Yet Another Suspect in CCD/Dwindling?

There have been discussions about neonicotinoids, poor nutrition, Nosema, and mysterious viruses. Now a soil pathologist points a finger at a suspect that's completely under our nose.

The following was written for the Center regarding CCD. Dr. Huber's profile is at the end of this article.

Is glyphosate a contributing cause of bee colony collapse disorder (CCD)?

(Submitted to the Center for Honeybee Research by Dr. Don D. Huber)

Introduction

Bee colony collapse disorder (CCD) is a growing threat to the efficient production of fruits, vegetables and nut crops, in addition to the critical role of bees as pollinators for numerous seed crops (Neumann and Carreck, 2009; Wines, 2013). CCD is characterized as a loss of adult (worker) bees from the hive that leaves the queen and immature bees (brood) inadequately attended even though there is adequate honey and other food present (van Engelsdorp et al, 2006; Wikipedia, 2013). The etiology (*reason)* of CCD is listed as unknown (NFIC, 2013) although neonicotinamid insecticides have been implicated in several studies through disruption of the endocrine hormone system (van Engelsdorp et al, 2006; Tapparo et al, 2012; Wikipedia, 2013) that causes bees to become disoriented and fail to return to the hive (NPIC, 2013).

Acute poisoning and disease leaving dead bees in and around the hive can generally be ruled out, although there is sometimes an increased incidence of *Nosema* and European foul brood (EFB) in stressed colonies that could be contributing factors in some cases (Pettis et al, 2012). Mineral nutritional deficiency is also suspected as a contributing stress factor in CCD (Ahmed, 2012) and malnutrition is the only universal condition found in all cases of CCD even though there is honey and bee-bread generally in the hive. This could be because of toxicity to the Lactobacillus and Bifobacterium species in the honey crop that digest the nectar and render the honey and bee-bread digestible (Ahmed, 2012).

Perhaps a more problematic cause of CCD has been over looked even though it is the most indiscriminately and extensively used chemical in agriculture and the environment. This organic phosphonate chemical that has been overlooked is the estimated 880 million pounds of the popular, broad-spectrum, systemic herbicide glyphosate (also marketed as Roundup®) used for broadcast weed control in general right-of-ways, home gardens, crop production, fallow fields, understory weed control in groves, vineyards, orchards, and parks; and for aquatic weed control in ponds and lakes. It is almost universally used on millions of acres of Roundup Ready® alfalfa, canola, corn, cotton, soybeans and sugar beets. An additional, more recent use has been as a crop desiccant prior to harvest for barley, beans, peas, peanuts, sugar cane, wheat, and for late season weed control in other crops.

These uses have created an extensive exposure level throughout the year with especially high concentrations in plants, air, water and soil during primary bee foraging periods. The exposure, physiological damage, and biological impact of glyphosate are consistent with all of the known conditions related to CCD as shown in Table 1. Of all of the potential individual factors implicated in CCD, glyphosate is the only compound extensively used world-wide where CCD occurs that impacts all of them. That compound, again, is the patented mineral chelator (USPTO, 1964), herbicide, and antibiotic (USPTO, 2000), glyphosate. New studies refer to this compound as the most biologically disruptive chemical in our environment (Samsel and Seneff, 2013). *(E. Note: Samsel and Seneff is worth reading the abstract on the link. You can download the entire PDF, which goes into the modern diseases glyphosate is creating.)*

Table 1. Common characteristics of glyphosate with CCD.

Glyphosate	CCD
Mineral chelator, lowers nutrients in plants	Malnutrition (the only universal condition for all CCD!
Antibiotic to beneficial bacteria (esp. *Lactobacillus* and *Bifidobacteria* spp.)	Loss of *Lactobacillus* and *Bifidobacteria* (critical beneficial bacteria for digestion)
(Low mineral content of plants)	
Neurotoxin	Neurological challenge
Endocrine hormone disruption	Disoriented
Immune suppressant	Suppressed immune system
Stimulates fungal pathogens	*Nosema* increased

Present throughout the foraging period High environmental exposure
Persistent, accumulative
Present in honey, nectar and other plant products

Glyphosate

Glyphosate is an organic phosphonate compound that was first patented as a broad-spectrum, cat-ionic metal chelator by Stauffer Chemical Company in 1964 (USPTO, 1964), as an herbicide by Monsanto Company in 1974 (USPTO, 1974), and as an antibiotic by Monsanto Company in 2000 (USPTO, 2000). All of these uses are based on its ability to 'grab onto' and form a chelate complex that immobilizes mineral nutrients such as Ca, Fe, Co, Cu, Mn, Mg, Ni, Zn, etc. (Glass, 1984). These metal nutrients serve as metal co-factors for various enzyme systems in plants, microorganisms, and animals. Once these metal nutrients are chelated by glyphosate in soil or plants, they become physiologically unavailable as co-factors for many enzymatic and other physiological functions.

The broad-spectrum toxicity of glyphosate to plants initially simplified weed control, especially with selectivity provided by genetically engineered glyphosate-tolerant (Roundup Ready®, RR) plants, so that glyphosate could be applied directly to the RR plants without killing them. This use has led to an estimated annual indiscriminate usage of 880 million pounds of this mineral-immobilizing herbicide and antibiotic in the US. There is nothing in the genetic engineering process, however, that does anything to the glyphosate that is applied to these plants that are foraged by bees.

Glyphosate is systemic in plants: As a phloem mobile chemical, glyphosate from foliar, stem, or root uptake is systemic in plants where it accumulates in flower and reproductive parts, root and shoot tips, and legume nodules (Huber, 2010; Johal and Huber, 2009). Much of the glyphosate will remain in the plant and it can accumulate over years in perennial plants such as alfalfa, vine, fruit, and nut crops and environmental perennial species. It is an active mineral chelator in the treated plant for as many as 8 to 15 days after application before becoming sequestered in flower parts, other meristematic tissues, or soil. As little as 12 gm/acre ($1/40^{th}$ of herbicidal rate and well below the general 12-16 % drift rate) inhibits root uptake and translocation of Fe, Mn, Zn and other nutrients so that plants exposed to glyphosate directly or through drift in air or water have lower nutrient content (Bellaloui et al, 2009, 2011; Bott et al, 2008, 2011; Cakmak et al, 2009; Eker et al, 2006; Huber, 2010, 2012; Zobiole et al 2012).

Minerals in glyphosate-tolerant plants may be impacted even more by glyphosate than those in non-tolerant plants since there is nothing in the genetic engineering that does anything to nullify the glyphosate and its chelating effect on mineral nutrients. Since plant products are the source of essential mineral nutrients, bees may become mineral deficient, malnourished, have a weakened immune system, and be more susceptible to infections and abiotic (*environmental*) stresses.

Direct toxicity of glyphosate: Glyphosate is not acutely toxic to bees, but is chronically toxic to animals, and, like the neonicotinamid insecticides, glyphosate is a neurotoxin and disrupts the endocrine hormone system at very low exposure rates (Antoniou et al, 2012; Gasnier et al, 2009) that are well below levels found in air, water, and, especially, plant tissues (Benbrook, 2012; Huber, 2012).

The 880 million pounds of glyphosate indiscriminately applied throughout the environment leaves glyphosate residues in plants and the environment that can lead to chronic diseases in animals such as autism, botulism, Parkinson's, difficale diarrhea (Clostridium difficile), immune suppression, *Salmonella* and numerous other diseases (Krueger et al, 2012; Shehata et al, 2012).

Disruption of the endocrine hormone system is associated with birth defects. The wide-spread cultivation of glyphosate-tolerant crops (alfalfa, canola, corn, cotton, sugar beets) since 1996 and use as a preharvest desiccant since 2000 have greatly increased the use of glyphosate (Benbrook, 2012; Yamada et al, 2009) and subsequent contamination of air, water, soil, and plant products consistent with the incidence of CCD (NPIC, 2013; Wikipedia, 2013).

Antibiotic activity of glyphosate: Glyphosate is a strong antibiotic and toxic to microorganisms possessing the Shikimate physiological pathway (Johal and Huber, 2009; Kremer and Means, 2009; Krueger et al, 2012; Shehata et al, 2012; USPTO, 2000)). Many of these sensitive microbes include beneficial bacteria such as *Lactobacillus* spp. and *Bifidobacterium* spp. that suppress pathogens such as *Clostridium*, *Salmonella*, *E. coli*, *Nosema*, and American (*Paenibacillus larvae*) and European foul brood (Ahmed, 2012; Clair et al, 2012; Krueger et al, 2012; Shehata et al, 2013). In the absence of these beneficial protective bacteria, the pathogens increase along with the toxins they produce (Krueger et al, 2012; Shehata et al, 2012).

Various fungal pathogens are especially increased in activity and virulence by glyphosate (Johal and Huber, 2009); Kremer et al, 2009; Krueger et al, 2012). All *Apis* species possess a similar *Lactobacillus* and *Bifidobacterium* species microbiota within the honey crop that is critical for collecting and transporting nectar to the hive as well as for the production of honey and bee-bread (Ahmed, 2012). Glyphosate is highly toxic to both of these bacterial species that are necessary for digestion of food and protection from pathogens (Ahmed, 2012; Wikipedia, 2013).

Exposure opportunity: Glyphosate is indiscriminately applied throughout the bee foraging period and is in significant amounts in air, water, and many plant parts frequented by bees. Although not highly volatile, it becomes airborne as drift and on particulate matter with significant levels detected in rain and ground water (USGS, 2012). It is highly water soluble and a common contaminate found in surface water from drift, run-off, or direct application to water for aquatic weed control. It is systemic and persistent in plants with as much as 80% accumulating in meristematic plant tissues such as flowers and buds frequented by bees and is found in honey collected by bees from contaminated flowers. The extensive cultivation of the many glyphosate-tolerant plants has permitted the application of glyphosate before, during, after, and throughout the foraging period of bees to greatly expand the environmental and plant exposure of bees to this organic phosphonate chemical.

This proposal is initiated to determine if glyphosate is a contributing factor in CCD by analyzing exposure of bees to this chemical and its effect on the two predominate bacteria that are essential for bee nutrition and health (Ahmed, 2012). The focus on insecticides and their acute toxicity may have resulted in over-looking the direct and indirect chronic effects of glyphosate as a contributing factor to bee colony collapse disorder.

Proposed Research (Analyses to compare healthy with CCD)

1. Analyze for glyphosate (and AMPA) in pollen, honey (already shown), bee-bread, nectar and bees
2. Determine toxicity of glyphosate (rates) to *Lactobacillus* and *Bifidobacterium species* (already shown for other animals)
3. Endocrine hormone disrupter, neurotoxin, immune suppressant (already shown for other animals)
4. Glyphosate in CCD compared with normal (healthy) hives
5. *Lactobacillus* and *Bifidobacterium* in CCD compared with healthy hives, bees, brood (already shown absent in CCD)

Profile:

- ✓ Dr. Huber is Professor Emeritus of Plant Pathology at Purdue University, West Lafayette, IN. He received B.S. and M.S. degrees from the University of Idaho (1957, 1959), a Ph-D from Michigan State University (1963), and is a graduate of the US Army Command & General Staff College and Industrial College of the Armed Forces. He was at the Department of Botany & Plant Pathology at Purdue University in 1971.

- ✓ His agricultural research the past 50 years has focused on the epidemiology and control of soil-borne plant pathogens with emphasis on microbial ecology, cultural and biological controls, and physiology of host-parasite relationships.

- ✓ He retired in 1995 as Associate Director of the Armed Forces Medical Intelligence Center (Colonel) after 41+ years of active and reserve military service.

- ✓ Dr. Huber is an active scientific reviewer; international research cooperator with projects in Argentina, Australia, Brazil, Chile, China, Costa Rica, Denmark, Germany, Japan, Mexico, and Russia

- ✓ He is internationally recognized for his expertise in the development of nitrification inhibitors to improve the efficiency of N fertilizers, interactions of the form of nitrogen, manganese and other nutrients in disease, herbicide-nutrient-disease interactions, techniques for rapid microbial identification, and cultural control of plant diseases.

- ✓ Dr. Huber teaches courses on anti-crop bioterrorism and serves as a consultant on biological weapons of mass destruction and emerging diseases.

- ✓ To get a more in-depth profile of Dr. Huber, visit: http://www.nvlv.nl/downloads/Dr_Huber_bio.pdf.

- ✓ His greatest accomplishment has been his marriage to Paula Huber and their 11 children and 42 grandchildren and 2 great-grandchildren.