidiating colonies (10–15 days old) over the course of about 30 days under laboratory conditions (23 ± 2 °C) on potato dextrose agar (PDA) for use in experiments. For plates inoculated in the larval development experiment, an agar plug was removed from the edge of the colony and placed on a new plate of almond PDA (Ampt et al. 2015). In the fungal metabolism experiment, plugs were instead dropped into Erlenmeyer flasks of liquid diet.

Development Bioassays We used third-instar caterpillars to test growth and survival because larvae add the majority of their body weight between the third and fifth instars. Four third-instar larvae were added to each assay plate and 10 plates were used per treatment. The plate was a Falcon plastic Petri dish (Corning Life Sciences, Corning, NY) containing almond PDA (Ampt et al. 2015). Ten plates (five inoculated with *A. flavus* and five uninoculated) were assigned to the following treatments: control (with 1 mL methanol and no other additives), xanthotoxin ground with mortar and pestle and then added to almond PDA (Sigma-Aldrich, St. Louis, MO; 4.52 mg/g), bergapten crystals ground with mortar and pestle and then added to almond PDA (Sigma-Aldrich, 6.48 mg/g), bifenthrin dissolved in methanol and then added to almond PDA (Sigma-Aldrich, 0.25 μg/g), and spinetoram dissolved in methanol and then added to almond PDA (Sigma-Aldrich, 2.32 μg/g). Prior to the addition of toxicants and streptomycin, liquid PDA was kept in a water bath slightly below 60° C; the final ingredients were then added, and the diet was poured into Petri dishes with constant stirring. The volume of almond PDA diet in each dish was ~46 mL (or ~43.9 g fresh weight). Toxicant concentrations were selected based upon earlier lethal concentration assays (Bagchi et al. 2016; Demkovich et al. 2015a, b), because the lethal effects of these concentrations have been established, and they provided a useful baseline for possible changes in toxicity due to fungal presence. Xanthotoxin and bergapten are both furanocoumarins; bergapten is found in some hosts of the navel orangeworm, including species in the families Moraceae and Rutaceae. For instance, it is present in figs in concentrations up to 26.8 μg/g dry weight, and in pulp up to 45.8 μg/g (in *Ficus carica*; Oliveira et al. 2009); bergapten is found at higher levels in citrus (up to 100 μg/g dry weight in Persian lime, *Citrus* × *latifolia*), while xanthotoxin is present only at low levels and is more abundant in plants outside of the navel orangeworm host range in California (Nigg et al. 1993). Bifenthrin is a pyrethroid insecticide, and spinetoram is a spinosyn; both are used throughout the Central Valley for navel orangeworm management. Bifenthrin is often added to the spray tank at 275–300 ppm active ingredient. The amount actually deposited on target is a matter of ongoing study and can range from 0.02–15 ppm in pistachios, and perhaps as high as 90 ppm on the entire almond hull. The concentration used in this study is 0.25 ppm and is realistic as a dose the larva would encounter in the field. Larval survival and development time were monitored under the aforementioned rearing conditions, and this assay was repeated three times.

Metabolism Bioassays We mixed a liquid minimal medium (Daniel Raudabaugh, personal communication) for *Aspergillus* incubation. Liquid minimal media contain only the basic nutrients needed for colony growth, and have a long history of use for selective culturing or experimental purposes (Kele and McCoy 1971); in our experiment, the purpose was to isolate the interaction of *A. flavus* with the toxicant of interest while excluding other sources of chemical activity. The medium was made using the following recipe (per 1 L of medium): 6 g $NaNO_3$, 0.52 g KCl, 0.52 g $MgSO_4 \cdot 7H_2O$, 1.52 g KH_2PO_4, 10 g glucose. Sodium hydroxide was used to adjust pH before addition of glucose. The minimal medium was mixed in ten Erlenmeyer flasks, and xanthotoxin, bergapten, bifenthrin, and spinetoram were added to two flasks each (the remaining two flasks were controls not amended with toxins). Agar plugs were taken from the edge of a conidiating *A. flavus* colony (10 days old, grown under the laboratory conditions listed above) and added to five of the flasks (one for each treatment); the other five were left uninoculated. After ~10 days of incubation at 28 ± 4 °C, the contents of each flask were vacuum-filtered to remove fungal matter, and the filtered liquid medium was collected. We add 1 mL from the liquid medium to wheat germ diet in plastic soufflé cups, with five cups per treatment. Our goal was to achieve a final concentration of each toxin approximately equal to the concentrations used for the development assays (described earlier). Four neonate larvae were then added to each cup and mortality was measured after 48 hr. There were a total of three replicates.

Statistical Analyses For all three data sets—time to pupation and mortality in the development assays and mortality in the metabolism assays–we wrote a three-way Analysis of Variance (ANOVA) model in R (RStudio v.0.98.1083, R Foundation, Vienna, Austria), with Strain (R347 or CPQ), Fungus (Presence or Absence), and Toxicant (insecticides and phytochemicals) as the three factors. A Tukey's least square means procedure was used for post hoc contrasts. We used a simple log transformation to produce normal and

homoscedastic data for time to pupation, although in the relevant figures we used unadjusted values for purposes of consistency and convenience.

Results

Development Bioassays We found that the three-way interaction effect was not significant for larval development or survival as the outcome, nor was the two-way interaction for fungus x strain. We removed these terms from the model, and the final results are shown in Table 1. The presence of *A. flavus* decreased development time for both strains, regardless of toxicant, particularly for larvae exposed to xanthotoxin, whose time to pupation was approximately halved (Fig. 1). In contrast to development, addition of *A. flavus* had a variable effect on larval mortality. Larvae fed diet containing xanthotoxin or bergapten survived at a much higher rate with *A. flavus* present. CPQ larvae on xanthotoxin-treated diet experienced a particularly notable 61.7 ± 1.9% increase in survival in the presence of *A. flavus*, whereas those on diet containing insecticide experienced reduced survival (Fig. 2). Strain R347 responded differently from the CPQ laboratory strain when exposed to the pyrethroid, as expected. On these diets, Strain R347 developed faster than Strain CPQ regardless of whether the fungus was absent ($t = 6.71$, $df = 40$, $P < 0.001$) or present ($t = 7.68$, $df = 40$, $P < 0.001$), and R347 mortality rates were unaffected by the presence/absence of *A. flavus*.

Table 1 ANOVA results for bioassays of *Amyelois transitella* development and mortality as a function of larval strain (pyrethroid-resistant vs. –susceptible), *Aspergillus flavus* presence/absence, and toxicant in the diet (including furanocoumarins xanthotoxin and bergapten, the pyrethroid bifenthrin, and the spinosyn spinetoram)

Effect	*F* value	DF	*P*
Larval development			
Strain	33.58	1	<0.001
Fungus	1072.52	1	<0.001
Toxicant	360.77	4	<0.001
Strain:Toxicant	20.23	4	<0.001
Fungus:Toxicant	76.70	4	<0.001
Larval mortality			
Strain	4.79	1	<0.05
Fungus	5.28	1	<0.05
Toxicant	21.01	4	<0.001
Strain:Toxicant	6.98	4	<0.001
Fungus:Toxicant	19.44	4	<0.001
Larval mortality after fungal incubation			
Strain	5.42	1	<0.05
Fungus	4.94	1	<0.05
Toxicant	32.70	4	<0.001
Strain:Toxicant	6.19	4	<0.001
Fungus:Toxicant	2.78	4	<0.05

Metabolism Bioassays We did not find a significant three-way interaction effect or a significant two-way interaction for fungus x strain, and we adjusted our model accordingly (Table 1). The 48-hr mortality rate was close to 50% for the four toxicants in the absence of *A. flavus*, demonstrating that we successfully approximated LC_{50} levels previously published (Bagchi et al. 2016; Demkovich et al. 2015a, b) (Fig. 3). Incubating the fungus in liquid medium containing spinetoram and bergapten did not affect larval mortality rates. However, when xanthotoxin was exposed to *A. flavus* for 10 days and the liquid incorporated into diet, both CPQ ($t = 4.35$, $df = 40$, $P < 0.001$) and R347 ($t = 4.52$, $df = 40$, $P < 0.001$) larvae experienced reduced mortality relative to medium to which fungus had not been added. CPQ larvae exposed to bifenthrin and fungus experienced a similar benefit ($t = 3.68$, $df = 40$, $P < 0.001$), but R347 larvae were unaffected by bifenthrin, as in the development bioassays.

Discussion

Many furanocoumarins can interfere with the survival and development of lepidopterans (Li et al. 2000; Mao et al. 2006; Niu et al. 2011). Bagchi et al. (2016) demonstrated navel orangeworm mortality following consumption of xanthotoxin and bergapten, despite the fact that this species has the capacity to detoxify some furanocoumarins (e.g., imperatorin) via cytochrome P450-mediated metabolism (Niu et al. 2011). However, in our study, by adding *A. flavus* to the diet, we reversed the negative effect of xanthotoxin and bergapten, and we increased larval survival and decreased development time to levels equivalent to those of larvae in control treatments (Fig. 2). Larval time to pupation decreased in the presence of *A. flavus*, although it still was prolonged for caterpillars consuming diets containing furanocoumarins compared with individuals on unamended diets. Our results strongly support the hypothesis that *A. flavus* increases navel orangeworm resistance to the deleterious effects of some phytochemicals present in some species utilized as hostplants.

Our findings from the assays determining the extent of fungal metabolism of toxins suggest that *A. flavus* metabolizes or sequesters xanthotoxin, making it significantly less toxic to navel orangeworm. Although this ability does not appear to directly benefit *A. flavus*, there are multiple examples of mutualistic associations in which a microbial partner detoxifies phytochemicals with the benefits accruing to the host insect. For instance, gut microbiota including *Pseudomonas* species

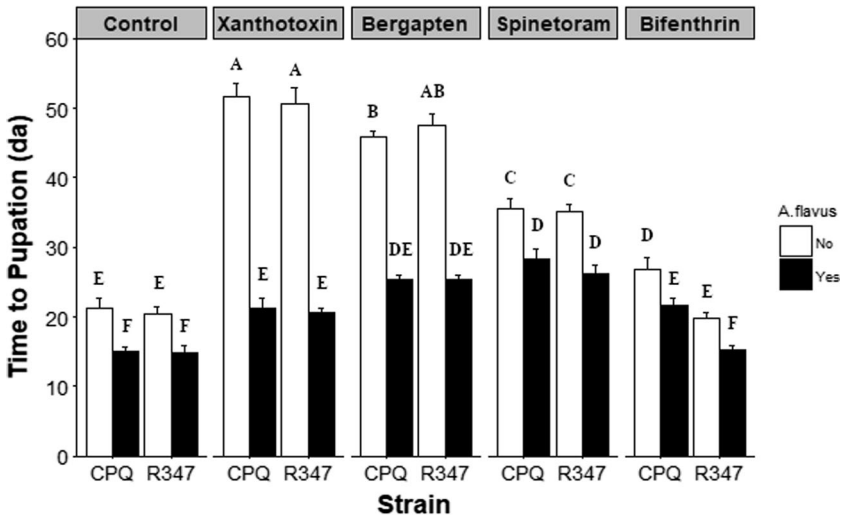

Fig. 1 Average time to pupation ± SE from third instar for navel orangeworm (*Amyelois transitella*) larvae from pyrethroid-susceptible (CPQ) and –resistant (R347) strains in the presence and absence of *Aspergillus flavus*, the furanocoumarins xanthotoxin and bergapten, the pyrethroid bifenthrin, and the spinosyn spinetoram. Data presented are non-normal and were adjusted via log-transformation prior to statistical analysis. Means with the same significance letter are not significantly different, where significance is $P \leq 0.05$

metabolize caffeine in plant matter ingested by the coffee berry borer *Hypothenemus hampei* (Ceja-Navarro et al. 2015). There is also evidence that *A. flavus* in particular is capable of biotransforming imperatorin (Teng et al. 2004), which is, like xanthotoxin and bergapten (Fig. 4), a plant furanocoumarin found in occasional hosts. We infer that *A. flavus* is likely capable of detoxifying xanthotoxin, with the associated navel orangeworm larvae benefiting from this metabolic capability. Although *A. flavus* does not appear to be capable of metabolizing bergapten in our assay system, navel orangeworm larvae still benefited from the presence of the fungus on bergapten-containing diets. The magnitude of this effect (38.1 ± 1.6% increased survival for CPQ; 42.2 ± 2.1% increase for R347) suggests that *A. flavus* improves diet quality in other ways, and to such a degree that the navel orangeworm was able to overcome the toxicological barrier posed by this furanocoumarin.

Navel orangeworm larvae presented with diet containing synthetic insecticides experienced significantly increased mortality, which was not moderated by the introduction of *A. flavus*; rather, the presence of the fungus actually increased mortality rates (although, among the survivors on pesticide diets, the presence of *A. flavus* still shortened developmental time, as in our other assays). These results suggest that larvae were stressed by exposure to the two insecticides in a way that left them vulnerable to other stress factors, such as fungal infection. Dead larvae were rapidly covered by fungal growth; they also displayed noticeable discoloration shortly before death, though this could have been a sign of sepsis resulting from some other source. Pyrethroids modify sodium channel activity to cause neurological damage (Lund and Narahashi 1983) and spinosyns disrupt the functions of nicotinic acetylcholine and γ-aminobutyric acid (GABA) receptors (Watson 2001). Conceivably, a combination of fungal detoxification, nutritional improvements, and larval cytochrome P450 activity is sufficient to allow the navel orangeworm to recover from the effects of furanocoumarins, but not from neurological damage caused by spinetoram and bifenthrin. Additionally or alternatively, these insecticides may disrupt navel orangeworm metabolism or immunity, making them

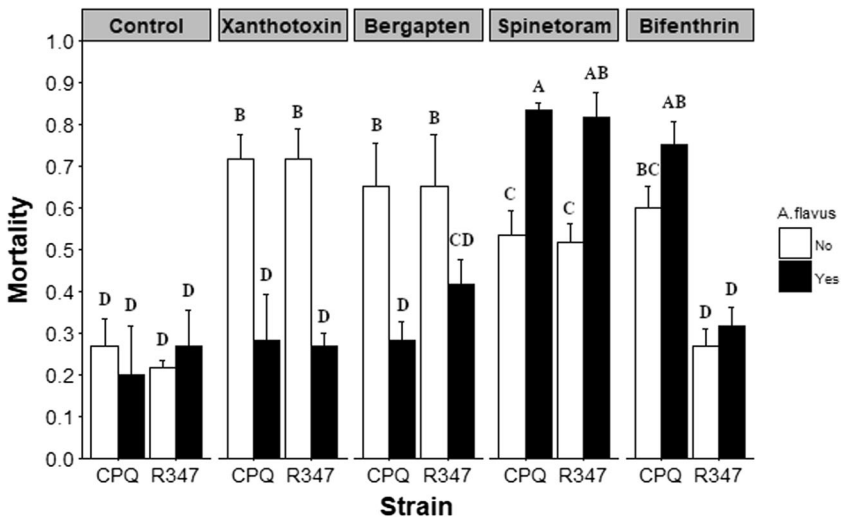

Fig. 2 Average mortality ± SE for navel orangeworm (*Amyelois transitella*) larvae from pyrethroid-susceptible (CPQ) and –resistant (R347) strains in the presence and absence of *Aspergillus flavus*, the furanocoumarins xanthotoxin and bergapten, the pyrethroid bifenthrin, and the spinosyn spinetoram. Means with the same significance letter are not significantly different, where significance is $P \leq 0.05$

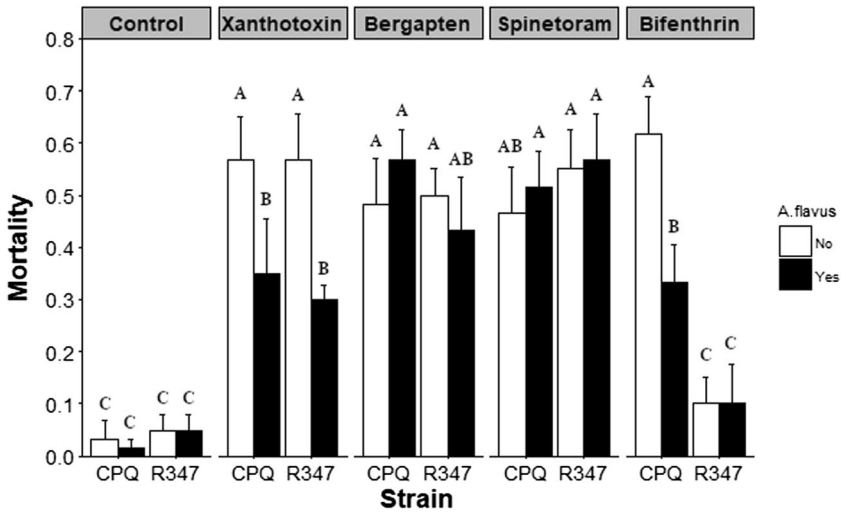

Fig. 3 Average mortality ± SE for navel orangeworm (*Amyelois transitella*) larvae from pyrethroid-susceptible (CPQ) and –resistant (R347) strains in the presence of the furanocoumarins xanthotoxin and bergapten, the pyrethroid bifenthrin, and the spinosyn spinetoram. Fungal presence denotes treatments previously exposed to fungal growth and metabolism. Means with the same significance letter are not significantly different, where significance is $P \leq 0.05$

susceptible to *A. flavus* infection. For example, the western honey bee, *Apis mellifera*, occasionally succumbs to infection by *Aspergillus* species (particularly *A. flavus*), causing a condition called stonebrood, despite the fact that *Aspergillus* species are predictably found in beebrood and elsewhere in hives (Gilliam et al. 1974, 1989). Whereas healthy bees are capable of coexisting with large concentrations of fungi (Seyedmousavi et al. 2015), bees experiencing stress can become susceptible to *Aspergillus* infection. The same may hold true for navel orangeworm larvae.

Our data indicate that *A. flavus* is capable of detoxifying bifenthrin; bifenthrin-containing diet caused lower larval mortality when the bifenthrin was first exposed to *A. flavus*. There is a precedent for the metabolism of pyrethroids by *Aspergillus* spp. A strain of *Aspergillus niger* (ZD11) is capable of using the pyrethroid permethrin as its sole carbon source via activity of a pyrethroid hydrolase (Liang et al. 2005), and other families of insecticide including organochlorines and organophosphates can be metabolized as well (Alvarenga et al. 2014; Mukherjee and Mittal 2005; Ramadevi et al. 2012). The R347 strain was not affected by bifenthrin at the concentration added to the diet, so we cannot claim that detoxification of bifenthrin was a benefit to these larvae. However, their development time decreased in the presence of *A. flavus*, which is a further indication of a nutritional benefit independent of detoxification effects. In addition, *A. flavus* presence reduced mortality for larvae on diet containing bergapten and xanthotoxin to the same levels that we observed in the control treatment and the bifenthrin treatment for R347 larvae (Fig. 2). These results indicate that the effectiveness of fungal detoxification mechanisms in reducing larval mortality due to dietary toxicants is comparable to the effectiveness of endogenous resistance mechanisms.

If our laboratory findings are applicable to agroecosystems, the ability of *A. flavus* to detoxify both phytochemicals and synthetic insecticides, combined with its strong association with navel orangeworm, presents potential problems for orchard growers in controlling these two pests. Pyrethroid usage is especially widespread in pistachios, as well as in some almond orchards in California (CDPR 2016), because of its low cost and efficacy against all stages of navel orangeworm. Our findings illustrate the potential for a double threat to nut growers, consisting of the continued development of pyrethroid resistance in the navel orangeworm combined with the ability of its mutualist partner fungus to metabolize or sequester bifenthrin or other pyrethroids, benefiting susceptible navel orangeworm larvae developing in infected nuts. Although the use of AF36 as a biocontrol agent for toxigenic strains of *A. flavus* has much promise as a means of reducing aflatoxin contamination, our study demonstrated the ability of AF36 to detoxify both phytochemicals and pyrethroids, making it an effective ally of the navel orangeworm. The use of

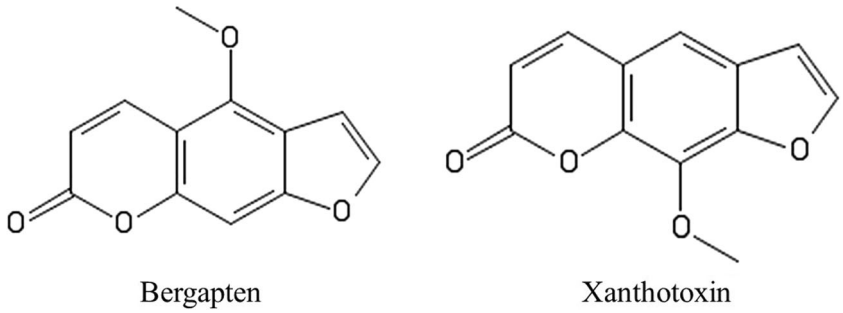

Fig. 4 Bergapten and xanthotoxin, two furanocoumarins found in some hosts of *Amyelois transitella*

AF36 formulated products does not increase the overall abundance of *A. flavus* in the application site (USEPA 2017), however, so its use is not expected to exacerbate problems due to its relationship with the navel orangeworm. We did not assess the ability of AF36 to detoxify diacyl hydrazine (IRAC 18) and diamide insecticides (IRAC 28) and, if *A. flavus* possesses a similar capacity to metabolize these chemicals, then control of navel orangeworm in tree nuts will be further complicated. Because *A. flavus* was unable to detoxify spinetoram in our study, this insecticide can be substituted for pyrethroids, but its substantially greater cost and shorter duration of control will likely limit its adoption. In conclusion, our study demonstrates the adaptive ability and broad metabolic activities of *A. flavus* and highlights the importance of understanding the biology of this fungus in tree nuts in order to develop a sustainable management strategy to control the navel orangeworm.

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