

# **Glyphosate and its Effects on Bee Gut Microbiomes and Bee Decline**

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## **Introduction**

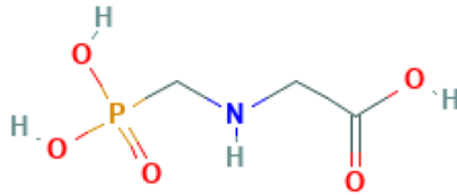
Glyphosate is a broad-spectrum herbicide primarily used in conjunction with genetically engineered crops and in urban landscapes for weed management (*Glyphosate*, 1993). Bumble bees and honey bees pollinate flowering plants and can be exposed to glyphosate while foraging. In this article, we summarized the results from the recent studies to explain how glyphosate's sublethal effects alter the bee's gut microbiome, impair cognitive and foraging abilities, and contribute to bee decline.

## **Glyphosate**

Glyphosate is a water soluble, non-volatile, corrosive, phosphonic acid derived from glycine that does not photochemically degrade (Fig. 1) (Tomlin, 2005). It is a white, odorless powder that is typically combined with other components such as polyethoxylated tallow amine (POEA) and alkyl polyglucoside (APG) (*PubChem* Database). These improve adhesion and absorption, and increase its toxicity to target plants and non-target organisms, like bees (Zhu et al., 2014). Glyphosate mimics and occupies the binding site of the second substrate, phosphoenol pyruvate (Schonbrunn et al., 2001), inhibiting the enzyme 5-enolpyruvyl-3-shikimate phosphate synthase (EPSPS). Such modes of action of Glyphosate has been found in plants and some microorganisms, including gut biota (Cerdeira et al., 2006; Motta et al., 2018). The inhibition of enzyme EPSPS activity in the plant and microorganisms can lead to organism death from lack of aromatic amino acid and subsequent protein formation.

Glyphosate is a fungicide, bactericide, and herbicide (Morjan et al., 2002; Motta et al., 2018; Tomlin, 2005). Its prolific use spans various applications in agricultural, forested, urban, and aquatic environments (Tomlin, 2005). A 2016 study showed that glyphosate is the most widely and heavily applied herbicide in US and global history, and will "likely

contribute to a host of adverse environmental and public health consequences.”  
(Benbrook, 2016).



**Figure 1.** The chemical structure of glyphosate ( $C_3H_8NO_5P$ ). The structure of glyphosate has an oxidative coupling between methylphosphonic acid and the glycine amino group  
(<https://pubchem.ncbi.nlm.nih.gov/compound/Glyphosate#section=2D-Structure>).

### **The effect of genetically engineered Canola for glyphosate resistance on bee decline**

Genetically modified (GMO) Canola (*Brassica napus*) was commercially released in 1998. It was engineered to be resistant against routine sprays of glyphosate (and additional component) herbicide (Schafer et al., 2011). Large monocultures of GMO Canola, and other modified products, have replaced natural habitats resulting in diverse floral resource loss (O'Brien, et al, 2018).

Approximately 2 million acres of Canola were planted in the US in 2019 (Acreage 2019); 93% being from genetically modified seed (Nestlé USA), and 10.1 Million hectares of GMO Canola were planted worldwide in 2018 (ISAAA 2019). In the US, almost 220,000 pounds of glyphosate was sprayed on GMO canola in 2014, however little or no governmental data has been published regarding glyphosate sprayed on GMO Canola since 2016 (Benbrook, 2016). Overspray, from glyphosate sprayed fields, kills beneficial organisms and vegetation around the fields, thereby additionally removing alternative food sources and nesting areas for bees (Blitzer et al., 2012; O'Brien et al., 2018). This lack of habitat and nutritional options may directly correlate to colony losses (Naug 2009).

While Canola doesn't require bees for pollination, the yield is significantly enhanced and its functional characteristics are beneficially modified (e.g., size and shape of the plant,

flower bloom timing, and root biomass) from bee pollination (Adamidis et al., 2019). Pollinators can increase Canola seed yield up to 46% when stocked with three hives per acre (Sabbahi et al., 2005). 55,000 – 75,000 Honey bee colonies are rented annually to pollinate Canola fields in Canada (Hoover et al., 2018).

Canola pollen and nectar are attractive, abundant, and robust food sources for pollinators (O'Brien et al., 2018). Canola pollen has an average 23% high, crude-protein content and in itself (Ellis et al., 2017). Genetically modified Canola pollen does not appear to harm bees (Huang et al., 2004). Bumble bees, ground-dwelling bees, and honey bees have all been shown to frequent Canola fields for food foraging (O'Brien et al. 2018; Galpern et al., 2017) where they can become exposed to Glyphosate in agricultural settings.



**Figure 2.** Canola flower. Canola pollen (*Brassica napus*) provides 23% crude protein to pollinators (Ellis et al., 2017). Photo credit: photograph by Honeyhuyue, distributed under CC BY-SA 3.0 license.

### **The impact of glyphosate on bee behavior**

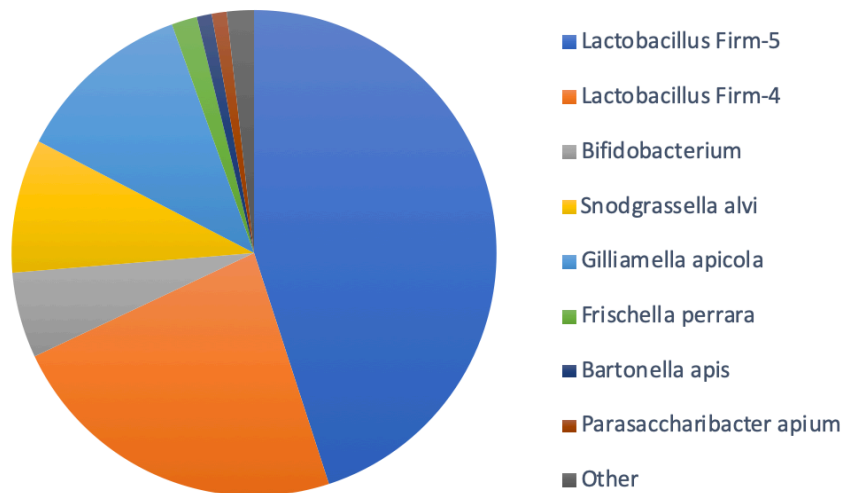
Honey bee navigation is affected by ingesting trace amounts of glyphosate. Foraging bees fed glyphosate were found to take more time returning to their hive compared to the control group (Balbuena et al., 2015). They also had significantly decreased short-term memory retention and diminished cognitive capacities (Herbert et al., 2014). Glyphosate is shown to cause impaired learning performance, diminished olfactory memory processes, and reduced sensitivity to sucrose (found in nectar). Bees also show a diminished capacity to associate an odor with a reward, raising concerns for food foraging ability and nectar processing (Herbert et al., 2014). These effects were not

immediately shown but more as a result of the glyphosate taken back to the hive, stored for periods of time, and fed to the young bees as they developed (Herbert et al., 2014).

### The dominant microbiome functioning in bee's gut

The honey bee gut microbiome is dominated by nine ubiquitous, gut-restrictive species clusters, accounting for over 98% of total microbes in bee's gut (Fig. 3) (Zheng et al., 2018). These bacteria regulate endocrine signaling, nutritional uptake, immune function, detoxification, and pathogen resistance (Johnson 2015; Zheng et al., 2018). Similar bacterial species have been found in western honey bee (*Apis mellifera*), black dwarf honey bee (*Apis andreniformis*), giant honey bee (*Apis dorsata*) and several bumble bee species (*Bombus spp.*) (Martinson 2010). These gut bacteria species are specialized, have a coevolved, symbiotic relationship with their bee host (Zheng et al., 2018), and are absent in solitary bee guts, leading to the understanding that they are spread through social transmission (Koch et al., 2011).

**Relative Abundance of Bacterial Species Found in the Honey Bee Gut**



**Figure 3.** The relative abundance of the primary host-adapted, bacterial taxa found in the guts of the honey bee (*Apis mellifera*). These clusters comprise 95-99.9% of all bacteria found in adult workers (Kwong et al., 2016). [Chart adapted from Kwong, W. K., & Moran, N. A. (2016)].

Bees undergo complete metamorphosis with four complete life stages (egg, larva, pupa, and adult). The bacterial community changes through the bee's lifecycle (Martinson et al., 2010). Egg cells, larval guts, and newly emerged worker bees are generally devoid

of bacteria and are socially colonized within the first three days of emergence (Martinson et al., 2012) (Zheng et al., 2019). Bees denied bacteria colonization show an increase in mortality (Raymann et al., 2017), pathogen susceptibility (Koch et al., 2011), hormonal signaling, and weight loss (Zheng et al. 2017).

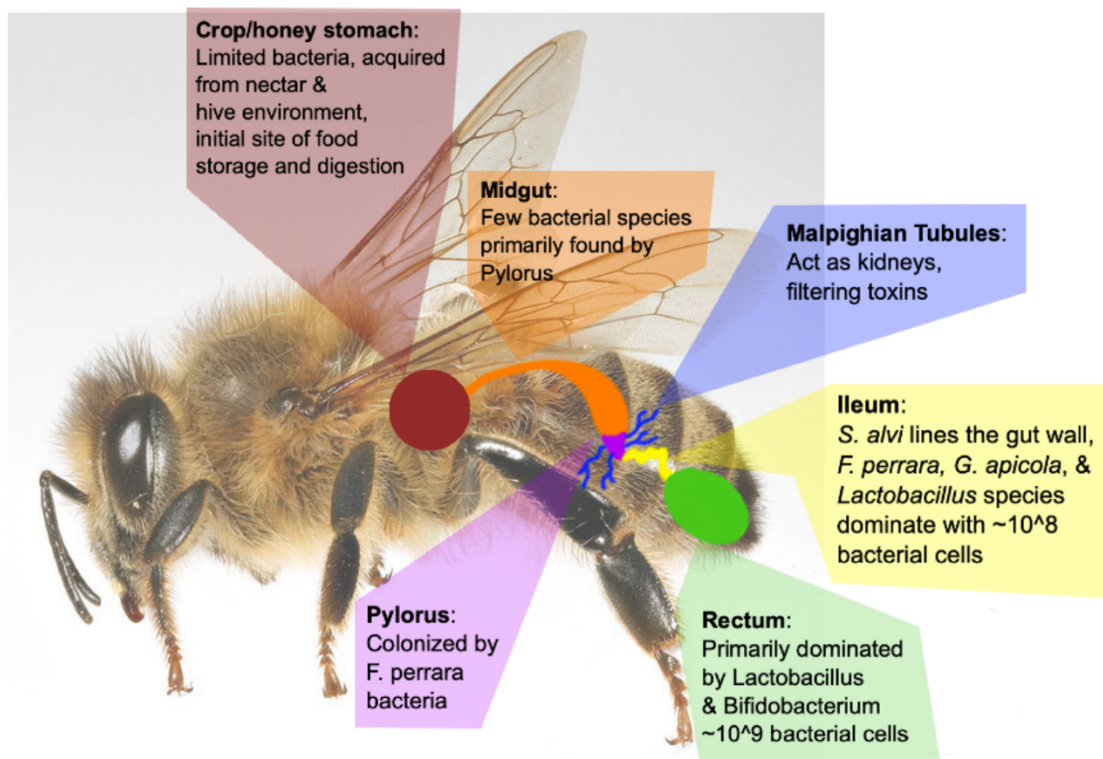
Poor nutrition results in negative effects on bee health and is understood to be a contributing factor in bee decline (Goulson et al., 2015). Gut bacteria, *Lactobacillus* and *Bifidobacterium* are required by bees for nutrient breakdown and bee bread processing (Vasquez et al., 2009). Bees denied these common gut bacteria showed nearly 50% in weight reduction. Bee gut bacteria would normally reduce the pH and redox potential of the bee gut, create an oxygen gradient along the gut lining allowing for anaerobic digestion, and excrete amino acids that increase the expression insulin and vitellogenin levels, all contributing to nutrition uptake and subsequent weight gain (Zheng et al., 2017).

Bees gather nectar for carbohydrates and water, and pollen for protein, free amino acids, lipids and other micronutrients (Hendriksma et al., 2016). Foragers primarily utilize nectar carbohydrates because of their increased metabolism (Crailsheim 1990). Young bees and developing larva require more protein-rich diets, like bee bread that has been processed and fermented by the bees and lactic acid bacteria within the bee's gut (Vasquez et al., 2009). Gut bacteria also degrade pollen polymers and complex carbohydrates, like pectin from the cell wall, releasing metabolites, that enable bees to intake nutrition (Zheng et al., 2017).

### **Glyphosate disrupts the enzyme activities in the gut microbiome of bees**

As described above, glyphosate targets the EPSPS enzyme required for amino acid and subsequent protein formation (Motta et al., 2018). This protein formation pathway is present in the majority of bacteria living in the bee gut (Motta et al., 2018). While glyphosate doesn't directly kill all bacteria in the bee's gut, it does create a domino effect that harms, or perturbs, the interdependency of the microbiome.

*S. alvi* and *Bifidobacterium* (Figure 4, Table 1) produce a glyphosate-susceptible EPSPS; their population numbers drop, showing no growth or a delay in growth when exposed to glyphosate. Conversely, Firm-4 and Firm-5 *Lactobacillus* strains lack the genes that encode for the aromatic amino acid biosynthetic pathway for EPSPS production (Kwong et al., 2016). (Figure 4, Table 1). However, the population of Firm-4 and Firm-5 were still negatively impacted when exposed to glyphosate. As Firm-4 and Firm-5 are unable to create the required amino acids themselves, it is believed they may instead require intake of the amino acids, released from other bacterial strains, from the bee hindgut environment (Motta et al., 2018).



**Figure 4.** Schematic showing an adult worker bee, *Apis mellifera*, and the distribution of the top five bacterial phylotypes. Figure adapted from Kwong, W. K., & Moran, N. A. (2016). *Apis mellifera* photo credit: [https://www.discoverlife.org/mp/20p?see=I\\_MWS100597&res=640](https://www.discoverlife.org/mp/20p?see=I_MWS100597&res=640)

Bee gut microbes biosynthesize a specific heme-thiolate enzyme group called cytochrome P450 monooxygenases (CYP) (Rand et al., 2015). CYP genes are upregulated in honey bees after honey, propolis, or pollen ingestion (Johnson, 2008), as both pollen and nectar sources have natural phytotoxins requiring breakdown before

ingestion by the bees (Kevan et al., 2005; Mesquita et al., 2010). These enzymes oxidize, lipids (as fatty acids), sterols, and xenobiotics.

Worker bees show significantly higher CYP enzyme expression in the Malpighian tubules; branches extending from the hindgut that absorb toxins and wastes from the hemolymph. Other members of the colony do not show this amplified CYP expression resulting in the inability to adequately filter out additional toxins brought back to the hive by the workers (Vannette et al., 2015).

Detoxification of phytotoxins and xenobiotics requires lipid-soluble substances to be converted into water-soluble substances that are then excreted as metabolites (Berenbaum et al., 2015). CYPs detoxify the lipophilic pyrethroid, Tau-fluvalinate, and organophosphate, and coumaphos, that are common miticides used by beekeepers to control the *Varroa destructor* mite, an invasive parasite of the honey bee (Johnson, 2008).

Without bee gut microbes, transcription of the genes for these enzymes is reduced, resulting in delayed molting and reduced weight in bee larva (Vazquez et al., 2018) and the inability to breakdown both natural, food source phytotoxins and anthropogenically produced xenobiotic compounds. Glyphosate acts as an antibiotic that has persistent effects on the size and composition of the gut microbiome; thereby increasing the bees' exposure to opportunistic pathogens and disabling their ability to breakdown toxins (Berenbaum et al., 2015).

**Table 1.** The top eight bacterial taxa living in bees' gut, the relative abundance of these taxa across different bee species, and their potential function. The top five taxa are considered the core gut microbiome in *A. mellifera*, which include two species of *Lactobacillus*, *Bifidobacterium*, *Snodgrassella alvi*, and *Gilliamella apicola* (Kešnerová et al., 2017; Kwong et al., 2016). Single bees contain various, numerous, and distinct gut bacteria phylotypes (Kešnerová et al., 2017; Moran et al., 2012).

\* No published data available for the relative abundance of in the bees' gut

\*\* Most affected by Glyphosate

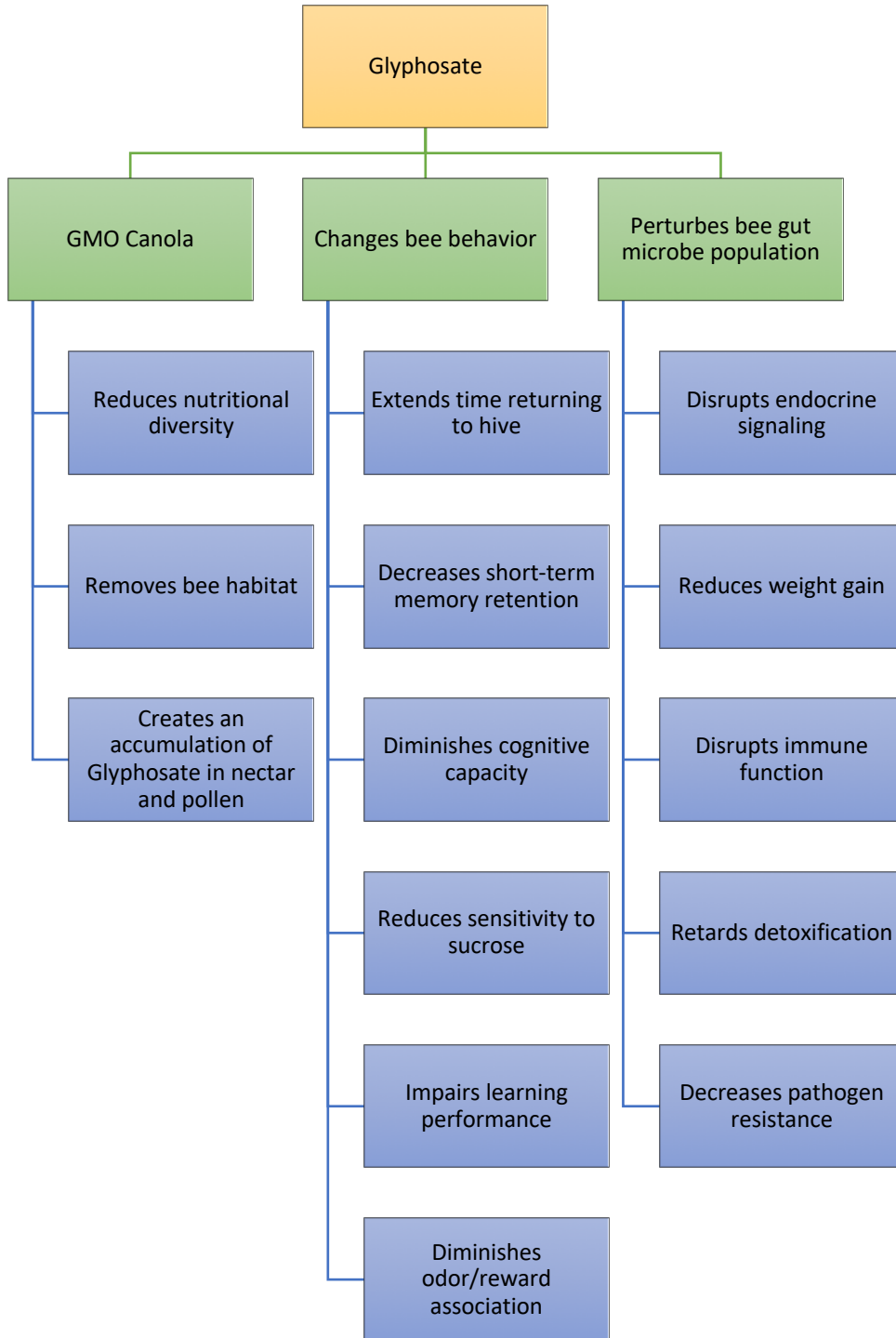
Ubiquitous Rank of gut microbiomes	Microbial Taxa	Scientific name of bee host	Phylum of microbial group & relative abundance (%) Found in gut	Function of specific taxa	Location	References
1	<i>Lactobacillus</i> sp. Firm-5: <i>L. apis</i> , <i>L. helsingborgensis</i> , <i>L. kimbladii</i> , <i>L. kullabergensis</i> , <i>L. melliventris</i>	<i>A. mellifera</i>	Firmicutes, 20-99%	<i>L. apis</i> , in vitro protective action against severe bee pathogens (American & European foulbrood), produces active proteins	Hindgut (ileum lumen)	Kešnerová et al., 2017; Killer et al., 2014; Moran et al., 2012; Olofsson et al., 2014; Raymann et al., 2018;
2	<i>Lactobacillus</i> sp. Firm-4: <i>L. mellifer</i> , <i>L. mellis</i>	<i>A. mellifera</i>	Firmicutes, 20-99%	Metabolize and digest recalcitrant plant carbohydrates	Hindgut (rectum)	Kešnerová et al., 2017; Moran et al., 2012; Raymann et al., 2018
3	<i>Bifidobacterium</i> sp.; <i>B. asteroides</i>	<i>A. indica</i> F, <i>A. mellifica</i> L., <i>Bombus</i> sp., <i>Xylocopa violacea</i>	Actinobacteria*	LAB, food production & perservation, honey & bee bread; Metabolize and digest recalcitrant plant carbohydrates; stimulate host hormone production	Hindgut (rectum), honey stomach	Alberoni et al., 2019; Bottacini et al., 2012; Kešnerová et al., 2017; Moran et al., 2012; Raymann et al., 2018;



4	** <i>Snodgrassella alvi</i>	<i>A. mellifera</i> ; <i>Bombus</i> sp.	Proteobacteria, 0.6-39%	Forms a protective biofilm on gut ileum; Consumes oxygen; maintains anoxic bee gut; modulate host immune function; upregulate antimicrobial peptides; increases resistance to infection.	Hindgut (ileum wall), epithelium	Kešnerová et al., 2017; Kwong et al., 2017; Moran et al., 2012; Motta et al., 2018; Raymann et al., 2018; Zheng et al., 2018
5	<i>Gilliamella apicola</i>	<i>Apis</i> & <i>Bombus</i> sp.	Proteobacteria, 0.6-30%	Metabolize and digest plant carbohydrates	Hindgut (ileum lumen)	Kešnerová et al., 2017; Moran et al., 2012; Raymann et al., 2018
6	<i>Frischella perrara</i>	<i>A. mellifera</i> , & <i>Apis</i> sp.	Proteobacteria*	Causes strong activation immune system response scab formation; Overgrowth influences dietary alteration & impairs host development	Hindgut (pylorus, ileum)	Kešnerová et al., 2017; Emery et al., 2017; Engel et al., 2015
7	<i>Bartonella apis</i> , Alpha 1	<i>A. mellifera</i> ; <i>B. lucorum</i> , <i>B. pascuorum</i> , <i>B. lapidarius</i>	Proteobacteria*	Increases in winter workers	Hindgut (Ileum)	Kešnerová, et al., 2017, 2019; Killer et al., 2010
8	<i>Commensalibacter</i> sp. "Alpha 2-1"	<i>A. mellifera</i>	Proteobacteria*	Increases in winter workers	Hindgut	Kešnerová et al., 2019

## Conclusion

Glyphosate is regularly used in agricultural and urban settings, further reducing areas where bees can safely forage and repopulate. Additionally, genetically modified Canola encourages bees to forage on flowers sprayed with Glyphosate. Glyphosate toxicity was originally tested safe for pollinators with a lethal dose of 50% for acute exposure (Herbert et al., 2014). More recent studies show low-dose, accumulated toxicity may perturb the bee gut environment and negatively impact a bee's ability to perform critical tasks within the colony (Figure 5). This directly and indirectly enhances the opportunity for colony collapse as worker bees bring trace amounts back to the hive, in nectar and pollen, that are unknowingly fed to the colony.



**Figure 5.** The potential effects of Glyphosate on bee decline.

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