Impact of Dietary Phytochemicals on Metabolism and Detoxification of Pesticides in Honey Bees

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Introduction

- Many fungicides applied to almond orchards during bloom are considered to be bee-safe. However, beekeepers have reported sporadic occurrences of high mortality in bees, especially in larval and pupal stages, after fungicide applications (Mussen, 2008). Thus, factors other than the fungicides might be involved.
- Bees often ingest pesticides along with phytochemical-rich food, which is detoxified predominantly by cytochrome P450 monooxygenases, potentially allowing toxicological interactions between pesticides and phytochemicals.
- According to our previous work, **consuming ubiquitous dietary phytochemicals from honey, including *p*-coumaric acid and quercetin, induces CYP450 gene expression** (Mao *et al*., 2011, 2013) and enhances detoxification of co-occurring tau-fluvalinate (Johnson *et al*., 2012), bifenthrin and β-cyfluthrin (Liao *et al*., 2017) by bees.

In silico High-Throughput Docking

class	CYP9Q 1	CYP9Q 2	CYP9Q 3	CYP 9Q 1, 2, and 3
insecticide	42	58	19	18
fungicide	22	28	13	12
herbicide	18	19	10	10
acaricide	9	10	7	7

- We used *in silico* high-throughput docking of 8757 chemical forms (118 unique prospective pesticides and metabolites) to identify possible substrates of CYP9Q1-3, known to metabolize quercetin.
- Using the Biovia software package, we identified 92 compounds that can dock in the active pocket of CYP9Q1, including 22 fungicides, 18 herbicides, and 9 acaricides.
- In the CYP9Q2 active pocket, 118 candidates dock, including 28 fungicides, 19 herbicides, and 10 acaricides.
- As for CYP9Q3, 51 candidates dock, including 13 fungicides, 10 herbicides, and 7 acaricides. Moreover, 48 candidates can dock in all three CYP9Q enzymes, including 12 fungicides, 10 herbicides, and 7 acaricides.

	CYP9Q 1	CYP9Q 2	CYP9Q 3
-44-dibromobenzophenone	v	v	v
-pp'-DDE	v	v	
acephate	v	v	v
acetamiprid	v	v	v
aldicarb-fulfone	v	v	v
aldicarb-sulfoxide	v	v	v
allethrin	v	v	
amicarbazone	v	v	v
amitraz	v	v	
atrazine	v	v	v
azinphosmethyl	v	v	v
azoxystrobin		v	
bendiocarb	v	v	v
bifenthrin		v	
boscalid		v	
captan	v	v	v
captan-THPI	v	v	v
carbaryl	v	v	v
carbaryl_1-naphthol	v	v	v
carbendazim	v	v	v
carbofuran	v	v	v
carbofuran-3-hydroxy	v	v	v
carfentrazone-ethyl		v	
chlorfenapyr	v	v	
chlorfenvinphos	v	v	v
chlorferone-coumaphos	v	v	v
chlorothalonil	v	v	
chlorpyrifos	v	v	
coumaphos	v	v	
coumaphos oxon	v	v	
coumaphos-potasan	v	v	v
cyfluthrin		v	
cyhalothrin		v	
cypermethrin		v	
cyprodinil	v	v	v
deltamethrin		v	
diazinon	v	v	v
dicofol	v	v	
dieldrin		v	
difenoconazole		v	
diflubenzuron	v	v	
dimethomorph	v	v	
diphenamid	v	v	v
diphenylamine	v	v	v
DMA-amitraz	v	v	v
DMPF-amitraz	v	v	v
endosulfan-I		v	
endosulfan-II		v	
endosulfan-sulfate		v	
esfenvalerate		v	
ethion	v	v	
ethofumesate	v	v	v
famoxadone		v	
fenamidone	v	v	
fenbuconazole	v	v	
fenhexamid	v	v	
fenoxaprop-ethyl	v	v	
fenpropathrin	v	v	
fipronil		v	

	CYP9Q 1	CYP9Q 2	CYP9Q 3
fluoxastrobin		v	
fluridone	v	v	
flutolanil	v	v	
heptachlor	v	v	
heptachlor-expoxide	v	v	
hexachlorobenzene	v	v	v
imidacloprid	v	v	v
imidacloprid-olefin	v	v	v
indoxacarb		v	
iprodione	v	v	
malathion	v	v	
metalaxyl	v	v	v
methidathion	v	v	v
methoxyfenozide		v	
methylparathion	v	v	v
metolachlor	v	v	
metribuzin	v	v	
myclobutanil	v	v	v
norflurazon	v	v	v
oxamyl	v	v	v
oxyfluorfen	v	v	
p-dichlorobenzene	v	v	v
pendimethalin	v	v	
permethrin	v	v	
phenothrin	v	v	
phosalone	v	v	
phosmet	v	v	
piperonyl-butoxide		v	v
pp'-DDD	v	v	v
pp'-DDT	v	v	
prallethrin	v	v	
pronamide	v	v	v
propanil	v	v	v
propiconazole	v	v	v
pyraclostrobin	v	v	
pyrethrin-I	v	v	
pyrethrin-II	v	v	
pyridaben	v	v	
pyrimethanil	v	v	v
pyriproxyfen	v	v	
sethoxydim	v	v	
simazine	v	v	v
spirodiclofen		v	
spiromesifen		v	
tebuconazole	v	v	
tebufenozide		v	
tebuthiuron	v	v	v
tefluthrin		v	
tetradifon	v	v	
tetramethrin	v	v	
thiabendazole	v	v	v
thiacloprid	v	v	v
thiamethoxam	v	v	v
triadimefon	v	v	
tribufos	v	v	v
trifloxystrobin		v	v
trifluralin	v	v	
Tua-fluvalinate		v	v
vinclozolin	v	v	v
Grand Total	**92**	**118**	**51**

Impacts of Dietary Phytochemicals on Honey Bee Longevity and Detoxification Capacity

- Overall, the fungicide/insecticide combination (hazard ratio, HR =1.09) and insecticide alone (HR=1.09) reduced honey bee survival rate but the fungicide ingested alone had no detectable effect on survival.
- At certain concentrations, quercetin (**orange**) and *p*-coumaric acid (**blue**) changed lifespan only at the low concentrations tested of propiconazole or chlorantraniliprole diets (Fig 1 A, C and D).
- Consumption of either *p*-coumaric acid or quercetin (500 uM and 12.5 uM, respectively) decreased the toxicity of the 0.9 ppm propiconazole/0.4ppm chlorantraniliprole combination, extending lifespan (by 15%, 60.3 h and 16%, 68.1 h, respectively) (Fig1 E and F).
- Similar lifespan extension occurred on the 50uM *p*-coumaric acid with 0.9 ppm propiconazole diets and 1000 uM quercetin with 0.4 ppm chlorantraniliprole diets.
- A synergistic effect was observed between *p*-coumaric acid and insecticide, whereby diets containing 1000 uM (164.16 ppm) *p*-coumaric acid and 0.4 ppm chlorantraniliprole reduced survival relative to diets containing the insecticide alone, causing a 7% reduction in lifespan (−31h).
- Consumption of the 90 ppm propiconazole/40 ppm chlorantraniliprole diet, with or without phytochemicals, reduced lifespan significantly (Fig 1G and H).
- In brief, these assays demonstrate that dietary phytochemicals influence lifespan and pesticide stress experienced by honey bees.

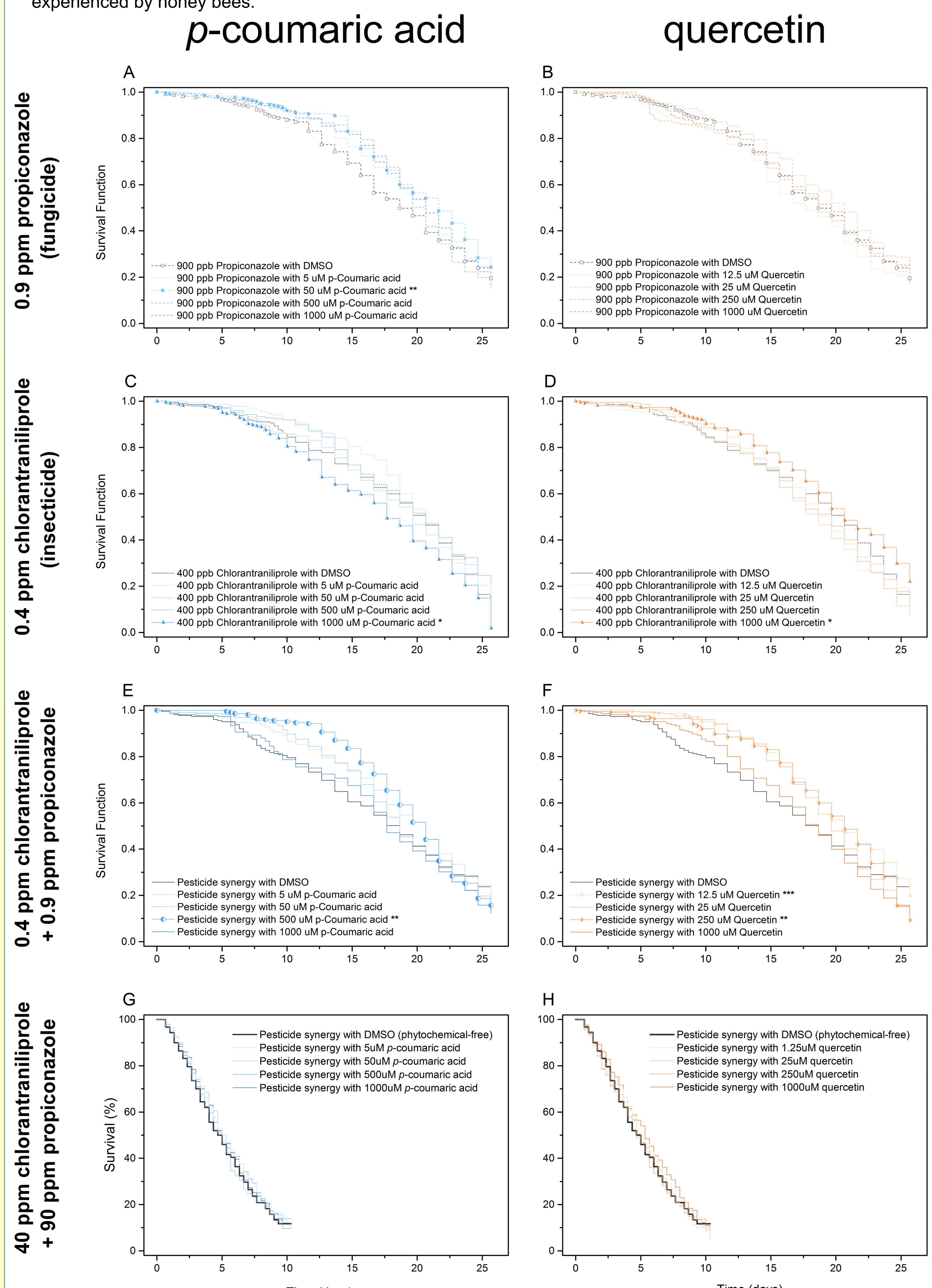

Fig1. Lifespan comparisons among honey bee workers fed different diets

Queen Flight Performance Assay (preliminary)

- We designed a "flight treadmill" to evaluate flight performance of queen bees reared by nurses consuming different pollen diets
- Queens reared by nurses consuming chlorantraniliprole-treated pollen diets exhibited the lowest frequency of wing-flapping (Fig2).
- However, no difference was seen in flight duration and total number of wing flaps.

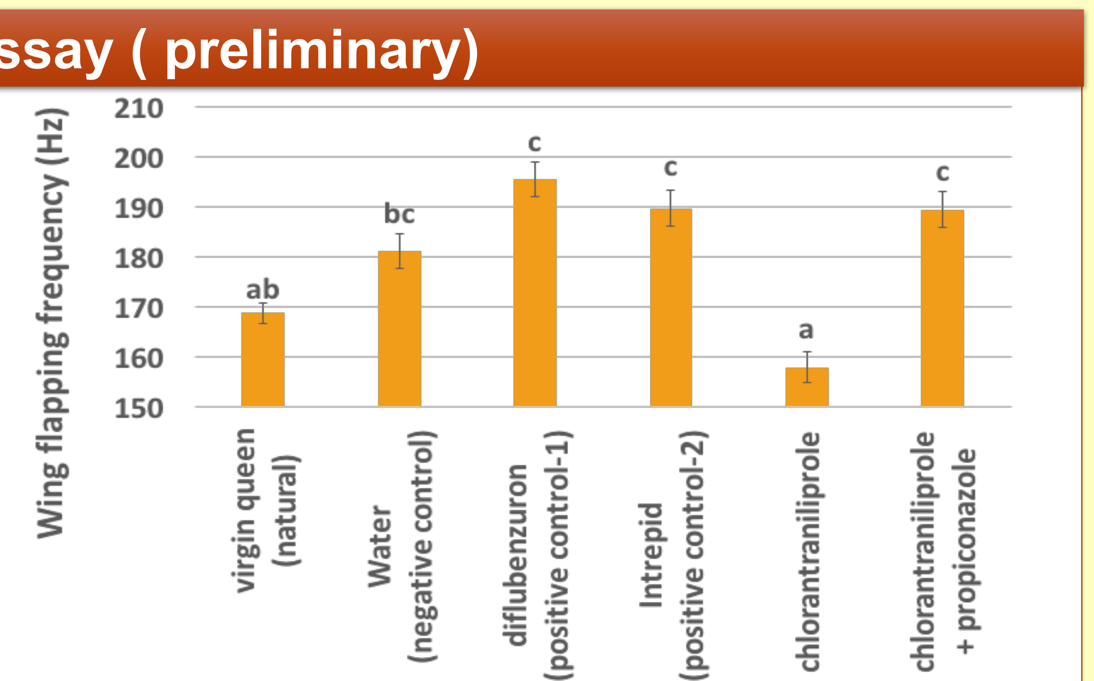

Fig2. Flight performance of queens.

Nursing Behavior Assay

Laboratory assay of worker nursing behavior

- The assay followed the method of Shpigler and Robinson (2015) with modification.
- Ten worker bees in each cage received one kind of treated-pollen diet and 50% sugar water.

Five pollen treatments

1. diflubenzuron (positive control)
2. water (negative control)
3. 40ppm chlorantraniliprole
4. 90ppm propiconazole
5. 40ppm chlorantraniliprole + 90ppm propiconazole

- Behavior associated with brood care of four- day-old queen larval cells was recorded.

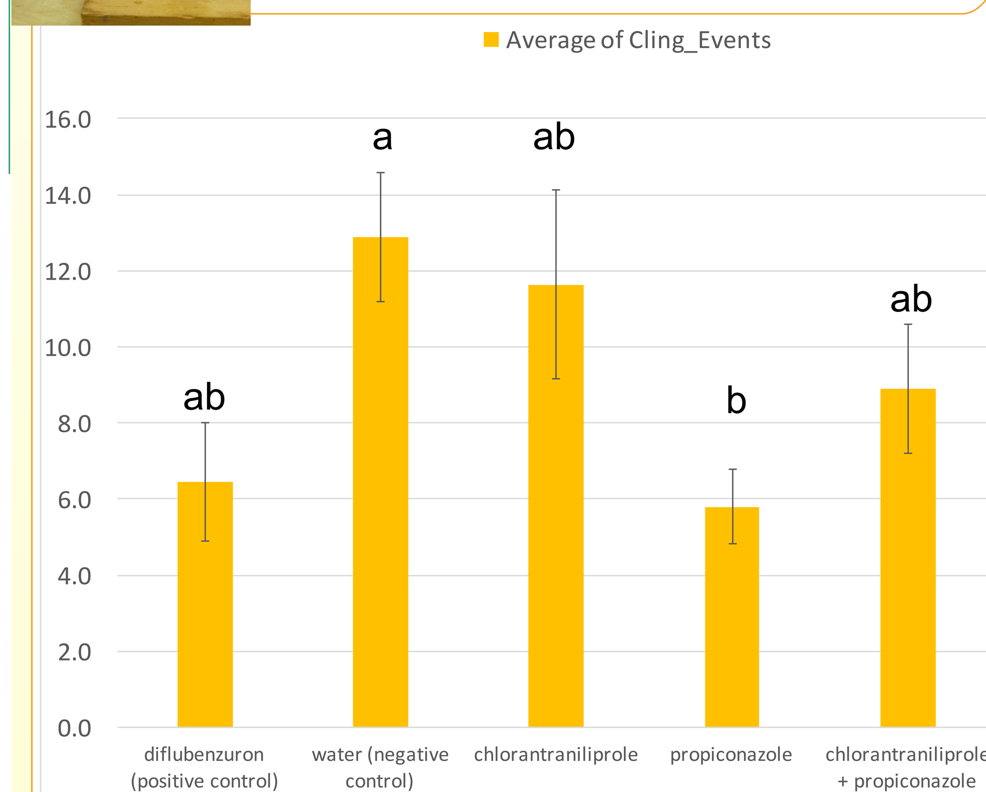

Fig3. One-week-old worker response to a queen cell with "clinging" behavior on different pollen diets. The number of clinging incidents recorded in 15 min. was significantly lower than control for bees consuming propiconazole-pollen (Data represent average ± SE)

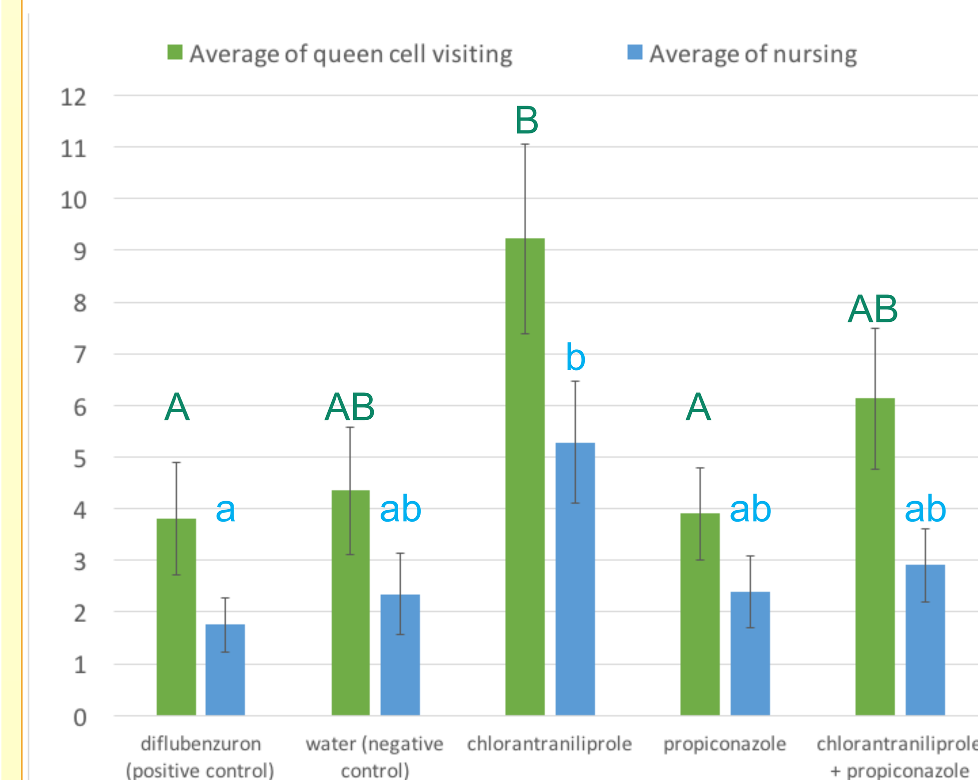

Fig4. One-week-old worker response to a queen cell with "visiting" or "nursing" behavior among workers consuming different pollen diets. Those consuming chlorantraniliprole pollen diet showed significantly more visiting and nursing behaviors. (Data represent average ± SE)

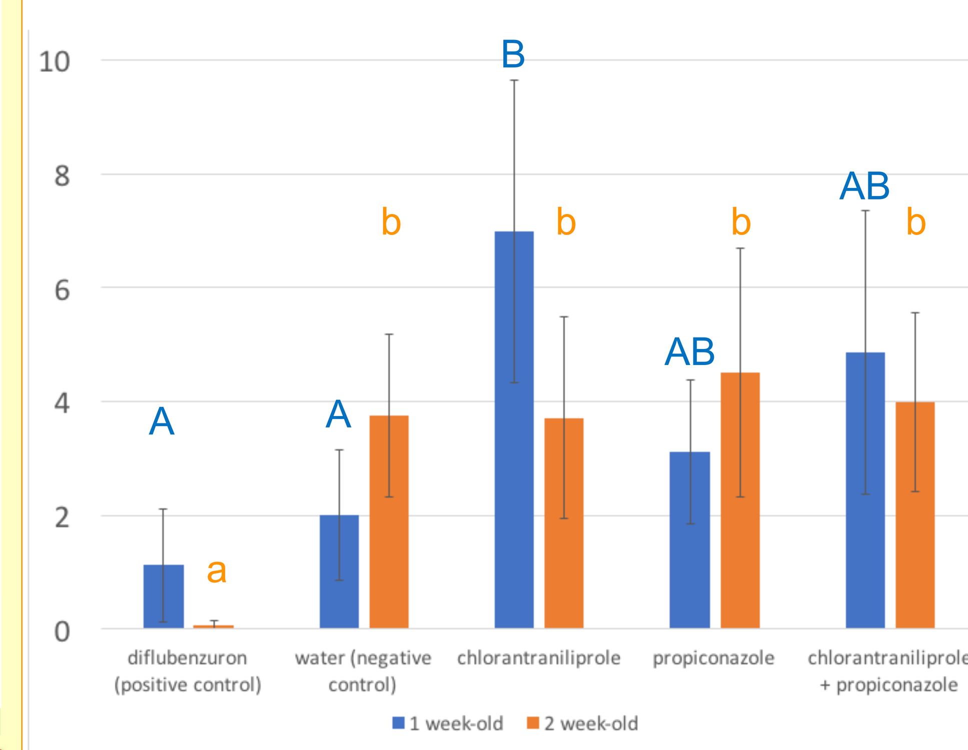

Fig5. Hive Y10 worker response to a queen cell with "visiting" behavior among workers consuming different pollen diets at one week of age or two weeks of age. (Data represent average ± SE)

Acknowledgments
We wish to express our hearty thanks to Edward Hsieh, Chia Lin, Ian Traniello, Adam Hamilton, Michael Wong, Alison Sankey, and Professor Gene Robinson. This project was funded by the National Honey Board and Almond Board of California.

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