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Scientific note

A scientific note on seasonal levels of pesticide residues in honey bee worker tissues

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Honey bees (*Apis mellifera* L.) provide essential pollination services for agricultural production worldwide. Recent declines in honey bee populations have been attributed to a multitude of potential stressors, such as pest pressure, nutritional stress, and exposure to pesticides (vanEngelsdorp et al. 2009b). One possible explanation for bee decline is the accumulation of pesticide residues in honey bee brood comb, resulting in chronic exposure to a suite of agrochemicals which may detrimentally influence honey bee health. Wu et al. (2011) demonstrated that bees reared in brood comb containing high levels of pesticide residues exhibit increased brood mortality and shorter adult longevity. Furthermore, Collins et al. (2004) and Pettis et al. (2004) demonstrated that wax impregnated with known quantities of coumaphos or fluvalinate can adversely impact queen health. Pesticide residues in honey bee brood comb are ubiquitous throughout many commercial beekeeping operations (Mullin et al. 2010; Wu et al. 2011). Understanding the potential long-term impacts of pesticide accumulation in wax on honey bee health may provide further insights into recent honey bee losses (Chauzat et al. 2009).

During the foraging season, the average lifespan of an adult worker honey bee is 4 to 5 weeks (Langstroth 1919). This short, seasonal lifespan limits exposure of an individual bee to pesticide residues in the comb. However, the lifespan of overwintering honey bees is significantly longer, naturally ranging up to 6 months (Maurizio 1950). These "winter" bees are subject to greater exposure to pesticides accumulated in brood combs, potentially leading to accumulation of pesticide residues in their tissue. Sublethal exposure to pesticides can result in physiological or behavioral changes (Johnson et al. 2009, Wu et al. 2011) and possibly influence colony performance and survival. Pesticide-induced changes in worker bee behavior have been shown to occur in short-lived, active season bees, and include impaired foraging and learning (Decourtye et al. 2005; El Hassani et al. 2005). This study reports the accumulation of pesticide residues in honey bee worker tissue during overwintering in migratory beekeeping equipment.

Eleven 1.4-kg packages of bees with sister queens were installed into bee hives borrowed from a western U.S. migratory beekeeping operation in Moscow, ID, during May 2010. Bees were managed using standard practices on a 2-ha organic farm and received no acaricide treatments for the duration of the experiment. They were fed ad libitum using a mixture of refined table sugar and water during spring and fall, which is a common practice employed by many U.S. beekeepers. Special care was made to ensure that all frames used in the study were fully drawn out and free of any honey or pollen from prior use.

All of the hive equipment had been previously exposed to typical migratory beekeeping practices, such as in-hive synthetic miticide applications and indirect environmental agrochemical exposure. In May 2010, prior to colony establishment, 2 g of wax were collected from

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the middle of each frame, pooled by hive, and submitted for chemical analysis. The brood comb samples were analyzed for a spectrum of 172 pesticide residues using gas chromatography coupled to mass spectrometry in order to provide a historical context for exposure to pesticides. The analysis revealed consistently high levels of commonly applied acaricides for *V. destructor* management, such as coumaphos, fluvalinate, and fenpyroximate (Online Resource Table I) in all brood comb samples examined.

This study tested whether the observed pesticide concentrations in wax might influence pesticide residue accumulation in worker honey bee tissue over time. Pre- and post-overwintering samples of approximately 100 bees were collected from the outermost frame of the top brood chamber of all the 11 hives during October 2010 and March 2011 for analysis of the same 172 agrochemicals tested for in the brood comb.

Four pesticides were found in the honey bee tissue, including coumaphos, fluvalinate, fenpyroximate, and cypermethrin (Online Resource Table I). Coumaphos, fenpyroximate, and cypermethrin were detected in small quantities (<10 μg/kg) and without any regularity in the 11 paired (October and March) tissue samples. Fluvalinate was detected in every one of the tissues samples collected from pre- and post-wintering bees. Additionally, the fluvalinate levels in worker tissue exhibited a significant mean increase of 60 % over the winter ($\bar{x}$ = 19.6 μg/kg in October 2010 and 32.0 μg/kg in March 2011), (t = 2.65, df = 10, P = 0.024; Online Resource Table I; Figure **1**). However, no correlation was determined between fluvalinate levels in the brood comb and worker tissues (Figure 1). Thus, fluvalinate quantities observed in the brood comb were not predictive of fluvalinate accumulation in bee tissue.

Most honey bee studies of sublethal pesticide effects have been conducted during the field season, rather than on overwintering bees in pesticide-exposed equipment. Surveys reported high winter colony losses (32, 36, and 29 %, respectively, for winters 2006–2008) (vanEngelsdorp et al. 2007, 2008, 2009a). Long-term sublethal exposure to pesticide residues in wax and subsequent impact on overwintering losses has not been determined.

This experiment suggests that accumulated pesticide residues in honey bee brood comb may influence pesticide residue expression in bee tissue. It is not clear whether the presence of pesticides at the levels detected can induce physiological and/or behavioral effects on the bees. The accumulation of pesticide residues in honey bee tissues may be of significance, due to a deficiency of detoxification enzymes coded by the honey bee genome (Evans et al. 2006). Johnson et al. (2009) also showed that a honey bee's ability to detoxify chemicals can be suppressed through exposure to

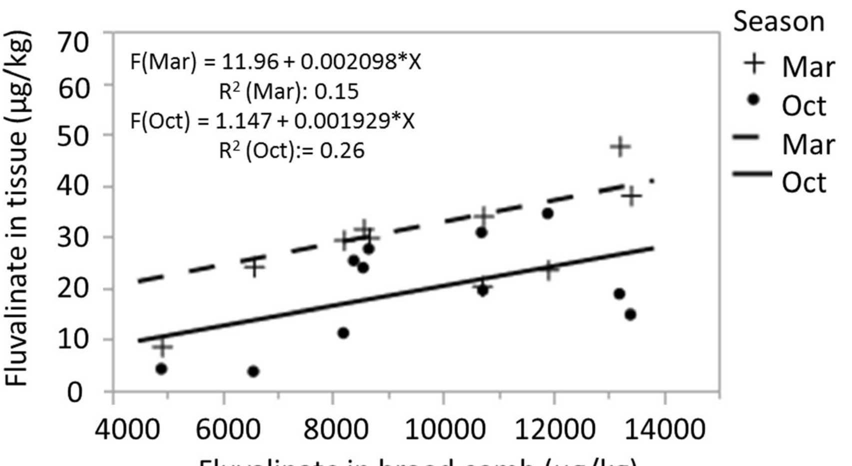

Figure 1. Fluvalinate concentrations in pre- and post-overwintering worker honey bee tissue samples, reported as a function of the fluvalinate concentration in associated brood comb samples. Fluvalinate levels in honey bee tissue do not appear correlate significantly with fluvalinate levels in the corresponding brood comb in October 2010 (Oct; t = 1.76; df = 10; P = 0.11) or March 2011 (Mar; t = 1.23; df = 10; P = 0.24). Fluvalinate residues in worker tissue increased significantly (n = 11, t = 2.65, df = 10, P = 0.024) over time.

multiple pesticides during a short period of time. Fluvalinate is widely recognized as a highly lipophilic and persistent pesticide found in honey bee brood comb (Martel et al. 2007, Lodesani et al. 2008; Bonzini et al. 2011; Hillier et al. 2013). However, further research would be required to determine whether detection of fluvalinate at the levels observed is adversely affecting overwintering honey bee health.

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Note scientifique sur les niveaux saisonniers de résidus de pesticides dans les tissus des ouvrières d 'abeilles.

Eine wissenschaftliche Notiz über die saisonale Höhe von Pestizidrückständen in Geweben von Honigbienen - Arbeiterinnen.

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