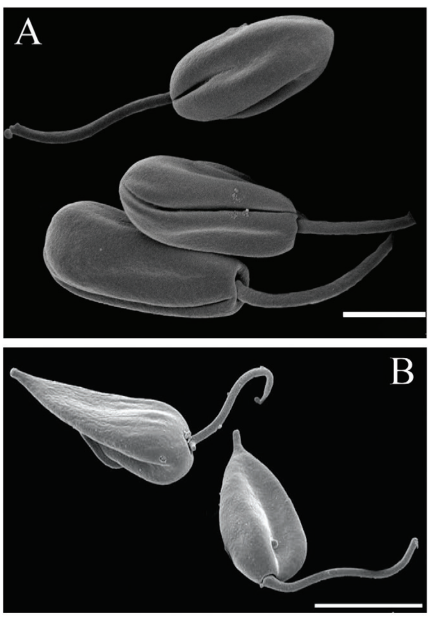
Notes from the Lab: The Latest Bee Science Distilled

by Scott McArt

Prospecting for plant toxins that will keep bees healthy

COVID has made me hypervigilant about the slightest tickle in my throat. But even in pre-COVID times, I and pretty much everyone I know had something they would take when they felt that tickle. Green tea, echinacea, garlic, cloves, perhaps a tincture of propolis?

Each of these herbal remedies has something in common: They contain phytochemicals (i.e., plant toxins) with known antimicrobial capabilities. Let's take cloves, for example. That strong "clove taste" comes from eugenol, an aromatic oil that can kill bad microbes, including *Leishmania*, a parasite that's transmitted by sand flies and causes Leishmaniasis in humans. If you didn't know, Leishmaniasis is a major threat to human health in the tropics, subtropics, and southern Europe.



Photos from Schwarz et al. (2015)

Photo 1 *Scanning electron micrograph (SEM) photos of the honey bee trypanosomatid parasites* Crithidia mellificae *(A) and* Lotmaria passim *(B). Scale bars are 2 µm and 4 µm for (A) and (B), respectively.*

As with anything in toxicology, the dose makes the response. If people consume too much eugenol, it will cause severe liver damage. But a small amount can be good, especially if you're trying to stay healthy by controlling a microbial infection.

What does this have to do with bees? Well, of course bees also have to ward off harmful microbes, including viral, bacterial, fungal, and protozoan parasites. Since bees are constantly consuming phytochemicals in pollen and nectar, is there any evidence some of these toxins are antimicrobial and therefore potentially ward off disease? If so, are there phytochemicals that are particularly promising in terms of their therapeutic potential for in-hive treatments? These are the topics for the fifty-fourth Notes from the Lab, where I summarize ***"Punch in the gut: parasite tolerance of phytochemicals reflects host diet,"*** written by Evan Palmer-Young and USDA Bee Research Lab colleagues and published in the journal *Environmental Microbiology* [2022].

For their study, Palmer-Young and colleagues focused on three trypanosomatid parasites: *Crithidia mellificae*, *Lotmaria passim*, and *Crithidia fasciculata*. *Crithidia mellificae* and *L. passim* are protozoan gut parasites of honey bees (see Photo 1) that are transmitted in feces. Infection is associated with reduced nutrient absorption and increased mortality in honey bee workers. *Crithidia fasciculata* is a parasite of mosquitoes and is similar to the bee-associated trypanosomatids in morphology and fecal-oral transmission. Many mosquitoes consume nectar as adults, potentially exposing them to phytochemicals. However, *C. fasciculata* also infects mosquito larvae and as a result, the parasite is likely exposed to a variety of phytochemicals in woody debris-rich aquatic breeding habitats.

The authors' goal was to test how a wide variety of plant phytochemicals did or didn't inhibit growth of each parasite. To do this, they cultured each parasite in the lab and performed inhibition assays using 25 different phytochemicals (see Photo 2, Table 1). Each phytochemical was chosen based on previously demonstrated inhibitory activity against *Leishmania*, which is the most well-studied trypanosome parasite due to its serious risk to human health.

To assess inhibitory activity, the authors measured parasite growth rates when each parasite was exposed to eight different concentrations of each of the 25 phytochemicals. Using these data, they were able to calculate IC50 values for each phytochemical-parasite combination. IC50 is the con-

Jay Evans

Photo 2 *Lead author Evan Palmer-Young (top left) and team working in the USDA Beltsville Bee Research Lab*

centration of a chemical that inhibits parasite growth by 50% compared to controls.

So, what did they find? Did any of the phytochemicals kill parasites? Yes. As seen in Table 1, 12 of the 25 phytochemicals (48%) inhibited growth of at least one of the three parasites. All chemicals that inhibited a parasite are colored yellow through orange, corresponding to increased inhibitory activity. Low numbers mean a small amount of the chemical was required to reach the IC50, high numbers mean a large amount of the chemical was required, and unshaded cells with a '>' sign followed by a number indicate there was no inhibition at the highest tested concentration.

The three chemicals with the strongest inhibitory effects (IC50 < 100 µg/ml for all species and strains) were the antileishmanial *Streptomyces* metabolite amphotericin B (IC50 range from 0.59 for *L. passim* to 2.32 for *C. mellificae*) and the terpenoids thymol (from 28.3 for *L. passim* to 54.1 for *C. mellificae*) and its isomer carvacrol (from 41.1 for *C. fasciculata* CFC1 to 65.7 for *C. mellificae*).

Were there differences between the honey bee vs. mosquito parasites in susceptibility to phytochemicals? Yes. Differences were found for the flavonoid chrysin (IC50 > 50 µg/ml for both *C. mellificae* and *L. passim* vs. 6.65 µg/ml for *C. fasciculata* CFC1 and 11.7 µg/ml for *C. fasciculata* Wallace), meaning there was more than a four-fold higher tolerance among the bee parasites. Interestingly, chrysin is a flavonoid found in nectar, pollen, and plant resin-derived propolis. Perhaps the bee parasites have evolved tolerance to chrysin since they're often exposed to this phytochemical in bee guts? Similarly, bee parasites were also more tolerant of the alkaloid piperine, which gives black pepper (i.e., the pepper on your dinner table) its biting taste.

In contrast, tolerance of cinnamic acid and vanillin were over two-fold

Class	Compound	*Crithidia mellificae*	*Lotmaria passim*	*C. fasciculata CFC1*	*C. fasciculata Wallace*
Polyene	Amphotericin B	2.32	0.59	1.5	1.29
Flavonoid	Chrysin	>50	>50	11.69	6.65
Flavonoid	Hesperetin	>50	>50	>50	>50
Flavonoid	Luteolin	>50	>50	>50	>50
Flavonoid	Naringenin	>50	>50	>50	>50
Flavonoid	Quercetin	>50	>50	>50	>50
Flavonoid glycoside	Hesperidin	>50	>50	>50	>50
Flavonoid glycoside	Rutin	>200	>200	>200	>200
Phenylpropanoid	Caffeic acid	>400	>400	>400	>400
Phenylpropanoid	Cinnamaldehyde	94.78	90.27	127.73	148.89
Phenylpropanoid	Cinnamic acid	194.89	413.84	>800	>800
Phenylpropanoid	Coumaric acid	>400	>400	>400	>400
Phenylpropanoid	Eugenol	264.23	175.95	146.89	163.53
Benzenoid	Gallic acid	>500	>500	>500	>500
Benzenoid	Methyl salicylate	>500	>500	>500	>500
Benzenoid	Vanillin	677.08	362.3	1487.36	1146.06
Terpenoid	Carvacrol	65.7	50.61	41.08	52.36
Terpenoid	Linalool	>500	>500	>500	>500
Terpenoid	Thymol	54.1	28.3	31.01	39.56
Alkaloid	Berberine	115.81	27.43	>100	186.47
Alkaloid	Piperine	45.3	60.56	6.09	9.22
Oxylipin	Methyl jasmonate	245.74	162.54	124.02	172.3
Extract	Clove	279.83	181.09	159.64	168.29
Extract	Eucalyptus	>500	>500	>500	>500
Extract	Tea tree	>500	>500	>500	>500

Table 1 *Estimates of 50% inhibitory concentrations (IC50, in µg/ml) for the tested trypanosomatids from honey bees (*Crithidia mellificae* and* Lotmaria passim*) and mosquitoes (*C. fasciculata*). To aid comparisons across strains and compounds, cells are shaded from yellow to red in order of decreasing IC50 value (i.e., decreasing tolerance of strains and increasing inhibitory activity of compounds). Cells of the table for which IC50 values could not be calculated due to insufficient inhibition are indicated by a '>' sign followed by the highest tested concentration; these cells are left unshaded.*

Peggy Grebs

Photo 3 *Part of the USDA Beltsville Bee Research Lab pollinator garden!*

higher among the mosquito parasites. Cinnamic acid is a product of lignin decomposition and is present in the woody debris-rich aquatic larval habitats of mosquitoes, perhaps suggesting a selective pressure for tolerance by the mosquito parasite.

One intriguing finding was the high tolerance of both bee and mosquito parasites to compounds that are highly toxic to *Leishmania* species parasites. These compounds include several flavonoids, which in addition to being found in tea and wine are abundant in nectar and (especially) pollen. Perhaps further study of the parasites from bees and mosquitoes, which are routinely exposed to such phytochemicals, could give us clues about how to prevent resistance to flavonoids (or drugs based on them) among *Leishmania* parasites of humans.

What about therapeutics? Do any of the phytochemicals show promise as a therapeutic that I can give to my bees? Yes. And this is where the mission of the USDA Bee Research Lab really starts to shine. Unfortunately, the chemical with the strongest parasite inhibition, amphotericin B, is also known to damage the membranes of insect cells. That's of course bad news for bees! But two of the phytochemicals — thymol and eugenol-rich clove oil — show good selectivity for honey bee parasites compared to honey bees themselves. In other words, the parasite IC50 for thymol is 18-35 times lower than thymol's LD50 for honey bees. This means a dose of thymol that will kill *C. mellificae* and *L. passim* is unlikely to harm honey bees. Similarly, the parasite IC50 for eugenol-rich clove oil is 28-43 times lower than its LD50 for honey bees.

Currently, some thymol products that target varroa are being sold by retailers. However, no product exists that uses thymol or eugenol to combat trypanosome microparasites. Clearly there's an opportunity for further development. And who knows, further research may reveal that these phytochemicals (or others) could be effective at controlling other microparasites such as nosema, chalkbrood, EFB, or AFB. Indeed, the study by Palmer-Young and colleagues is a very nice start on this topic, but as I think about the amazing amount of plant diversity and even more amazing amount of phytochemical diversity in the world, I'd say there's quite a bit more bioprospecting that can be done to keep our bees healthy.

Until next time, bee well and do good work.

Scott McArt

References:

Palmer-Young, E. C., R. S. Schwarz, Y. Chen & J. D. Evans. 2022. Punch in the gut: parasite tolerance of phytochemicals reflects host diet. *Environmental Microbiology*. **https://doi.org/10.1111/1462-2920.15981**

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For a video summary of the research paper by the authors, see **https://www.youtube.com/watch?v=u_HY0VakaDo**

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