



**BOARD OF
PESTICIDES CONTROL**

DEPARTMENT OF AGRICULTURE,
CONSERVATION & FORESTRY

Neonicotinoids: potential health and environmental risks

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European bee study fuels debate over pesticide ban

Neonicotinoids harm domesticated bees and wild cousins in study, but leave some honey bee colonies unaffected.

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“We learn again: It's complicated,” says biologist Tjeerd Blacquièrè

Insecticides have varying and contrasting risks

		Application rate	Human acute Ref. dose	Acute toxicity (LD ₅₀)			Soil affinity	Leaching potential	Bioaccum. factor	Soil half-life
		kg/h	aRfD	rat	bee (contact)	trout	Koc	GUS	BAF	DT50
carbamates	carbaryl									
	carbofuran									
	methomyl									
Neonics	clothianidin									
	imidacloprid									
	thiamethoxam									
Organo-P	chlorpyrifos									
	acephate									
	dimethoate									
Pyrethroids	cypermethrin									
	bifenthrin									
	cyhalothrin									

(data were manually assembled from EcoTox and Pesticide Properties Database)

Risk Quotient is calculated to *estimate potential* risk

$$RQ = \text{exposure} / \text{toxicity}$$

$RQ = \text{max application rate per hectare} / LD_{50} \text{ in rats} * 1,000,000 \text{ (kg-mg conversion)}$

	Raw value risk quotient	Log tranformed RQ (log)
carbaryl	4435	3.6
carbofuran	176000	5.2
methomyl	12000	4.1
clothianidin	20	1.3
imidacloprid	91	2.0
thiamethoxam	69	1.8
chlorpyrifos	8296	3.9
acephate	419	2.6
dimethoate	6833	3.8
cypermethrin	80	1.9
bifenthrin	1296	3.1
cyhalothrin	208	2.3

Possible unintended consequences of a neonicotinoid ban: Corn

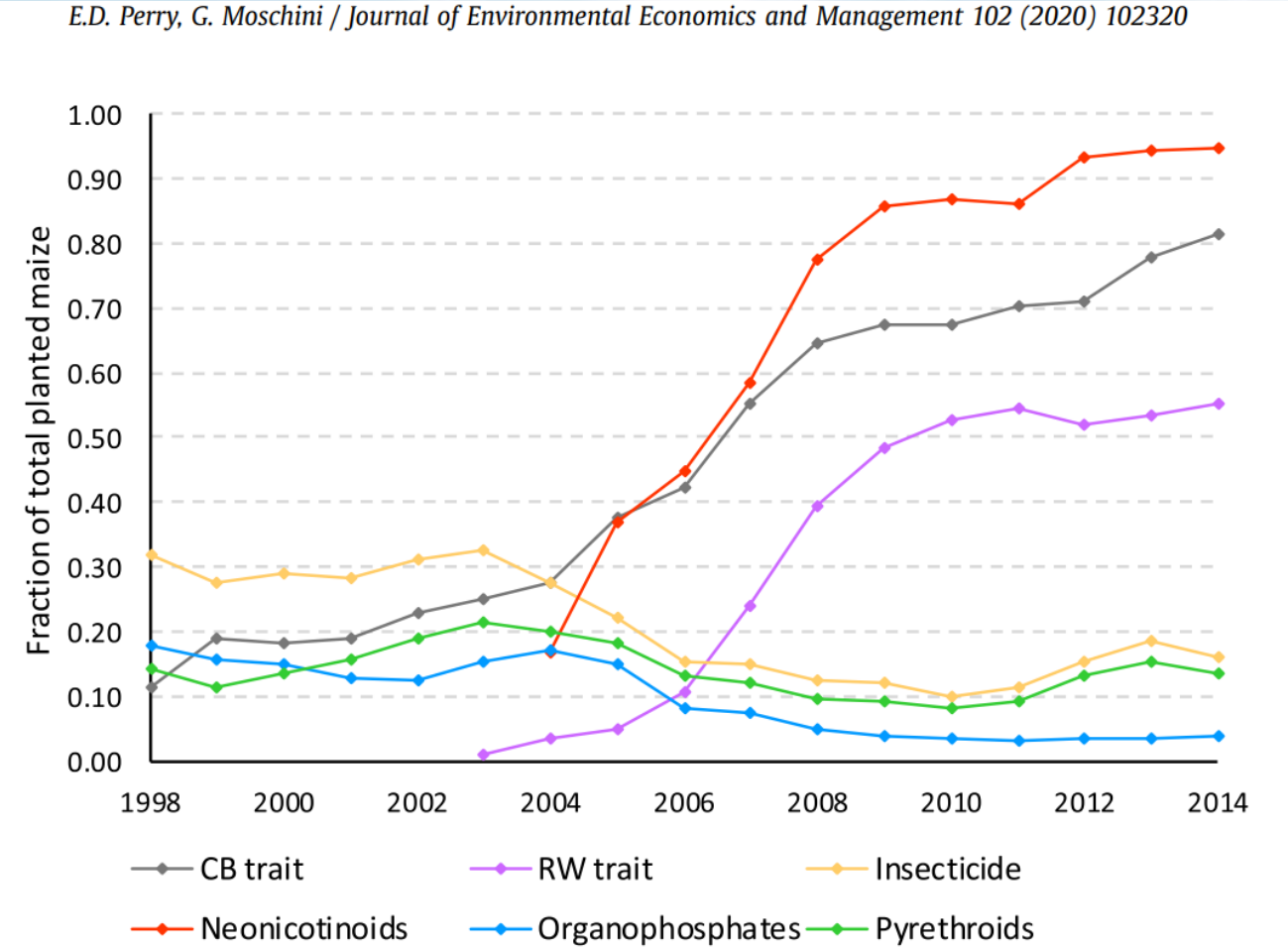


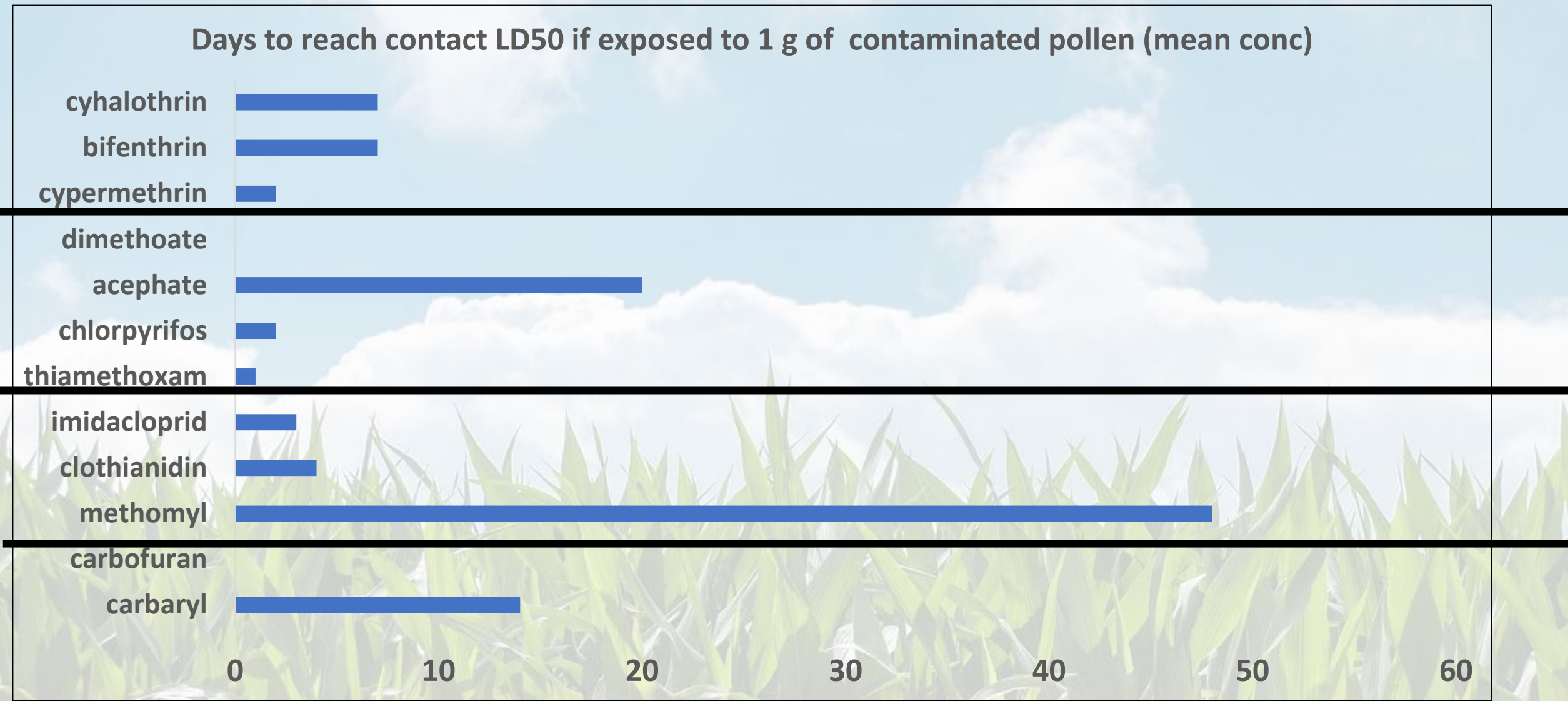
Table 5
Predicted impacts of a NeoST ban: Insecticide use and hazard quotients in 2014.

	Baseline	NeoST Ban	Change (%)
Organophosphate Adoption ^a	0.04	0.10	174.7%
Pyrethroid Adoption ^a	0.13	0.21	54.9%
Category I Insecticide Adoption ^a	0.05	0.11	106.9%

Corn

Various methods exist to assess the risk of pesticide residues in honey bees.

(These data are from Sanchez-Bayo and Goka, 2014. Estimates reflect environmentally relevant exposures in the field).



Risk of neonicotinoids to honey bees in Maine

Pollinator Ecology and Management 2018

Research

Exposure of Honey Bee (*Apis mellifera* L.) Colonies to Pesticides in Pollen, A Statewide Assessment in Maine

Francis A. Drummond,^{1,2,5} Elissa S. Ballman,¹ Brian D. Eitzer,³ Brianne Du Clos,⁴ and James Dill²

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Bees are most frequently exposed to fungicides and herbicides, but insecticides pose greatest risk.

Insecticides detected in pollen at 9 of 32 sites.

Neonicotinoids detected in pollen at 2 out of 32 sites.

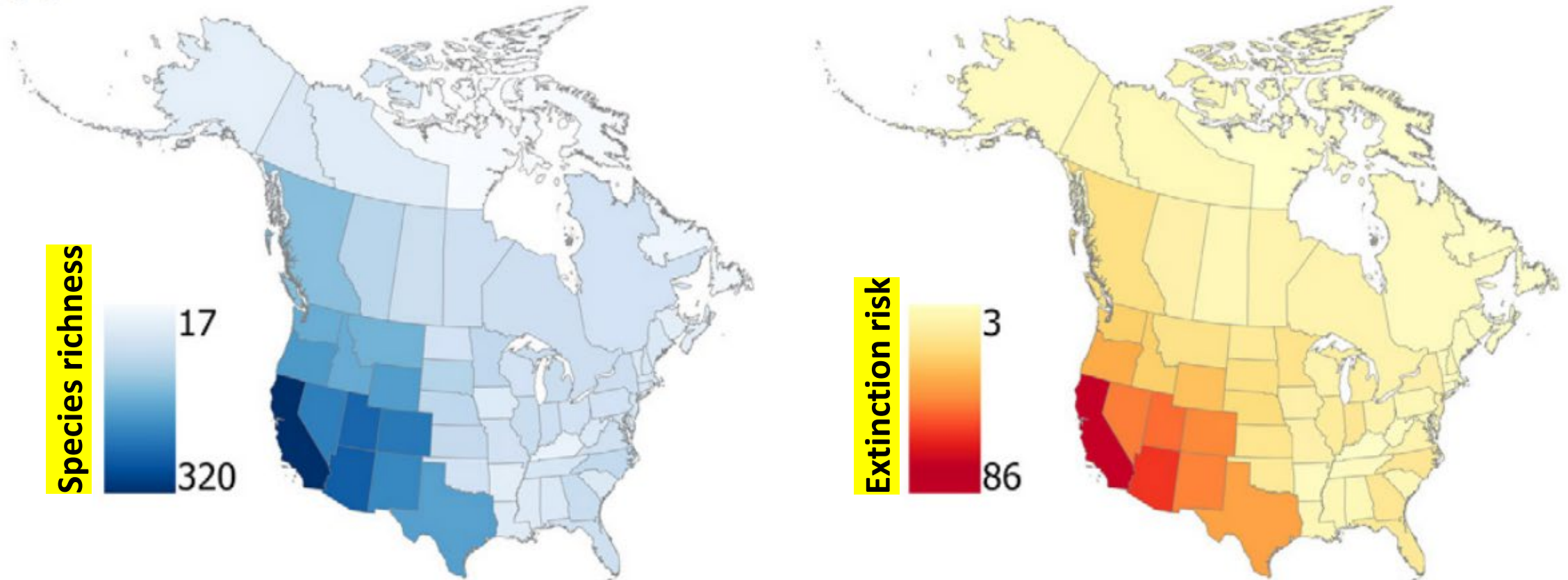
Only one site has a risk quotient at a level of concern (phosmet in apple orchard).

Exposure predicted by land use (blueberries followed by conifer forest)

2025

Elevated extinction risk in over one-fifth of native North American pollinators

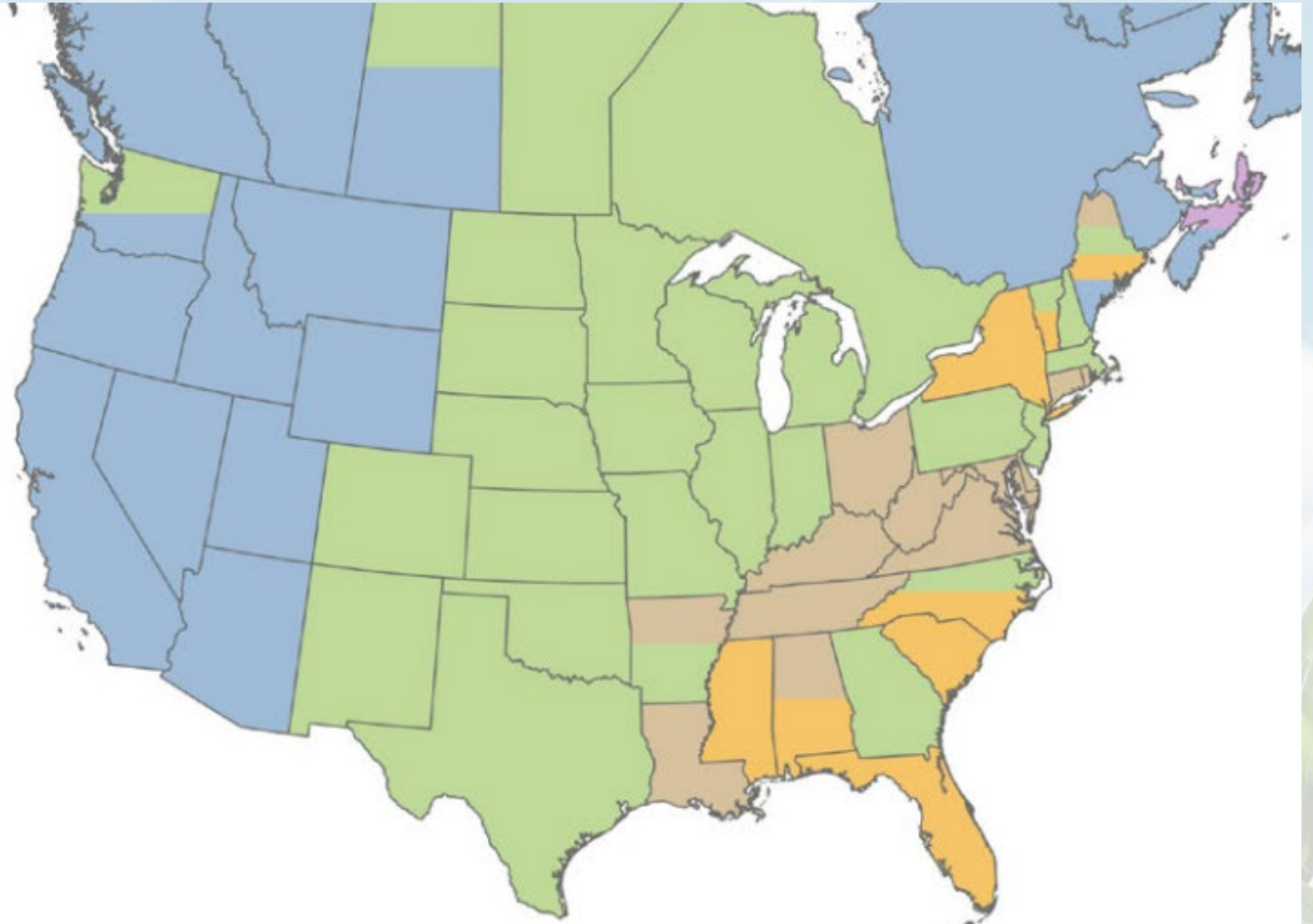
Bees



Elevated extinction risk in over one-fifth of native North American pollinators

Major Threat

- Agriculture
- Climate change
- Diseases
- Housing/urban development
- Pollution



2017

Article

Bee Community of Commercial Potato Fields in Michigan and *Bombus impatiens* Visitation to Neonicotinoid-Treated Potato Plants

Amanda L. Buchanan, Jason Gibbs [†], Lidia Komondy and Zsolia Szendrei *

Department of Entomology, Michigan State University, East Lansing, MI 48824, USA;

No significant difference in bee visitations between treated and untreated potato fields.

- # of trips/day
- Length of time/trip

Exposure of Honey Bee (*Apis mellifera* L.) Colonies to Pesticides in Pollen, A Statewide Assessment in Maine

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Abstract

In 2015, we conducted a statewide assessment of honey bee exposure to pesticides with assistance of volunteer beekeepers. Pollen trapping was conducted at 32 sites in the spring, summer, and early fall. Apiary locations ranged from unmanaged natural landscapes to managed agricultural or urban landscapes. Pollen samples at each site were aggregated over the collection dates and chemical residue analysis was conducted on each pollen sample for 190 pesticides and metabolites using HPLC/MS. Twenty-five different residues were detected for an average of 2.9 detections per site. Detections were dominated by fungicides, but risk, calculated as: ppb residue concentration/LD₅₀, was mostly due to insecticides. Beekeeper perceived land-use in the vicinity of each apiary was associated with significant differences in the number of detections and residue concentrations, agricultural landscapes greater than nonagricultural. However, there was no significant difference in oral or contact risk quotients due to land-use type. The landscape composition surrounding apiaries, derived with GIS, determined pesticide exposure for honey bees when total detections, log pesticide residue concentration, and log contact risk quotients were used as measures. Partial least squares explained 43.9% of the variance in pesticide exposure due to landscape composition. The best predictors describing pesticide exposure were: area (ha) of blueberry, coniferous forest, and urban/developed land cover types. Maine is the most forested state in the United States (as determined by % land area forested, 93%) and a negative exponential decay was observed between land area in conifer forest and the number of pesticide detections per apiary.

Key words: risk quotient, pollen trapping, citizen science, landscape analysis

Honey bees (Hymenoptera: Apidae, *Apis mellifera* L.) are exposed to a variety of pesticides in agricultural, residential, and rural settings (Mullin et al. 2010, Stoner and Eitzer 2013). Many of these pesticides are known to be highly toxic to honey bees (Greigsmith et al. 1994, Mineau et al. 2008, Johnson et al. 2010, Zhu et al. 2014, Kiljanek et al. 2016). Those that are not acutely toxic can still have detrimental impacts on honey bee colony health. Sublethal doses of pesticides can affect foraging and grooming ability, immunology, and parasite load (Desneux et al. 2007, Vidau et al. 2011, James et al. 2012, Wu et al. 2012, Sandrock et al. 2014, Schmehl et al. 2014, Doublet et al. 2015, Sánchez-Bayo et al. 2016). Honey bee colonies have been declining by 30% or more over the last several years (Lee et al. 2015, Seitz et al. 2016) and pesticides are thought to contribute to this decline.

However, much of the focus on pesticide exposure has been concentrated on exposure and risk assessment of neonicotinoid

insecticides on honey bees. This is not surprising due to the low concentrations that are biologically active in honey bees (≤ 50 ppb, Yang et al. 2008) and also because of their ubiquitous presence in many geographic regions. Lu et al. (2016), in Massachusetts, found at least one neonicotinoid present in 73% of their pollen samples and 57% contained imidacloprid. A study in France revealed that half of all pollen samples tested positive for imidacloprid (Chauzat et al. 2006). Toxicology and acute and chronic effects of several neonicotinoids has been intensively studied (Guez et al. 2001, Suchail et al. 2001, Iwasa et al. 2004, Nguyen et al. 2009, Cresswell 2011, Henry et al. 2012, Di Prisco et al. 2013, Dively et al. 2015).

While many studies have investigated the effects of individual and simultaneous exposures of 2–3 pesticides on honey bees, multiple exposures to several pesticides may be a more realistic exposure scenario (Mullin et al. 2010). This is a complex undertaking not only due to the number of simultaneous pesticides that a colony can be

exposed to, but also due to the variety of exposure routes involved in a colony. A number of honey bee colony constituents can be and have been tested for pesticides to estimate exposure including the bees themselves, wax, honey, and pollen (Al Naggat et al. 2015). Pollen trapping has been a common route of exposure explored since residues on pollen brought into a hive can be linked directly to what bees are being exposed to while foraging (Chauzat et al. 2006, Stoner and Eitzer 2013, Lu et al. 2016). Pollen is an important food source for bees and therefore pesticide levels in pollen can have a direct negative effect on the bees feeding on it, especially the brood (Brodschneider and Crailsheim 2010). Because the honey bee is such an important beneficial insect, contact, and oral LD_{50} 's are required for registration of pesticides in the United States. The U.S. EPA has compiled these LD_{50} values in their ecotoxicity database (US EPA). Indices of risk to exposure are critical in assessing continued health of honey bee populations (Alix et al. 2014). A risk factor for honey bees can be calculated by measuring the amount of pesticide that bees are exposed compared to their associated LD_{50} values (Stoner and Eitzer 2013, Ostiguy and Eitzer 2014). These risk factors can help beekeepers understand the risk their bees are facing in different environments (Stoner and Eitzer 2013), although synergy, and effects of multiple modes of action are not currently addressed by this approach.

Although pesticide exposure assessments for honey bees have been conducted in several states and countries (Chauzat et al. 2006, Škerl et al. 2009, Drummond et al. 2012, Pettis et al. 2013, Stoner and Eitzer 2013), there is no baseline data for pesticide exposure to honey bees in Northern New England. This study examines pesticide exposure in pollen in Maine. We designed an assessment representing common ecosystems ranging from natural relatively undisturbed landscapes to residential and agricultural landscapes across the state of Maine. It was our goal to compare exposure rates among ecosystems within Maine and also to compare our findings in Maine to agricultural or nonagricultural landscapes previously reported from other regions in the United States.

Materials and Methods

Assessment

During the winter of 2015, beekeepers throughout Maine were solicited to volunteer their time and colonies to assist in trapping pollen throughout the state. We initially selected beekeepers who had at least two colonies and represented a diversity of geographic regions in the state and a diversity of landscapes within which their apiaries were embedded. However, poor overwintering success in many apiaries across the state necessitated finding additional volunteers just prior to the spring. A total of 26 volunteers/sites were involved in this project. In addition, colonies located in six lowbush blueberry fields were sampled season long by the Drummond laboratory, for a total of 32 sites (Fig. 1).

Each volunteer beekeeper was requested to describe the surrounding land use in the foraging radius of their apiary (ca. 3.2 km). The volunteers were provided with a front entrance pollen trap (Anatomic Front Mount Pollen Trap, Fig. 2), instructions for use, and collecting cups. Tape was suggested for use by beekeepers to provide a good seal around the edges of the pollen trap (Fig. 2). Instructions were to collect pollen from a single colony for a week in the spring (May–June), summer (July–August), and fall (September–October) during a period of warm sunny weather. Collected pollen was stored in the beekeepers freezers until collecting the final sample. Pollen from the three collection periods was sent overnight via

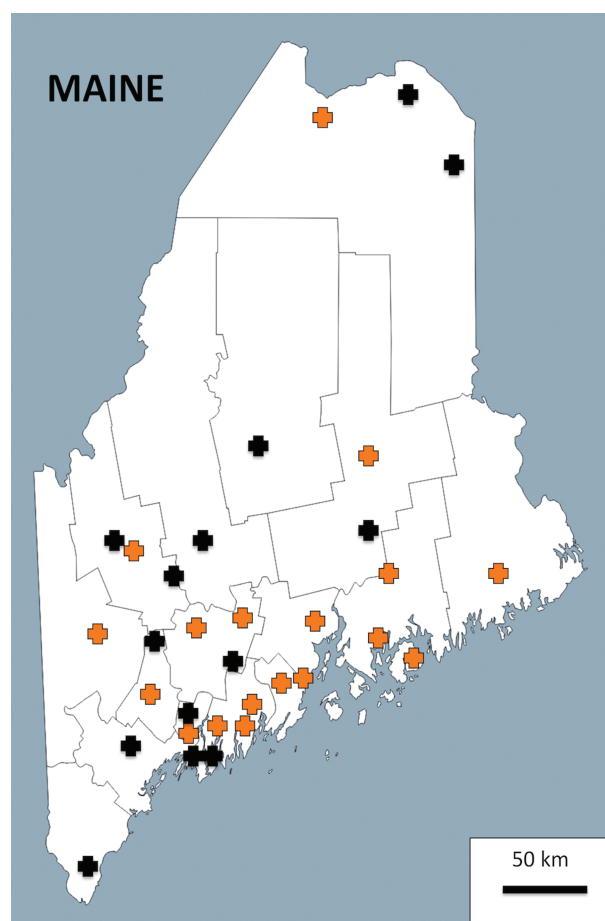


Fig. 1. Locations of honey bee colony apiary sites in 2015. Orange symbols represent the locations of agricultural sites and black symbols, nonagricultural sites.

Fed-Ex to the University of Maine where each site's pollen samples were aggregated over the three sample dates.

Analytical Chemistry

The 32 pollen samples were sent to the Connecticut Agriculture Experiment Station where Dr. Brian Eitzer ran a screen for 166 different pesticides and metabolites using HPLC and mass spectrometry with a modified QuEChERS (for Quick, Easy, Cheap, Effective, Rugged and Safe) procedure (Stoner and Eitzer 2013). The analytical procedures that we followed are not sensitive to pyrethroids unless detections are at high levels. Therefore, pyrethroid detections are probably under estimated in our study. The detection limits for the compounds that were included in our screen mostly ranged from 0.5 to 2 ppb, but some were as high as 10–30 ppb (Stoner and Eitzer 2013). In brief, 5 g pollen was spiked with 100 ng of isotopically labeled (d-4) imidacloprid (Cambridge Isotope Laboratories) as an internal standard. They were then combined with water to a total mass of 15 g. Next 15 ml of acetonitrile, 6 g magnesium sulfate, and 1.5 g sodium acetate were added. After shaking and centrifuging, 10 ml of the supernatant was combined with 1.5 g magnesium sulfate, 0.5 g PSA, 0.5 g C-18 silica, and 2 ml toluene. The samples were shaken and centrifuged and 6 ml of the supernatant was concentrated to 1 ml for instrumental analysis. Samples were analyzed using a Dionex 3000 LC interfaced to a Thermo Velos Pro Mass Spectrometer using an Agilent SB-C18-RRHD-2.1 mm × 150 mm 1.8 μ column and on a Agilent 100 LC interfaced to a Thermo



Fig. 2. Front entrance pollen trap that was distributed to volunteers and used by the University of Maine research team.

Exactive Mass spectrometer using a Hypersil Giold aQ-C-18 2.1 mm $\times$ 100 mm 1.9 μ column. Both instruments used a gradient elution program. The Velos Pro was operated in an MS/MS mode and was the primary quantitation instrument while the Exactive used the high resolution mass spectrometry data for confirmation of pesticide residues. The average quantitation limit (QL) for all compounds and metabolites ranged between 0.5 and 20 ppb. Hundred and fifty-three of the compounds had a QL of less than 5ppb with 88 compounds at 1 ppb or less.

Exposure and Risk Metrics

After the pollen residue results were obtained, a summary of the pesticide exposure by site was conducted. Concentration over the season for each site was expressed as ppb residue. Other measures used for assessment of exposure were the number of pesticide residue detections per site, and the diversity of exposure derived as the Shannon diversity index using concentration of each detection at each site.

We quantified risk by hypothesizing exposure through contact with the outside body of the bee (based upon the contact LD_{50}) and also through feeding on contaminated pollen (based upon the oral LD_{50}). To calculate contact and oral risk quotients, lethal dose 50th percentile values (LD_{50}) were compiled based upon available literature and public databases: [Helson et al. 1994](#), [Nauen et al. 2001](#), [Stoner and Eitzer, 2013](#), [US EPA 2008](#), [US EPA ECOTOX Database](#), and [Agritox](#). We calculated a bee colony's risk quotient by dividing the concentration of each pesticide quantified in trapped pollen for a given hive/site by the contact and/or oral LD_{50} estimated for honey bees. If LD_{50} values differed among literature sources, the value provided by the [EPA ECOTOX Database](#) was used; if more than one LD_{50} value was reported in this database, the lowest value was used ([Table 1](#)). The LD_{50} value for the parent compound was used, unless information specific to a metabolite was available. Oral and contact LD_{50} values reported in terms of μ g/bee were converted to ppb relative to body weight (ng pesticide per g bee) by multiplying each value by a factor of 10,000; this is an approximate equivalent to 1,000 ng per μ g $\div$ mean bee weight of 0.1 g ([Page and Metcalf 1984](#)). Therefore, a risk quotient of 1.0 suggests that, on average, the exposure level either by a contact or oral pathway will result in 50% mortality to honey bee populations. Risk quotients greater than 1.0 represent a high colony expectation of acute mortality. Based upon these risk quotients, we assessed risk both at the individual pesticide compound level and also

additively across all pesticides detected, a total colony risk. The total colony risk assumes that effects due to pesticides are additive and this is most likely not the case based upon several studies showing synergy. However, feel that this is acceptable as we use total colony risk only as a relative means of comparison among geographic locations and not as an absolute estimate of potential mortality.

Statistical Analysis

A general linear model, using data representing each apiary site as a stratum, was used to determine if differences existed between contact and oral risk quotients. Linear regression was used to assess if a constant ratio in difference between contact and oral risk quotients existed. In all cases, logarithmically transformed risk quotients were used in our analyses to meet the assumptions of homoscedasticity and normality. General linear models were also used to test if estimated proximate land-use type determined by the volunteer beekeepers (i.e. wild blueberry, other agriculture, and nonagriculture) and geographic location in the state (represented by latitude, longitude, and the interaction of the two coordinates) determined pesticide and metabolite concentration, contact risk quotient, and oral risk quotient. We relied upon the beekeepers to use their own methods of quantifying the land-use type composition about their apiaries, although we did tell them to confine their assessment to a 3.2 km radius of their apiary. The radius was described as an average foraging distance from the hive for worker bees ([Drummond et al. 2012](#)). The dependent variables were logarithm transformed (base 10) to meet the assumptions of the analyses of variance ([Zar 2010](#)). Poisson regression was used to test the effect of land-use type on the mean number of pesticide and metabolite detections and the Shannon diversity index ([Shannon 1948](#)) of pesticide contamination in trapped pollen. To test the hypothesis that apiary sites close in geographic distance are more likely to be exposed to similar measures of pesticide exposure (# detections, ppb, diversity, oral, and contact risk quotients), we used a Mantel test. The geographic distance matrix was a squared Euclidean distance and the pesticide exposure matrix with the 5 pesticide exposure measures (defined above) used a Sorenson similarity metric. Both asymptotic and randomization tests were performed (PC-ORD, version 6, [McCune and Mefford 1999](#)).

To determine the effects of the GIS digital landscape ([MELCD 2004](#)) surrounding each apiary on pesticide exposure, landscape

Table 1. Pesticide residues in trapped pollen in Maine, 2015^{a,b}

Pesticide group	Chemical name	Mean pollen concentration ppb/hive (s.d.) ^c	Mean pollen concentration detected apiaries only ppb/hive	Apiaries detected	Contact LD ₅₀ (ppb)	Oral LD ₅₀ (ppb)	LD ₅₀ Source ^d
Fungicide	Carbendazim	1.6 (4.3)	4.9	11	5.00E+05	5.00E+05	1
	Propiconazole	3.4 (13.1)	12.6	9	2.50E+05	1.00E+06	1,2
	Pyraclostrobin	11.5 (122.3)	47.7	8	1.00E+06	7.31E+05	1,2
	Boscalid	27.8 (145.8)	183.6	5	2.00E+06	2.00E+06	1,2
	Thiabendazole	3.8 (70.1)	42.0	3	4.00E+04	3.40E+05	2
	Thiophanate-methyl	6.0 (110.9)	66.0	3	1.00E+06	1.00E+06	1
	Cyprodinil	147.9 (3408.3)	2440.0	2	8.00E+06	8.00E+06	1
	4-Hydroxychlorothalonil	3.2 (15.6)	53.0	2	1.81E+06	1.81E+06	1 ^e
	Azoxystrobin	0.1 (na)	0.9	1	2.00E+06	3.00E+05	1,2
	Difencconazole	0.5 (na)	18.0	1	1.00E+06	2.00E+06	1
	Fludioxonil	496.9 (na)	16400.0	1	1.00E+06	1.00E+06	1
	Propamocarb	0.1 (na)	0.7	1	na	na	na
	Atrazine	1.9 (12.5)	6.5	10	9.70E+05	9.70E+05	3
	Pendimethalin	0.8 (2.5)	3.3	8	4.98E+05	4.98E+05	1
Herbicide	Diuron	0.9 (6.9)	5.0	6	1.00E+06	1.00E+06	1
	Metolachlor	0.2 (0.6)	0.9	6	1.10E+06	1.50E+06	3
	Hexazinone	3.6 (47.0)	29.9	4	na	na	na
	Metribuzin	0.1 (na)	3.4	1	6.04E+05	2.00E+06	1,2
	Metalaxyl	0.1 (na)	0.6	1	1.00E+06	2.50E+05	1
	Sethoxydim	1.5 (na)	51.0	1	1.00E+05	1.00E+05	1
	Simazine	0.6 (na)	21.0	1	9.67E+05	9.67E+05	1
	Phosmet	85.6 (1066.3)	706.5	4	1.06E+04	3.70E+03	1
	Carbaryl	1.8 (19.5)	19.6	3	1.10E+04	2.31E+03	1
	Acetamiprid	0.8 (na)	27.0	1	8.10E+04	1.45E+05	1,2
Insecticide	Indoxacarb	0.1 (na)	3.7	1	1.06E+04	3.70E+03	1

^aBolded pesticides comprise five most frequently detected.

^bSources for honey bee LD₅₀ values are provided in methods, na means not available.

^cStandard deviation in parentheses, na means not applicable because only 1 detection from 32 apiaries.

^dSources for honey bee LD50 values: 1 = US EPA, 2 = AGRITOX, 3 = Stoner and Eitzer (2013).

^eLD₅₀ for Chlorothalonil.

composition was examined using a statewide map developed to assess bee habitat across Maine (Groff et al. 2016). This digital land cover has 5 m spatial resolution and seven land cover types: nonblueberry agriculture, wild blueberry field, coniferous forest, deciduous/mixed forest, emergent wetland, urban/developed, and wetland/open water. The proportion of these seven land cover types in the estimated foraging area (3.2 km radius, Drummond et al. 2012) around each of the 32 sites was calculated using an ArcGIS-derived Python script (ArcGIS version 10.2, Esri, Redlands, CA, United States; Python 2.7, Python Software Foundation, <https://www.python.org/>; Kaszas 2012). The area of each cover class (km²) was then used in latent structure projection or partial least squares (Wold 1966) to model the effect of surrounding landscape on pesticide exposure. All land cover types ($n = 7$) were used for the predictor matrix and all exposure metrics (number detections, diversity, log (ppb), log (contact risk)) were used for the dependent matrix. Our estimate of oral risk quotient was not used in the analysis since it was highly correlated to the contact risk quotient and did not add any additional power in preliminary modeling trials. The model was fit with the statistical software JMP (2015) using the NIPALS algorithm and van der Voet's T² test was used to assess the number of extracted factors to include in the model (van der Voet 1994).

Results

In our assessment in 2015, 25 pesticides or their metabolites were detected in pollen at the 32 sites (Table 1). There were 94 total residue detections (total number detections across all pesticides, i.e.

many detections were the same pesticide) or 2.9 detections per site. The average of the mean (per hive) concentration of all pesticides detected in pollen samples aggregated over the entire spring–fall season per site was 32.04 ± 102.37 (SD) ppb (parts per billion). There were 5 sites (15.6%) that had no pesticides detected.

Detections by pesticide class can be seen in Fig. 3a. Fungicides and herbicides constituted the majority of the detections, while insecticides only comprised 9.6% (of all detections (Table 1, $n = 94$). The top five pesticides detected (in terms of frequency of detections) are also shown in Table 1. The fungicide, carbendazim was the most commonly detected pesticide, however, thiophanate-methyl rapidly breaks down to form carbendazim—so the presence of carbendazim could also be from use of thiophanate-methyl. The other most frequently detected pesticides are the herbicide, atrazine; the fungicide, propiconazole; the fungicide, pyraclostrobin; and the herbicide, pendimethalin. Of these, propiconazole is a common fungicide used in wild blueberry production almost exclusively for the control of mummy berry disease (incited by the fungus, *Monilinia vaccinii-corymbosi*), formulated as Orbit and Tilt. When exposure was assessed in terms of concentration (ppb) and not detections, a slightly different picture emerges. Fungicides make up the majority of exposure with herbicides almost being imperceptible and insecticides about 11% of the total residue concentration (Fig. 3).

In our study, risk was measured as the exposure concentration (ppb) of a specific pesticide or metabolite in pollen divided by the concentration that is expected to kill 50% of the exposed worker bees (oral or contact LD₅₀). Risk quotients 1.0 or greater should be

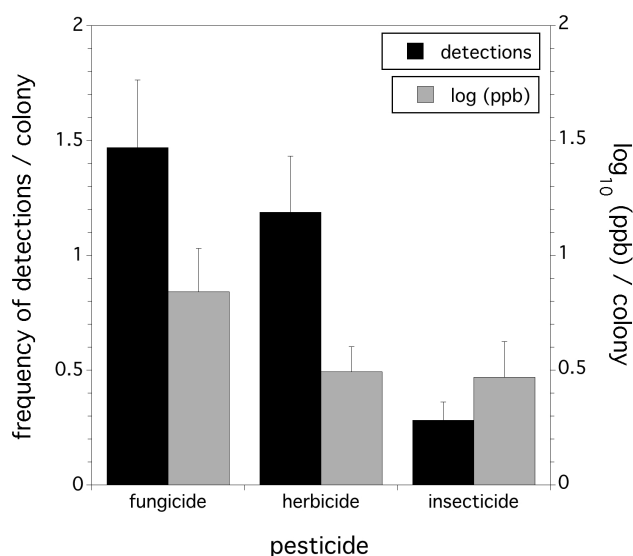


Fig. 3. Frequency of detections and concentrations ($\log_{10}$ (ppb)) per colony of insecticides, fungicides, and herbicides in the 2015 honey bee hive assessment in Maine.

of concern because we would expect 50% or more bees exposed to that level to die. A Risk Quotient of 0.2 suggests crudely (assuming the dose–mortality response is linear), that the level of exposure to that particular pesticide is expected to kill 10% of the colony work force ($0.5 \times 0.2 = 0.1$). Therefore, a risk quotient of 0.2 might also be considered a significant risk to the beekeeper. Figure 4 shows that both contact and oral risk is due to exposure, almost entirely to insecticides. This is important to realize, considering that Fig. 3 shows that detections and concentrations of pesticide residues in pollen were primarily represented by herbicides and fungicides.

Overall, oral risk quotients were numerically higher than contact risk quotients (oral = 0.025 ± 0.019 vs contact = 0.009 ± 0.007). Figure 4 shows that for both contact (Fig. 4A) and oral (Fig. 4B) risk quotients, insecticides constitute almost all the risk proportionately, despite fungicides and herbicides constituting most of the pesticide detections and concentrations of residues in pollen (Fig. 3). Figure 5A and B depict the colony frequency distributions of logarithm transformed oral and contact risk quotients. Considerable orders of magnitude variation in risk quotients can be seen for both oral and contact exposure. A general linear model stratified by apiary site did not provide evidence that mean risk differed by oral compared to contact exposure ($F_{(1,24)} = 0.3174$, $P = 0.578$). We found a linear relationship between oral and contact risk quotients (intercept = -0.631 ± 0.182 , slope = 0.8531 ± 0.042 , $P < 0.0001$). This regression suggests that very little difference in the ratio between oral and contact risk coefficients exists across the range of contact risk quotients (-6.22 to -0.208). There is a tendency for oral risk quotients to be less than contact below -4.0 and higher than contact risk quotients above -4.0 . Table 1 shows that, on average, detections and concentrations of insecticides were very low, resulting in low potential risk, despite insecticides making up the majority of oral risk in the 2015 pollen samples.

We assessed whether the number of detections and diversity of pesticide exposure (Shannon diversity index) was determined by average foraging distance within the estimated beekeeper proximal land-use type (i.e. wild blueberry, other agriculture, and nonagriculture) as defined by the beekeepers' knowledge of their sites through a written description. Diversity of trapped pollen pesticide contamination

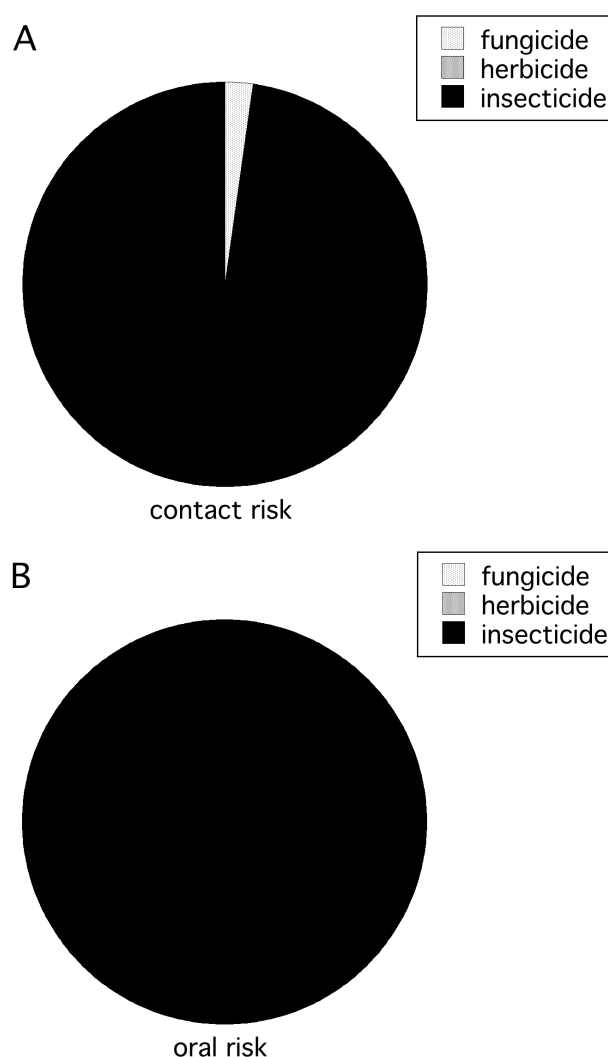


Fig. 4. Proportional risk by pesticide type due to contact (A) and oral risk (B) exposures throughout Maine in the 32 apiaries.

was not determined by land-use ($\chi^2_{(2)} = 3.854$, $P = 0.146$). However, the number of detections was determined by land-use ($\chi^2_{(2)} = 29.108$, $P < 0.001$). Figure 6 shows the mean number of detections by beekeeper-assessed land-use type and the ranking of the means by single degree of freedom contrasts. When concentration and risk quotient were summarized by the beekeeper-assessed land-use (Fig. 7), the concentration of pesticide residues in pollen (Fig. 7A) was significantly greater in wild blueberry and other agricultural areas by an order of 1.5 magnitude difference ($F_{(2,29)} = 6.094$, $P = 0.006$). Risk quotients (Fig. 7B), both contact and oral were not significantly different between landscape types with both separate analysis of variance or when risk quotients were analyzed together with a multiple analysis of variance ($P > 0.10$); although, a trend in increasing average risk quotient in agricultural land-use types compared to nonagricultural land-use types can be seen. Pesticides that were unique (>2 detections) to agricultural land-use types were four; the fungicide thiophanate-methyl (Topsin M, among others, three detects), the insecticide phosmet (Imidan, four detects), the herbicide metolachlor (Bicep, six detects), and the fungicide pyraclostrobin (Insignia, eight detects). There were no pesticides or metabolites detected in nonagricultural areas, but not found in agricultural areas. We did not find any pattern in logarithm ppb concentrations, log contact

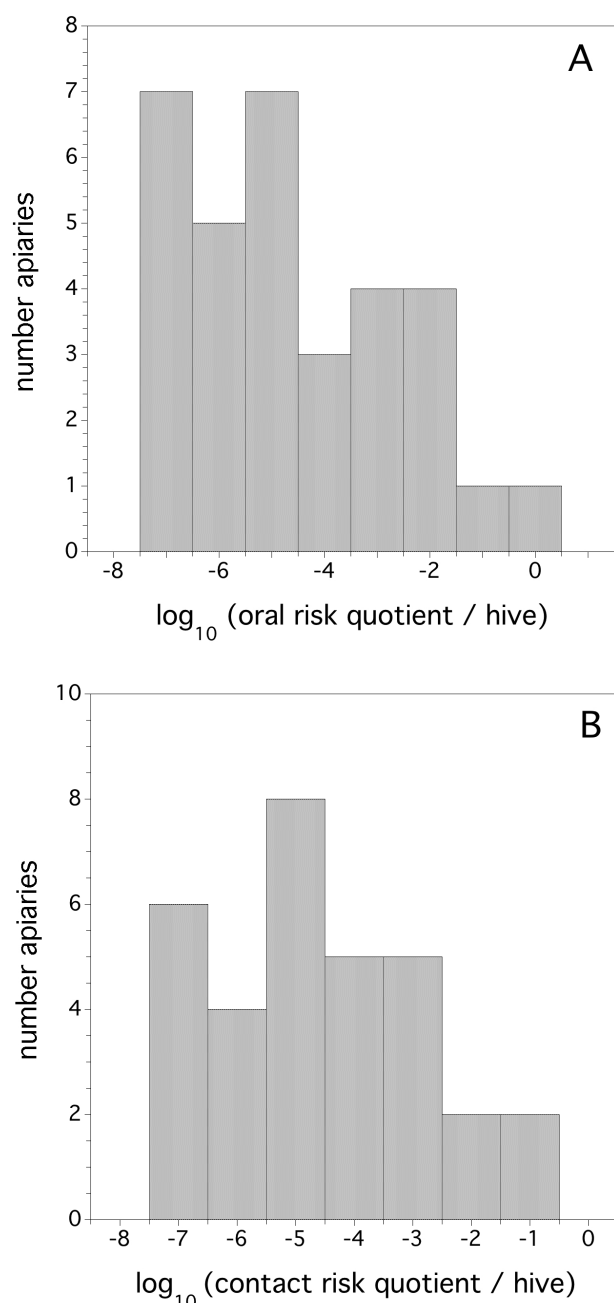


Fig. 5. Frequency distribution of log transformed oral risk quotients (A) and contact risk quotients (B) across all 32 apiary sites.

risk quotients, or log oral risk quotients (residuals from land-use general linear model above) across latitude or longitude. However, a trend was exhibited ($F_{(1,22)} = 2.639$, $P = 0.109$) in oral risk quotient across latitude, possibly decreasing from southern Maine to northern Maine. We did not find a significant correlation between geographic distance between apiaries and similarity in exposure and risk ($P > 0.05$ with both an asymptotic approach and a randomization approach ($n = 999$)).

Landscape composition of the honey bee foraging area about each apiary determined by GIS analysis of each apiary site explained 43.93% of the variance in pesticide exposure according to a two-factor partial least squares model. The most important predictors describing pesticide exposure were the area (ha) of blueberry, coniferous forest, and urban/developed land cover types. The mean

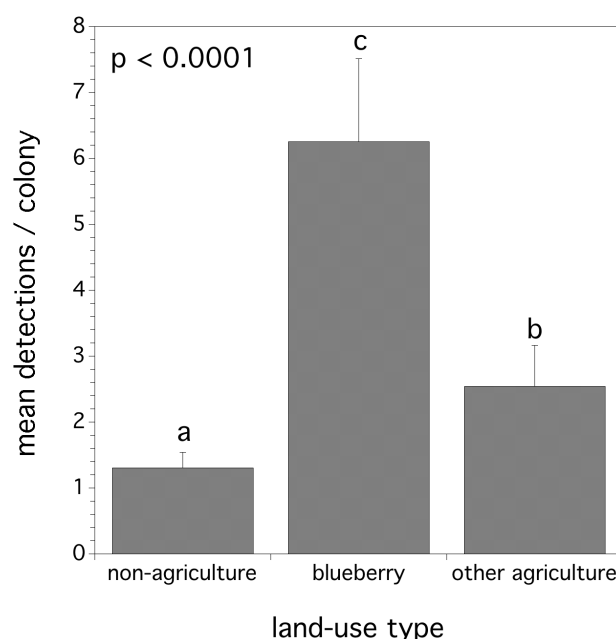


Fig. 6. Mean number of pesticide and/or metabolite detections per apiary for each of the three land-use types suggested as foraging habitat around an apiary by the beekeepers. Bars with the same letters are not significantly different (Poisson regression, single degree of contrast comparisons). Error bars are standard errors.

of the coefficients for the three pesticide exposure measures are (blueberry: 0.359 ± 0.097 (se); coniferous forest: -0.170 ± 0.091 ; urban/developed: -0.173 ± 0.019). A positive coefficient suggests that as land area of that GIS-derived landscape type increased, so did pesticide exposure in pollen. A negative coefficient represents the opposite relationship. Therefore, more exposure is expected when the apiary is within 2 miles of a large area of blueberry land cover and less within 3.2 km of a large area of coniferous forest. Figure 8A–C depicts the landscape predictions in exposure as measured by the number of detections, total logarithm (ppb) concentration, and logarithm contact risk quotient compared to the observed pollen samples. In all three cases (a–c), significant relationships are represented between the observed measures and the model predictions (detections: slope = 0.700 ± 0.095 , $P < 0.0001$, $r^2 = 0.687$; log (ppb): slope = 0.406 ± 0.102 , $P = 0.0006$, $r^2 = 0.380$; log (contact risk quotient): slope = 0.211 ± 0.085 , $P = 0.021$, $r^2 = 0.177$). It is also apparent that in all three of our measured exposure measures, the model predictions underestimate the observed measures. Figure 8D shows, as an example, the relationship between the land cover area in conifer forest ($\log_{10}$ transformed) within 3.2 kms of the sampled apiary sites and the number of total pesticide detections in sampled pollen ($F_{(1,30)} = 10.969$, $P = 0.002$, $r^2 = 0.243$).

Discussion

This study is one of the first in the United States that provides a baseline pesticide exposure to honey bees statewide, not pertaining specifically to agricultural landscapes, although Stoner and Eitzer (2013) did assess five locations in Connecticut over several years from 2007 to 2010. It is important to note that our estimates of pesticide exposure and risk to honey bees are only a relative measure of exposure and most likely underestimate the total seasonal exposure in Maine. This is because we only pollen trapped for three 1-week periods during the spring, summer, and fall. Future studies

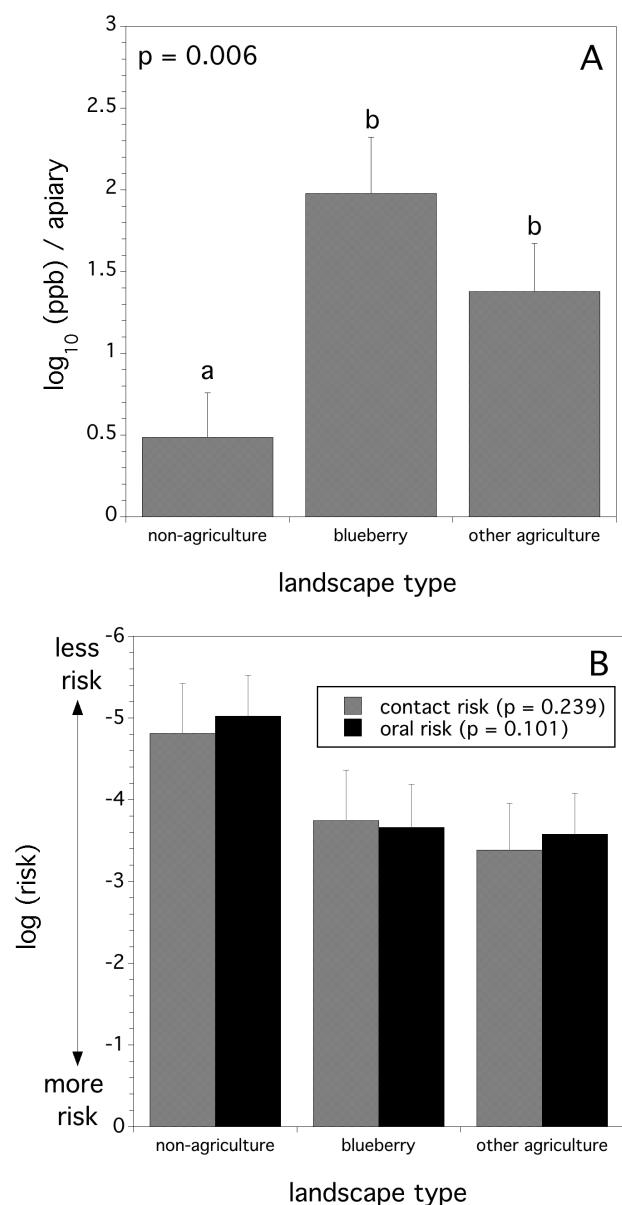


Fig. 7. Concentration (log ppb) (A) and logarithm transformed contact and oral risk quotients (B) for the three beekeeper-assessed land-use types (wild blueberry, other agriculture, and nonagricultural) suggested as foraging habitat around an apiary by the beekeepers. Bars with the same letter are not significantly different, Tukey test. Error bars are standard errors.

might involve a more rigorous sampling over time. However, when utilizing volunteers to conduct research, there is always a tradeoff between consistent, uniform, and careful collection of data and quantity of data collected (Dickinson et al. 2010). It is certainly likely that because of our sampling protocol we might have missed pulse exposures of pesticides that dissipate quickly in the environment (Tuzimski 2012). In addition, de Oliveira et al. (2016) showed that pollen has differential affinity and sorption potential for various pesticides and so actual tallies of compound-specific exposure only provides a relative estimate of risk to honey bees.

The total number of individual pesticides or metabolites detected in pollen was 25. This is in contrast to pollen contamination found in Connecticut by Stoner and Eitzer (2013), who found 60 pesticides and metabolites from five locations, but over a 2–5 yr period. In Connecticut, residues ranged from 1 to 16,556 ppb with a mean

of 69.4 ppb averaged over all sites, years, and compounds. In our study, detection rate per apiary site and concentrations were of similar ranges (range = 0.6 to 16,400 ppb, mean = 27.1 ppb) and were dominated by the generally less toxic fungicides and herbicides. However, fungicide exposure to honey bees has been suspected of synergizing insecticide toxicity (Thompson and Wilkins 2003, Iwasa et al. 2004, Johnson et al. 2013) in the honey bee and resulting in sublethal physiological impairment (Vandame and Belzunces 1998, Desneux et al. 2007). This was a different trend than that reported in a study conducted by Chauzat et al. (2006) in five regions in France. They found only 20 pesticide compounds in 36–81 samples analyzed and the contaminant levels were dominated, in frequency of detection, by the four insecticides/metabolites: imidacloprid (49.4%), 6-chloronicotinic acid (44.4%), fipronil (12.4%), and fipronil desulfinyl (11.1%). They also found that insecticides and miticides were also the dominant contaminants from the perspective of concentration. Mean concentrations ($\mu\text{g}/\text{kg}$) for the four largest contaminants in their pollen analyses were: coumaphos (925 $\mu\text{g}/\text{kg}$), Tau-fluvalinate (487), carbaryl (219), and endosulfan (81). In Maine, insecticides, while at low concentrations, constituted the highest risk of the three pesticide classes, but the individual apiary site risk of exposure was very low. There was only one of 32 sites that resulted in a summed risk quotient that was of concern (0.22 contact and 0.64 oral). This site was close to an apple orchard and phosmet (Imidan) exposure was relatively high. Phosmet is a common insecticide applied to both tree and small fruits. It was also shown to be a common contaminant of pollen trapped from honey bee colonies in Maine blueberry landscapes (Frazier et al. 2015). In our study, we also found that nonagricultural sites as assessed by beekeepers, did have significantly lower exposure concentrations of pesticide residues in pollen than agricultural landscapes, but overall risk to colonies did not differ significantly due to the variability between sites. We also found that based upon a digital land cover data base, apiaries within foraging distances of urban/developed, and conifer forested landscapes had fewer pesticide residue detections in pollen, lower residue concentrations and lower risk quotients as land area of these land cover types increased. The relationship between urban/developed land cover type and pollen contamination by pesticide residues was a surprise as several researchers have suggested that residential and urban areas in the United States tend to be characterized by significant pesticide contamination and exposure to children (Racke and Leslie 1993, Lu et al. 2001, Lu et al. 2008).

Neonicotinoid insecticide exposure has been implicated as a serious threat to bee health (Goulson 2013, Lundin et al. 2015). We found that neonicotinoids were not an exposure risk to honey bees in Maine in 2015 and are probably not a threat most years in most parts of the state, based upon the proportion of land area that is nonforested (<8%, Huff and McWilliams 2016). This was not the case in France, Connecticut, or Massachusetts (Lu et al. 2016). In Massachusetts, 73% of all sampled pollen contained at least one neonicotinoid and the spatio-temporal variation was characterized by peak neonicotinoid detections in April through August, depending upon the geographic sampling site. This does suggest that colonies in Maine could have been exposed to one or more neonicotinoids at times that pollen was not collected, although we did collect during August, the month that colonies in Massachusetts were exposed to the highest concentrations of neonicotinoids. Although it is important to note that our detection limits for neonicotinoids ranged from 1 to 2 ppb (metabolites of imidacloprid from 3 to 10 ppb), while those of Lu et al. (2016) for their Massachusetts study were an order of magnitude lower at 0.1 ppb.

Odoux et al. (2014) and Sponsler (2016) (in France and Ohio, USA, respectively) found that forest land cover in agricultural landscapes are correlated with colony productivity. Unfortunately, these

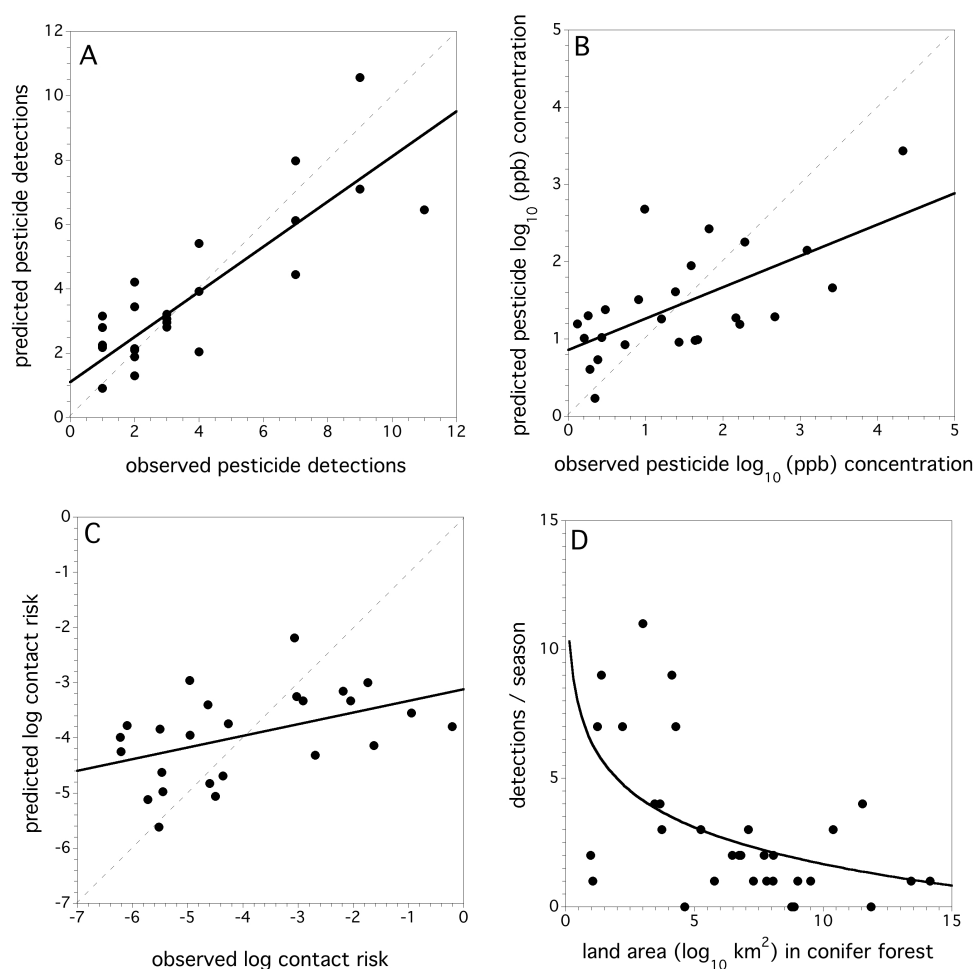


Fig. 8. Partial least squares model predictions and observed pesticide exposure measures for total detections (A), log pesticide concentration ppb (B), log contact risk quotient (C). Solid lines are least square regressions and dashed lines represent a slope of 1.0 or perfect prediction. (D) shows the relationship between conifer land area (logarithmically transformed) and the number of pesticide detections.

authors did not specifically classify the forest stands and so it is not known if the majority of forest stands were deciduous or coniferous. Sponsler (2016) also found that honey production was negatively correlated with urban landscapes. He suggests that this is a result of a lack of forage in urban landscapes, relative to agricultural landscapes. Does landscape result in a similar pattern for pesticide exposure? Our study in Maine suggests that honey bees foraging in agricultural land cover increases the likelihood of pesticide exposure via pollen, while conifer forest and urban/developed land cover decreases the likelihood of exposure. While herbicides are used in Maine forest management (usually glyphosate), the application is generally only once immediately after a clearcut operation in order to reduce deciduous tree competition to emerging stands of coniferous timber and pulp species being managed (Lough-Guiseppa et al. 2006). This one time application is low in frequency relative to the 60–80 year stand management horizon (LeVert et al. 2007).

We were not able to find many studies that assessed the indirect effects of land cover on pesticide exposure to honey bees. Heimbach et al. (2016) attempted to standardize land cover in a study on the impact of clothianidin on insect pollinators and thus no environmental effect on risk could be determined. Native bee pesticide exposure studies appear to have focused more on land cover. Hladik et al. (2016) showed that pesticide exposure in native bee communities was not related to land cover types (compared agricultural,

grasslands, and open/developed land cover types) in Colorado. Park et al. (2015) showed that the native bee community pollinating apples in New York had less risk to pesticide exposure when the landscape surrounding the apple orchards was comprised of higher amounts of natural landscape. Whether this type of pesticide risk mitigation occurs with honey bees in lowbush blueberry is not explored in our study due to a lack of sample size ($n = 6$ blueberry landscapes sampled), but is an intriguing research question to pursue.

Within agricultural landscapes, Barmaz et al. (2010) found that perennial crop agricultural ecosystems increased pesticide exposure to honey bees relative to annual crop systems and that the exposure was greatest in the spring. A somewhat similar pattern was observed in Maine. We found that both beekeeper-assessed land-use type and our estimates of land cover type derived from a digital land cover data base, suggests that lowbush blueberry, a perennial crop system, had significantly higher detection frequency of pesticides in trapped pollen compared to other agricultural landscapes (mostly annual cropping systems). However, mean pesticide concentration in pollen and exposure risk was not significantly different between lowbush blueberry and other agricultural landscapes.

In summary, based upon our assessment, honey bees do not appear to be at great risk to pesticide exposure, even in agricultural landscapes. This appears to be related to Maine's landscape composition. Maine is estimated to be about 93% forested (McCaskill

2014) based upon a total land area of 91,633 km² (US Census Bureau 2012). Approximately 50% of this land area is conifer forest (O'Connell et al. 2014), a very poor bee habitat in Maine (Groff et al. 2016). The urban/developed and crop landscape areas each only comprise about 2.0–2.5% of Maine land area (Plantinga et al. 1999). Thus, it can be seen why pesticide exposure to honey bees would be low, on average, across the state. Even industrial chemical pollution would be estimated to be low, given the percent of land cover in urban/developed landscapes (2.5%).

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