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

**Project No.:** **16-POLL18-Berenbaum**

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**Objectives:**

1. Determine if consuming dietary phytochemicals results in enhanced survival and longevity in the presence of pesticides, including fungicides, used in almond production.
2. Determine how fungicides used in almond production influence toxicity of dietary phytochemicals by conducting adult rearing assays with the phytochemicals quercetin and *p*-coumaric acid in the presence and absence of pesticides likely to be inhibitory and measuring ATP generation and flight capacity.
3. Use *in silico* high-throughput screening of a diversity of both pesticides and phytochemicals to develop a predictive algorithm for *a priori* prediction of risks associated with application during bloom.

**Interpretive Summary:**

Although formulated to target metabolic processes in fungi, fungicides used in almond production can have adverse effects on honey bee health. Boscalid, a constituent of the fungicide Pristine (along with pyraclostrobin), is a carboxamide that owes its fungicidal activity to its ability to inhibit succinate dehydrogenase and thus interfere with energy production via the mitochondrial electron transport chain. Honey bees exposed to this fungicide show signs of dysfunction of mitochondrial electron transport, including reduced production of ATP in thoracic (wing) muscles and wing-flapping activity. The tank mix of the insecticide chlorantraniliprole and the triazole fungicide propriconazole (which targets sterol metabolism) is itself toxic to bees, significantly reducing the lifespan of adult workers. The fact that detoxification of multiple classes of pesticides depends on a limited number of cytochrome P450 enzymes suggests that antagonistic interactions among insecticides and fungicides are very likely to occur. Phytochemical constituents of honey, however, including quercetin, can ameliorate the adverse effects of tank mixes on longevity. In terms of recommendations, beekeepers may benefit by trying to ensure that bees in danger of fungicide exposure have access to phytochemicals in their natural foods, including honey and beebread to enhance worker capacity to cope with pesticide stress.

**Materials and Methods:**

Objective 1. determine if consuming dietary phytochemicals results in enhanced survival and longevity in the presence of pesticides, including fungicides, used in almond production:

For each assay, brood frames were provided from three hives and kept in an incubator (34 °C, 50% relative humidity) for collecting one-day-old bees daily. Observation cages were constructed and provisioned with diets differentially amended with test compounds with a basic sugar water diet to assay survivorship. The assays were carried out in 32 oz. observation chambers containing 25 bees; fresh sugar water and distilled water were available *ad libitum*. All assays used the basic diet comprising 50% sugar water, 0.25% DMSO and protein (casein)/carbohydrate ratio of 1:12.

The phytochemicals quercetin and *p*-coumaric acid were used in combination with the insecticide chlorantraniliprole and the fungicide propiconazole. There were 56 diets tested in total across two summers. In the first trial, due to seasonal limitations, six replicates were carried out with a three-hive mixed population. Two phytochemicals in 4 combinations (control, 500 µM p-coumaric acid, 250 µM quercetin and 500 µM p-coumaric acid + 250 µM quercetin) and 3 pesticide combinations (2 ppm chlorantraniliprole, 30 ppm propiconazole, and 2 ppm chlorantraniliprole + 30 ppm propiconazole), thus constituting 12 diets in total, were tested for their effects on performance. In the second set of assays, for each treatment, three hives were tested, and three to four replicates were carried out per hive. Two phytochemicals, p-coumaric acid and quercetin, in four concentrations based on naturally occurring levels in nectar and pollen were tested. Also, we followed the ratio (9:4) of the pesticide combinations usually suggested for field tank-mixed applications and used in almond orchards. For assays with phytochemicals at low concentrations, 3 pesticide combinations (0.9 ppm propiconazole, 0.4 ppm chlorantraniliprole and both) were tested. For assays with phytochemicals at high concentrations, the combination of 90 ppm propiconazole and 40 ppm chlorantraniliprole were tested.

Objective 2: Determine how fungicides used in almond production influence toxicity of dietary phytochemicals by conducting adult rearing assays with quercetin and other phytochemicals in the presence and absence of pesticides likely to be inhibitory and measuring ATP generation and flight capacity:

The fungicide boscalid, frequently found as a hive contaminant, interferes with fungal energy production, specifically via inhibiting succinate dehydrogenase in mitochondrial complex II. Quercetin, a flavonol ubiquitous in honey and pollen, also may affect energy-linked mitochondrial metabolism in honey bees. The effects of dietary quercetin on levels of ATP in flight muscles of foragers as well as the effects of ingesting quercetin and boscalid together on forager flight performance were investigated. The tested honey bees were collected from colonies from the UIUC Bee Research Facility in Urbana, IL, split from the same colony; initially, each tested colony was composed of 6,000 adult bees, one young larval brood frame and a newly introduced laying queen. These queens were naturally mated and were sisters. The test colonies were kept separately in outdoor flight chambers and provided with water and 25% sucrose syrup feeders along with pollen patties placed on the top of frames and under the inner

cover weekly. Foragers from each colony were trained to visit a syrup feeder and caught as required for experiments.

2a. Measuring ATP levels: Proteins and adenosine triphosphate (ATP) levels of the flight muscles of foragers were measured from paired hives, a quercetin-treated colony and a solvent control colony modified from Costa et al. 2013). Individual foragers were collected on the feeders when they finished feeding and were then immediately placed into liquid nitrogen. The thorax of each collected bee was placed on dry ice and transferred to a 1.5 ml Eppendorf tube. The thoraces of three bees were ground together with an extraction buffer on ice. After grinding, the homogenates were placed in water at 95$^{o}$C for one minute and returned to ice. The samples were centrifuged at maximum speed and the supernatant was collected to measure the protein and ATP levels in each sample. The total protein concentration of each sample was determined by the Bradford method (500-0201, Bradford Protein assay kit, Bio-Rad, Hercules, CA) and the ATP level was measured with the CellTiter-Glo Luminescent Cell Viability Assay System (G7570, Promega Life Sciences, Madison, WI) according to the manufacturer's protocol. The bioluminescence signal was used to calculate ATP levels based on the ATP standard curve. Each experiment had a concurrent negative control, a blank, and an ATP standard curve. The values.  expressed as picomoles ATP per milligram protein (pmol ATP /mg protein), were calculated from the ATP and total protein concentrations measured in each sample. ATP concentrations among treatments were compared by Mann–Whitney *U* test.

2b. Measuring of flight capacity: Two sets of colonies were tested; the first set of experiments compared a quercetin-treated colony with a control colony (treated with solvent only) and the second set compared a boscalid-treated colony with a colony treated with both boscalid and quercetin and with a control colony. Quercetin and boscalid were dissolved in dimethyl sulfoxide (DMSO) as stock solutions; the quercetin, boscalid, and boscalid-quercetin stock solutions as well as DMSO were incorporated into 25% sugar water at sublethal concentrations, as determined previously (control: 0.25% DMSO; quercetin: 0.25 mM quercetin with 0.25% DMSO; boscalid: 10 ppm boscalid with 0.25% DMSO; boscalid with quercetin: 10 ppm

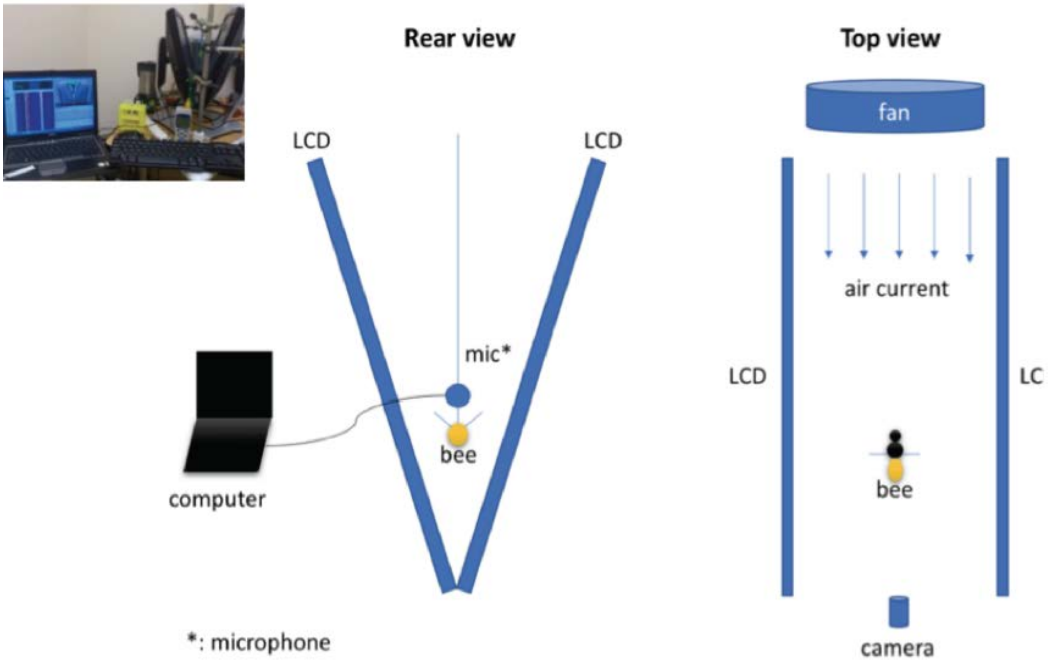


**Figure 1.** Flight treadmill for testing the flight characteristics of bees

boscalid with 0.25 mM quercetin and 0.25% DMSO). A flight treadmill was constructed to assay flight ability **(Figure 1**). Two LCD monitors were arranged face-to-face in a vertical 36-degree V-shape arrangement to provide bees with visual and optical flow stimuli from the lateral and ventral sides. Optical flow provided by each side monitor was a moving vertical black-and-white striped pattern generated by a custom-written LabVIEW program; the horizontal spatial frequency of the pattern was 0.016 cycles degree$^{-1}$ and the speed of the optical flow was about 209.4 degrees s$^{-1}$ as seen from the center point of the fixation position of the tested bee. Also, an in-line fan blower was set in front of the V-shaped monitors to provide a constant velocity air flow (3.6 m s$^{-1}$) stimulus. A metal rod was positioned vertically at ¾ of the longitudinal axis of the V-shaped "LCD valley". A small magnet was attached to the end of the rod for tethering the bee.

Each forager for the flight treadmill experiment was caught when it was feeding at a syrup feeder, placed in a vial, chilled down on ice until it was incapable of moving, and anesthetized. Hairs on the dorsal thorax of the bee were shaved using a razor blade and a 2-mm diameter piece of carbon steel plate was glued on the middle of the notum of the bee using a cyanoacrylate adhesive. Then the bee was set in a quiet humidified box for at least 30 minutes to recover from stress before the first flight in the flight treadmill (designated as a "restorative time-out"). Without supplemental feeding, after a restorative time-out, the bee was placed on the flight treadmill for a flight to exhaust her energy and stored sugar fuel (designated as a "depletion flight"). Generally, a depletion flight required at least 20 minutes and could take as long as four hours.

After the depletion flight, the tested bee was fed 10 µL of 25% sucrose syrup with the treatment chemical for its group. The bee was then positioned in the flight treadmill and allowed to hold onto a stick to rest for five minutes. The optic flow stimulus was turned off and the screens of the monitors were set to all-white during the resting period. After the resting period, the stick was removed to trigger a tarsal reflex and the optic flow was started to induce another trial of flight of the bee. Three to five trials were conducted per honey bee to reduce inter-animal variability. Between each trial, the tested bee was also fed 10 µL of 25% sucrose syrup with the treatment chemical and then held a five-minute restorative period.

The sound of wing-flapping was recorded using a case-removed electric condenser microphone attached on the metal rod. The recorded signal was digitized at 32 kHz sampling rate and 16-bit depth by the built-in sound card in a laptop. The wing-flapping frequency was estimated through measuring the fundamental frequency of the recorded buzzing sound. The fundamental frequency was measured every 0.1 second during a flight using custom-written LabVIEW programs. The searching band for the fundamental frequency was set between 0 to 320 Hz to avoid unnecessary interference from environmental high-frequency noise. Furthermore, because the sampling of the recording microphone was limited, and the air blower fan produced a large amount of low-frequency noise, the measured fundamental frequencies equal to or lower than 50 Hz were also ignored. Therefore, the duration of flight was calculated as the total time in a flight shown by the fundamental frequency at levels higher than 50 Hz. The averaged wing flapping frequency was obtained from the average of the fundamental frequencies higher than 50 Hz, and total wing-flapping was calculated as 1/10$^{th}$ the sum of the measured fundamental frequencies higher than 50 Hz during a flight. Data were tested for their normality, and equality of their means was compared by two-sample *t* test or by Mann–Whitney *U* test.

Objective 3. use in silico high-throughput screening of a diversity of both pesticides and phytochemicals to develop a predictive algorithm for a priori prediction of risks associated with application during bloom:

Beginning August 1, 2017, postdoctoral associate Daryl Meling focused his attention on this component of the project, using a slight modification of the methods used in the laboratory previously to model catalytic activity of honey bee and lepidopteran cytochrome P450 monooxygenases (Mao et al. 2009, 2011). Basically, the sequence of the target P450 was aligned with 8 class II human sequences and with the sequence of CYP3A4, all with known pdb structures. Primary sequence alignments were also done with the same set of P450 sequences for the B region, F-G loop region, and B4 region. A series of 3D model was made using the HOMOLOGY function of MOE. Among these, the model with the best packing score was kept. After incorporation of heme coordinates, this model was subjected to further energy minimization in MOE using the CHARMM27 force field. A Ramachandran plot was used to verify that psi-phi torsion angles of the backbone are in the normal ranges with few outliers. Docking was performed initially with relevant phytochemicals and with pesticides that are most frequently encountered in almond orchards. Up to 100 docking conformations were generated in three independent docking simulations for each molecule. All binding modes with the reactive hydrogen less than 7 Angstroms from the heme oxygen were selected for further energy minimization in which the protein could move freely and the heme remain rigid. The final optimal binding modes were selected based on both proximity of the reactive hydrogen to the heme oxygen and binding energy.

**Results and Discussion:**

Objective 1. Determine if consuming dietary phytochemicals results in enhanced survival and longevity in the presence of pesticides, including fungicides, used in almond production:

Effects of dietary phytochemicals in presence of 30 ppm propiconazole and/or 2 ppm chlorantraniliprole on survivals of honey bees: In evaluating a residues exposure risk trial, the 30 ppm fungicide propiconazole (hazard ratio (HR) = 1.18) and the fungicide/insecticide combination (2 ppm chlorantraniliprole plus 30 ppm propiconazole; HR =1.44) reduced honey bee survival rate but 2 ppm chlorantraniliprole ingested alone had no detectable effect on survival. Moreover, ingesting 0.5 mM quercetin plus 0.25 mM *p*-coumaric acid diet improved tolerance of 30 ppm fungicide propiconazole (**Figure 2A**). However, when these two phytochemicals were ingested individually with 30 ppm propiconazole, the survival rate was unaffected. Phytochemicals ingested at a lower concentration increased the mortality of workers when the diet was also amended with 2ppm chlorantraniliprole plus 30 ppm propiconazole (**Figure 2B and C**). During this trial, seasonal variation might explain some of the phytochemical effects on bee lifespan. This trial was conducted in late summer when honey bees experience a change in their physiological condition to transition from summer demands to overwintering demands. Moreover, limitations on food availability during late summer may have caused a decline in hive health.

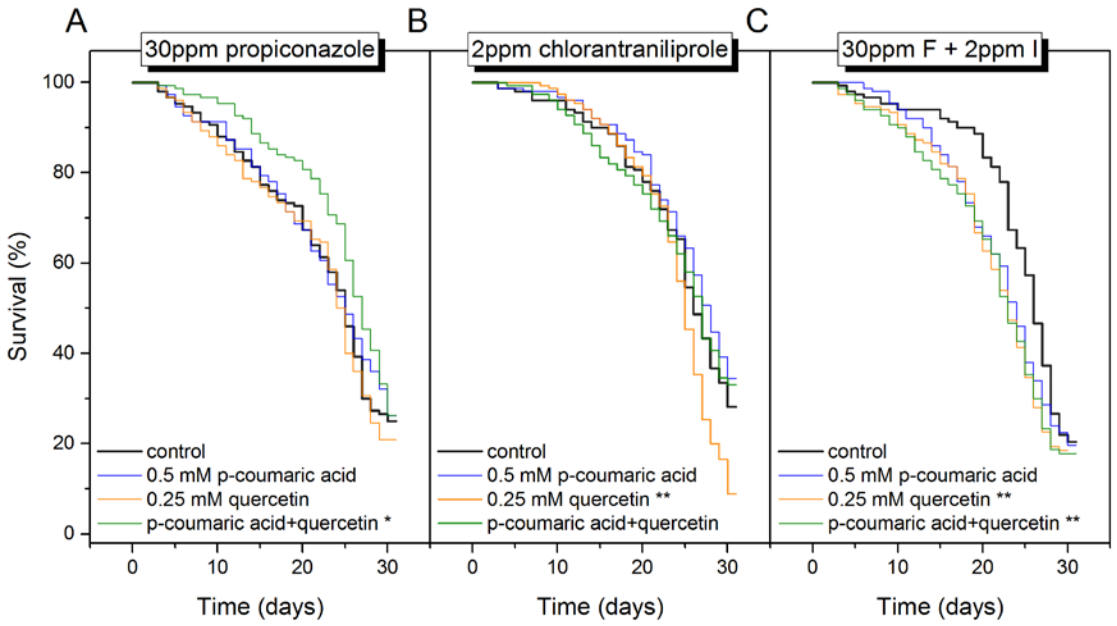


**Figure 2.** Kaplan–Meier plots of honey bee longevity on diets varying in phytochemical content (0.5mM quercetin, 0.25mM *p*-coumaric acid and the combination of both phytochemicals),as well as pesticide content: (A) 30 ppm fungicide propiconazole amendment, (B) 2 ppm insecticide chlorantraniliprole amendment,(C) 30 ppm propiconazole plus 2 ppm chlorantraniliprole amendment (log-rank test between the treatment and control, ** = $p < 0.01$, * = $p < 0.05$).

<u>Effects of dietary phytochemicals in different concentrations with the tank-mixed ratio propiconazole and chlorantraniliprole on survivals of honey bees:</u> We tested two phytochemicals in various concentrations and their interaction with pesticides to evaluate their optimal concentrations in terms of honey bee longevity. In the absence of pesticides, consuming dietary phytochemical *p*-coumaric acid alone at 0.05 mM and 0.005mM (**Figure 3A**) as well as quercetin alone at 0.25 mM, 0.025 mM and 0.0125mM (**Figure 3B**) results in enhanced survival rates of honey bees during the test period. These results are

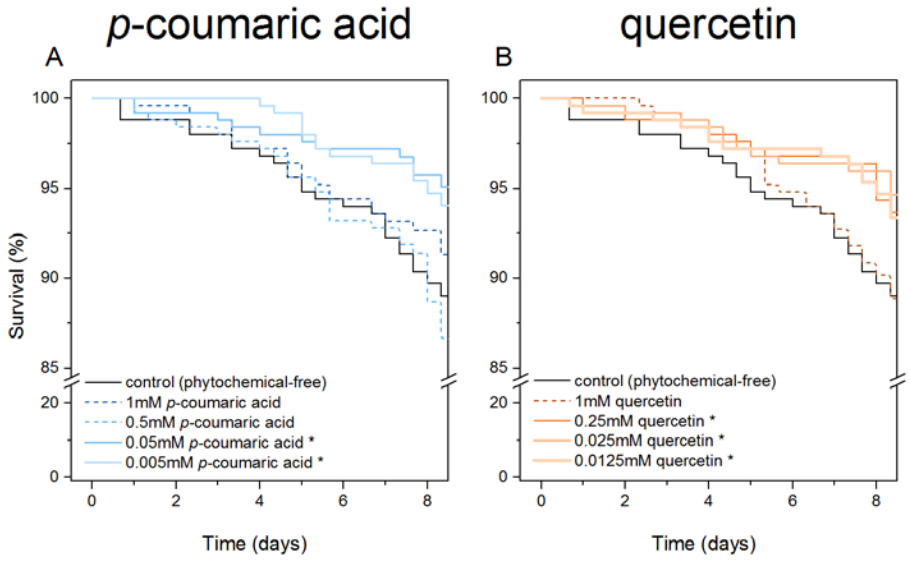


**Figure 3.** Kaplan–Meier plots of honey bee longevity on with phytochemical (A) *p*-coumaric acid supplements and (B) l quercetin supplements in different concentrations (log-rank test between the treatment and control, * = $p < 0.05$).

consistent with our previous finding that dietary phytochemicals enhance longevity (Liao et al, 2017). In addition, fungicide propiconazole and insecticide chlorantraniliprole were tested in a tank-mix ratio, 9:4. This same ratio was used by our collaborators Reed Johnson and colleagues at Ohio State University for a series of parallel experiments. Two tested sublethal concentrations of pesticides, 90+40 ppm and 0.9+0.4 ppm, were based on the OSU team results.

Moreover, consuming propiconazole and/or chlorantraniliprole in at the two test concentrations reduced longevity (**Figure 4**). Although our results are generally consistent with those of OSU, they reported that low concentration of pesticides 0.9ppm propiconazole + 0.4ppm chlorantraniliprole, did not differentially affect survival. This difference may reflect methodological differences: whereas we mixed the pesticides into sugar water, the OSU team applied the pesticides on pollen. Honey bees usually consume sugar water faster and in greater amounts than they consume pollen, a difference that may have altered the nature of pesticide exposure (reviewed in Liao et al., 2017.

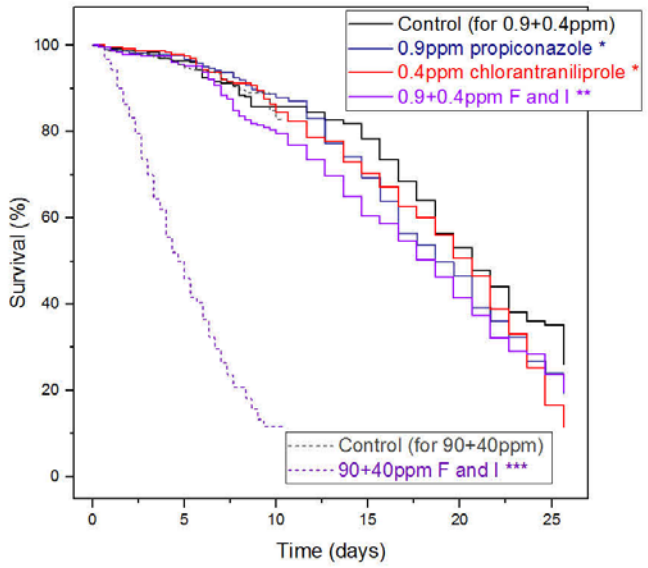


**Figure 4**. A Kaplan–Meier plot of honey bee longevity on diets with 0.9 ppm fungicide propiconazole, 0.4 ppm chlorantraniliprole amendments, their combination, or the high concentration combination amendment, 90 ppm propiconazole and 40 ppm chlorantraniliprole (log-rank test between the treatment and control, *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$). Bees consuming pesticides displayed reduced longevity relative to those on control diets.

With respect to the interaction between phytochemicals and pesticides, when consumed in diets containing low concentrations of pesticides (0.9 ppm propiconazole alone and 0.4 ppm chlorantraniliprole alone), quercetin and *p*-coumaric acid increased longevity (**Figure 5A, C, and D**). This phytochemical rescye effect was not detected in the presence of 90 ppm propiconazole/40 ppm chlorantraniliprole (**Figures 5G and 5H**). 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 (**Figures 5E 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 chlorantraniliprole, 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 diets containing 90 ppm propiconazole/40 ppm chlorantraniliprole, with or without phytochemicals, reduced lifespan significantly (**Figures 5G and H**). In brief, these assays demonstrate that dietary phytochemicals influence longevity and pesticide stress experienced by honey bees.

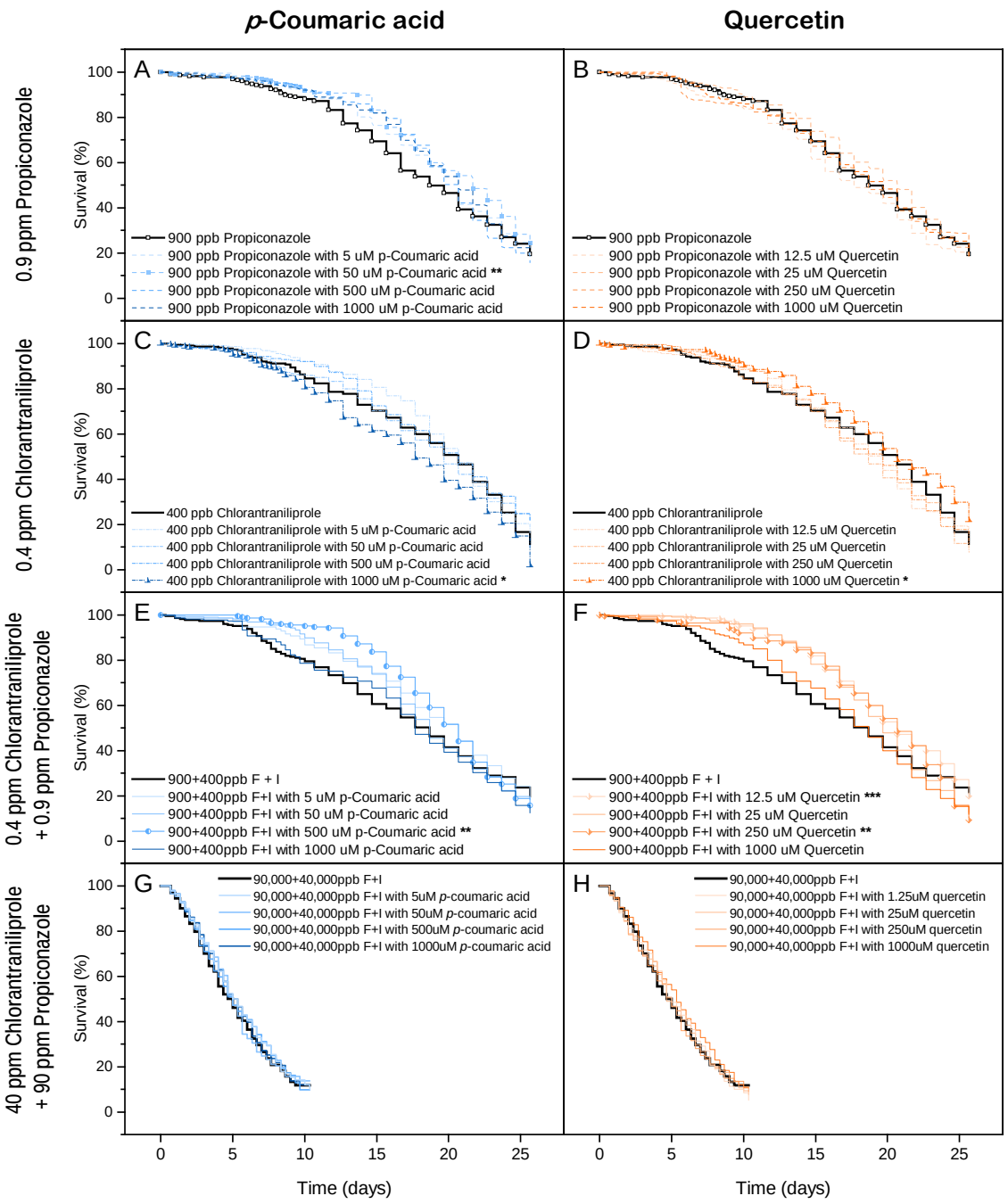


**Figure 5.** Kaplan–Meier plots of lifespan of honey bees on diets with phytochemical *p*-coumaric acid and quercetin supplements as well as the fungicide propiconazole and the tank-mixed insecticide chlorantraniliprole. These diets were (A) 0.9ppm propiconazole amendment with *p*-coumaric acid at different concentrations, (B) 0.9 ppm propiconazole amendment with quercetin at different concentrations, (C) 0.4 ppm chlorantraniliprole with *p*-coumaric acid at different concentration supplements, (D) 0.4 ppm chlorantraniliprole with quercetin at different concentrations, (E) combination of 0.4 ppm chlorantraniliprole and 0.9 ppm propiconazole with *p*-coumaric acid at different concentrations, (F) a combination of 0.4 ppm chlorantraniliprole and 0.9 ppm propiconazole with quercetin at different concentrations, (G) a combination of 40 ppm chlorantraniliprole and 90 ppm propiconazole with *p*-coumaric acid at different concentrations, and (H) a combination of 40 ppm chlorantraniliprole and 90 ppm propiconazole with *p*-coumaric acid at different concentration supplements (Log-rank test, *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$).

Objective 2: Determine how fungicides used in almond production influence toxicity of dietary phytochemicals by conducting adult rearing assays with quercetin and other phytochemicals in the presence and absence of pesticides likely to be inhibitory, measuring ATP generation and flight capacity.

2a. Effects of quercetin on ATP concentration of flight muscle of foragers:

The average value of ATP concentrations in flight muscles of foragers from the colony experiencing long-term dietary exposure to quercetin was 37.46 ± 4.67 pmol/mg protein (mean ± SE), which was ca. four times higher than concentrations from individuals from the solvent-control colony (10.29 ± 1.99 pmol/mg protein) {(**Figure 6**) U = 64, Z = -4.61, p < 0.001). The result indicated that the quercetin-treated foragers had higher ATP stores in general and may also have had a higher metabolic rate.

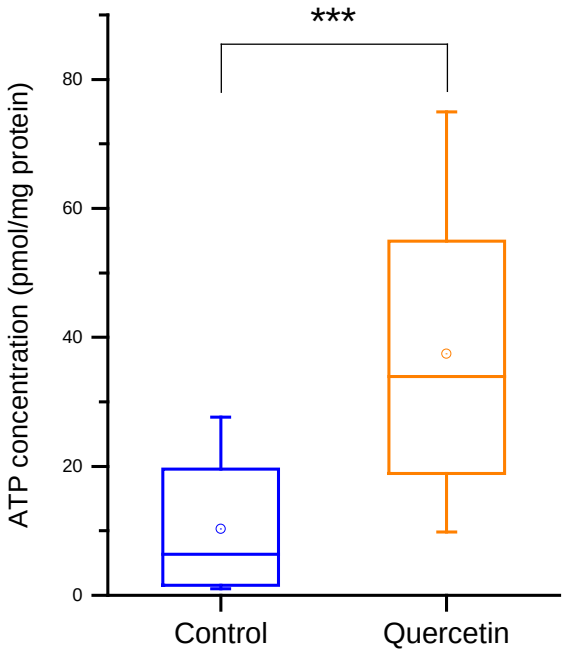


**Figure 6**. Box plots showing the level of ATP in the flight muscle of foragers from the quercetin-treated and control colony. The circle with a central point indicates the mean. The middle line of box shows the median value, the box delimits the 25th and 75th percentiles. The ends of the whiskers indicate the minimum and maximum of all the data. The asterisk indicates significant difference between the two means (*** = $p < 0.0001$, Mann–Whitney $U$ test).

2b.Effects of the phytochemical quercetin on fungicide suppression of flight performance: Consuming quercetin potentially facilitates faster flight. The foragers from the long-term dietary quercetin colony exhibited a higher wing-flapping frequency (183.27 ± 2.93 Hz, mean ± SE) than did the foragers from the solvent control colony (171.65 ± 2.48 Hz, mean ± SE; t = 3.03, df = 20, p < 0.01) in the same environment and with the same level of stimulation (**Figure 7A**). However, the duration of each flight trial was not affected by long-term exposure to dietary quercetin (t = -1.20, df = 20, p =0.25 > 0.05) (**Figure 7B**), although there was a trend for foragers from the quercetin-treated colony to fly for a shorter period (1499.13 ± 46.65 s, mean ± SE) than did foragers from the control colony (1589.61 ± 59.60 s, mean ± SE). Also, the total number of wing flaps per flight was not affected by the quercetin treatment (268592.86 ± 8365.25 vs 267909.08 ± 11280.10, mean ± SE; t = 0.05, df = 20, p = 0.96 > 0.05) (**Figure 7C**).

In contrast, foragers consuming boscalid alone exhibited the lowest frequency of wing-flapping {(**Figure 8A**) boscalid: 180.85 ± 3.31 Hz; control:190.98 ± 1.88 Hz, mean ± SE; boscalid vs control: U = 154, Z = 2.12, p < 0.05}. Consuming quercetin eliminated the adverse effects of boscalid on flight performance of foragers (boscalid plus quercetin: 198. 29 ± 2.04 Hz, mean ± SE). Consuming food containing both boscalid and quercetin appeared to increase wing-flapping frequency (boscalid plus quercetin vs. control: U = 62, Z = -2.07, p < 0.05; boscalid plus quercetin vs. boscalid: U = 24, Z = -3.51, p < 0.001). Foragers consuming diets with boscalid

plus quercetin displayed the highest wing- flapping frequency among the three tested groups. Those on a solvent diet showed a mid- range wing-flapping frequency.
In addition, neither the foragers from the colony consuming boscalid (1154.53 ± 46.34 s, mean ± SE) nor those from the colony consuming boscalid plus quercetin (1068.94 ± 27.92 s, mean ± SE) demonstrated any significant differences in terms of flight duration (control: 1154.53 ± 46.34 s) (**Figure 8B**).

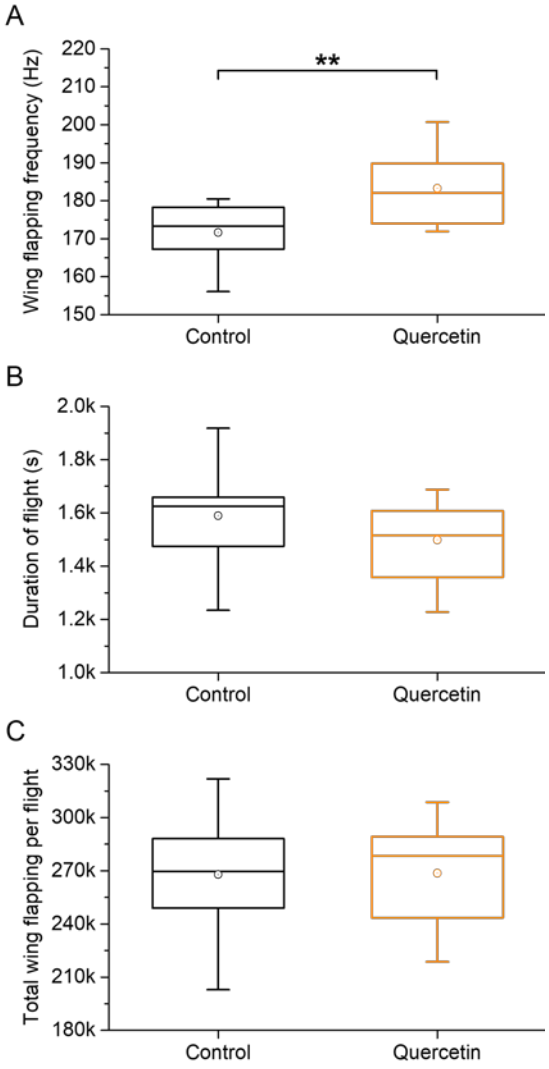


**Figure 7.** Effect of quercetin-treated foragers on the flight performance of foragers. (A) wing-flapping frequency; (B) duration of flight performance; (C) total wing flapping per flight performance. In each panel, the circle with a central point indicates the mean, the middle line of each box shows the median value, and the box delimits the 25$^{th}$ and 75$^{th}$ percentiles. The ends of the whiskers indicate the minimum and maximum of all the data and the asterisk indicates a significant difference between the two means (** = $p < 0.01$, two-sample *t*-test).

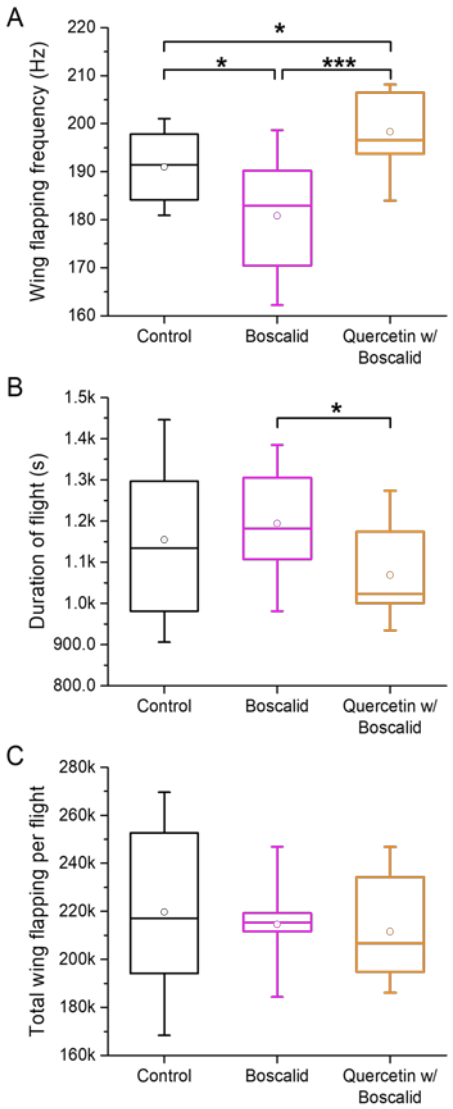


**Figure. 8.** Effect of dietary boscalid, quercetin and quercetin plus boscalid on flight performance of foragers. (A) wing-flapping frequency; (B) duration of flight; (C) total wing-flapping per flight. In all panels (A, B and C), the circle with a central point indicates the mean, the middle line of the box shows the median value, and the box delimits the 25th and 75th percentiles. The ends of the whiskers indicate the minimum and maximum of all the data and the asterisk indicates a significant difference between the two means (* = $p < 0.05$, *** = $p < 0.0001$, Mann–Whitney *U* test).

However, flight duration was significantly shorter in foragers that consumed both quercetin and boscalid than in those consuming boscalid only ($U = 164$, $Z = 2.553$, $p = 0.011 < 0.05$). Despite the difference in flight duration, the mean value of wing flaps per flight was unaffected by consuming either boscalid or boscalid plus quercetin (**Figure 8C**).

The reduction in flight performance caused by boscalid could be due to mitochondrial dysfunction in flight muscle. DeGrandi-Hoffman *et al.* (2015) found that bees fed treated pollen with fungicide (Pristine®), which includes boscalid as an active ingredient, had lower ATP concentrations in their flight muscles, which suggests fungicidal interference with mitochondrial respiration. Additionally, Campbell *et al*. (2016) found that Pristine® inhibits mitochondrial function *in vitro*. These findings are consistent with our results; moreover, our study provides evidence that quercetin may rescue foragers from energy deficiencies caused by boscalid and suggests that compensatory changes may occur in the mitochondria of bees consuming boscalid-quercetin sugar water.

Objective 3. Use in silico high-throughput screening of a diversity of both pesticides and phytochemicals to develop a predictive algorithm for a priori prediction of risks associated with application during bloom: We used *in silico* high-throughput docking of 8757 chemical forms (118 unique pesticides and metabolites) to identify possible substrates of CYP9Q1-3, known to metabolize quercetin (Mao et al., 2011). Using the Biovia software package, we identified 92 compounds that can dock in the active pocket of CYP9Q1, including 23 fungicides, 18 herbicides, and 12 acaricides. In the CYP9Q2 active pocket, 118 candidates could be docked, including 29 fungicides, 19 herbicides, and 13 acaricides. As for CYP9Q3, 51 candidate compounds dock, including 14 fungicides, 10 herbicides, and 9 acaricides. Moreover, 48 candidates can dock in all three CYP9Q enzymes, including 13 fungicides, 10 herbicides, and 9 acaricides.

**Table 1.** Summary of docking results for pesticides and metabolites in *Apis mellifera* CYP9Q1, CYP9Q2, and CYP9Q3

| class | CYP9Q 1 | CYP9Q 2 | CYP9Q 3 | CYP 9Q 1, 2, and 3 |
|---|---|---|---|---|
| **insecticide** | 46 | 64 | 23 | 21 |
| **fungicide** | 23 | 29 | 14 | 13 |
| **herbicide** | 18 | 19 | 10 | 10 |
| **acaricide** | 12 | 13 | 9 | 9 |

**Table 2.** Docking results for pesticides and metabolites in *Apis mellifera* CYP9Q1, CYP9Q2, and CYP9Q3

| Pesticides/metabolites | CYP9Q1 | CYP9Q2 | CYP9Q3 |
|---|---|---|---|
| -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 | |

| Pesticides/metabolites | CYP9Q1 | CYP9Q2 | CYP9Q3 |
|---|---|---|---|
| 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 | |
| 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 | |

| Pesticides/metabolites | CYP9Q1 | CYP9Q2 | CYP9Q3 |
|---|---|---|---|
| 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 |

**Recent Publications Related to this Project:**

Liao L.H., W.Y. Wu, M.R. Berenbaum (2017) Impacts of dietary phytochemicals in the presence and absence of pesticides on longevity of honey bees (*Apis mellifera*). Insects 8:22.

Liao L.H., Wu W.Y., M.R. Berenbaum MR (2017) Behavioral responses of honey bees (*Apis mellifera*) to natural and synthetic xenobiotics in food. Sci. Rep. 7(1):15924, doi: 10.1038/s41598-017-15066-5.

**References Cited:**

Campbell J.B., R. Nath, J. Gadau, T. Fox, G. DeGrandi-Hoffman, J.F. Harrison. (2016) The fungicide Pristine® inhibits mitochondrial function *in vitro* but not flight metabolic rates in honey bees. J. Insect Physiol. 86: 11-16.

Costa A.C., S.H. Loh, L.M. Martins. (2013) *Drosophila Trap1* protects against mitochondrial dysfunction in a PINK1/parkin model of Parkinson's disease. Cell Death Dis. 4: e467.

DeGrandi-Hoffman G., Y. Chen, E. Watkins Dejong, M.L. Chambers, G. Hidalgo. (2015) Effects of oral exposure to fungicides on honey bee nutrition and virus levels. J. Econ. Entomol. 108(6): 2518-2528.

Liao L.H., W.Y. Wu, M.R. Berenbaum (2017) Impacts of dietary phytochemicals in the presence and absence of pesticides on longevity of honey bees (*Apis mellifera*). Insects 8:22.

Mao, W., R. M. Johnson, S. Rupasinghe, M. A. Schuler and M. R. Berenbaum (2009) Quercetin-metabolizing CYP6AS enzymes of the pollinator *Apis mellifera* (Hymenoptera: Apidae). Comp Biochem Physiol C: Toxicology and Pharmacology 154: 427-434.

Mao W., M.A. Schuler, M.R. Berenbaum. (2011) CYP9Q-mediated detoxification of acaricides in the honey bee (*Apis mellifera*). Proc. Natl. Acad. Sci. USA 108(31): 12657-12662.