Effects of altered lipid metabolism in navel orangeworm (*Amyelois transitella*) on cuticle permeability, and the use of kaolin as an insecticide and synergist of chlorantraniliprole

Esther Ngumbi, Mark Demkovich, Daniel Bush, Bernarda Calla*, Joel Siegel†, and May Berenbaum*
*Department of Entomology, University of Illinois at Urbana-Champaign
† USDA, Agricultural Research Service, Parlier, CA

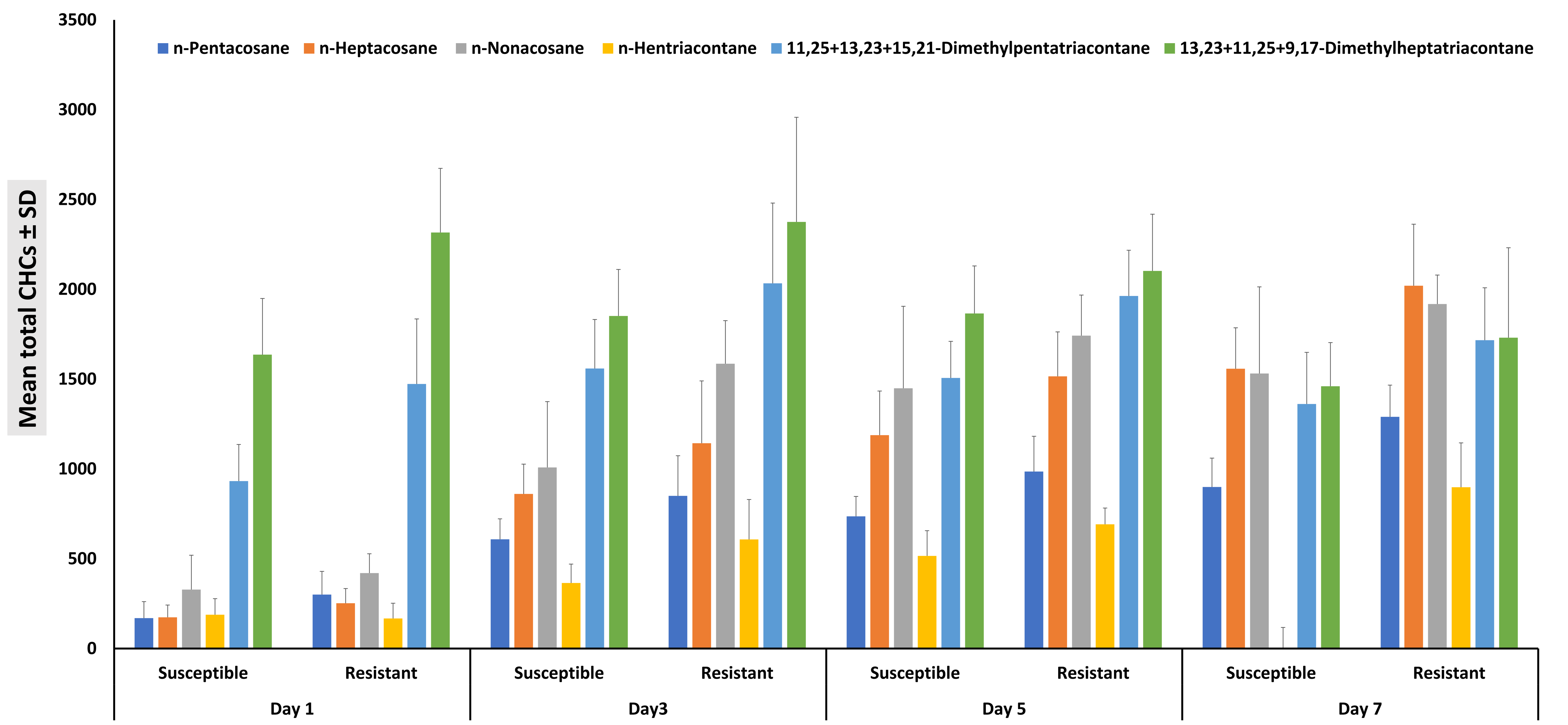
Project ID: ENTO1-Berenbaum

Cuticular hydrocarbons: Factors associated with their variation in the navel orangeworm

Background: Cuticular hydrocarbons (CHCs) are the main components of the epicuticular wax layer in many insects. Functioning primarily as a barrier against desiccation, CHCs play other roles, including serving as sex pheromones, kairomones, and colony-, caste-, species-, and sex-recognition signals in social insects. More recently, they have been implicated in resistance to insecticides in many insects, including the navel orangeworm (NOW). The content and composition of CHCs can vary with age, diet, development stage, environment, and sex. In our study, we investigated the effect of age and strain on the composition of CHCs in NOW adults.

Methods: CHCs were extracted from resistant and susceptible adults of different ages (1, 3, 5, and 7 d) following methods by Nelson and Buckner[1] with modifications. Individual adults were submerged for 10 min in 200 µl hexane containing 1-bromooctadecane as the internal standard: 25 ng/µl. Extracts were transferred to clean glass vials. The adults were rinsed with an additional 200 µl of hexane containing the internal standard, which was combined with the initial extract. We analyzed ten replicates of extracts using gas chromatography-mass spectrophotometry (GC-MS).

Figure 1. Effect of age on the composition of cuticular hydrocarbons in susceptible and resistant navel orangeworm strains



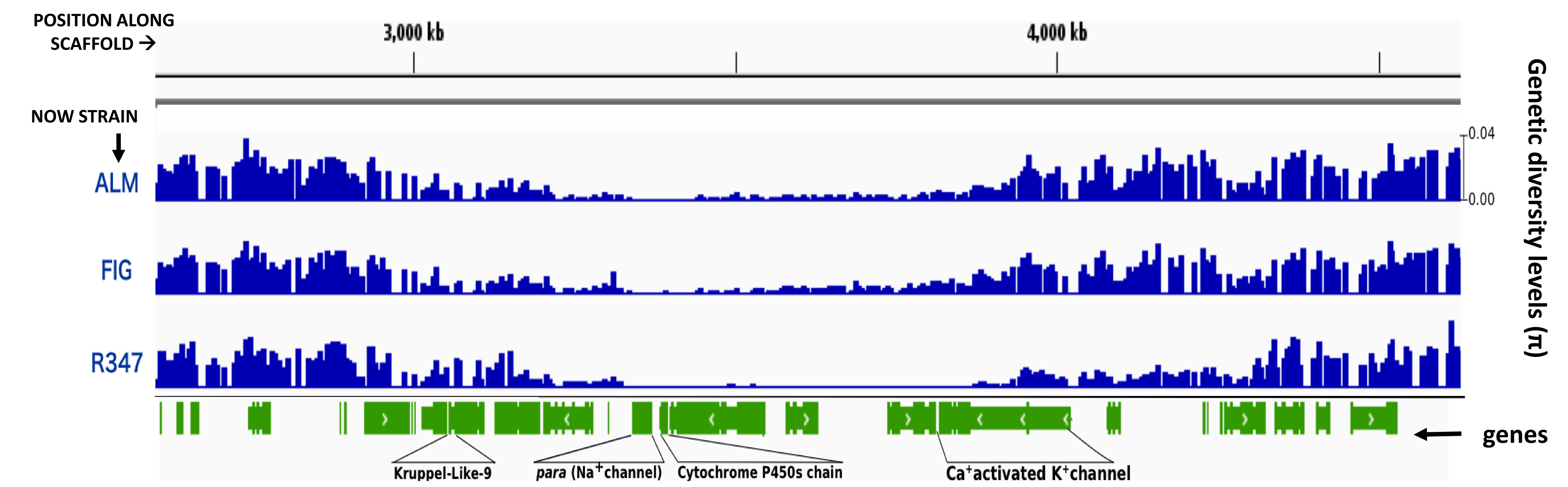
Results: There were significant age-based differences between pyrethroid-resistant and susceptible NOW strains. Post-eclosion, total CHCs increased with adult age. In general, on day 1 adults had significantly lower amounts of the most abundant hydrocarbon constituents compared to days 3, 5, and 7 (Figure 1). In addition, within each age class, adults from resistant populations had greater quantities of CHCs in total than those from susceptible strains (Figure 1).

Significance: CHC profiles in NOW vary by age and strain. Our findings are consistent with others in Lepidoptera and other insects. Our results suggest that CHC profiles may be useful as biomarkers to differentiate between insecticide-resistant and susceptible populations and adults of different ages. Knowledge of the factors associated with variation in NOW CHC profiles is an important step in enhancing monitoring and management strategies.

Table 1. Pooled navel orangeworm mortality data 1, 7, and 12 days after applications of Altacor with adjuvants Kinetic, Vintre, and kaolin (Surround). Application rates:
Insecticide: Altacor, at 4.5 oz/acre
Adjuvant 1: Vintre at 16 oz/100 gal (0.125%)
Adjuvant 2: Kinetic at 4 oz/100 gal (0.031%)
Adjuvant 3: Kinetic at 4 oz/100 gal (0.031%) + Surround at 15 lbs/acre (1.875%)
Spray Rig: AiroFan PTO with multinozzles (GB36); Speed: 2 mph; Volume: 100 gal/acre
There were 6 rows per treatment, separated by 4 row buffers. The middle two rows were used for each treatment to hang hooks.

Selective sweeps in the genome of pyrethroid-resistant navel orangeworm

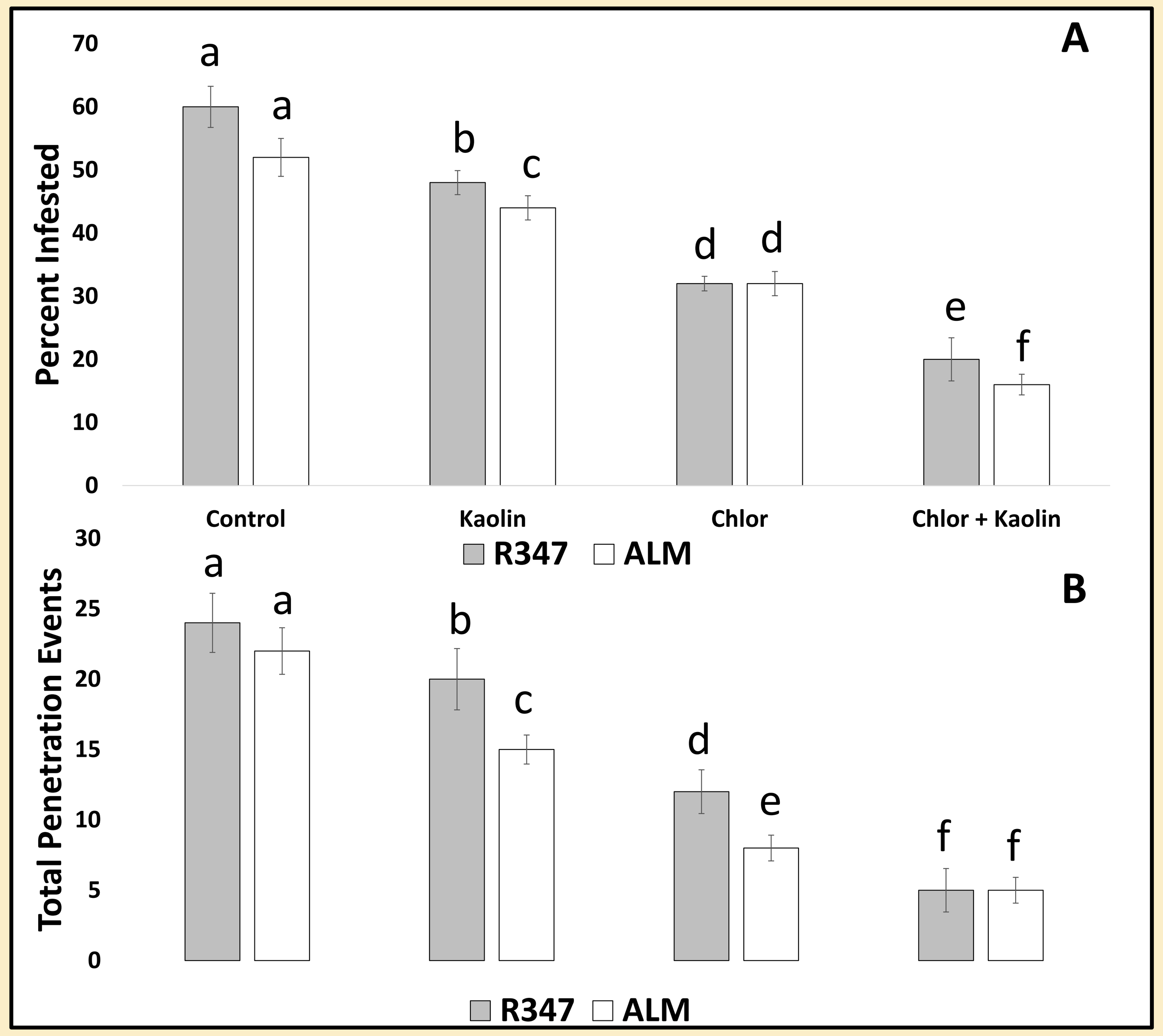
Figure 2. Genomic diversity levels for three strains of navel orangeworm (NOW): ALM (pyrethroid-susceptible NOW from almond orchards), FIG (NOW from figs with some resistance to pyrethroids), and R347 (pyrethroid-resistant NOW from Kern County)



Background: Selective sweeps are regions in the genome that display abnormally low levels of nucleotide variation, which result from intense selection on a specific trait that promotes survival.

Results and Significance: In the case of NOW, the target of selection could be in the ***para*** gene with the mutation "*kdr*" (knock-down-resistance), which confers resistance to pyrethroids, and/or in a chain of cytochrome P450s (***CYP6AB54*, *CYP6AB55***, and ***CYP6AB66***) situated in the center of the sweep. CYP6ABs have been previously implicated in pyrethroid resistance in other lepidopterans (e.g., cotton bollworm, *Helicoverpa armigera*).

Insecticidal and synergistic activity of kaolin against navel orangeworm



Treatment	Sig	Mortality Effect
Altacor Mortality ± SD Kinetic Alone		
83.25% ± 0.086. (N=59) (2,456/2,950)	A	
Altacor Mortality ± SD Vintre Alone		
86.80% ± 0.083. (N=60) (2,604/3,000)	B	4.3% Increase over Kinetic
Altacor Mortality ± SD Kinetic + **Surround**		
88.80% ± 0.066. (N=60) (2,664/3,000)	**C**	**6.7% Increase over Kinetic**

Background: Kaolin clay hampers feeding and survival in some insect pests via an unknown mechanism; one suggested mechanism is abrasion or disruption of CHCs.

Methods: We treated almond kernels with kaolin, chlorantraniliprole, or both together. We exposed larvae to kaolin concentrations of ~15 lb/acre. We also added the registered kaolin product Surround to Altacor + Kinetic applications in contact toxicity field trials in pistachios and compared the effects to the adjuvant Vintre.

Results: Kaolin and chlorantraniliprole reduced penetration of NOW larvae into almonds and the percentage of kernels infested after one week. Kaolin and chlorantraniliprole interacted synergistically. R347 was more resistant to all treatments but was still significantly adversely affected (Fig. 3). In field trials in pistachios, adding kaolin to Altacor + Kinetic resulted in a 6.7% increase in mortality, a greater increase than that caused by the addition of Vintre (Table 1).

Significance: These results indicate that kaolin can improve insecticide efficacy, perhaps disrupting CHCs.

References
1. Nelson DR, Buckner JS (1995). The surface hydrocarbons of larval *Heliothis virescens* and *Helicoverpa zea*. Comp Biochem Physiol 111B: 681-689

Contact Information
Esther Ngumbi – enn@Illinois.edu ; Mark Demkovich – mdemkov3@illinois.edu
Daniel Bush – dsbush2@illinois.edu; Bernarda Calla – calla2@illinois.edu
Joel Siegel – joel.siegel@ars.usda.gov; May Berenbaum – maybe@illinois.edu

Acknowledgments
We thank the Almond Board of California and California Pistachio Research Board for research funding and Jocelyn Millar, UC-Riverside, for advice on GC-MS analyses.