The Effect of Herbicide Application on Lifespan Duration of European Honeybees (*Apis mellifera*)

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Abstract

Honeybees (*Apis mellifera*) are extremely important contributors to agriculture worldwide. In North America and parts of Europe the population decline of these insects as a result of Colony Collapse Disorder (CCD) is attributed to several potential causes, including foraging stress however there are still many questions to be answered as to the cause of CCD. The present study uses captive bees to determine if a potential factor of honeybee decline is herbicide application to their food resources. Days alive since capture were averaged per treatment group as a measure of lifespan or cumulative effects of three common herbicide chemicals, concentrations mirrored those used in a typical household. Control honeybees lived up to 12 days. Herbicide treatment did not significantly change the number of bees alive per day except at high concentrations of EcoSMART Organic Weed and Grass Killer (active ingredient: rosemary oil, 1%). Although the two other herbicides did not affect lifespan length there were observed effects such as leg spasms that may affect their ability to survive. Further study is needed to determine how herbicides may affect honeybee populations.

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Introduction

Anthropogenic change is impacting organisms at a global level. One such impact is the transport of foreign organisms that has been linked to frequent and accessible travel by humans (Work, McCullough, Cavey, & Komsa, 2005). Of most concern when discussing transport of organisms, are species that have the potential to become, or have become invasive. Invasive species consist of 10-15% of introduced species (Kenis et al., 2009; Simberloff, 1996). These species may cause genetic limitation of indigenous species, may take over a niche that causes native species to vacate, or may introduce new diseases to the native populations (Abrol, 2012; Goulson, 2003; Kenis et al., 2009). Some species however are not considered invasive despite having these effects on ecosystems because of the interdependence between man and the organism. Organisms in this category include domesticated animals such as cows and sheep, and the European honeybee (*Apis mellifera*) (Turpin, 1999). Although these species have the same potential to cause harm to native species, the resources that they provide to humans is considered to be worth the risk (Moisset, 2011).

With human population rates exponentially increasing, the demand for new resources is ever-present (Nekola, 2013). To meet this demand, agriculture has been a primary resource for centuries. The need for successful agricultural endeavors brought about the transport of animals that settlers from Europe were previously familiar with, such as pigs and honeybees (Nekola, 2013; Turpin, 1999). Honeybees spread across North America quickly and have most likely had detrimental effects on the native bee populations (Moisset, 2011; Turpin, 1999). Honeybees have also become extremely important pollinators and are the only organism that can produce honey. The estimated economic benefit of honeybees in the United States alone is approximately $15 billion ("Fact Sheet: The Economic Challenge Posed by Declining Pollinator Populations," 2014). Such economic importance and the sudden decline of populations of honeybees which has been termed Colony Collapse Disorder has caused concern about long-term reliance on these organisms as pollinators and producers in North America ("Honey Bee Health and Colony Collapse Disorder," 2015). In Colony Collapse Disorder ridden hives, the honeybees disappear en masse leaving only the queen and a few workers along with most of the food resources (Bekić, Jeločnik, & Subić, 2014). The particular scientific struggle with Colony Collapse is that there is no trace of dead bees, so there is no evidence that researchers can track back to a single or several causes (Bekić et al., 2014). Colony Collapse Disorder is an ongoing area of research with many cited potential causes including *Varroa* mites, gut fungus, pesticide use, relocation of honeybees by humans, and environmental stressors, specifically foraging stress ("Honey Bee Health and Colony Collapse Disorder," 2015). However there is no consensus as to one leading cause of Colony Collapse Disorder (Hou, Rivkin, Slabezki, & Chejanovsky, 2014).

Concerning pesticide use, neonicotinoids specifically, have been cited as likely the most significant chemical cause for Colony Collapse (Fairbrother, Purdy, Anderson, & Fell, 2014; "Honey Bee Health and Colony Collapse Disorder," 2015). The hypothesis is that the neonicotinoid pesticides are weakening the bees and are indirectly causing starvation or immune deficiency allowing the other cited factors such as the *Varroa* mite or gut fungus to further weaken the bees and cause collapse (Alaux et al., 2010; Fairbrother et al., 2014). Several other pesticides have been marketed as bee safe and research has suggested that they may not cause immediate or direct effects (Abramson, 2004). However there may detrimental effects in learning ability that may threaten individual bee survival (Abramson, 2004; Alaux et al., 2010; Stone, 1997). Other chemicals that honeybees come into contact with, such as herbicides on their food source flora may cause shortened lifespans within individual bees, may cause cumulative internal effects or may weaken the honeybees in the same manner that neonicotinoids are. The present study attempts to understand how individual honeybees may be affected long-term by cumulative herbicide consumption via contaminated food sources using captive honeybees.

Methods

Two bee cages were constructed with mesh siding to limit potential escape and to allow air flow and light exposure (Figure 1A) (Evans, Chen, di Prisc, Pettis, & Williams, 2009). Both cages were constructed with a cloth opening to allow placement or extraction of honeybees. Honeybees were all from neighboring domestic hives located in Stillwater, OK. Honeybees were collected at a feeder approximately 20m from the hives. The feeder was periodically filled with 50% sucrose/ water solution (Figure 1B). Immediately after bees were caught they were placed into a cage and the number of bees in each cage was counted. Bees were then taken to an indoor space where temperature was controlled and lights were on a 12 hour light-dark schedule (Evans et al., 2009). Upon arrival all honeybees were given 50% sucrose/water solution in shallow bowls. Bowls were lined with paper towels to limit drowning risk (Figure 1A).

Figure 1A: Example of honeybee cage and food source methodology
Figure 1B: Honeybee feeder used for capture

The number of dead bees was counted every 12 hours for the duration of the experiment. Both treatment and control cages were given new sucrose solution every 24 hours.

Treatment group food sources were applied with chemical in either high or low dosages daily starting 24 hours after bees were caught. Chemicals were applied to each new food source bowl for treatment groups and control groups were given the same sucrose solution without added chemicals. Chemicals used included, EcoSMART Organic Weed and Grass Killer (active ingredient: rosemary oil, 1%), Ortho Weed B Gon (active ingredient: Triclopyr trimethylamine salt, 8%), and Round-Up Concentrate Plus (active ingredient: Glyphosphate isopropylamine salt, 18%). Sprays were administered using the same spray bottle to maintain similar dosages while retaining the standard application practice used for herbicides. Low dose was considered a single spray and high dose was considered two sprays to mimic the prescribed use by the manufacturers and retain ecological relevance. Chemicals were diluted according to the packaging directions to maintain similarity to household scenarios.

The percentage of bees alive at the end of each day was calculated for each group and plotted against the number of days since treatment was applied. The first 24 hours was excluded for all treatment groups because chemicals were not applied until 24 hours after catching to eliminate any travel effects mistakenly considered chemical effects. Uneven sample size t-tests were run to analyze significance between the control groups and treatment groups.

Results

On average the control groups survived longer than all treatment groups though not significantly longer. Ortho Weed B Gon low dosage however had individuals survive on average one day longer than control bees. All treatment groups had a more rapid decline of the average percentage of honeybees than the control groups despite the Ortho-low bees living longer. EcoSMART which uses rosemary oil as the active ingredient demonstrated the shortest average lifespans in the captive honeybees. The results for EcoSMART high dosage were significantly different for the first three days from the control bees, day 1, p<.001, day 2, p<.01, and day 3, p<.05 (Figure 2).

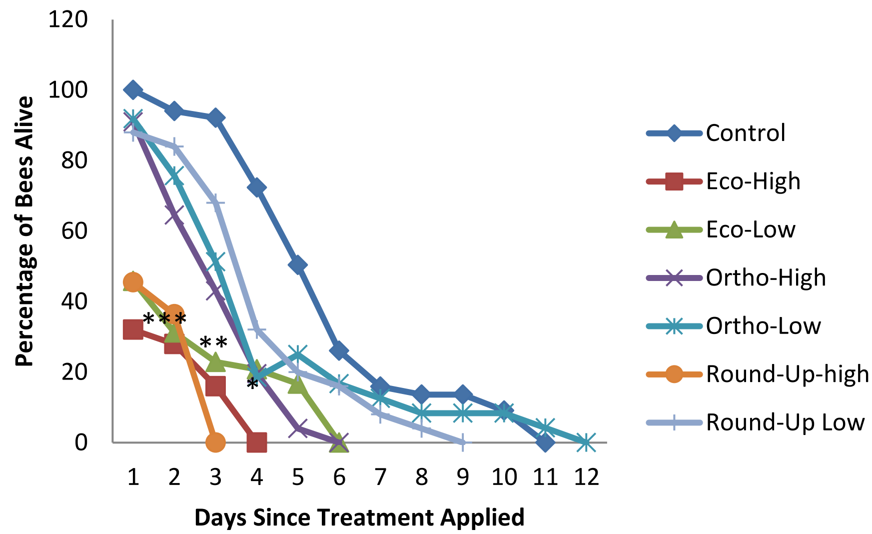

Figure 2: Average percentage honeybees alive per day after treatment was applied separated by treatment group (***p<.001, **p<.01, *p<.05)

Discussion

The effect of herbicides on the lifespan of captive honeybees was not determined to be significant by the present study. The effects that were shown are dependent on the type of herbicide. Further study into the internal effects of herbicides on honeybees is needed to determine if the chemicals could be contributing to Colony Collapse Disorder. However, this study suggests that herbicides that are marketed as safer to organisms that come in contact with the chemicals such as EcoSMART may be a significant problem to the wellbeing of the honeybees. For all the administered herbicides the active ingredient consisted of less than a fifth of the contents of the bottle and for EcoSMART specifically the active ingredient only consisted of 1% of the contents of the bottle. Research into how component ingredients in the chemicals may affect the lifespan of the honeybees may be needed to investigate which aspects of the chemicals could contribute to the faster decline of the honeybees.

Considering secondary effects of the chemicals, although Ortho Weed B Gon treatment groups lived on average minimally longer than the control groups there were observed negative effects that may cause the honeybees to be less successful in their pollen or nectar collection. General observation of the honeybees suggested flight directedness impairment approximately 48 hours before death. Approximately 24 hours before death the honeybees were immobilized with the exception of their legs which were twitching uncontrollably. This suggests a toxic effect of the Ortho Weed B Gon that may not shorten their captive lifespan but would be detrimental to the hive. In an environment where the honeybees need to return to the hive to stay warm overnight the bees may not survive.

The active ingredient in Round-Up (Glyphosphate isopropylamine salt) has been shown to negatively affect endocrine systems in some studies but in others has not shown inherently negative results (Bicho et al., 2013; Folmar, Sanders, & Julin, 1979). This is consistent with the present study. Although the honeybees did not survive as long in Round-Up treated groups there was not a significant difference in the lifespan from the control honeybees. There was a difference in the rapidity of death between the concentrations which is also consistent with previous research, although there may be effects when in ecologically relevant dosages Round-Up may not disrupt the ecosystem (Folmar et al., 1979).

This methodology has been used in the past to study the effects of chemicals on honeybees (Kulinčević & Rothenbuhler, 1975). However studies have shown that supplying the honeybees with protein and honeycomb food sources increases their lifespan when captive. The present study did not supply the honeybees with these items and it may have shortened their potential lifespans (Evans et al., 2009). The standard captive methodology is often used when trying to keep the honeybees alive as long as possible for potential use in other experiments. For the present experiment it was not entirely necessary but may have changed the results if comb or protein sources were supplied.

The present study does not support the hypothesis that herbicides are a potential cause of Colony Collapse Disorder, however further study is needed to determine how to potentially rectify the deadly incidence of CCD. Chemical application is a known factor of Colony Collapse and needs to be regulated and monitored to maintain our populations of bees in North America. Considering honeybees as invasive organisms they have limited the spread and viability of native bees (Abrol, 2012; Goulson, 2003; Moisset, 2011; Simberloff, 1996; Turpin, 1999). The ability of native bees to take over for pollination duties is therefore unknown and unlikely because they have been so drastically limited by honeybees and by human activity (Goulson, 2003; Kremen, Williams, & Thorp, 2002; Moisset, 2011). This limitation could create bigger risk if the honeybees are fully lost to Colony Collapse. Colony Collapse should continue to be a serious concern to agricultural industry as well as to individuals who rely on honeybees as resources and the further study of this issue is imperative.

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