**Abstract**

Bee declines have been reported at concerning rates world-wide for the last decade. These losses have been referred to as colony collapse disorder, which consists of multiple pressures affecting the survival of economically important bees. A common thread among contributing factors to colony collapse disorder is the presence of agrochemicals. Researchers have had difficulty quantifying the impact of agrochemicals on pollinators, particularly in super organisms like bumble bees and honey bees. We, therefore, investigated the sublethal impacts of agrochemicals on ritualistic behaviors in two bee species: *Apis mellifera* and *Bombus impatiens*. We specifically analyzed broad concentrations of two of the most popular pesticides used in the Midwest: (1) the insecticide thiamethoxam, and (2) the herbicide glyphosate. We tested each chemical independently in both species and then in combination in one species to get a complete understanding of the agrochemical effect the bees,. Additionally, we tested different casts of bees throughout the study to gain broader insights on the exposures. The findings of this study suggest that there is no immediate threat to the ritualistic behavior or survival of either species if exposed to sublethal concentrations of thiamethoxam or glyphosate.

**Introduction**

Western honey bees, *Apis mellifera*, are the most commercially managed bees due to their high value to agriculture world-wide. Specifically, honey bees are highly regarded for their honey, and depended upon for [pollination](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/pollination) of many important crops.1 Following *A. mellifera* in value are wild pollinators like bumble bees (*Bombus impatiens*), which are equally important to agriculture for their highly efficient sonication pollination style.2 During sonication pollination, bumble bees bite the stamen of the flower and vibrate to release pollen. This behavior, unique to bumble bees, is extremely efficient and highly beneficial to nectarless plants such as tomatoes and peppers.2 Because of their ability to transfer larger quantities of pollen more efficiently, bumble bees have secured themselves an even more effective pollinator than honey bees.2

Unfortunately, bumble bee and honey bee populations have been declining in North America and many other parts of the world. Colony declines are being recorded worldwide over the last few years.3 Many causal theories for bumble bee and honey bee colony declines have been postulated, however, in the absence of a single known cause the syndrome has been named colony collapse disorder (CCD).4 The hallmark of CCD has been missing adult workers among hives.4 The risk factors associated with CCD affecting both bumble bees and honey bees include (1) pathogens, such as Nosema, American/European foulbrood, and chalk brood which are typically spread throughout hives by contaminated food; (2) parasites, like *Varroa jacobsoni*, originally found in Asia and first characterized in1904.5

Two separate species of Varroa mites have been identified through genetic analysis, the first being the original species (*V. jacobsoni*), and the second classified as *Varroa destructor*.5 The latter has been identified as the species involved in European honey bee decline affecting both Europe and North America*. V. destructor* parasites reportedly attach themselves to honey bees during development and sucks the hemolymph of the bees, like ticks, consequently transmitting deadly viruses such as deformed wing.6 Astonishingly, honey bee drone (male bee) brood cells are infested with mites at a greater frequency than others of the same colony.5 Furthermore, while Varroa mites have been recorded residing and feeding on bumble bees, they are not known to successfully reproduce in bumble bee hives.7 Finally, further contributing to the colony declines are pesticides: namely the neonicotinoid imidacloprid, which was patented by Bayer in 1985.8 Imidacloprid became a popular choice of pest control by the 1990s, and two more neonicotinoids, clothianidin and thiamethoxam, followed also with tremendous market success.8 Bayer purchased the herbicide glyphosate from Monsanto in 2018.

Pathogens, parasites, and pesticides have synergistic effects on bees. For example, *V. destructor* weakens bee immune systems by giving them viruses that in turn make the bees vulnerable to other risk factors associated with CCD.4 Recent research shows that several bacteria species exist in the guts of honey bees that help reduce pathogen susceptibility, but which are also significantly reduced when exposed to field-realistic concentrations of glyphosate, thereby increasing pathogen load and mortality rates among the adult workers in the hive.9

Pollinators, especially bees have become a model organism for assessing xenobiotics in the environment because they are constantly surveying the landscape. Sublethal levels of pesticides like glyphosate, found in gathered resources like pollen and nectar, have been shown to have neurological affects in the brains of worker honey bees, disrupting key processes like navigation and memory.10 Bishop et al.11 detected 18.4 ng/g of imidacloprid in pollen collected from bumble bees foraging in conventionally sprayed blueberry fields, and from bees foraging in organic sites near the conventionally sprayed blueberry fields.

**Neonicotinoids**

One contributing factor in the increased use of agrochemical is the widespread usage of the neonicotinoid class of pesticides.12 Specifically since the market introduction of imidacloprid, neonicotinoids have become the fastest growing class of insecticides.12 The tremendous commercial success of the neonicotinoids can be explained by their unique chemical and biological properties, such as (1) broad-spectrum insecticidal activity, (2) low application rates, and (3) excellent uptake and translocation in plants.12 As a result of neonicotinoid chemistry, the chemical can be directly applied to the seed as a coating and taken up through the plant, which protects the plant for its entire life cycle while minimizing the amount of pesticide drift from the target site.13 Imidacloprid thiamethoxam and clothianidin neonicotinoids are thus the most widely used pesticides in the nation and are primarily used on corn and soybeans to target white grubs and scarab beetles.13 However, because these pesticides are such a popular choice among farmers, they have become a problem for non-target organisms like bees, which provide important pollination services in agriculture.

Neonicotinoids are, for instance, highly water soluble selective agonists of nicotinic acetylcholine receptors, which are important excitatory neurotransmitter cells, making them very toxic to insects but not mammals and birds.14 Neonicotinoids further have a similar structure to nicotine and can therefore bind directly to the nicotinic acetylcholine receptors on the postsynaptic neuron. This opens voltage-gated ion channels allowing the ions to flow freely across the neuromuscular junction, depolarizing the cell. Unlike acetylcholine, neonicotinoids are not degraded by the acetylcholinesterase enzyme, allowing for continuous synaptic stimulation and causing muscle cramps and paralysis in insects.8,15

Earlier studies show that neonicotinoids impact bees’ ability to forage, learn and remember navigation routes to and from food sources. Namely, as little as 0.1nM of neonicotinoid treated sugar solution is enough to confuse a worker bee concerning how to get back to its hive.16 Also, honey collected from beekeepers in the upper Midwest had as much as 10.0 ng/g of thiamethoxam in the honey.17

 Meanwhile, the effect of various insecticides on the reproductive systems of agriculturally important insects is poorly understood but becoming clearer. Yi-hua gu et al. 18 has, for example, shown that spermatozoa directly exposed to imidacloprid and acetamiprid during capacitation for 30 min significantly reduced fertilization ability, and Burley et al.19 reported drone spermatozoa exposed to miticide coumaphos (a non-neonicotinoid organophosphate insecticide) during development showed significantly reduced survival when compared to control samples. The findings from both of these studies support the report of Straub et al.,20 who demonstrated that agrochemicals and other environmental contaminants act as inadvertent contraceptives among bees by reducing sperm viability, among other reproductive mechanisms They specifically found that the neonicotinoids thiamethoxam and clothianidin reduced bee sperm viability by up to 39%.20

**Organophosphate Glyphosate**

N-(phosphonomethyl)glycine (Glyphosate) is a broad-spectrum organophosphate herbicide that was brought to market in the 1970s and steadily and increasingly used on in agriculture over the past five decades.21 It functions by inhibiting enzyme 5-enolpyruvylshikimate-3-phosphate (EPSPS) synthase (EC 2.5.1.19) production in the shikimate pathway, which is an aromatic amino acid pathway specific to plants and some microorganisms.9