

An Evaluation of the Honey Bee (Hymenoptera: Apidae) Safety Profile of a New Systemic Insecticide, Flupyradifurone, Under Field Conditions in Florida

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Abstract

Flupyradifurone (Sivanto) is a novel systemic insecticide from the butenolide class developed by Bayer. Based on available data (USEPA 2014), this insecticide appears to have a favorable safety profile for honey bee colonies. As a result, the label permits the product to be applied during prebloom and bloom in various crops, including citrus, except when mixed with azole fungicides during the blooming period. We placed 24 honey bee (*Apis mellifera* L.) colonies adjacent to eight flowering buckwheat (*Fagopyrum esculentum* Moench) fields that either had been sprayed with the maximum label rate of flupyradifurone or with water only (control fields), with three colonies placed adjacent to each field. We conducted colony strength assessments during which the number of adult bees, eggs, uncapped brood cells, capped brood cells, food storage cells, and weights of honey supers and brood chambers were determined prior to, during, and after the flowering period. We also analyzed bee-collected pollen and nectar for flupyradifurone residues. Overall, there were no differences in any colony strength parameter for colonies placed at control and flupyradifurone-treated buckwheat fields. Residue analyses showed that pollen ($x = 565.8$ ppb) and nectar ($x = 259.4$ ppb) gathered by bees on fields treated with flupyradifurone contained significantly higher flupyradifurone residues than did bee bread and unprocessed nectar collected by bees from control fields (75% of samples < LOD). Within the conditions set forth by our experimental design, our collective data suggest no adverse effects of flupyradifurone on honey bee colonies when following label directions.

Key words: honey bee, systemic pesticide, pollinator, buckwheat, flupyradifurone

Sucking insect pests (e.g. aphids, whiteflies, leafhoppers, psyllids etc.) can be difficult to control in various agricultural crops. Pesticides are a primary tool used to control such pests. However, there can be unintended consequences associated with pesticide use in cropping systems. These include pest development of resistance to a given pesticide (Ahmad et al. 2003), loss of biological control agents (Geiger et al. 2010), and killing of bees and other pollinators (Johansen 1977, Brittain et al. 2010). As an example, the Asian citrus psyllid (*Diaphorina citri* Kuwayama), first introduced into Florida in 1998, has devastated Florida's citrus groves by vectoring a bacterium (*Candidatus Liberibacter* spp.) that causes huanglongbing (HLB) disease, also known as citrus greening disease (Halbert and Manjunath 2004). In the case of the Asian citrus psyllid, biological control has shown some successes (Qureshi et al. 2009), but insecticides have been the main component of psyllid management programs (Tiwari et al. 2011). Citrus, like many crops that harbor sucking insect pests, also attract a multitude of bees and other pollinators. Unfortunately most insecticides recommended for

sucking insects may impact pollinators negatively; in fact, many of the pesticides recommended for controlling Asian citrus psyllids are considered highly toxic to bees (Rogers et al. 2006). Consequently, care must be taken during the pesticide application period to mitigate any potential impacts on nontarget organisms (Rogers et al. 2006).

Potential pesticide impacts on nontarget organisms create a difficult situation for the grower who relies on pollinators to enhance crop production and pesticides to control crop pests. Knowledge on the potential impacts of pesticides on pollinators, especially managed species like the honey bee, is an important component of crop sustainability and the pesticide registration process. Given that pesticides can impact bees and other pollinators negatively (Johnson 2015), finding pesticides that are safe for pollinators is a critical research need under the paradigm of sustainable agriculture.

Flupyradifurone (4-[(2,2-difluoroethyl)amino]-2(5H)-furanone) is a novel systemic insecticide from the butenolide class and is the active ingredient in Sivanto (Bayer Crop Science, Research Triangle

Park, NC). This pesticide gained EPA approval and was registered in the United States in January 2015 for use against Asian citrus psyllid and other piercing-sucking insect pests. It is considered a versatile insecticide because it can be applied to a range of crops in a variety of ways (e.g., foliar spray, chemigation, seed treatment, etc.) and controls numerous pest insect species (primarily Hemiptera/Homoptera), including neonicotinoid-resistant populations (Jeschke et al. 2015). Flupyradifurone works by reversibly binding to insect nicotinic acetylcholine receptors (nAChR), similar to what neonicotinoids and sulfoximines do, but at a different site of action, resulting in different structure-activity relationships (Jeschke et al. 2015), which means that flupyradifurone is different in terms of how it binds to the receptor and to the extent to which it is metabolized. Flupyradifurone has been shown to be effective against Asian citrus psyllid and will give farmers an additional insecticide that can be rotated with other labeled products (Qureshi et al. 2012).

Flupyradifurone was found to be highly toxic to honey bees in lab-based studies via acute oral exposure ($1.2 \mu\text{g ai/bee}$ for the technical grade ai and $3.2 \mu\text{g ai/bee}$ for the 200 SL formulation = Sivanto) but nontoxic to adult honey bees by acute contact exposure (USEPA 2014). Although, semifield (confined tunnel tests) and field studies (higher-tier studies) showed that short-term, sublethal effects may occur in foragers, overall, these studies showed no long-term effects of the compound at the colony level and therefore concluded that flupyradifurone poses little risk to the honey bee (USEPA 2014, EFSA 2015). The higher-tier studies were accomplished on winter canola at two locations (six colonies/location) and had foliar application ($\sim 2\times$ label rate) but also seed and soil treatment (Rexer 2012a,b). The purpose of our study was to expand the USEPA studies to include more sites and determine if flupyradifurone application at label rate to another bee-attractive crop would cause deleterious effects to neighboring honey bee colonies allowed to forage on the treated crop. Herein, we present data from honey bee colonies that were either allowed to forage on flowering buckwheat (*Fagopyrum esculentum* Moench) that was sprayed during bloom with flupyradifurone at the maximum seasonal application rate permitted by the label, or control fields of buckwheat to which no pesticide was applied. Buckwheat is well known for its attractiveness to honey bees and its ability to yield a large amount of nectar (Myers and Meinke 1994). Buckwheat was selected to represent a worst-case exposure scenario because it is both, a pollen and a nectar source for honey bees, unlike some citrus crops that only provide nectar.

Materials and Methods

Study Sites and Experimental Setup

Eight, 2-hectare buckwheat fields separated by at least 5 km were established in north-central Florida (Fig. 1) from seed (Hancock Seed Company, Dade City, FL) between 15–29 June 2015. The seeding rate was 113.4 kg/field (56.7 kg/ha) per label instructions and the seeds were the cultivar “Mancan”. Nitrogen fertilizer is needed to improve growth of buckwheat in soil that is nitrogen limited (Myers and Meinke 1994). Hence, nitrogen fertilizer was added to two of the eight fields that showed poor growth shortly after planting. Four out of the eight fields selected at random were treated with flupyradifurone while the other four fields were used as untreated controls and received water only applications. Three colonies of managed honey bees were placed adjacent ($<5 \text{ m}$) to each of the eight study fields ($n = 24$ colonies). The total study time including the placement of colonies in the fields and their relocation to a common apiary after buckwheat bloom was approximately three months.

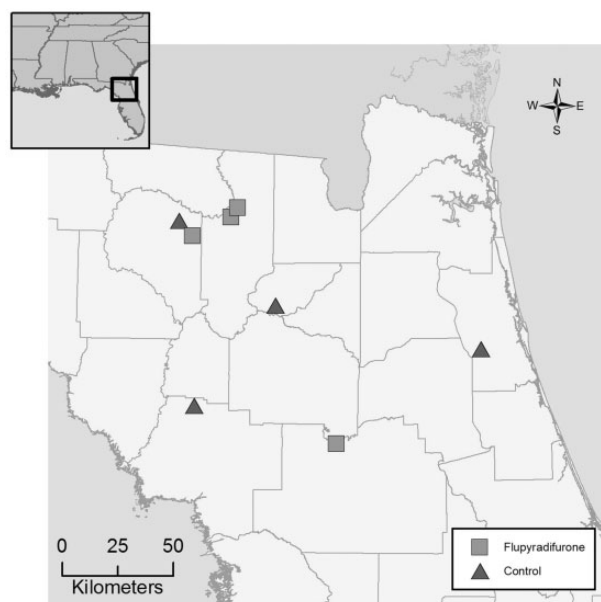


Fig. 1. Schematic map of north-central Florida depicting the locations of the control and flupyradifurone-treated buckwheat fields used in the study.

The 24 study colonies (three colonies per field) were selected from 30 total colonies using the data generated from an initial colony condition assessment (CCA; not included in the analysis). This allowed us to use colonies that were of similar size and weight and with at least five brood frames, a minimum of 8,000 adult bees and a marked queen. Three preweighed colonies consisting of one hive body and one medium super were placed on each field. The super contained five empty (new foundation) and five full honey frames. The honey super and brood chamber were separated by a queen excluder.

The four buckwheat treatment fields were treated twice with flupyradifurone via foliar spray at the highest single application rate for buckwheat, which corresponds to 205 g ai/ha per individual foliar application. As two sequential applications were made, the buckwheat crop also received the maximum cumulative seasonal application rate allowed by the label, which corresponds to 410 g ai/ha . The application volume was $\sim 94 \text{ L/ha}$, as recommended in the Sivanto label for foliar ground application of cereal grain crops, including buckwheat. This cumulative application rate of flupyradifurone is also the maximum allowed for all other crops, including citrus and other bee attractive crops.

We used the extended Biologische Bundesanstalt, Bundessortenamt and CHemical (BBCH)-scale to determine growth stages of buckwheat (Hack et al. 1992). The first foliar flupyradifurone or water application occurred at prebloom, between BBCH 55 and 59 (after the first individual closed flowers emerged, but before the first flower petals were visible). This occurred about 4–5 wk after planting. The honey bee colonies were placed at the edge of their corresponding field at $\sim 20\%$ bloom (BBCH 62; 3–5 d after the first flupyradifurone application), and allowed to adapt to their new surroundings before the second flupyradifurone application. The second application occurred at full bloom (BBCH 65), at least 6–7 d after the placement of the colonies beside the buckwheat fields; the interval between the two pesticide applications was at least 10 d due to a minimum of 7 d between sprays as stated on the label. The treatment and control applications were made before dawn or near/after dusk to minimize direct spraying of foraging bees.

Colony Condition Assessments

Colony condition assessments (CCAs) were performed on all colonies at five time points (per Delaplane et al. 2013): 1) ~4 wks after initial establishment of the colonies in a single apiary (Gainesville, FL), 2) ~1 wk before placing the colonies at the experimental fields (pre-exposure period), 3) during the exposure period, close to the end of the blooming period (BBCH 67–69), 4) 1 mo after relocation of the colonies to the post exposure apiary, and 5) 2 mo after relocation to the postexposure apiary. At each CCA, pictures were taken of each frame from the brood chamber in every colony to assess the area covered with capped brood. Each picture was analyzed using the automated software Indicoounter (WSC Regexperts, Germany) and verified independently by a laboratory technician. The assessment of adult bees, eggs, uncapped brood and food stores was done visually as suggested by Delaplane et al. (2013). To minimize bias two researchers separately approximated the percent coverage of adult bees, eggs, uncapped brood and food stores within frames. The two researchers' approximations were then averaged and converted to area (cm²) and then to number of adult bees and number of cells containing eggs, uncapped brood, or food stores (Delaplane et al. 2013). During each CCA, brood chambers and honey supers from all colonies were weighed separately using a digital scale. All CCAs were conducted on colonies in a randomized order at each assessment date to reduce bias.

The study colonies remained on their respective buckwheat fields until the end of the flowering period. After the flowering period, the colonies were transferred to a common apiary (Gainesville, FL) where adequate forage was available. The colonies at the postexposure apiary were placed ~3–5 m apart and remained there for the rest of the study.

Foraging Activity and Pollen and Nectar Collection

Honey bee forager flight activity surveys started when at least 20% of a field was flowering (BBCH 62). Forager flight activity was monitored along two 100-m transects of the blooming buckwheat, twice per week during the flowering period. During the visits, researchers walked two random 100-m linear transects within a field, counting the number of honey bees observed landing on a buckwheat flower within one meter on either side of the transect line. Each 100 m transect walk lasted 30 min. To minimize edge effects, transects were begun ~10 m from the field edge and directed toward the center of the field. During the flowering period (20 July–19 August 2015), we conducted 54 transect walks (27 total hours) within the 8 buckwheat fields. All transects were accomplished between 09:00–16:00 on days for which weather was appropriate for pollinator activity during this time of year (i.e. no rain and temperatures above 30 °C). Forager flight activity also was monitored by observing and counting bees returning to each colony for 5 min on the same day and time that transects were monitored.

The percentage of all incoming pollen (corbicular) collected by bees that was buckwheat pollen was estimated twice. This was done by trapping corbicular pollen for ~4 h at colony entrances 2 d after placing the colony in the buckwheat fields, but before the second flupyradifurone application, and 2–3 d after the second flupyradifurone application. Pollen slides were prepared following Kearns and Inouye (1993) and Erdtman (1969). Palynological analysis of these pollen samples was used to identify buckwheat pollen grains (compared to a reference hand-collected pollen sample from buckwheat) and other pollen grains. Composite samples of pollen (bee bread) and nectar (unprocessed) from colony stores (i.e. from the wax comb) were collected during peak bloom (i.e. about 5 d after

the second application with flupyradifurone) to characterize in-hive residue levels of flupyradifurone. To gather composite samples, all three colonies per study field were opened and approximately five cells of unprocessed nectar or bee bread (minimum of 3 g) were taken from each of the colonies and placed into a single collecting container. Four composite samples of unprocessed nectar and bee bread were taken per study field (64 samples overall). Samples were transported to the laboratory as soon as possible and stored at –20 °C until shipped to the USDA NSL Laboratory for residue analysis.

Statistical Analyses

We performed all analyses on the colony assessment data using Dell Statistica (data analysis software system), version 13. We analyzed the seven dependent variables (number of adult bees, eggs, uncapped brood cells, capped brood cells, food storage cells, and weights of honey supers and brood chambers) by treatment (with or without flupyradifurone), using an univariate approach, since the relatively few fields did not provide sufficient degrees of freedom for a multivariate approach. Since we needed to apply univariate repeated measures ANOVA tests, we first tested for sphericity using Mauchly's W tests for all seven dependent variables. In all cases, the *p* values were greater than the critical α -value of 0.05, so the sphericity assumption was met. We used a within subjects general linear model with four repeated measures representing the four censuses. To avoid inflating Type I error, we applied a Bonferroni's correction ($\alpha/7 = 0.007$). Differences were determined using t-tests (Statistix 9.0 Analytical Software, Tallahassee, FL) on both buckwheat pollen and nectar and flupyradifurone residue levels between flupyradifurone-treated and control fields. We also conducted separate t-tests on the relative amounts of buckwheat pollen collected by honey bees between flupyradifurone-treated and control fields, as well as between forager flight activity at the colony entrances between flupyradifurone-treated and control fields. All data were tested for normality and log transformed if needed. A Wilcoxon Rank Sum test (Statistix 9.0 Analytical Software, Tallahassee, FL) was used to test differences between forager flight surveys within the flupyradifurone and control fields.

Results

Colony Condition Assessments and Foraging Activity

There was a significant repeated measures effect for five of the seven dependent variables (number of adult bees, uncapped brood cells, capped brood cells, and weights of honey supers and brood chambers), meaning that these measurements changed significantly over time, as would be expected for normal colony development. There were no significant interactions between the treatment and time or the treatment main effect (Table 1). This means that the presence of flupyradifurone had no statistical significant effect on these variables throughout the course of the study. It may therefore be concluded that in this study buckwheat treatment with flupyradifurone did not impact any strength parameter of colonies located in the treated fields relative to those located in untreated ones. It is important to note that the power of the tests ranged from 0.05 to 0.285, likely due to the small sample size (4 treated fields vs. 4 controls) and large variances. So, given our sample size, the tests failed to reject the null hypothesis that there were no differences between control and treatments.

Honey bees accounted for 5,300 flower visits during our survey work. Overall, each survey walk documented an average of 98

Table 1. Mean (\pm SE) number (No.) of adult honey bees, eggs, uncapped brood cells, capped brood cells, food store cells, and hive super weights measured during the four CCA (Colony Condition Assessments)

Parameter w/Test statistics	CCA 1		CCA 2		CCA 3		CCA 4	
	Control	Flupyrad.	Control	Flupyrad.	Control	Flupyrad.	Control	Flupyrad.
No. adult honey bees ($F = 0.102$, $df = 1, 6$, $P = 0.76$)	11,604 (1,078)	9,832 (629)	13,114 (1,218)	12,245 (1,270)	10,785 (927)	10,184 (1,685)	5,175 (2,128)	6,344 (1,325)
No. eggs ($F = 1.15$, $df = 1, 6$, $P = 0.33$)	3,954 (559)	3,331 (692)	4,521 (796)	2,682 (972)	4,047 (534)	3,572 (755)	2,113 (616)	1,913 (764)
No. uncapped brood cells ($F = 0.47$, $df = 1, 6$, $P = 0.51$)	7,128 (1,879)	7,802 (1,096)	8,048 (852)	6,067 (1,389)	4,851 (639)	4,104 (1,376)	2,329 (747)	1,279 (408)
No. capped brood cells ($F = 0.18$, $df = 1, 6$, $P = 0.69$)	12,837 (1,882)	12,407 (1,317)	11,700 (1,815)	11,572 (1,058)	7,830 (11,820)	6,451 (2,166)	3,390 (1,481)	2,493 (953)
No. food store cells ($F = 1.87$, $df = 1, 6$, $P = 0.22$)	21,897 (5,318)	12,680 (1,479)	13,863 (3,267)	10,149 (1,261)	16,186 (1,904)	11,826 (1,177)	13,982 (2,812)	1,5921 (3,597)
Hive body weight (kg) ($F = 1.74$, $df = 1, 6$, $P = 0.24$)	17.7 (0.68)	15.9 (0.71)	15.6 (0.59)	15.0 (0.34)	14.9 (0.29)	14.0 (0.74)	13.9 (0.63)	13.7 (0.94)
Hive super weight (kg) ($F = 0.14$, $df = 1, 6$, $P = 0.72$)	14.3 (0.44)	14.6 (0.30)	12.1 (0.25)	11.8 (1.2)	11.2 (0.50)	10.9 (1.2)	9.4 (0.95)	11.2 (1.7)

No significant differences were detected between hives placed adjacent to control fields ($N = 4$) or flupyradifurone-treated fields ($N = 4$) within each CCA for any parameter at $\alpha = 0.05$.

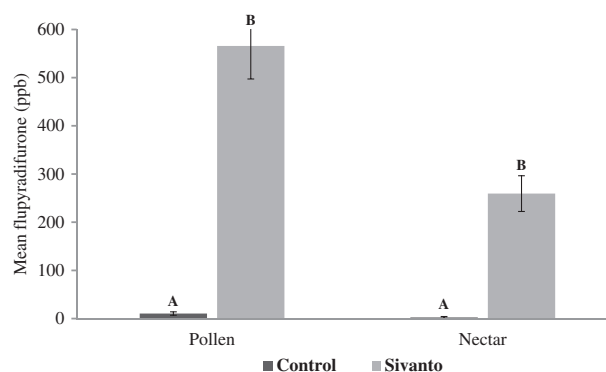


Fig. 2. Mean flupyradifurone (ppb) found in bee-collected pollen and nectar from hives placed adjacent to fields sprayed with Sivanto or control fields. Within food type (pollen or nectar), there were more flupyradifurone residues in hives placed adjacent to fields sprayed with Sivanto than in hives placed adjacent to control fields (pollen: $F = 8.07$; $df = 1, 15$; $P \leq 0.0001$; nectar: $F = 6.92$; $df = 1, 15$; $P \leq 0.0001$). Columns within food type (pollen or nectar) with different letters are significantly different at $P \leq 0.05$.

honey bees/100 m transect/30 min walk ($N = 54$). Honey bees primarily utilized buckwheat during morning hours then became noticeably absent during the afternoon (McGregor 1976). Although the flupyradifurone-treated fields significantly contained more foragers visiting buckwheat ($z = 2.16$, $P = 0.003$), this was attributed to more surveys being conducted in the morning hours on flupyradifurone-treated fields when bees were foraging whereas more control field surveys were conducted in the afternoon. Both control and flupyradifurone-treated fields were visually observed to be highly attractive to honey bee foragers during morning hours. Forager flight activity at the colony entrances was not significantly different ($t = 0.62$, $df = 6$, $P = 0.56$).

Pollen Collection and Flupyradifurone Residue Analysis

Honey bees gathered abundant buckwheat pollen during the morning hours. Two pollen slides per colony were made and these data were averaged. Palynology analysis (minimum of 600 grains counted) revealed that an average of $67.9 \pm 6.0\%$ ($N = 24$) of all pollen collected by honey bees was buckwheat pollen. Bees in both control fields and flupyradifurone-treated fields collected similar relative amounts of buckwheat pollen (67.6 ± 9.3 and $68.3 \pm 8.3\%$ of all pollen collected, respectively). No significant difference in relative amounts of buckwheat pollen between control and flupyradifurone-treated fields were observed ($t = -0.04$, $df = 6$, $P = 0.97$). Overall, bee bread ($t = -8.07$; $df = 15$; $P \leq 0.0001$) and unprocessed nectar ($t = -6.92$; $df = 15$; $P \leq 0.0001$) collected by bees in flupyradifurone-treated fields contained significantly more flupyradifurone than the bee bread and unprocessed nectar collected by bees in untreated fields (Fig. 2), confirming that treatment and control fields were isolated from each other. The level of detection for the residue analysis was 20 ppb. Table 2 contains summary statistics for residue values as well as estimation of risk quotients (RQs) for acute and chronic flupyradifurone exposure, relevant to risk assessment. A risk quotient value puts into perspective the exposure and the hazard of a pesticide, thus providing a relatively conservative evaluation of the risk of a pesticide to honey bees.

Discussion

Honey bees can be exposed to pesticides via multiple routes in agricultural settings (Krupke et al. 2012). Our study was designed to

Table 2. Flupyradifurone residue levels (ppb) in bee-collected nectar and pollen samples from control and flupyradifurone-treated buckwheat fields

Field Type	Plant matrix	Min.	Max	Mean	Median	90th percentile	% Samples positive for residue	Acute RQ	Chronic RQ
Control	Nectar*	<LOD	20	<LOD	<LOD	<LOD	12.5 (2/16)	—	—
Flupyradifurone	Nectar*	126	541	259.4	201.5	499	100	0.1214	0.0745
Control	Pollen*	<LOD	34	<LOD	<LOD	31.5	37.5 (6/16)	0.0003	—
Flupyradifurone	Pollen*	225	1,170	565.8	512.5	929.5	100	0.0074	0.0062

Risk quotients for acute (Acute RQ) and chronic exposure (Chronic RQ) were obtained using the BeeREX V.1 model developed by EPA, PMRA, and CDPR. These regulatory agencies have established levels of concern of 0.4 and 1.0 for acute and chronic risk quotients, respectively. A potential risk is presumed to occur if the RQ exceeds the level of concern; and conversely, a minimal risk is indicated if the RQ is below the level of concern. The adult honey bee acute oral LD₅₀ is 1.2 µg flupyradifurone/bee and the adult chronic oral NOEC is 0.79 µg flupyradifurone/bee/day. An * in front of plant matrices indicate a significant difference between control and flupyradifurone mean residue levels within the matrix at $P \leq 0.05$.

determine honey bee colony-level impacts resulting from short term (~3 wk) exposure to flupyradifurone via nectar and pollen from buckwheat treated fields over a time period of ~2 mo post pesticide exposure. Within the parameters of our study, exposure to flupyradifurone-treated buckwheat did not impact any of the variables measured by the colony condition assessments within the honey bee colonies in any measurable way. This was found considering the fact that the test buckwheat was treated twice with flupyradifurone at the highest single foliar application rate. This finding is reinforced by our RQ calculations, which suggest that the residue levels of flupyradifurone in colonies located adjacent to flupyradifurone-treated fields were below levels which would give rise to concern (USEPA 2014, Table 2). In general, many of the colony strength parameters decreased over the course of the study for colonies adjacent to both types of fields. This, we believe, was normal reduction for the time of year the study was conducted. Honey bee colonies commonly show decreases in strength as summer wanes and fall nears (McLellan 1978).

Pesticide residue analyses of colony matrices from managed honey bees near agricultural fields showed that pesticide residues and other chemicals (e.g. herbicides, fungicides, etc.) can be detected in bee-gathered pollen and in wax (Mullin et al. 2010). Some bee species, honey bee included, cannot detect many pesticides and, therefore, cannot actively avoid plants to which pesticides have been applied (Kessler et al. 2015). Though our study design does not permit us to know if honey bees can detect flupyradifurone or not, bee visitation rates of buckwheat were high within the flupyradifurone-treated fields, suggesting that flupyradifurone does not repel honey bees under field conditions.

Interestingly, a few of the bee bread and unprocessed nectar samples taken from colonies on control fields contained trace or very low levels of flupyradifurone. Honey bees rarely fly over 3 km from their hive when food sources are nearby (Eckert 1933) and our control fields were substantially further from flupyradifurone-treated fields. Sivanto was registered in the US in January 2015 and most of the fields we used in our study were near other locations used for agricultural purposes. Therefore, it is possible that the bees whose colonies were located adjacent to control fields were exposed to small amounts of flupyradifurone from flupyradifurone-treated crops located in the general vicinity of our research fields. Our bee bread and unprocessed nectar samples were taken from colonies ~10 d after the second flupyradifurone application and on areas of the frame that did not contain bee bread or unprocessed nectar prior to placement in the fields. Thus, we considered the residues present in these matrices indicative of flupyradifurone translocation to the bee bread and unprocessed nectar within the treated plants and direct spraying onto anthers and nectaries subsequently visited by bees.

In our study, bee-collected pollen (bee bread) contained significantly higher levels (~2x) of flupyradifurone compared to levels in bee-collected (unprocessed) nectar. This trend also was noted by the USEPA (2014), based on studies submitted by the registrant in most residue studies with various crops. We did not observe any behavioral differences or mortality between bees from colonies located adjacent to control or treated fields during the bee foraging surveys. The USEPA (2014) established the LD₅₀ for acute oral toxicity as 1.2 µg ai/bee for the technical grade ai and 3.2 µg ai/bee for the 200 SL formulation (=Sivanto). The chronic adult oral NOEL (no observed effect level) established by the USEPA was 0.79 µg ai/bee. To reach the chronic no effect level intake of 0.79 µg flupyradifurone per day, a honey bee feeding on pollen with a concentration of 1,170 ppb (µg/kg), the maximum level measured in this study, would need to ingest 675 mg of pollen per day. However, this far exceeds the maximum daily intake of pollen (9.6 mg per day) estimated by EPA for honey bees in the BeeREX model. Similarly, to reach the chronic no effect level of 0.79 µg flupyradifurone per day, a honey bee feeding on nectar with a concentration of 541 ppb (µg/kg), the maximum level measured in this study, would need to ingest 1,460 mg of nectar per day. Again, this far exceeds the maximum daily intake of nectar (292 mg) estimated by EPA for honey bees in the BeeREX model. Using the BeeREX model and inputting acute and chronic endpoints, as well as our nectar or pollen residue levels, risk quotients for acute and chronic exposure were calculated for the exposed colonies in our study and none surpassed the levels of concern established by the USEPA (acute RQ ≥ 0.4, chronic RQ ≥ 1.0; see Table 2).

Pesticides have been considered to be one of several factors associated with honey bee annual losses in recent years (e.g. Potts et al. 2010; Henry et al. 2012; Krupke et al. 2012). Pesticides can move to pollen and nectar from indirect routes of application, allowing for nontarget, chronic exposure events to occur (Johnson et al. 2010). Developing systemic pesticides with a favorable safety profile for honey bees and other pollinators will contribute to agricultural sustainability and will benefit growers and beekeepers. Overall, our collective data suggest no adverse effects of flupyradifurone on honey bee colonies when following label directions. Our results and findings were similar to other studies that tested flupyradifurone under field conditions (USEPA 2014), reinforcing a favorable honey bee safety profile for flupyradifurone.

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