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

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Background Introduction

- Dietary phytochemicals serve important functions in insect herbivores (Nishida, 2014). Functions of phytochemicals in pollinators are largely unknown.
- According to our previous work, consuming certain **phytochemicals from honey, including *p*-coumaric acid and quercetin, induces CYP450 gene expression** (Mao *et al.*, 2011, 2013).
- Bees rely on cytochrome P450 monooxygenases for detoxifying pesticides and phytochemicals. Thus, **phytochemicals may interact with pesticides and lead to toxicity changes.**
- In acute toxicity bioassays, phytochemicals synergistically reduced the toxicity of tau-fluvalinate to bees (Johnson *et al.*, 2012).
- **The use of fungicides while almond flowers are in bloom thus likely presents a toxicological challenge to honey bees by compromising their ability to process quercetin and other biologically active components of their pollen, honey, and beebread diet.**

Object 1. *In silico* High-Throughput Docking

Results

Docking results of of CYP 9Q1

PubChem CID	Name	Class
91768	tau-fluvalinate	pyrethroid insecticide
10342051	esfenvalerate	pyrethroid insecticide
443046	cyhalothrin	pyrethroid insecticide
104926	**cyfluthrin**	**pyrethroid insecticide**
2912	cypermethrin	pyrethroid insecticide
40585	deltamethrin	pyrethroid insecticide
6442842	**bifenthrin**	**pyrethroid insecticide**
4767	phenothrin	pyrethroid insecticide
40326	permethrin	pyrethroid insecticide
47326	fenpropathrin	pyrethroid insecticide
11442	allethrin	pyrethroid insecticide
11534837	tefluthrin	pyrethroid insecticide
6433155	pyrethrin-II	pyrethroid insecticide
9839306	prallethrin	pyrethroid insecticide
83975	tetramethrin	pyrethroid insecticide
5281045	pyrethrin-I	pyrethroid insecticide
13709	methidathion	organophosphate insecticide
3286	ethion	organophosphate insecticide
4793	phosalone	organophosphate insecticides and miticide
12901	phosmet	organophosphate insecticide
2268	azinphosmethyl	organophosphate insecticide
4130	methylparathion	organophosphate insecticide
2871	coumaphos	organophosphate insecticide
56840790	coumaphos-potasan	coumaphos metabolite
9453	coumaphos oxon	coumaphos metabolite
5355079	chlorferone-coumaphos	coumaphos metabolite
91754	pyridaben	heterocyclic insecticides and miticide
36324	amitraz	acaricide and insecticide
86418	imidacloprid	neonicotinoid insecticide
	imidacloprid-olefin	metabolite of Imidacloprid
213021	acetamiprid	neonicotinoid insecticide
107720	indoxacarb	oxadiazine insesticide
91753	pyriproxyfen	pyridine-based insecticide
91773	tebufenozide	insecticide as the agonist of ecdysone
105010	methoxyfenozide	insecticide as the agonist of ecdysone
9907412	spiromesifen	insecticide
91778	chlorfenapyr	pro-insecticide, inhibition of ATP production
3352	fipronil	phenylpyrazole insecticide
2314	bendiocarb	carbamate insecticide
2566	carbofuran	carbamate insesticide
37123	diflubenzuron	benzoylurea-type insecticide
177863	spirodiclofen	tetronic acid acaricide
5794	piperonyl-butoxide	P450 inhibitor
86173	difenoconazole	fungicide
213016	trifloxystrobin	fungicide
5463781	dimethomorph	fungicide
213031	fenhexamid	fungicide
6422843	pyraclostrobin	fungicide
3034285	azoxystrobin	fungicide
11048796	fluoxastrobin	fungicide
86138	fenbuconazole	fungicide
86102	tebuconazole	fungicide
213032	famoxadone	fungicide
213013	**boscalid**	**fungicide**
39385	triadimefon	fungicide
6336	myclobutanil	fungicide
47898	flutolanil	fungicide
43234	**propiconazole**	**fungicide**
10403199	fenamidone	fungicide
86367	cyprodinil	fungicide
37517	iprodione	fungicide
39327	oxyfluorfen	herbicide
86222	carfentrazone-ethyl	herbicide
43079	fluridone	herbicide
47938	fenoxaprop-ethyl	herbicide
33360	ethofumesate	herbicide
52923	sethoxydim	herbicide
33775	norflurazon	herbicide

- We performed *in silico* high-throughput docking into the active pocket of CYP9Q1 and identified 68 compounds that could be docked.
- **We have further tested the pesticides**, which are listed with yellow background, **with dietary phytochemicals on honey bee longevity, detoxification capacity and flight performance.** (Please check the results in Objectives 2 and 3).

Object 2. Impacts of Dietary Phytochemicals on Honey bee Longevity and Detoxification Capacity

Methods

- Newly emerged workers from a single hive were collected over a 3-day period.
- Caged bees (25 bees/cage with 5 replicates for each treatment) were kept in an incubator in a dark room at 33 °C and 50% RH.
- Bees in each cage received one kind of treated 50% syrup with casein diet (protein : carbohydrates = 1 : 12; casein was used as a phytochemical-free protein source).
- Syrup diets were made fresh and replaced daily. Survival rate of each cage were recorded daily.

Results

Exp. 1

- **Quercetin and *p*-coumaric acid enhanced worker longevity** (Hazard ratio < 1), a finding that reinforces the importance of naturally occurring phytochemicals in the diet of honey bees.
- **Quercetin improved tolerance of two tested pyrethroids in workers**.
- ***p*-Coumaric acid also had a protective effect on workers exposed to bifenthrin**; however, the effect was small in magnitude in other subgroups.
- Bifenthrin and β-cyfluthrin at sublethal concentrations had negative effects on worker lifespan (Hazard ratio > 1).

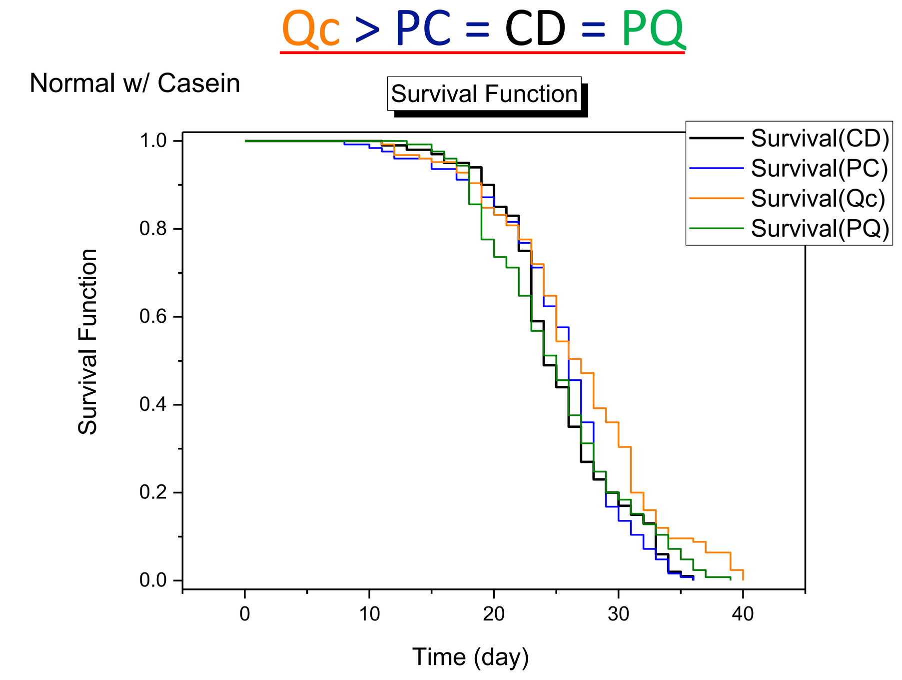
Exp. 2

- This experiment is still in progress. Preliminary results suggest that ***p*-coumaric acid has a protective effect on workers exposed to chlorantraniliprole (Fig B).**
- **Quercetin plus *p*-coumaric acid diet improved tolerance of the tested fungicide, propiconazole (Fig C).** However, when these two phytochemicals are ingested individually with propiconazole, the survival rate was unaffected.
- Moreover, **phytochemicals increased the mortality of workers when the diet was also amended with both chlorantraniliprole and propiconazole diet (Fig D).**

Exp. 1 – Phytochemicals and Pyrethroids

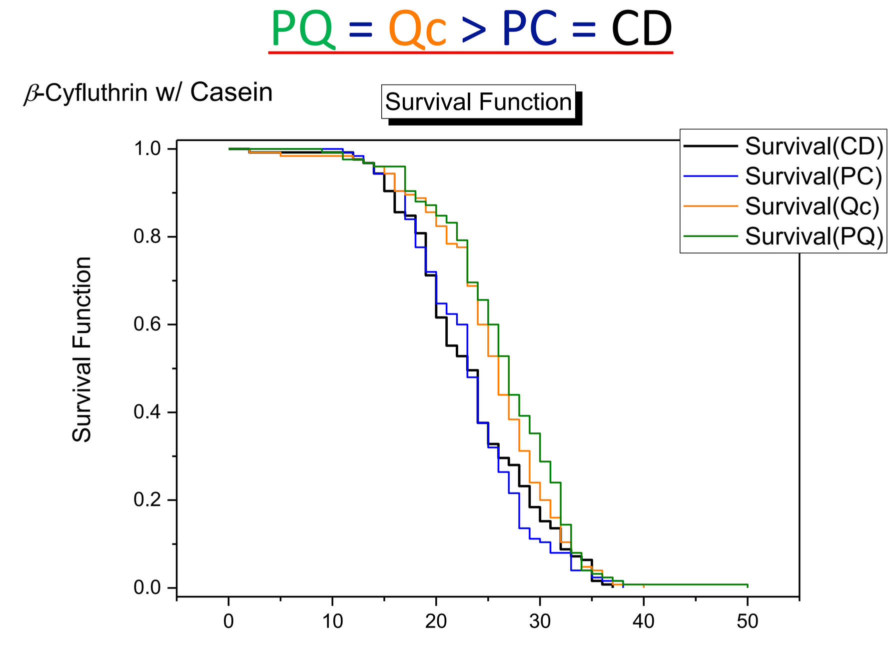
Pesticide-free diet

Qc > PC = CD = PQ



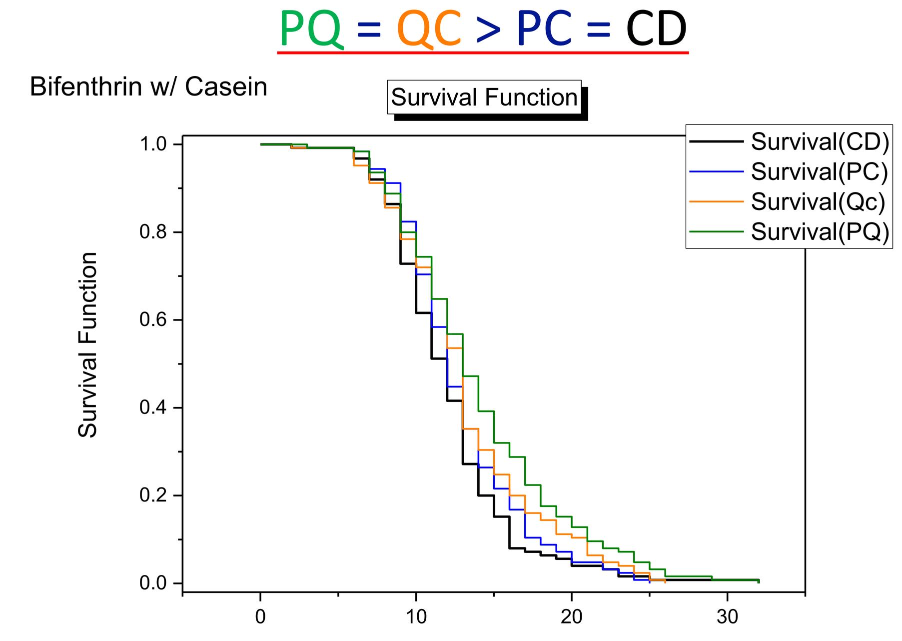
0.5 ppm β-cyfluthrin diet

PQ = Qc > PC = CD



4 ppm bifenthrin diet

PQ = QC > PC = CD

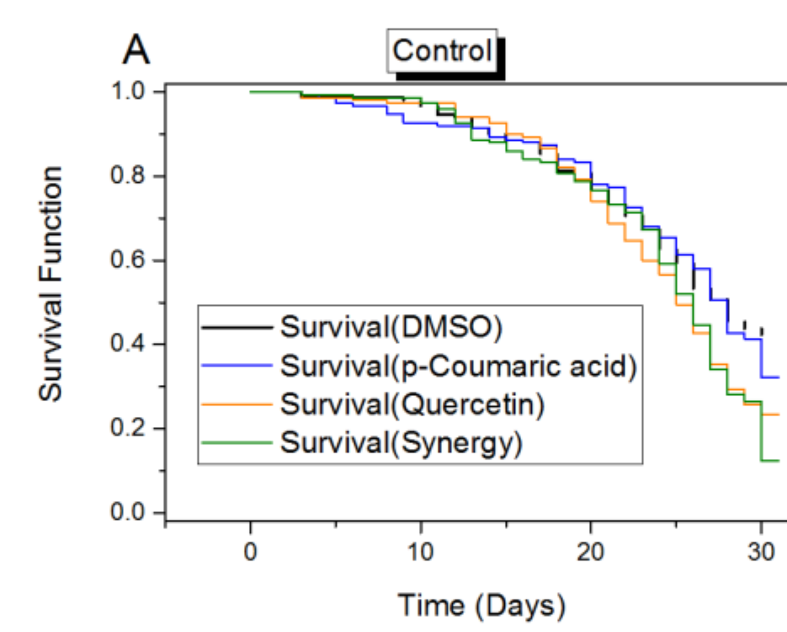


[a] The comparison symbols with a red underline indicate the comparison results among phytochemical subgroups, which were calculated by the log-rank test. (< or > was regarded as statistically significant, $p < 0.05$; = indicated no significant different.)

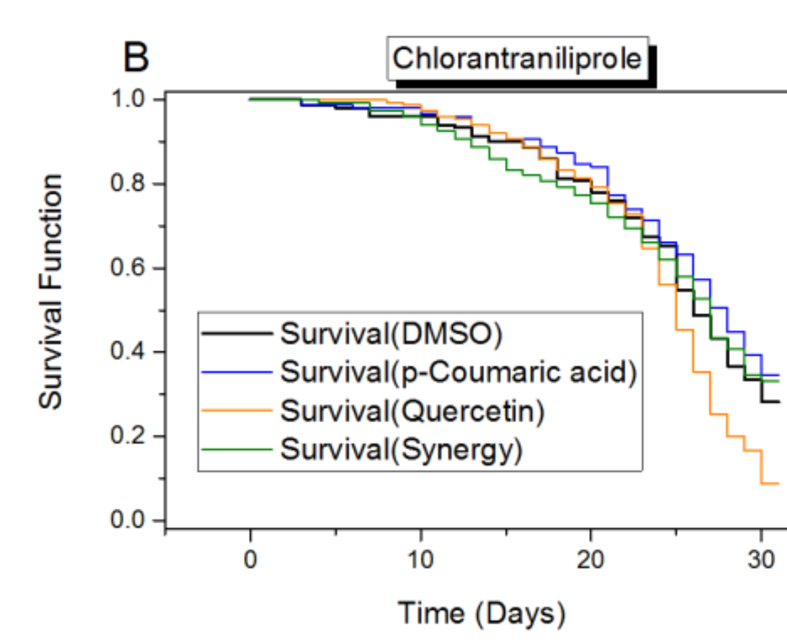
[b] phytochemical subgroup:
CD: 0.25% DMSO, 50% control syrup;
PC: 0.5 mM *p*-coumaric acid syrup;
Qc: 0.25 mM quercetin syrup;
PQ: *p*-coumaric acid plus quercetin syrup.

Exp. 2 – Phytochemicals, a fungicide and a insecticide

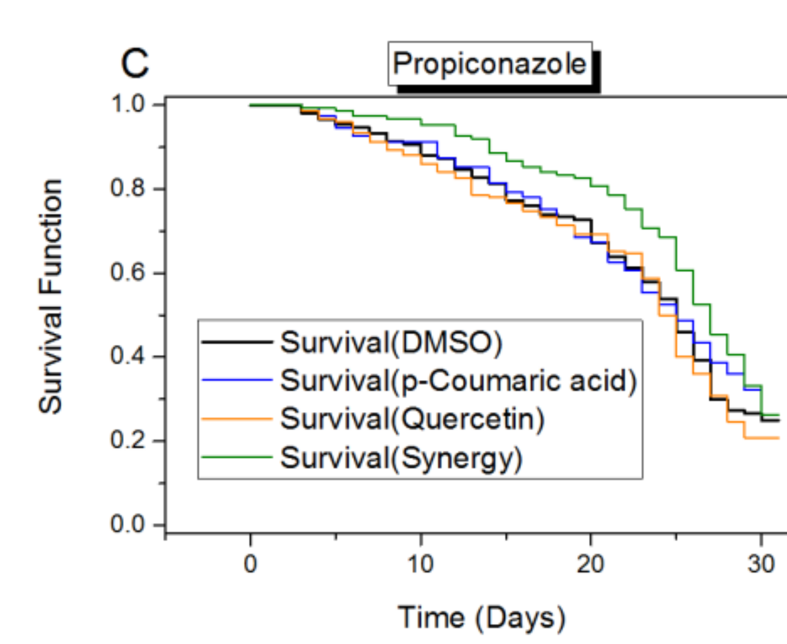
Pesticide-free diet



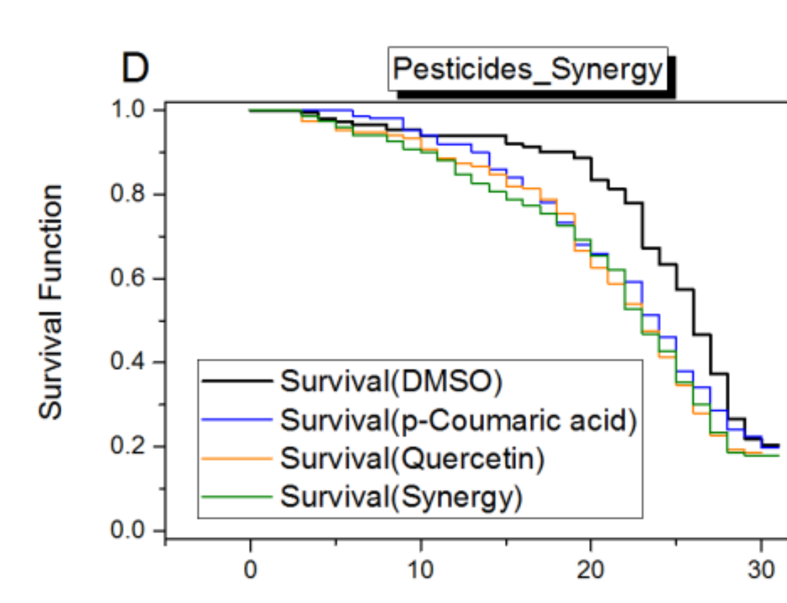
2 ppm chlorantraniliprole diet



30 ppm propiconazole diet



Chlorantraniliprole plus Propiconazole diet



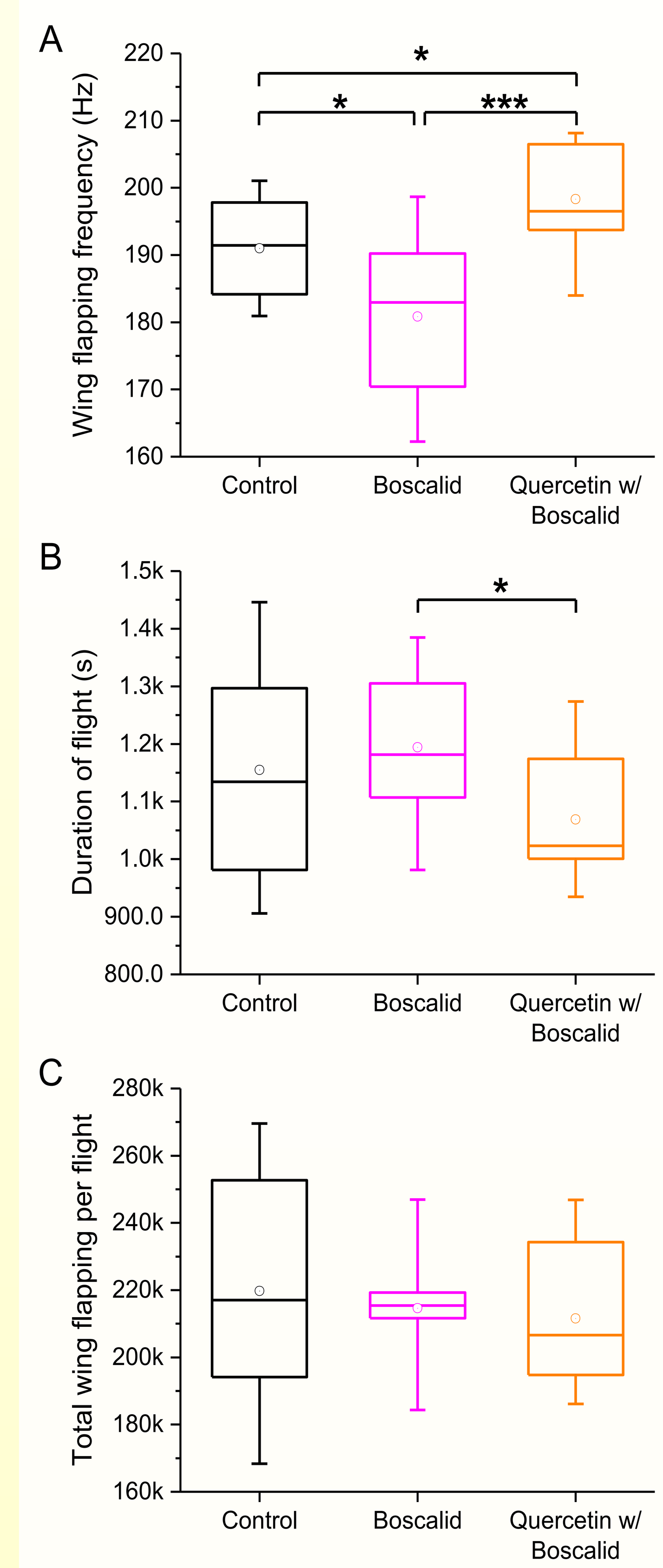
Object 3. Effects of Quercetin on Fungicide Suppression of Flight Performance

Methods

- The "flight treadmill" was designed and used to evaluated the flight performance of bees.

Results

- Consuming quercetin potentially facilitates faster flight. In contrast, foragers consuming boscalid alone exhibited the lowest frequency of wing flapping.
- Consuming quercetin eliminated the adverse effects of boscalid on flight performance of foragers.



- In all figures (A, B and C), the circle with a central point indicates the mean. The middle line of box shows the median value; the box delimits the 25th and 75th percentiles. The ends of the whiskers indicate the minimum and maximum of all of the data. The asterisk indicates significant difference between the two means (*: $p < 0.05$, ***: $p < 0.0001$, Mann–Whitney *U* test).

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