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Lobster capture. (Photo: Stock File)

Pesticide effect on lobster in the Northumberland Strait analysed

CANADA
Wednesday, August 26, 2015, 02:40 (GMT + 9)

A team of researchers is developing a study on the potential impact of agricultural pesticide run-off on lobsters in the Northumberland Strait.

The three-year study is the result of the joint work carried out by scientists of the University of Prince Edward Island (UPEI), the PEI Fishermen's Association (PEIFA), and Homarus Inc.—a non-profit organization managed by the Maritime Fishermen's Union.

Funds for the analysis were provided by a Strategic Partnership Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC).

"The lobster populations in the Northumberland Strait have been collapsed for quite a number of years, and no amount of fisheries measures seem to have improved that," points out Dr. Michael van den Heuvel, UPEI's Canada Research Chair in Watershed Ecological Integrity and principal investigator of this project.

"The conclusion is that there are other influencing factors that are not coming from the strait itself. They're, in fact, coming from the surrounding land," the scientist adds.

The researcher explains that estuaries and coastal zones are under environmental pressure due to urban development, agriculture, and climate change. In the southern Gulf of St. Lawrence, this is of special concern.

For his part, PEIFA president Craig Avery stressed that the uniqueness of that area is mainly because of the proximity to shore on both sides and said that among those contaminants are pesticides that are highly toxic to invertebrates, especially crustaceans such as lobster.

Referring to the study, a research scientist with Homarus Inc., Dr. Dounia Daoud, highlighted: "We are interested in any effect that could potentially be an explanation. It could be temperature. It could be pesticides. It could be acidification. We are studying all of those aspects."

"I am proud that the fishermen are taking care of their resource. I think it's important. They are at the origin of our involvement of this project. It's original and new that fishermen are taking care of the future," Daoud concluded.

The project will also establish new long-term monitoring methods to improve our understanding of how the environment changes in response to activity on land.

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CALS researchers deploy insect 'birth control' to protect cranberries

Aug. 20, 2015 by Nicole Miller

Sundance cranberries at a farm near Wisconsin Rapids, Wis.

Photo: Jeff Miller

It's no fun being a male moth in one of Shawn Steffan's cranberry research plots in central Wisconsin. When the time comes to mate, it's tough to find a partner.

Here's why: Using an approach known as pheromone-based mating disruption, Steffan and his team dot their test fields with hundreds of dollops of pheromone-infused wax — known as SPLAT for short — that give off the scent of female moths ready to mate.

The males can't tell the difference between the pheromone plume emanating from the SPLAT versus the real thing — and many die before they are able to home in on a real partner.

"We throw a wrench into their communication system with lots of false plumes. In essence, it's moth birth control," explains Steffan, a UW-Madison College of Agricultural and Life Sciences professor of entomology and a USDA entomologist.

Wisconsin is the nation's leading producer of cranberries, growing more fruit than all other states combined. Insect pests are a perennial problem, and while growers have insecticide sprays that largely do the job, Steffan notes, there's room for improvement — especially in the interest of saving pollinators, including honeybees.

"One of the typical spray-timings for the cranberry fruitworm is when the adult moths are flying, which is right during bloom when the honey bees are out," explains Steffan. "That's one of the huge drivers behind pheromone-based mating disruption — to avoid spraying when pollinators are active."

In addition to such environmental benefits, this approach could also have a major impact on growers' bottom lines. By doing fewer pesticide applications, the state's cranberries should have an easier time entering European and Asian markets, which have stricter rules about pesticide residue levels.

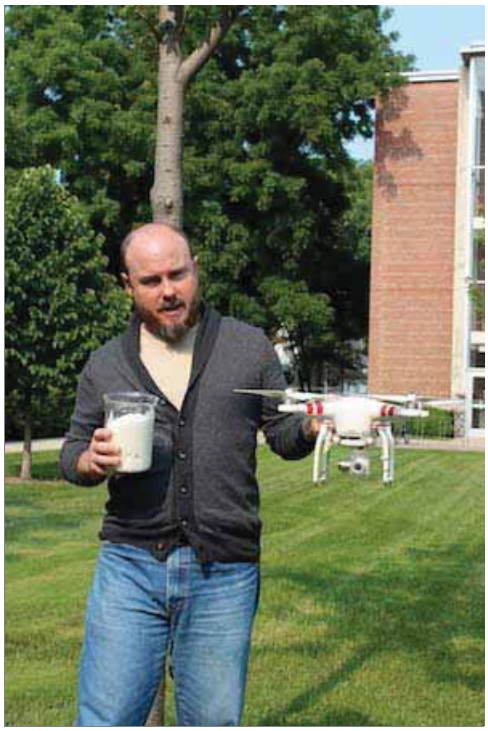
"Wisconsin fruit has sometimes failed to meet those standards," says Steffan, "but mating disruption is poised to change that."

Growers of all stripes are eager to get their hands on this new option, including organic growers, who need more pest control options. "This will give them a powerful new tool," Steffan says.

To speed things along, Steffan and his team are hard at work trying to mechanize the application of SPLAT. They are particularly excited about the potential of unmanned aerial vehicles (UAVs, better known as drones) and are working with Brian Luck, a CALS/UW-Extension professor of biological systems engineering, to build the perfect UAV for the job.

Steffan's team is also exploring reformulating the SPLAT recipe. It currently works against two of the state's top three cranberry pests: the cranberry fruitworm and the blackheaded fireworm. But they want to go for the trifecta by adding the pheromone for the sparganathis fruitworm.

"I think this would be the first-ever three-species mating disruption blend," says Steffan. "That's what I dream about."



Shawn Steffan holding a jar of pheromone-infused wax known as SPLAT, and an unmanned aerial vehicle. Steffan is working to develop a much larger UAV system to deliver SPLAT to cranberry fields. Photo: Joan Fischer

Genetically engineered potato gets federal OK

pressherald.com/2015/08/28/genetically-engineered-potato-gets-federal-ok/

By KEITH RIDLER The Associated Press

BOISE, Idaho — The U.S. Department of Agriculture has approved a potato genetically engineered by Idaho-based J.R. Simplot Co. to resist the pathogen that caused the Irish potato famine and that still damages crops around the world.

“For historical reasons and current agriculture reasons, this is an important milestone,” said Haven Baker, vice president of plant sciences at Simplot. “The Irish potato famine did change a lot of Western history. Even today – 160 years later – late blight is a $5 billion problem for the global potato industry.”

The USDA made the announcement Friday on its website.

The Russet Burbank variety the USDA approved is the second generation of Simplot’s Innate potatoes and also includes the first generation’s reduced bruising and a greater reduction in a chemical produced at high temperatures some studies have shown can cause cancer.

The second generation potato also includes an additional trait the company says will allow potatoes to be stored at colder temperatures longer to reduce food waste.

Baker notes that the modifications were made by silencing existing genes or adding genes from other types of potatoes.

The late blight resistance, he said, came from an Argentinian variety of potato that naturally produced a defense to late blight.

“It’s potato genes in the potato,” he said. “There are clear benefits for everybody, and it’s just a potato.”

However, one of the company’s oldest business partners – McDonald’s – has already rejected using the company’s first generation Innate potato. The company didn’t immediately respond to calls for comment about the Russet Burbank potato.

Shut out of that market, the company is focusing on grocery stores.

The first generation of the Innate potatoes, approved by the Food and Drug Administration as safe for consumers in March, were marketed as White Russets. Doug Cole, the company’s director of marketing and communications, said about 400 acres worth sold out last summer in grocery stores in 10 states in the Midwest and Southeast. The company plans to market about 2,000 acres of potatoes next summer.

“Our focus is on the fresh market for the coming year,” Baker said. “We think the benefits are clear. We’ve got customers and it’s a place that we’re excited to be. To some degree I think we need to prove that consumers are willing to buy White Russets, and they know what they are and that they see the benefits. Then I think the other parts of the industry will come.”

For the second generation Innate potato, the next step in the process is approval by the FDA as safe for consumers. The potatoes must also be approved by the U.S. Environmental Protection Agency, which regulates genetically modified organisms.

The company said they expected those approvals within a year. Commercial planting would likely begin in 2017, with the second generation potatoes available to consumers that fall. Cole said it hasn't been decided how the potatoes will be marketed.

Baker said the company is currently working on the third generation of Innate potatoes that will have a resistance to a type of virus that can make potatoes unmarketable.

He said the company hopes to eventually have potatoes that require less water and can better survive heat and drought stress, a benefit Baker said that could be important as climates appear to be more volatile.

"I think that from a scientific perspective, these biotechnology tools have a lot of promise," he said.

The New York Times http://nyti.ms/1JGTrWu

SCIENCE

Replacing Pesticides With Genetics

By DEVIN POWELL AUG. 31, 2015

GENEVA, N.Y. — Every spring, a host of unwelcome visitors descends on the Hansen farm in upstate New York. Diamondback moths blown in from the South threaten rows of cabbages to be sold for slaw and sauerkraut.

The moths can't be fought off with a single insecticide. Workers must spray a series of chemicals throughout the growing season to keep the moths' numbers in check.

"You have to rotate what chemical you use so you don't get a buildup in resistance," said Ed Hansen Jr., whose family has farmed the land for nearly a century. This adaptability, he said, makes the moths one of the worst pests to deal with each season.

At a university laboratory down the road, scientists are hoping to substitute sex for sprays. They have genetically modified thousands of diamondback moths, infusing them with DNA designed to kill female larvae. In August, the researchers began introducing the altered moths into outdoor cages in a field, where their mating habits will be monitored.

If the results are promising, the transgenic moths will be released into a small cabbage patch next summer. It would be the first experimental release on American soil of insects genetically engineered to self-destruct.

A plan to let similar transgenic mosquitoes loose in Key West, Fla., has met with strong opposition amid fears about being bitten. But federal regulators seem to have few worries about the safety of the moth experiments.

"Our goal as a community is to reduce the amount of pesticides used in agriculture," said Anthony Shelton, the entomologist running the experiments at the Cornell University Agricultural Experiment Station. "Why not use genetics to accomplish this?"

An invasive species, the diamondback moth was once a minor nuisance. It became an agricultural headache in the late 1940s as chemical pesticide use exploded. The moth, the first crop pest to evolve resistance to DDT, multiplied as feebler competitors died off.

Today, the pest is found where kale, broccoli, Chinese cabbage and other cabbage cousins grow. Hungry caterpillars that hatch from eggs laid on the plants cost farmers an estimated $5 billion a year worldwide. And the diamondback moth continues to adapt to new generations of pesticides. In Malaysia, it is immune to

all synthetic sprays.

In the 1990s, scientists searching for alternatives to pesticides bombarded diamondback moths with gamma rays to sterilize them. This tactic had eradicated from the United States a parasitic fly larva called the screwworm; for decades, hordes of radiation-sterilized male flies were released in the wild, outcompeting fertile males and reducing the population.

But the diamondback resisted even radiation. So Oxitec, the British biotechnology company working with Dr. Shelton, found another way to sabotage diamondback reproduction. The company, an Oxford University spinoff, stitched together scraps of DNA from a virus and a bacterium to make a gene deadly to female insects.

A female larva harboring the gene is dependent on regular feedings of the antibiotic tetracycline to survive. Out in the wild, she dies long before reaching adulthood.

In a study by company scientists published in BMC Biology in July, male moths carrying the gene wiped out communities of normal moths living in small cages. Females mating with transgenic males had as many offspring as those coupling with unaltered males, but the female offspring died before being able to reproduce.

Only some of the male offspring inherited the synthetic gene, which tends to disappear after a few generations. So thinning the moth population required multiple waves of assault by fresh males bred in the lab.

Dr. Shelton's outdoor cages, also stocked with wild moths, will test how well the genetically engineered males compete in a bigger arena. The release next summer into the field would take the technology one step closer to being used on farms.

The strategy has drawn criticism. Groups opposed to the use of genetically modified organisms worry that the protein made by the synthetic gene could harm wildlife that eat the moths.

"We would argue that more information should be collected," said Helen Wallace, the director of GeneWatch U.K.

Haydn Parry, the chief executive of Oxitec, says the company addressed this concern and others in data submitted to the Department of Agriculture.

"We fed the protein to mosquitoes, fish, beetles, spiders and parasitoids," he said. "It's nontoxic."

After weighing the evidence, the department decided the planned experiments would have no significant effect on the environment.

A public letter signed in June by the Northeast Organic Farming Association of New York protested any outdoor trials. The association cautioned that escaping moths could contaminate nearby farms and endanger their organic certification.

Yet studies suggest the likelihood of diamondback moths straying is low. Wild moths released into the

open tend to stay put as long as they have food and company. Any that do venture farther afield are likely to be wiped out by New York's cold winter.

Even if strays are found, legal experts say that national organic standards penalize only the deliberate use of a genetically modified organism.

"If these moths came across into an organic field inadvertently, that would not be a problem for the farmer," said Susan Schneider, a professor who specializes in agriculture and food law at the University of Arkansas School of Law.

Insects that do wander into other fields can be identified by their red glow under ultraviolet light — caused by another gene inserted into their DNA, this one from coral.

Even if the moths in Dr. Shelton's experiments pass muster, there is still no guarantee that farmers will use them.

"At the end of the day, the technology may not go forward for commercial reasons," said Mark Benedict, an entomologist at the Centers for Disease Control and Prevention.

Other weapons developed for combating diamondbacks — larva-eating wasps, for instance — have struggled to compete with cheap chemical pesticides.

"What almost always happens is the pesticides take precedent," said Michael Furlong, an entomologist at the University of Queensland. "The growers can't resist spraying, as it's the easiest thing to do."

As for Mr. Hansen, he says he has not ruled out using the genetically engineered moths one day in the continuing battle to save his cabbages.

"I'm glad they're doing these experiments," he said. "But it's really early days."

Correction: September 1, 2015

An earlier version of a photograph with this article carried an incorrect picture credit. The photograph of two mating moths was by Dan Olmstead of Cornell University, not the Cornell Alliance for Science.

A version of this article appears in print on September 1, 2015, on page D3 of the New York edition with the headline: Replacing Pesticides With Genetics.

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BIOPESTICIDE-INDUCED BEHAVIORAL AND MORPHOLOGICAL ALTERATIONS IN THE STINGLESS BEE *MELIPONA QUADRIFASCIATA*

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Abstract: Because of their natural origin, biopesticides are assumed to be less harmful to beneficial insects, including bees, and therefore their use has been widely encouraged for crop protection. There is little evidence, however, to support this ingrained notion of biopesticide safety to pollinators. Because larval exposure is still largely unexplored in ecotoxicology and risk assessment on bees, an investigation was performed on the lethal and sublethal effects of a diet treated with 2 bioinsecticides, azadirachtin and spinosad, on the stingless bee, *Melipona quadrifasciata*, which is one of the most important pollinators in the Neotropics. Survival of stingless bee larvae was significantly compromised at doses above 210 ng a.i./bee for azadirachtin and 114 ng a.i./bee for spinosad. No sublethal effect was observed on larvae developmental time, but doses of both compounds negatively affected pupal body mass. Azadirachtin produced deformed pupae and adults as a result of its insect growth regulator properties, but spinosad was more harmful and produced greater numbers of deformed individuals. Only spinosad compromised walking activity of the adult workers at doses as low as 2.29 ng a.i./bee, which is 1/5000 of the maximum field recommended rate. In conclusion, the results demonstrated that bioinsecticides can pose significant risks to native pollinators with lethal and sublethal effects; future investigations are needed on the likelihood of such effects under field conditions. *Environ Toxicol Chem* 2015;34:2149–2158. © 2015 SETAC

Keywords: Native pollinators Bioinsecticides Sublethal effects Behavioral impact Meliponini

INTRODUCTION

Pesticides have been considered potential stressors for insect pollinators, such as bees [1–3]. Although there has been a consensus that other factors, such as parasites, pathogens, poor nutrition, habitat fragmentation, and beekeeper practices may also impact bee populations [4–6], some scientific and political opinions consider that insecticides such as fipronil and systemic neonicotinoids constitute one of the main causes contributing to honey bee decline [7–9].

Pesticides may cause mortality or have sublethal effects on bees. The latter may include immune system weakness [10], neural and locomotive disorders [11], and impaired learning, memory, and foraging [12,13]. Such sublethal effects have been considered very harmful for individual fitness in a bee colony, eventually potentiating the colony's collapse. However, up to now, mortality has been one of the most commonly used parameters for comparison of the potential risks of pesticides to pollinators [3,14]. To ensure more sustainable agricultural practices that can minimize the impact of pesticides on bees, the use of molecules of natural origin has been widely encouraged, with the assumption that such compounds are less harmful to beneficial insects [15,16]. Such compounds, which are labeled as biopesticides or biorational pesticides, have received considerable attention in organic production systems, but have expanded to conventional cultivation systems as well, as they aim for more sustainable production [17,18].

Spinosad is a prominent bioinsecticide derived from fermentation of the actinomycete *Saccharopolyspora spinosa* [19]. It shows a reduced spectrum of toxicity and low potential risk to mammals compared with synthetic compounds, and thus it has been recognized by the US Environmental Protection Agency as a reduced-risk insecticide [20,21]. As a compound of natural origin, spinosad was originally considered less harmful for nontarget arthropods compared with older compounds, and thus its use for plant protection and insect vector control quickly expanded [22,23]. However, the selectivity of this neurotoxic bioinsecticide to natural enemies and pollinators has been questioned [24–27].

Azadirachtin is another bioinsecticide that has been widely used for crop protection. This compound is derived from the Indian plant *Azadirachta indica* (Meliaceae) and shows high efficacy against arthropod pest species [28,29]. Azadirachtin may cause feeding deterrence, behavioral changes, incomplete ecdysis, altered developmental time, morphological deformities, and hormonal disruption leading to sterility [28,29]. However, opinions regarding its selective action in nontarget organisms have been mixed, because of divergent research outcomes [25,29–32].

Faced with the (misguided) notion that the origin of the molecule drives the potential for toxicity on (nontarget) organisms, more studies regarding the unwanted effects of natural insecticides are necessary. In the present study, we investigated the toxicity of spinosad and azadirachtin to workers of the stingless bee species *Melipona quadrifasciata* Lepeletier 1836, (Hymenoptera: Apidae, Meliponini), which is an important pollinator of native and cultivated plants (e.g., tomatoes and green pepper, among others) in the Neotropics [33,34]. *Melipona quadrifasciata* is phylogenetically and

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biologically related to *Melipona capixaba* Moure & Camargo, another Neotropical stingless bee species formally recognized as an endangered species [35]. These factors emphasize the importance of *M. quadrifasciata* as a model pollinator in insecticide risk assessments [36,37]. We suspected that exposure to a bioinsecticide-contaminated diet might impair the development and survival of bee larvae, and we tested this hypothesis using an in vitro rearing technique suitable for these pollinator species [11]. We also assessed the emerged worker bees to confirm our predictions on the sublethal impacts of the biopesticides in terms of morphology and behavior.

MATERIALS AND METHODS

Stingless bee colonies

Colonies of *M. quadrifasciata* were collected in Viçosa County (State of Minas Gerais, Brazil; 20°45′S, 42°52′W) and maintained at the Experimental Meliponary/Apiary of the Federal University of Viçosa (Viçosa, Minas Gerais State, Brazil), which is over 2 km away from the closest open field with agronomic cultivation and pesticide application. All colonies were collected from different fields to guarantee a representative genetic variability.

Insecticides

Commercial formulations of the bioinsecticides azadirachtin (Azamax, emulsifiable concentrate at 12 g a.i./L, DVA Agro Brasil) and spinosad (Tracer, suspension concentrate at 480 g a.i./L, Dow AgroScience) available and registered for agricultural use in Brazil were used in the bioassays [38]. The bioinsecticides were used at increasing doses based on the maximum field recommended rate of each compound (azadirachtin: 30 g a.i./ha; spinosad: 81.6 g a.i./ha) for control of the tomato leafminer *Tuta absoluta* (Meyrick; Lepidoptera: Gelechiidae). This choice was made because *M. quadrifasciata* is a particularly important pollinator of tomatoes in Neotropical America, where both bioinsecticides are frequently used [33,34]. For both bioinsecticides, the maximum spray volume (water carrying insecticide) applied per hectare of tomato fields (1000 L/ha) was used as the basis for dose calculation. Such rates and the spray volume of both bioinsecticides follow the recommendations of the Brazilian Ministry of Agriculture [38].

In vitro rearing

Stingless bee workers were reared as described by Tomé et al. [11], by transferring egg-containing brood combs to artificial honey bee wax cells filled with 140 μL of diet (130 μL of larval food + 10 μL of distilled and deionized water), which provided enough sustenance for full larval development. The wax was obtained from the Experimental Meliponary/Apiary of the University and had no evidence of pesticide residue contamination, whereas pollen and honey were obtained from commercial beekeepers in Viçosa. Distilled and deionized water was used as the carrier of the insecticides into the larval food. The artificial cells were placed into the wells of polyethylene 24-well microplates and individually closed with honey bee wax caps. Each microplate received 24 eggs (i.e., 1 egg/artificial cell) from a single field colony and was maintained at 28 ± 1 °C, 95 ± 5% relative humidity in continuous darkness until the end of the feeding period. The pupae were transferred to new artificial brood combs (containing new wax cells) at the beginning of the pupal period (white eyed-pupae) and maintained at 28 ± 1 °C, 70 ± 5% relative humidity in continuous darkness, as in natural field conditions. After emergence, the adult workers were marked with different colors using a nontoxic water-soluble paint (Acrilex) for age monitoring. These adults were maintained in Petri dishes (9 cm in diameter and 2 cm high), where they were supplied daily with a 50% honey and 50% pollen-based diet. Each Petri dish received the emerged bees from the same microplate and was kept at 28 ± 1 °C and 70 ± 5% relative humidity in continuous darkness.

Larval exposure to bioinsecticides

The stingless bee larvae (i.e., 24 larvae/colony) were chronically exposed to azadirachtin or spinosad via contaminated diet, and 4 different colonies (i.e., replicates) were used for each combination of bioinsecticide and dose (i.e., 96 larvae/bioinsecticide dose). The exposure lasted the entire larval feeding period, approximately 20 d. Each compound was independently mixed into 240 μL of distilled and deionized water, which was added to 3120 μL of larval food. Then, the total amount of treated larval diet (3360 μL) was distributed in 24 equal portions (140 μL) within the artificial wax cells in the microplate. Azadirachtin was applied at increasing doses of 42 ng, 210 ng, 420 ng, and 840 ng a.i./bee, which were based on dilutions of 1/100, 1/20, 1/10 and 1/5 of its maximum field recommended rate (30 ng a.i./μL), respectively. The higher dose of azadirachtin (4200 ng a.i./bee) was not considered for the bioassays because at this dose the insecticide solution floats to the surface of the diet, preventing the eggs from standing upright and killing the embryos. Spinosad was applied at increasing doses of 0.57 ng, 1.14 ng, 2.29 ng, 11.4 ng, 22.9 ng, 114 ng, 228 ng, 1142 ng, and 11 424 ng a.i./bee, which represented dilutions of 1/20 000, 1/10 000, 1/5000, 1/1000, 1/500, 1/100, 1/50, 1/10, and 1/1 of its maximum field recommended rate (81.6 ng a.i./μL), respectively. Only distilled and deionized water was used in the larval diet for the untreated control treatment. The full doses of both azadirachtin and spinosad were known because each larva consumed the entire amount of food provided. The average weight of the bees in the entire experiment was 96.80 ± 0.97 mg (bees were weighed at the white-eye pupa stage).

Insect survival, developmental time, fresh body mass, and external morphology

Survival of stingless bee larvae was recorded by daily monitoring of each individual throughout its development, which encompassed the period from egg hatch until death or adult emergence. The observations were carried out by briefly removing the wax caps from the wax cells during the assessment and placing them back afterward. Dead larvae and pupae were identified by the absence of spiracle movement (in larvae) or by the presence of darkened tegument (larvae and pupae), and then removed. Each dose of azadirachtin or spinosad was represented by 96 larvae, and sets of 24 larvae from the 4 different colonies were used for each compound.

Developmental time (days) from egg hatch until adult emergence was also recorded for each insect. All insects that survived the bioinsecticide exposure were weighed on an analytical scale (Sartorius BP 210D) to determine fresh body mass at the white-eye pupa stage (up to ~4 d after pupation). This stage is closest to the end of the feeding period when bees had already defecated and can be manipulated without causing damage. The pupae and adults were visually inspected for external morphological deformities, and the deformed individuals, who usually exhibited malformed appendages (i.e., contorted or nonstretched wings, legs, proboscis, or tarsi),

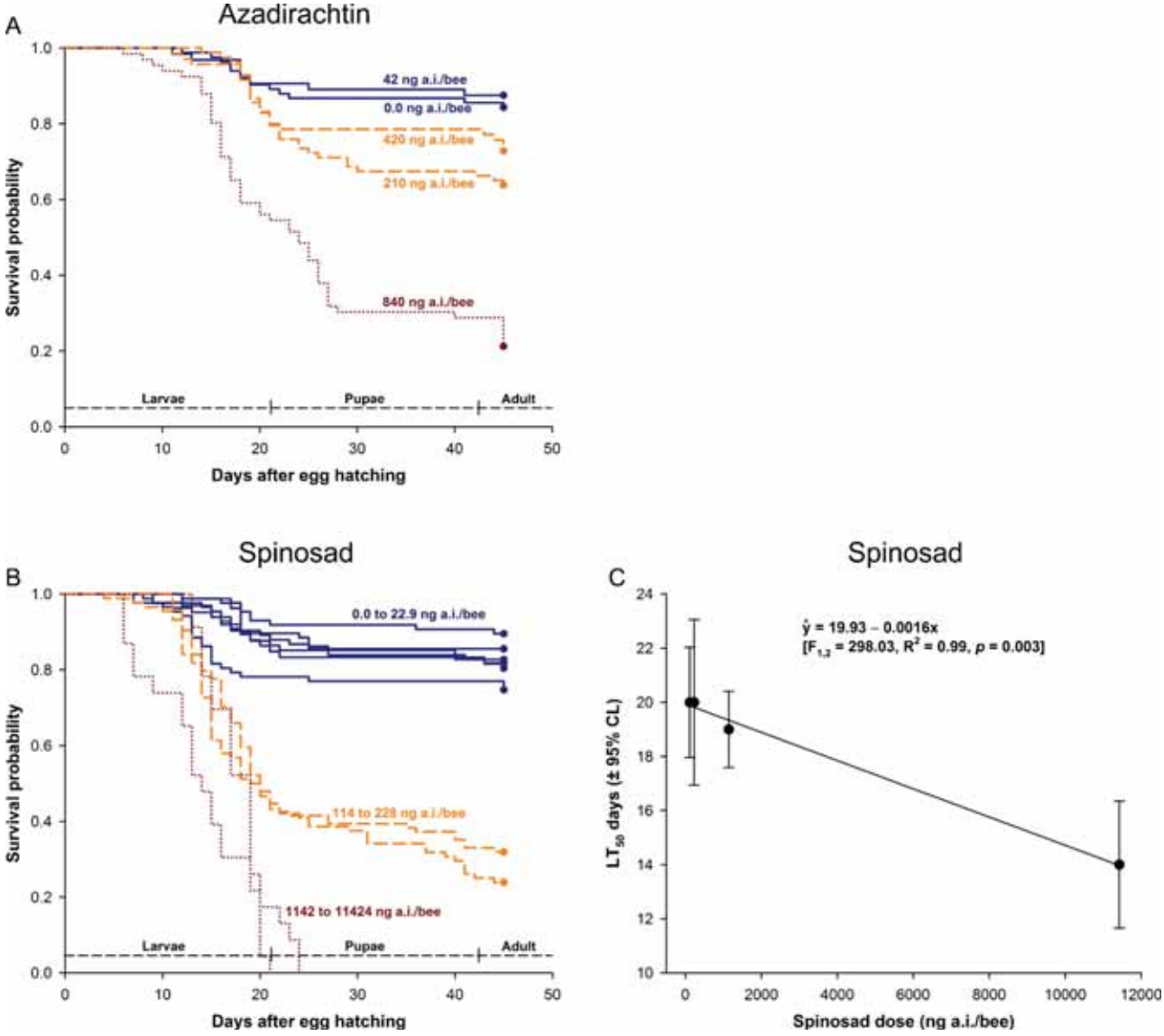

Figure 1. Survival plots of stingless bee larvae (*Melipona quadrifasciata*) reared either on azadirachtin- (**A**) or spinosad- (**B**) contaminated diet. (**C**) Linear regression estimated for the median lethal times (LT50s) of stingless bee larvae reared on spinosad contaminated-diet. No LT50 regression was estimated for azadirachtin because of the lack of suitable LT50 estimates for this insecticide. (**A**) Survival curves coded with the same color and shape were not significantly different by the Bonferroni method ($p > 0.05$); closed circle indicates censored data. Vertical bars in (**C**) indicate 95% confidence intervals.

were thus recorded for subsequent analysis. When queens and drones were identified at their adult stage (less than 1% of the insects), they were removed from subsequent analyses (i.e., immature queens and drones were not considered). Queens were recognized by the absence of corbicula at their hind tibia and reduced compound eyes compared with workers. Drones were recognized by the absence of corbicula at their hind tibia and the different external morphology of their abdomen compared with workers.

Walking behavior

Surviving adult workers of 0, 3, 6, 9, and 12 d old, which were previously exposed to azadirachtin or spinosad via the contaminated diet, were subjected to bioassays to evaluate their ability to walk before they were able to fly [11,39]. No deformed workers, queens, or drones were used for this bioassay. Each worker was individually transferred to an arena consisting of an open Petri dish (9 cm in diameter and 2 cm high) lined at the bottom with filter paper (Whatman no. 1) and with the inner walls coated with Teflon PTFE (DuPont) to prevent their escape. The movement of each worker within the arena was recorded for 10 min and digitally transferred to a computer using an automated video tracking system equipped with a CCD camera (ViewPoint LifeSciences). The variables recorded in each arena included the number of stops, resting time (s), distance walked (cm), and velocity (cm/s). The behavioral bioassays were carried out in a room with artificial fluorescent light at $25 \pm 2\ ^{\circ}C$ and $70 \pm 5\%$ relative humidity. Five workers from each of the 4 colonies were used per dose (i.e., 20 workers/dose). The average of 5 workers from a single colony was considered a replicate (i.e., 4 colonies/dose).

Statistical analyses

The data from the survival bioassays were subjected to survival analyses in which survival curves were obtained using Kaplan–Meier estimators (PROC LIFETEST in SAS) [40]. As the workers emerged at different times (39–45 d), the survival curves were standardized by censoring the data when the insects

were 45 d old (counted from egg hatch). The overall similarity among survival curves was tested by the χ^2 log-rank test, and pairwise comparisons between curves were tested using the Bonferroni method. Insect body mass, developmental time, and number of deformed individuals were subjected to regression analyses in the software TableCurve 2D Ver 5.01 (Systat) with bioinsecticide doses (azadirachtin or spinosad) as independent variables. The data from the walking bioassays were subjected to multiple regression analyses in the software TableCurve 3D Ver 4.0 (Systat), with bioinsecticide dose and age after emergence as independent variables. The models used to describe the effect of both azadirachtin and spinosad on the measured variables were selected based on parsimony, high F values, and steep increases of R^2 with model complexity, and plotted using the software SigmaPlot 12.0 (Systat).

RESULTS

Survival of stingless bee larvae

Larval survival of *M. quadrifasciata* was significantly impaired after ingestion of increasing doses of azadirachtin (log-rank $\chi^2 = 112.44$, $df = 4$, $p < 0.001$; Figure 1A). Azadirachtin at 42 ng a.i/bee resembled the untreated control, but the highest dose, of 840 ng a.i./bee, caused high mortality throughout development, reaching nearly 80% at the end of the bioassay with a median lethal time (LT50 ± standard error [SE]) estimate of 24.0 ± 2.5 d (Figure 1A).

Survival of spinosad-exposed larvae was also impaired, with dose-dependent mortality (log-rank $\chi^2 = 369.57$, $df = 9$, $p < 0.001$; Figure 1B and C). Spinosad at 0.57 ng to 22.9 ng a.i./bee did not cause significant mortality in the immature stingless bees, resembling the untreated control (Figure 1B). However, spinosad significantly reduced the larval survival rate at 114 ng a.i./bee and higher doses (Figure 1B). More than 80% of the individuals died before reaching the pupal stage at doses of 1 ng, 142 ng, and 11 424 ng a.i./bee (Figure 1B). A negative relationship between insecticide dose and median survival time was also evident (Figure 1C).

Developmental time of stingless bee larvae

Ingestion of either azadirachtin ($F_{1,15} = 3.98$, $p = 0.06$) or spinosad ($F_{1,29} = 2.28$, $p = 0.14$) during the larval stage did not significantly affect the developmental time of the insects (period from egg hatch to worker emergence). The average developmental time among the doses for the larva-adult period (± SE) was 41.81 ± 0.59 d for untreated larvae, and 42.24 ± 0.30 d and 41.15 ± 0.35 d for azadirachtin- and spinosad-treated larvae, respectively.

Fresh body mass of stingless bee pupae

Azadirachtin ($F_{1,17} = 13.34$, $p = 0.002$) and spinosad ($F_{1,27} = 27.93$, $p < 0.001$) significantly reduced pupal body mass (Figure 2A and 2B), as reflected in the regression analysis for each insecticide. The reduction in body mass was not the result of food rejection, because all of the diet was ingested by the larvae. This was the case for all treatments as well as the untreated control.

External morphology in pupae and adult bees

Ingestion of both azadirachtin and spinosad produced deformed pupae (Figure 3A and 3B), which in turn resulted in adults with external deformities (Figure 3C and 3D). Deformed pupae exhibited contracted appendages, including antennae, legs, wings, and mouth parts (Figure 3B). All deformed adults were derived only from deformed pupae (i.e., never from healthy pupae). The wings of the deformed workers showed no stretching, appearing as pupal wing pads. Deformed adults also exhibited contorted legs or antennae and color patterns that were different from the control bees (Figure 3D).

Eighteen deformed pupae (out of 205 total pupae) were found in the azadirachtin treatments, and only 2 of them reached the adult stage. These numbers from the azadirachtin-exposed bees allowed for the adjustment of a linear model for the appearance of deformed pupae in terms of percentage (% deformed individual = [no. deformed insects/total no. insects] × 100) (Figure 4A, pupae: $F_{1,17} = 5.71$, $p = 0.03$), but no significant regression was adjusted to the percentage of deformed adults (Figure 4A, $F_{1,15} = 0.21$, $p = 0.65$). For spinosad, the number of deformed pupae was 51 of 426, and the number of deformed adults was 23 of 387. The appearance of deformed individuals increased in a dose-dependent manner at both the pupal (Figure 4B, $F_{2,30} = 42.13$, $p < 0.001$) and adult stages (Figure 4B, $F_{1,19} = 48.75$, $p < 0.001$).

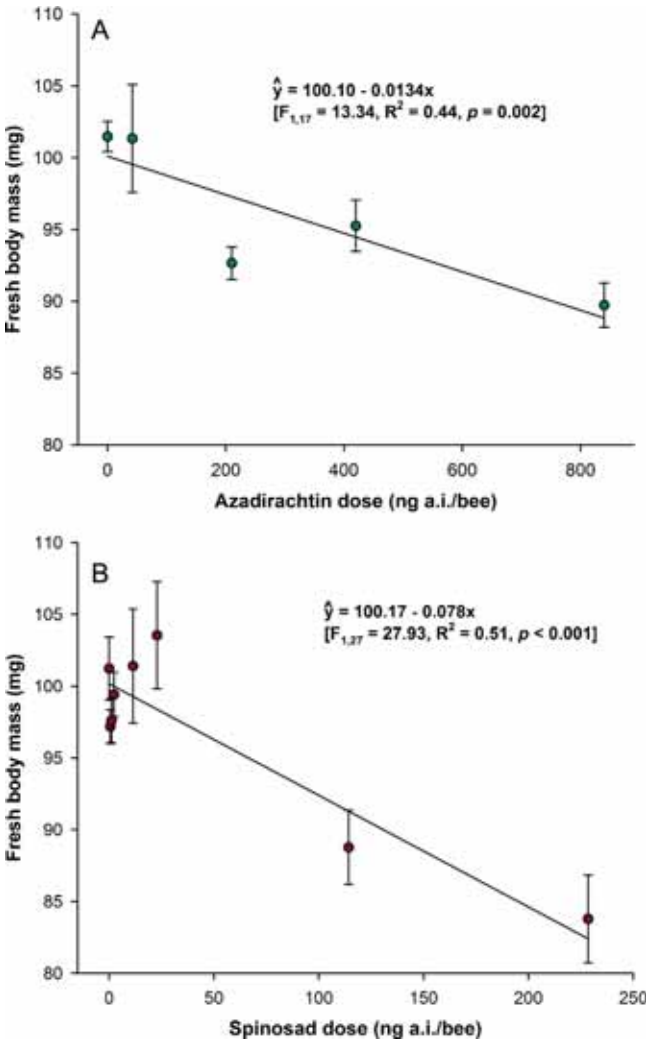

Figure 2. Fresh body mass (± standard error) of the white-eye pupae of the stingless bee (*Melipona quadrifasciata*) reared on azadirachtin- (**A**) or spinosad- (**B**) contaminated diet. The symbols represent the mean of adult workers from 4 independent replicates (i.e., colonies).

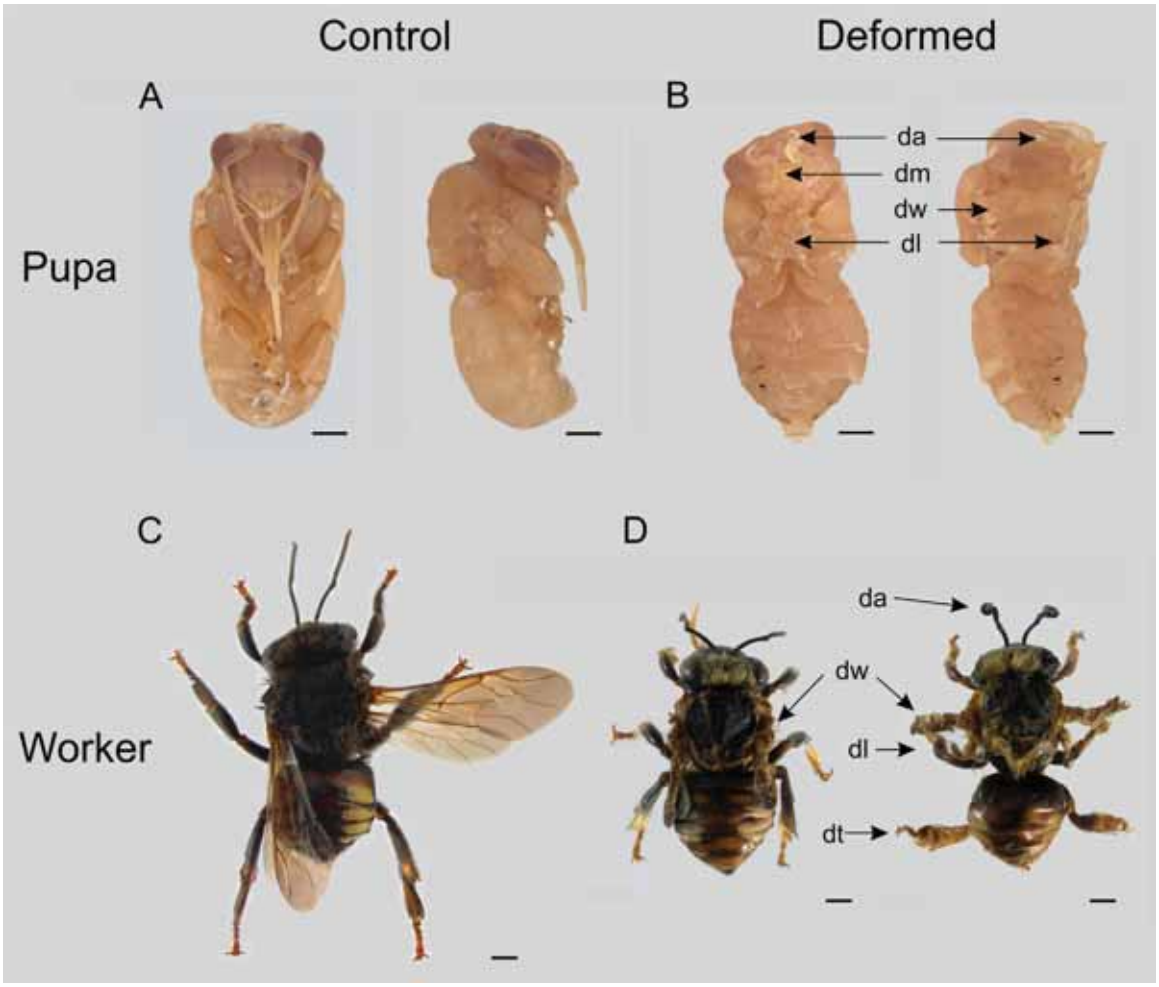

Figure 3. Overview of the worker pupae and adults of the stingless bee *Melipona quadrifasciatha* reared on control or (azadirachtin or spinosad) contaminated diet. (**A**) Ventral (left) and lateral (right) views of a control pupa with well-formed appendages. (**B**) Ventral (left) and lateral (right) views of an azadirachtin-treated pupa with deformed (contracted) appendages. (**C**) Dorsal view of a control worker with well-formed antennae, wings, and legs. (**D**) Dorsal views of an azadirachtin- (left) and spinosad- (right) treated worker with deformed antennae, wings, and legs. Arrows indicate deformities on antennae (da), wings (dw), mouth parts (dm), legs (dl), and tarsi (dt). Scale bar = 1 mm.

Walking behavior of adult workers

Dependent variable trends associated with walking behavior (numbers of stops, resting time, distance walked, and walking velocity) of young adult workers that originated from larvae orally exposed to azadirachtin were described by multiple regression models (Table 1), which are plotted in Figure 5. Azadirachtin dose did not contribute to the best adjustment in 3 of 4 equations; this is shown by the absence of the independent variable that represents the dose (variable x) in the equations (Table 1). In addition, even for resting time (Table 1), the azadirachtin dose only slightly deformed the shape of the response surface curve, whereas age of workers (independent variable y) caused a substantial reduction in resting time (Table 1, Figure 5B). Therefore, the plotted equations showed that worker age was the prime source for shape variation in the response surface curves (Table 1, Figure 5). For the walking variables, increasing worker age caused a linear decrease in the number of stops and a curvilinear increase in the distance walked and walking velocity with a stabilization tendency of these dependent variables.

In contrast to azadirachtin, the walking behavior of adults that emerged from spinosad-treated larvae depended on insect age and insecticide dose (Table 2, Figure 6A–6D). The highest doses of spinosad (1142 ng and 11 424 ng a.i./bee) were not represented because they killed all individuals before they reached the adult stage. The response surface curves for spinosad showed that increasing age (in days) decreased the number of stops (Figure 6A) and resting time (Figure 6B), but the distance walked (Figure 6C) and walking velocity (Figure 6D) increased with age. The overall shape of each response surface curve reflected the interaction between the independent variables (age and dose) on the walking parameters measured, with high spinosad doses compromising walking activity (Figure 6A–6D).

DISCUSSION

After over a decade of debate, the impact of pesticides is still considered one of the possible causes for the decline in bee abundance and diversity [1,6,37]. The heated debates concerning bee decline are, however, frequently restricted to a few synthetic insecticides, such as neonicotinoids and fipronil, and to a limited group of pollinators, with a great emphasis on the honey bee, *Apis mellifera* L., 1758 (Hymenoptera: Apidae, Apini) [7–9,37,41]. This skewed focus diverts attention from other pollinators, such as stingless bees, which are key pollinator species in both wild and cultivated crops in the

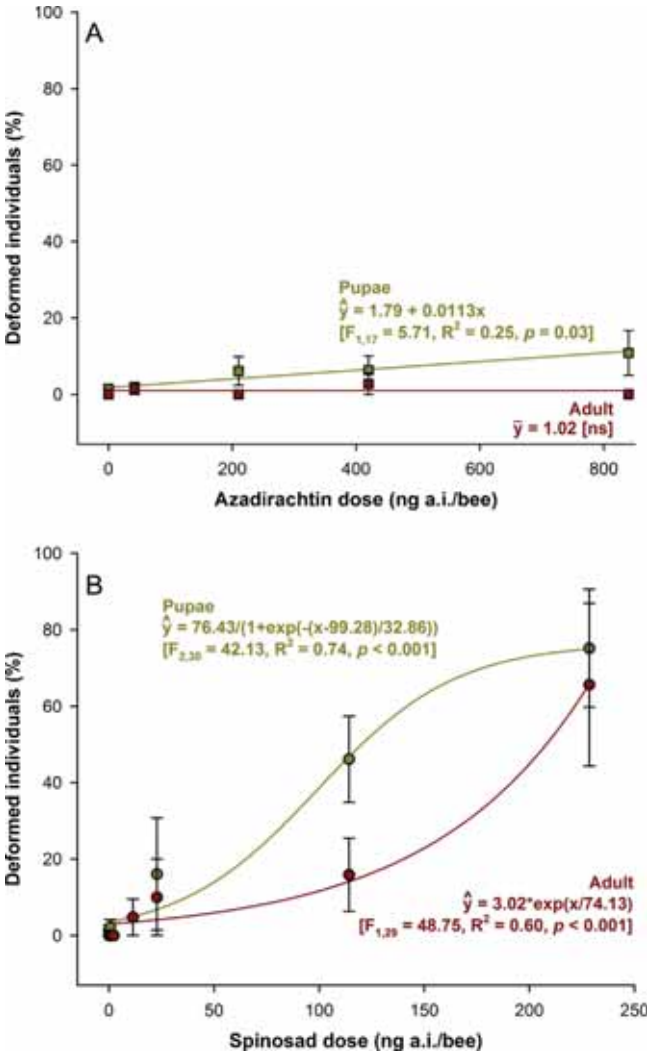

Figure 4. Percentage (± standard error) of deformed pupae and workers of the stingless bee (*Melipona quadrifasciata*) reared on azadirachtin- (**A**) or spinosad- (**B**) contaminated diet.

Neotropics [33,34,37], and from biopesticides, whose potential risk to pollinators is still poorly understood [22,24,27,37,42]. It is well known that pesticide residues are found in bee hives [43,44] and that they have the potential to damage the brood, with lethal and sublethal effects [3,11,14,45]. Nonetheless, most of the studies addressing the effects of pesticides on bees are directed only to the adult stage, and neglect the immature larval stages. Considering the above-mentioned lack of information on risk assessment of pesticides for pollinators, we carried out successful in vitro rearing of the stingless bee *M. quadrifasciata* and assessed the lethal and sublethal effects on the brood and surviving adults after larval exposure to the bioinsecticides azadirachtin and spinosad.

Overall survival and median survival time of the larvae were used as initial signs of susceptibility of the stingless bees to larval exposure to both azadirachtin and spinosad. Chronic ingestion of these bioinsecticides impaired larval survival in a dose-dependent manner but only showed significant toxicity at doses higher than 210 ng a.i./bee for azadirachtin and 114 ng a.i./bee for spinosad. Afterward, as a sublethal effect, the fresh body mass of the pupae was negatively correlated to the ingested dose of each bioinsecticide (azadirachtin and spinosad). Such a decrease in pupal body mass was not an effect of a behavioral feeding impairment because no residual diet was observed after the end of the feeding period within the artificial cells, which contained enough food (140 μL of diet) for full development of the workers. Therefore, neither azadirachtin nor spinosad stopped the feeding behavior of the exposed stingless bee larvae; azadirachtin, which is well known for its antifeedant activity, also did not stop feeding behavior [28,29].

Altered developmental time was not observed with either bioinsecticide, but, as expected, azadirachtin caused deformities in the pupal stage, which also resulted in deformed adults after molting. This effect is probably the result of disturbances in the juvenile hormone and/or ecdysteroid titers, which are related to the insect growth regulator activity of azadirachtin [28,29,46]. Surprisingly though, deformed pupae and adults also appeared as a consequence of spinosad exposure at higher proportions than those found with exposure to azadirachtin. Such a response is certainly not because of the well-established spinosad mode of action, which is to hyperexcite neurons, leading to insect paralysis and subsequent death [19,47]; it is probably the result of another physiological impairment possibly involving hormonal disturbances such as those described for insect growth regulators [28,48]. However, no mechanism has been described in the literature to explain how spinosad may physiologically trigger hormonal disorders in insects and consequently lead to deformities. It is obvious that deformed workers are less able, or even unable, to perform the variety of tasks that the colonies depend on to survive because essential activities related to locomotion, foraging, feeding, and olfactory perception and communication are compromised.

Locomotor behavioral abnormalities were also noted as sublethal effects. Newly emerged adults were virtually inactive regardless of the bioinsecticide used, because locomotor activity

Table 1. Summary of regression analyses for the walking activity variables (Figure 5) of stingless bee *Melipona quadrifasciata* workers after exposure to increasing doses of azadirachtin during the larval stage[a]

Walking variables	Model[b]	Coefficient estimates (± standard error) a	b	c	d	df_{error}	F	p	Adjusted R^2
Number of stops	$z = a + by^3 + cy^{0.5}\ \ln y$	545.62 ± 27.90	0.12 ± 0.05	−54.99 ± 10.73	–	78	26.03	<0.001	0.38
Resting time	$z = a + bx\ln x + cx^2 + de^{-y}$	72.03 ± 13.10	0.04 ± 0.02	−0.0004 ± 0.0002	298.65 ± 20.62	77	71.93	<0.001	0.72
Distance walked	$z = a + by + cy\ln y$	134.86 ± 65.53	256.64 ± 41.91	−75.15 ± 16.42	–	78	53.05	<0.001	0.56
Walking velocity	$z = a + by + cy^{1.5}$	0.60 ± 0.09	0.35 ± 0.06	−0.07 ± 0.02	–	78	46.78	<0.001	0.52

[a]All coefficient estimates (a, b, c, and d) of the equations were significant at $p < 0.05$ by Student's t test.
[b]In the regression models, z indicates the dependent variable (walking variables), x indicates the first independent variable (azadirachtin dose), and y indicates the second independent variable (age after emergence).
df = degree of freedom.

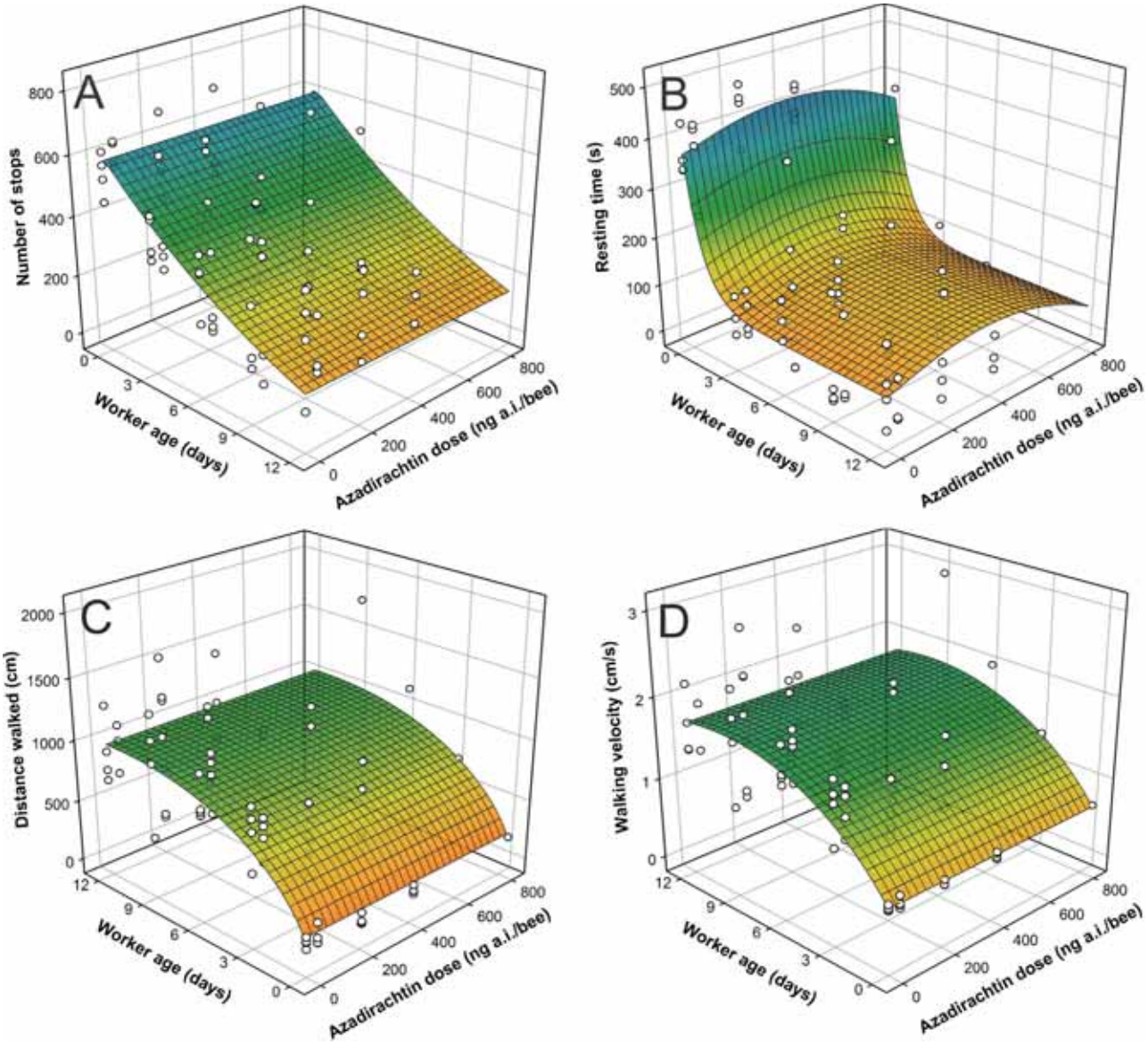

Figure 5. Walking behavior plots of workers of the stingless bee *Melipona quadrifasciata* after larval exposure to azadirachtin. (**A**) Number of stops. (**B**) Resting time. (**C**) Distance walked. (**D**) Walking velocity. Changes in the color pattern of the surface curve indicate changes in the range of values in the walking variables (i.e., the dependent variable in the z-axis of each plot).

normally increases with age [49]. However, adults up to 3 d old or older exhibited distinct walking activity after exposure to azadirachtin or spinosad during the larval stage. Walking activity of azadirachtin-exposed workers increased with age in a way that resembled the untreated insects. However, spinosad at doses as low as 2.29 ng a.i./bee compromised the gradual increase in walking activity. Such a divergence in walking response for azadirachtin and spinosad can probably be

Table 2. Summary of regression analyses for the walking activity variables (Figure 6) of stingless bee *Melipona quadrifasciata* workers after exposure to increasing doses of spinosad during the larval stage[a]

		Coefficient estimates (± standard error)						
Walking variables	Model[b]	a	b	c	df_{error}	F	p	Adjusted R^2
Number of stops	$z = a + bx/\ln x + cy^3$	442.28 ± 21.98	4.83 ± 1.60	−0.06 ± 0.02	124	9.51	<0.001	0.11
Resting time	$z = a + bx^{0.5} + ce^{-y}$	129.26 ± 12.03	13.21 ± 2.39	208.27 ± 20.68	124	74.86	<0.001	0.54
Distance walked	$\ln z = a + bx^{0.5} + ce^{-y}$	6.94 ± 0.05	−0.14 ± 0.02	−0.91 ± 0.30	124	45.17	<0.001	0.41
Walking velocity	$\ln z = a + bx^{0.5} + ce^{-y}$	0.54 ± 0.03	−0.08 ± 0.01	−0.69 ± 0.12	124	53.97	<0.001	0.45

[a]All coefficient estimates (a, b, and c) of the equations were significant at $p < 0.05$ by Student's t test.
[b]In the regression models, z indicates the dependent variable (walking variables), x indicates the first independent variable (spinosad dose), and y indicates the second independent variable (age after emergence).
df = degree of freedom.

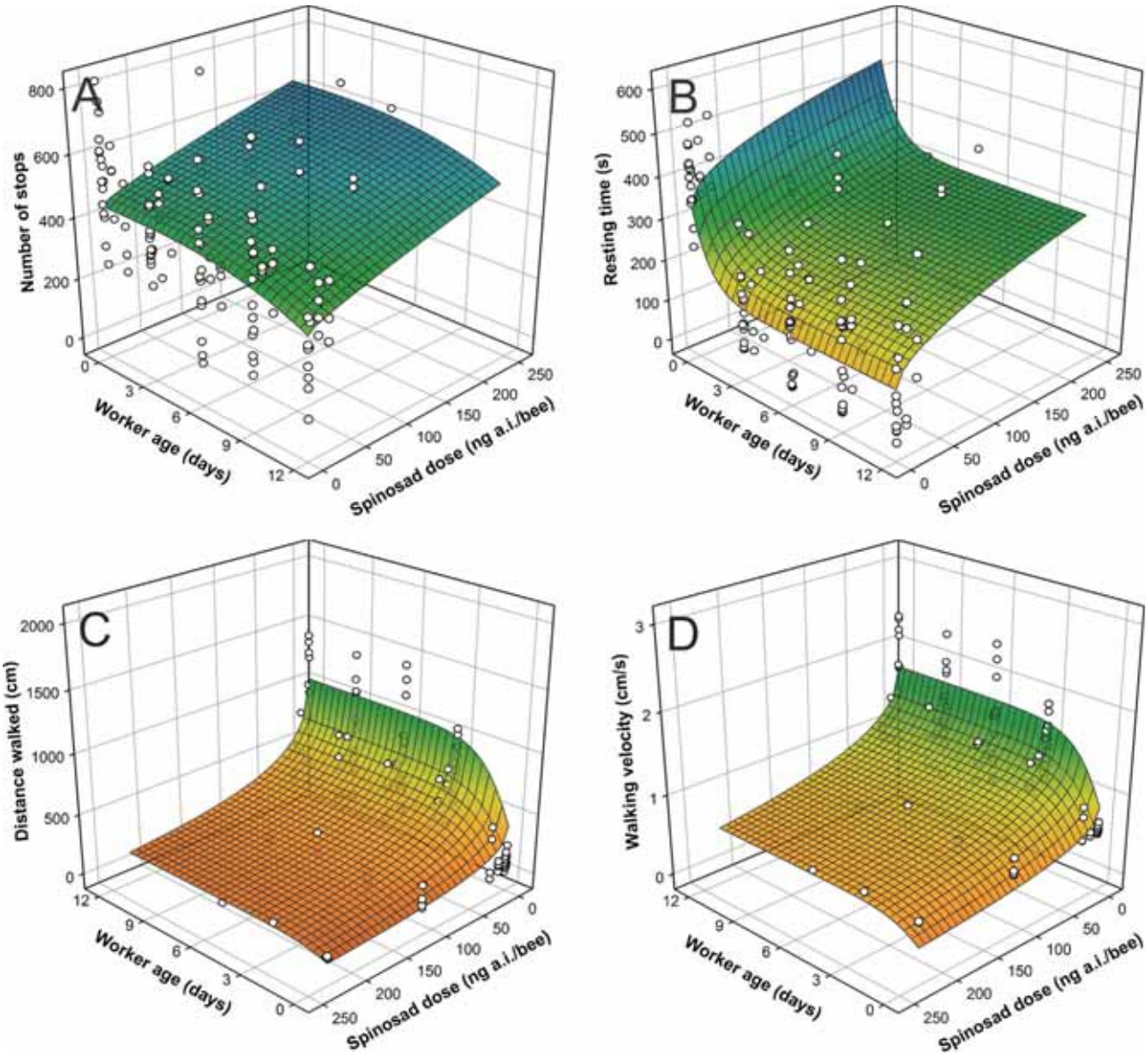

Figure 6. Walking behavior plots of workers of the stingless bee *Melipona quadrifasciata* after larval exposure to spinosad. (**A**) Number of stops. (**B**) Resting time. (**C**) Distance walked. (**D**) Walking velocity. Changes in the color pattern of the surface curve indicate changes in the range of values in the walking variables (i.e., the dependent variable in the z-axis of each plot).

explained by the different mode of action of these bioinsecticides. Whereas azadirachtin acts primarily as an insect growth regulator, causing hormonal disturbances, and to a lesser extent by interfering with the insect nervous system through impairment of brain neuropeptides [28,29,50], spinosad acts on the insect nervous system primarily as an agonist of nicotinic acetylcholine receptors (nAChRs), and secondarily as an agonist of γ-aminobutyric acid (GABA) receptors; the latter is known to be involved in the regulation of mobility [19,47].

Behavioral impairment after spinosad exposure is corroborated by another study showing a similar effect of the synthetic insecticide imidacloprid on walking activity of *M. quadrifasciata* workers reared in the same way as in the present study [11]. Like spinosad, imidacloprid interferes agonistically with nAChRs in the insect nervous system, but at a different target site [51,52]. In addition, morphometric analyses revealed that imidacloprid impaired the development of mushroom bodies in *M. quadrifasciata* brain [11], and this activity was also confirmed with Kenyon cell cultures from other pollinators such as bumblebees [53]. Mushroom bodies are brain structures related to the processing and integration of multisensory information, learning, and memory in bees [54,55]. These structures show volumetric plasticity dependent on the age and experience of adult workers [49,56].

We hypothesize that the impairment in worker walking activity may also be related to developmental restriction of the mushroom bodies, as spinosad and imidacloprid share a similar target receptor in the insect nervous system, and the mushroom bodies include motor control as part of their sensory integration. A direct effect on worker locomotor activity by spinosad is also possible because GABA receptors are found in motor neurons, as observed in cockroaches [57]. After compromise of walking activities in the stingless bees, further impairment would be expected in flight behavior with spinosad exposure. Therefore, every task necessary for the maintenance, survival, and reproduction of the stingless bee colonies could be impaired, including, for instance, hygienic behavior, comb production, food storing, larval feeding, guarding, and foraging [39,58].

The difference in the magnitude of the observed effects between the bioinsecticides indicates that spinosad is a greater threat to stingless bees than azadirachtin. However, it is worth mentioning that the results for both compounds reaffirm that pesticide origin is not a relevant determinant of toxicity (unlike the structure and relative physicochemical properties of the compounds), and, therefore, their assessment and potential impact on native pollinators such as *M. quadrifasciata* should not be neglected. To confirm the differences observed in the present study with bioinsecticide-exposed stingless bees, more complex experimental setups, including semifield and field studies, need to be performed. Such setups will allow the assessment of more complex behaviors, such as foraging, which are very important for colony survival [59]; they also follow recent regulatory guidelines (e.g., the 2012 guidelines of the European Food Safety Authority [60]). In addition, an eventual reduced persistence of bioinsecticides may minimize potentially harmful effects detected under laboratory conditions [61–64], emphasizing the need for semifield and field studies for a more comprehensive and realistic assessment of the risks imposed by bioinsecticides to native stingless bees.

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Data availability—The full dataset is available on request to the first author of the manuscript (barbosawf@gmail.com).

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VARIETY

Appeals court overturns approval of pesticide over concerns about declining honey bee numbers

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SAN FRANCISCO — A federal appeals court Thursday blocked the use of a pesticide over concerns about its effect on honey bees, which have mysteriously disappeared across the country in recent years.

The U.S. Environmental Protection Agency did not adequately study the pesticide sulfoxaflor before approving its use in 2013 on a wide variety of crops, including citrus and cotton, the 9th U.S. Circuit Court of Appeals said.

Initial studies showed sulfoxaflor was highly toxic to honey bees, and the EPA was required to get further tests, Circuit Judge Mary Schroeder said.

"In this case, given the precariousness of bee populations, leaving the EPA's registration of sulfoxaflor in place risks more potential environmental harm than vacating it," she wrote.

EPA spokeswoman Laura Allen said the agency is reviewing the decision but had no further comment.

Sulfoxaflor is part of a group of insecticides known as neonicotinoids (NEE-OH-NIC-DUH-NIDES), according to the 9th Circuit ruling. Neonicotinoids are suspected of being among several factors that have contributed to the collapse of honey bee colonies throughout the U.S.

Bees, especially honeybees, are needed to pollinate crops, and they are considered essential to the U.S. food supply.

But a disorder has caused as much as one-third of the nation's bees to disappear each winter since 2006. A 2013 report issued by the EPA and U.S. Department of Agriculture cited a parasitic mite, multiple viruses, bacteria, poor nutrition, genetics, habitat loss and pesticides as factors for the bees' disappearance.

"We're certainly extremely happy," said Greg Loarie, an attorney with the group Earthjustice, which challenged the EPA's approval of sulfoxaflor on behalf of groups in the beekeeping industry. "It means that sulfoxaflor comes off the market while the EPA does the work it should have done a long time ago."

Loarie said the pesticide was used on cotton in southern states, but it had only been approved on an emergency basis for one crop in California.

The 9th Circuit overturned the EPA's unconditional registration of sulfoxaflor and ordered it to get additional studies and data about the pesticide's effect on bees.

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ENVIRONMENTAL HEALTH

RESEARCH Open Access

Municipal bylaw to reduce cosmetic/non-essential pesticide use on household lawns - a policy implementation evaluation

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Abstract

Background: Pesticide use on urban lawns and gardens contributes to environmental contamination and human exposure. Municipal policies to restrict use and educate households on viable alternatives deserve study. We describe the development and implementation of a cosmetic/non-essential pesticide bylaw by a municipal health department in Toronto, Ontario, Canada and assess changes in resident practices associated with bylaw implementation.

Methods: Implementation indicators built on a logic model and were elaborated through key informant interviews. Bylaw impacts on awareness and practice changes were documented through telephone surveys administered seasonally pre, during and post implementation (2003-2008). Multivariable logistic regression models assessed associations of demographic variables and gardening season with respondent awareness and practices.

Results: Implementation indicators documented multiple municipal health department activities and public involvement in complaints from commencement of the educational phase. During the enforcement phases only 40 warning letters and 7 convictions were needed. The number of lawn care companies increased. Among survey respondents, awareness of the bylaw and the Natural Lawn campaign reached 69% and 76% respectively by 2008. Substantial decreases in the proportion of households applying pesticides (25 to 11%) or hiring lawn care companies for application (15 to 5%) occurred. Parallel absolute increases in use of natural lawn care methods occurred among households themselves (21%) and companies they contracted (7%).

Conclusions: Bylaws or ordinances implemented through education and enforcement are a viable policy option for reducing urban cosmetic pesticide use.

Background

Growing concern has been expressed by environmental scientists, health researchers, clinicians, and the public about the widespread use of pesticides for lawn and garden applications regarded as "non-essential" i.e. not related to the growing of food, or "cosmetic" i.e. for aesthetic appearances only. These uses contribute to broad exposure to these chemicals as documented by environmental scientists in pesticide run-off in surface waters [1,2] and contamination of groundwater intended for drinking [3]. Human exposure studies have found pesticides among other household exposures [4] and documented pesticide residues associated with pesticide use in homes [5] and on lawns [6]. Potential effects of widespread exposures to pregnant women or children in urban settings are a particular concern [7].

Health researchers have made links between household pesticide use and children's illnesses [7], including cancer [8,9] and developmental impacts [10] from perinatal exposure. Although epidemiological studies are often limited in their ability to interpret cause or ascribe increased risks to specific pesticides, the evidence has been judged to be sufficient to prompt applications of precaution in legislation and regulation to address

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children's particular vulnerability to pesticides at both national [11,12] and state [13] levels.

Cosmetic pesticide use behaviour is governed by a complex mixture of social and environmental factors, and may be difficult to influence at an individual level [14]. Intensive consumer marketing of the ideal of "the perfect lawn" and of the pesticides needed to achieve it have fostered deeply-entrenched behaviours, neighborhood norms and even municipal ordinances requiring certain standards of lawn and garden care [15]. US surveys have found between one half and three quarters of households use pesticides and/or fertilizers outdoors [16,17] to create and maintain these outdoor spaces according to expectations, while in Canadian surveys prior to bylaw (ordinance) activity, approximately one third to one half of homeowners maintaining lawns and gardens reported using pesticides [18]. Similarly, many lawn care and landscaping companies retained in urban and suburban areas apply pesticides routinely as part of their service packages offered to clients to maintain weed- and insect-free lawns.

In light of the complex array of determinants of householder use of pesticides on lawns, jurisdictional efforts have often begun with programs to reduce the non-essential use of pesticides on public lands (i.e. areas under the direct control of government). For example, between 1995 and 2002 Danish municipalities, with support from the national government, achieved a remarkable reduction of 78% in the tonnes of active ingredients applied in public areas [19]. In a 2001 survey of 448 Ontario municipalities, more than one third did not use pesticides and nearly all had taken steps to substantially reduce or minimize pesticide use on public lands in the previous decade [20]. Approaches taken by nine jurisdictions in the USA, Canada and Europe to reduce residential pesticide use either by households or hired lawn care companies were reviewed by the Canadian Centre for Pollution Prevention in 2004 [21]. Reductions in pesticide use were estimated based on a combination of qualitative and quantitative data, and ranged from marginal (< 10%) to high (> 50%). However, the authors cautioned that "none of the communities had as strong and reliable data as [they] would have liked".

Municipal initiatives to reduce both public and private pesticide use through programs and policies are population-level health interventions that attempt to reduce health risks by changing the social, economic and/or environmental contexts contributing to those risks [22]. Evaluation of municipal initiatives to reduce pesticide use is part of the growing field of population health intervention research in which the ways they bring about change, the value of the interventions, and their effectiveness are all examined.

This paper describes a case where a systematic policy exploration, subsequent bylaw enactment, public education process and enforcement were led by the public health department of a metropolitan North American municipality. In terms of stance, several co-authors have been active participant-observers throughout the process as staff of the health department (more "insiders") while others connected with the university have been involved primarily in evaluation (more "outsiders"). We aimed to document indicators of the policy implementation, which combined both environmental health protection and health promotion components. Our specific research question was "To what extent did resident attitudes and practices change during pesticide bylaw implementation, controlling for demographic and location characteristics?"

Methods

Policy & Program Development

Setting

Toronto is Canada's largest city and the province of Ontario's capital city. It has a population of approximately 2.5 million people, of which 25% is less than 20 years old, and 20% older than 60 [23]. About half of Toronto's population was born outside of Canada. Just under 50% report a mother tongue other than English or French (the official languages). Income is distributed unevenly, and disparities between rich and poor are growing.

Administratively, the current City of Toronto was created in 1998 by the amalgamation of six former municipalities: the mostly "urban" former city of Toronto; three more "suburban" municipalities (Etobicoke, Scarborough, and North York), and two municipalities with a mix of urban and suburban areas (East York and York) [24]. The only remaining agriculturally zoned areas are in Etobicoke, North York and Scarborough, although small scale growing of fruits and vegetables in home gardens and community garden plots does occur over the summer months in all former municipalities. Visible private and public space is dominated by pavement and grass/lawn coverage on which, historically, the majority of pesticides have been applied.

Public health matters are handled by Toronto Public Health (TPH) under the direction of the Toronto Board of Health (BOH). The BOH is made up of elected officials, citizen and school board representatives, with the Medical Officer of Health (MOH) as the Executive Officer. It sets public health policy and advises City Council, Toronto's main governing and legislative body.

Impetus

In Toronto, reductions in pesticide use on public spaces began in the 1980s. Spurred on by concerns of both parents' associations and TPH staff, the Toronto Boards of

Education discontinued pesticide spraying on school properties. With the support of environmental organizations (led by the Toronto Environmental Alliance), municipal unions, and staff from multiple City divisions, Toronto City Council adopted the MOH's recommendation in 1998 to restrict pesticide use on all City property. A 97% reduction of herbicide use on general parklands, sports fields and road sides was achieved by 2001 [25].

In 2001, the Supreme Court of Canada upheld the right of a municipality, Hudson Quebec, to restrict pesticide use [26], observing that a 'general enabling clause' in the relevant provincial legislation gave municipalities in Quebec the ability to make bylaws related to health and general welfare. The Ontario *Municipal Act, 2001* granted municipalities general powers to pass bylaws regulating health and safety concerns when the provincial government does not have legislation governing the activity [27]. Following the Supreme Court of Canada decision, the federal Pest Management Regulatory Agency has also acknowledged the role of municipalities in regulating pesticide use by citizens.

In this permissive federal and provincial context, active municipal councilors championed consideration of a bylaw, the Canadian equivalent to an ordinance. Pressure also came from environmental and health groups, e.g. Toronto Environmental Alliance, the Ontario College of Family Physicians and the Canadian Environmental Law Association. The BOH asked TPH to prepare a document that would generate public discussion on the development and feasibility of various strategies to reduce pesticide use in Toronto and inform policy development.

TPH staff reviewed and synthesized the evidence on potential adverse health effects of lawn and garden pesticides [28]. Based on their findings, the resultant report argued for precautionary actions and for limiting unnecessary uses of commonly used pesticides. In addition, TPH incorporated information from a 2000 telephone survey regarding Toronto residents' awareness about, uses of and attitudes towards lawn pesticides [29] and a 2002 public opinion poll that gauged support for different options to reduce pesticide use [30]. Whether they used pesticides or not, over three quarters of respondents to the poll supported restrictions on pesticides and welcomed information that would help them use safer alternatives.

Generation of policy options

TPH incorporated information from an environmental scan of initiatives in other jurisdictions into a policy document [31] with four options: A) public education only, as carried out in Seattle, King County [32]; B) voluntary compliance approach, as in most recycling programs; C) bylaw (all properties), such as Oregon state's Pesticide Tracking Law, also called a "right-to-know" Law [33] and D) bylaw (vulnerable populations only) as in Washington State's children's law around notification of pesticide spraying in schools [34] or New York's Neighbor Notification Law [35].

TPH retained external consultants to undertake a stakeholder consultation on these options in early 2002. They conducted a workshop which brought together 65 stakeholders from pesticide manufacturing companies, lawn care companies, golf course associations, community garden groups, regional conservation authorities, environmental non-governmental organizations, health care provider organizations, school boards, ratepayer groups and governments (provincial Ministry of the Environment, Environment Canada). Workshop results informed six evening public meetings held across the city in the spring of 2002. Approximately 400 people signed in at the meetings and engaged in lively, small group discussions moderated by professional facilitators. A follow-up stakeholder meeting examined the key challenges/barriers the City would face with either a voluntary industry-led initiative or some type of bylaw. Upon consideration of the consultation report, the BOH recommended that Toronto adopt a pesticide bylaw to best protect public health [36].

Policy enactment & Program design

In May 2003, Toronto City Council passed a bylaw that "restricts the outdoor use of pesticides on all public and private properties in Toronto." It applied to anyone who might use pesticides outdoors, including homeowners, renters, lawn care companies, golf courses and cemeteries [37]. Pesticides composed of specific low-risk active ingredients such as soaps or oils, biologicals (such as nematodes) or acetic acid, among others, were exempted from the bylaw and had no municipal restrictions on their use (though federal authorities do place some use restrictions). In addition, certain uses of restricted pesticides were permitted under the bylaw: control of pests which infested property or uses related to health protection. Note, the pesticide bylaw did not govern the selling or buying of pesticides, as this falls under provincial jurisdiction. Crop Life Canada, a plant science industry trade association, challenged the City of Toronto's bylaw in court but their case was rejected by successive provincial and federal courts [38,39]

City Council recognized the need to limit commercial difficulties for lawncare and gardening businesses and to support residents in changing their long-standing methods for lawn and garden care. While a visible enforcement presence in the community was deemed critical to motivate behaviour change among both lawncare professionals and residents, the enforcement strategy was phased-in from 2004 to 2007 (Figure 1), granting time for those accustomed to using pesticides to learn about

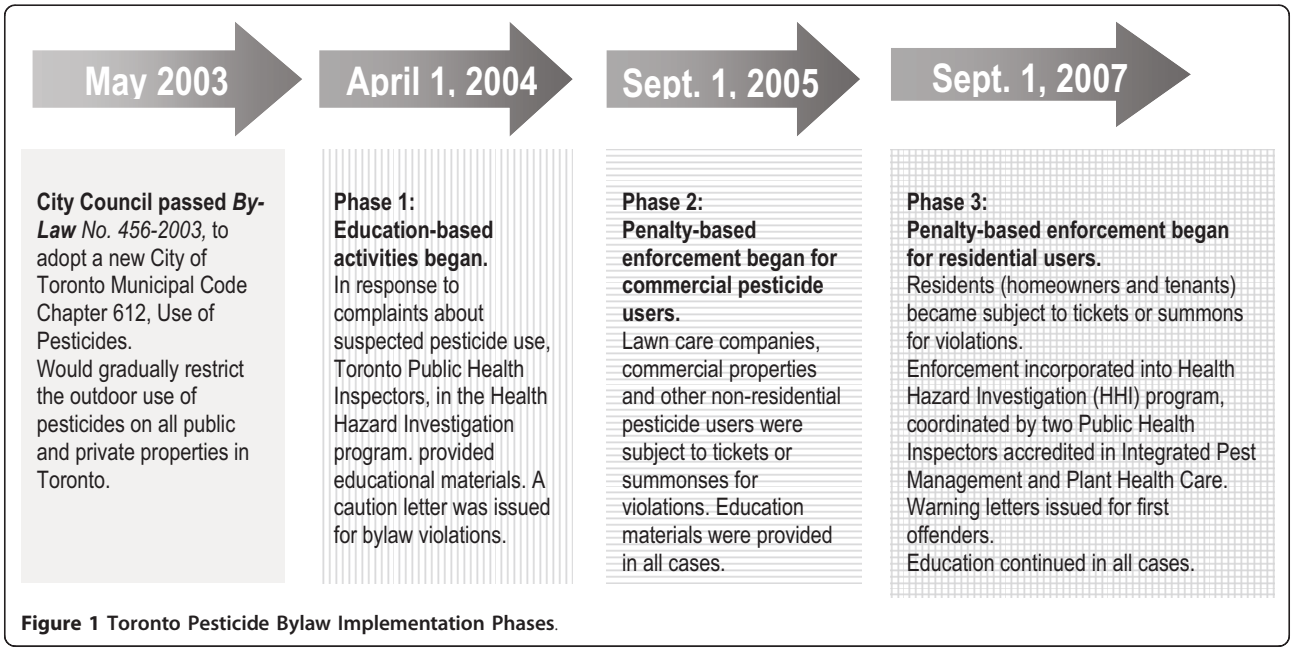

Figure 1 Toronto Pesticide Bylaw Implementation Phases.

the restrictions and to adopt alternative methods for lawn and garden care.

A pre-bylaw education program promoting natural lawn and garden care methods such as aerating the lawn, leaving grass clippings on the lawn, spreading organic fertilizers like compost, and applying mulch in garden beds and around trees [40], which can prevent pest problems and minimize the need for pesticides and chemical fertilizers, was substantially expanded. Other City divisions joined and supported the outreach campaigns. For example, Toronto Water contributed funds for advertisements through their drinking water campaign in 2003 and put a reminder in water bills. In keeping with existing evidence on effectiveness of environmental health awareness programs, traditional education/health promotion activities were complemented with more intensive interventions using multiple methods and settings [41]. TPH's education campaign aimed for variety and adaptability, delivering a combination of simple tips and more comprehensive advice, to both residents and commercial users; information through various media, in stores and in several languages and reminders throughout the gardening season to influence key decision points, such as what to do or purchase in spring and fall for a healthy lawn or garden (Table 1).

Initial implementation costs projected for the 2004 season [42] were approximately CDN$220K covering seasonal staff time for both bylaw and education work, another CDN$150K for advertising and another CDN $70K for workshops, expert consultation and other expenses (total CDN$450K or approximately US$425K).

Implementation Indicator Selection

To inform monitoring of bylaw implementation, we developed a logic model to lay out in diagrammatic form how the policy was intended to produce results and achieve an overall goal [see Figure 2]. Our logic model identified education and enforcement as two key components, outlined activities for each of these components, and predicted short- and medium-term results.

We also supported a structured literature review to learn from other experiences in documenting household pesticide use practices and evaluating initiatives to reduce such use [14]. Building on this, we interviewed 14 key informants from a wide range of sectors as to potential indicators for evaluating bylaw implementation. The following five potential indicator domains emerged from the literature review and key informants: 1) Enforcement/Legal; 2) Education and Outreach activities, and associated community responses; 3) Economic; 4) Environmental testing; and 5) Medical/Public Health, including urine bio-monitoring and clinical visits.

Data on Enforcement/legal (1) and Education/outreach implementation indicators (2) were most readily available as TPH staff implemented the program. TPH tracks bylaw complaints and the details of complaint investigations through its Toronto Healthy Environments Information System (THEIS) database. TPH also tracked activities relevant to its education and outreach activities (as outlined in Table 1).

The potential economic impacts of a bylaw were an important concern expressed by key informants from landscaping and lawn care companies. Since actual sales data on pesticides or services were not available, we

Table 1 Public Education and Outreach Campaign

Means by which particular audiences were reached with appropriate information:	
Advertising	in spring and fall - when people are thinking most about their lawns and gardens - served to remind residents of the bylaw, to balance marketing of traditional pesticides, and to support community acceptance of natural lawn care. In collaboration with Toronto Water and Parks, Forestry and Recreation, 300-500 advertisements were created and placed in major newspapers, community and ethno-cultural newspapers, City guides and newsletters, family and lifestyle magazines, transit shelters and on recycling bins [71].
City of Toronto website [72]	had the text of the bylaw, answered frequently-asked questions, included guidance for professional users, provided complaint forms, and made links to relevant information from other City divisions and community organizations. Given the ethnic diversity of Toronto, some material appeared in the City's most commonly spoken non-English languages (French, Spanish, Italian, Portuguese, Russian, Tamil, Chinese and Farsi).
Toronto Health Connection	staff responded to public telephone inquiries, processed complaints, sent educational material and provided basic advice on natural lawn care.
Brochures, fact sheets and lawn signs	were designed to appeal to residents at all stages of awareness and activity. They contained general information, lawn care and gardening tips, information on how to prevent and deal with specific pest problems; bylaw information; questions to ask a lawn-care company, and information about the lower risk pest control products with no restrictions on use.
Information in stores	as both restricted and exempted pesticides remained available for purchase and residents mistakenly assumed that products for sale were "approved" by the City. In consultation with retailers, a "Go Natural" in-store education program was launched in 2005. Go Natural brochures, tear-off sheets, staff aprons, posters and banners were voluntarily posted on store shelves or at cash registers and directed consumers to lower-risk products for certain lawn or garden problems.
Regular communication	with professional stakeholders, including landscapers, lawn care companies, arborists and other horticultural professionals to support compliance and their transition to sustainable pesticide reductions.
Community partnerships	included 16 environmental and cultural organizations funded to deliver innovative outreach such as workshops, garden tours and radio shows in eight languages. Toronto Public Health also collaborated with academic and community partners to identify communication barriers and explore opportunities to improve multicultural outreach [54].
Presentations	by City staff included expert advice through health promotion consultants, Public Health Inspectors, Parks, Forestry and Recreation staff and the Toronto Environmental Volunteers.
Public events	included both small community gatherings and large events such as Toronto's Community Environment Days, the Canadian National Exhibition, Canada Blooms, and the Toronto Renovation Forum.

used data on companies in business available from Statistics Canada [(Business Register, Canadian Business Patterns (1998-2005) & (2001-2006)]. These provided a rough indicator of potential economic impacts before and during bylaw implementation (3).

Environment Canada, Ontario Ministry of Environment and Toronto Works and Emergency Services colleagues had done some short-term surface water testing from 1998 to 2000, particularly during high run-off events (4) [43]. Unfortunately, the costs of systematic, regular, long-term surface water monitoring were unsupported at the time the bylaw was implemented. Further, we were unable to implement indicators for domain 5 for several reasons. For bio-monitoring, we were concerned that intra- and inter-individual variation could potentially swamp an exposure reduction effect, the substantial costs involved (minimally an estimated $125K annually over at least five years) were beyond municipal resources, and ethical concerns had been raised during the evaluation of environmental health interventions [44,45]. We explored clinical data systems but those available were incomplete, focused on hospital visits only, and did not adequately identify pesticide exposure in routinely coded data, unlike pesticide exposure incident reporting systems specifically designed for such purposes which have proved very useful in evaluating reductions in other jurisdictions [46].

Repeat Surveys

Design

To track community responses via resident awareness and behaviour over time (domain 2), we turned to the Rapid Risk Factor Surveillance System (RRFSS), a set of ongoing monthly surveys designed to monitor community trends in health risk behaviours among the Ontario population. RRFSS surveys are administered independently by the Institute for Social Research, York University, and consist of questions organized into core and optional modules [47]. TPH led development of optional "pesticides and lawns", "pesticide bylaw" and "pesticide reduction education" modules that were conducted on a monthly basis from 2003 to 2008. The repeated surveys were an appropriate evaluation tool given the phased in approach of pesticide bylaw implementation (See Figure 1). This design approximates the one-group pretest-posttest design most frequently used to evaluate the impact of health programs, though with more 'during' measures, given the phased implementation [48].

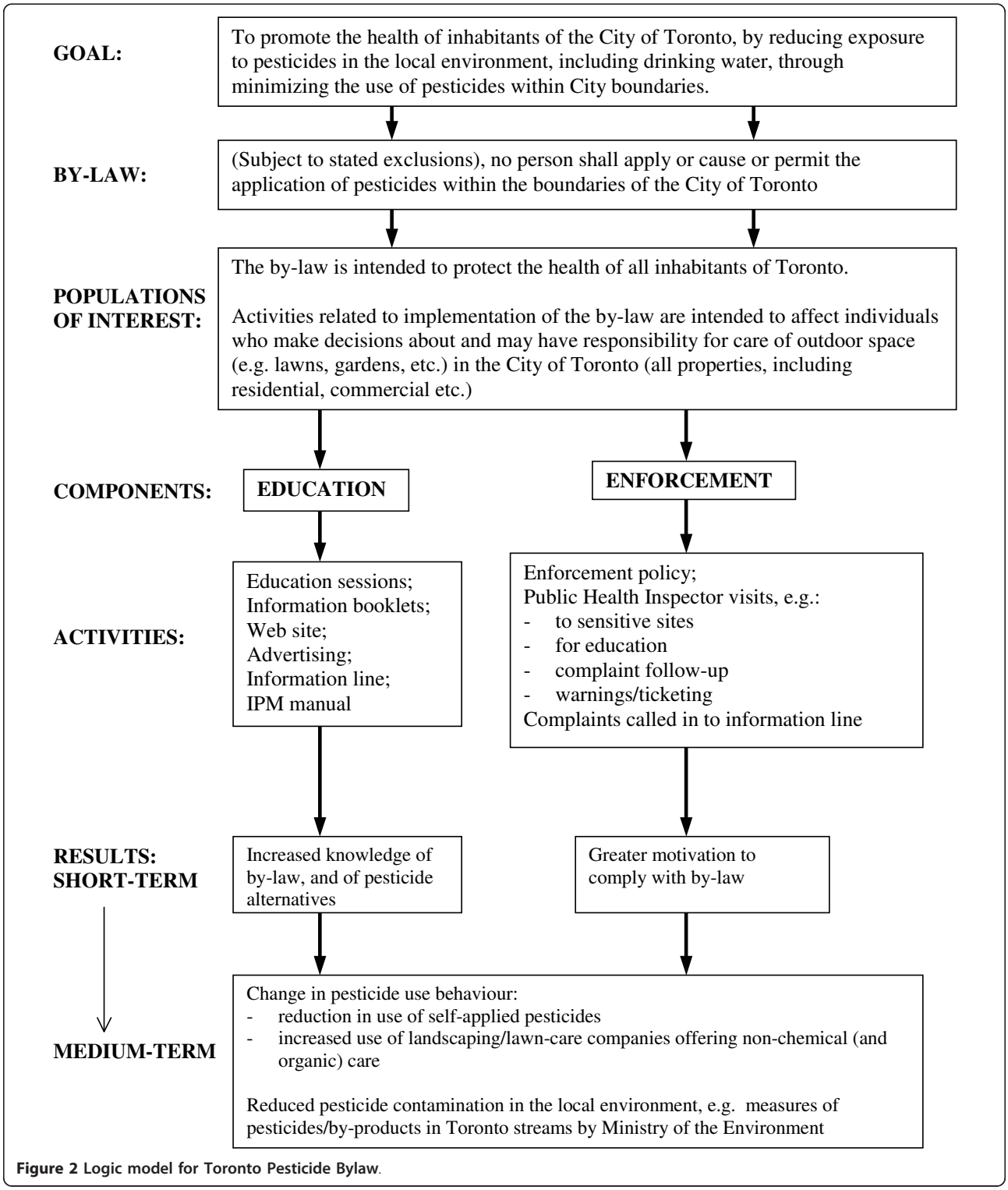

Figure 2 Logic model for Toronto Pesticide Bylaw.

Household selection was via random digit dialing procedures. A phone number was called repeatedly until either the survey was completed or the maximum number of 14 calls had been made, at which time the number was considered a 'dead' sample. Within households, the adult with the most recent birthday was selected with no substitutions of more willing household members. Attempts were made to encourage those

individuals who initially refused to participate by calling them at least once after they first refused. Consent to participate was verbal, with no address, name or other identifying information collected (i.e. anonymous) as approved by York University's Research Ethics Board, Human Participants Review Subcommittee for generation and use of RRFSS data.

All interviews were completed in English, using Computer-Assisted Telephone Interviewing techniques, which greatly assists in expediting the data editing and cleaning process because logical and quality control checks can be programmed into the questionnaire design [49]. Respondents were asked about either the current (for surveys conducted from April to October) or most recent past (for surveys conducted from October to following spring) gardening seasons. Although this may create some uncertainty in assignation of the relevant gardening season, it was required when surveys were administered in winter months when no gardening was occurring. RRFSS data had indicated that about 50% of households in Toronto had lawns. Because questions about pesticide use were only asked of this subsample of residents, an oversample was implemented in some seasons to ensure a subsample of sufficient size to allow for more detailed analyses of the data.

Socio-demographic variables available included: respondent's gender, pre-tax household income (twelve categories), respondent's highest level of education (four categories from did not graduate from high school to college or university degree) and household location within the City (as defined by the household's municipality prior to amalgamation). Missing data, in addition to item refusals or "don't know", varied from 0 (gender) to 27% (household income). Household size, asked in some years, was on average 2 adults, permitting the estimation of a low income cut-off (LICO) as CDN$27,601 [50]. As this fell towards the upper limit of an income category on the survey ($20,000 to $29,999), all respondents in this category or the lowest (< 20,000) were designated as below the LICO.

For bylaw awareness, all respondents from 2005 on were asked "Some communities have bylaws that limit the outdoor use of pesticides, some are thinking about it and others do not. Do you think that the City of Toronto currently has a bylaw that limits the outdoor use of pesticides?" Further, respondents were asked "Have you seen or heard anything about the Naturally Green/ Simple and Effective Lawn Care Campaign in your community? ...includes lawn signs, brochures, and ads in the newspaper which encourage people to avoid pesticides and try pesticide free methods." Responses (Yes/No) became the pesticide bylaw and Natural Lawn Care Campaign awareness dependent variables. Additional questions were asked to understand reasons for reducing pesticide use, using more natural methods and obtaining information on each of these, particularly among the 2005 and 2006 oversamples [51].

For household practices, across all years, respondents were first asked "Does your home have a lawn that you or someone else in your household is taking care of?" If yes, then "Did you or someone else in your household hire or pay a lawn care company to treat your lawn?" and, if yes, then "Did the lawn care company use any pesticides on your lawn to kill weeds or insects?" and "Did they offer to use any natural lawn care/pesticide-free methods such as aeration, over-seeding, hand weeding, or products such as corn gluten?" Similar questions were also asked of respondents with a lawn about applications by "you or someone else in your household" (except for natural lawn care methods in the first year of interest). These became the practices or use dependent variables.

Analysis

Data distributions were analyzed with STATA/IC statistical software version 11.0 (2009). Individual level variables (gender, education, pesticide bylaw awareness and Natural Lawn Care Campaign awareness) were weighted to account for the unequal probabilities of selection of one-adult households [52]. Two weights were created for gender and education (gardening 2003-2008), and for pesticide bylaw and Natural Lawn Care Campaign awareness (gardening season 2005-2008), to account for the different time periods in which the questions were asked. Oversamples were given separate values for gardening years, as demographic characteristics of these oversamples varied systematically from regular samples; i.e., more women, greater education, and different location distribution. For key practice variables, confidence intervals were calculated on the first and last year proportions. Bivariate associations were assessed with chi-square statistics.

We then constructed multivariable logistic models with gardening year as the primary independent variable of interest and demographic variables as covariate independent variables (to control for differences across years), with one awareness or use variable as the dependent variable in each model. As associations were observed between awareness variables and between them and gardening year (i.e. increased awareness as bylaw enforcement and education programs rolled out), variable selection was required. The latter examined associations between gender, level of education, income, location, and gardening year as independent variables and respondent awareness or household practice dependent variables. Given the mix of individual and household variables, we conducted sensitivity analyses to examine the effect of using weights that account for individual-level variables versus household variables, and

weights that account for different time periods in which the questions were asked. These weights are available from authors upon request. Eventually we settled on application of individual weights as the most inclusive option. Changes in adjusted odds ratios (OR) with 95% confidence intervals were displayed graphically.

Results

Implementation indicators

Enforcement/legal - Public Health Inspectors with special training in integrated pest management/plant health care led an enforcement strategy that included proactive visits to schools, golf courses and other properties, participation in educational events in the community and at garden centres, and in-person response to over 3,000 complaints of suspected violations, most occurring early on (see complaint investigations, Table 2). From initiation in 2004 to the first full season of enforcement on lawn care companies and commercial properties (2006), complaints decreased over 80 per cent. This decrease and the low number of convictions required suggest that enforcement and education messages reached much of the professional sector and most came into compliance [53].

Education/outreach - As can be seen in Table 2, the effort to make residents aware was substantial. In addition to those methods listed, city staff conducted 291 proactive information visits to sensitive sites, such as child care centers and hospitals, and all public and private golf courses and bowling greens. Informal feedback from the community helped identify the need for expert resources on plants and gardens not just lawns. TPH responded by partnering with the Toronto Master Gardeners to produce a series of fact sheets on natural care of flowers and vegetable gardens and promote the information via the internet, during lectures, community events and a telephone information line. Community feedback also resulted in new retail materials, information for the lawn care sector, and particular efforts with ethno-cultural partners [54].

Economic - From 2001 to 2006, the number of landscaping and lawn care sector businesses located in the City of Toronto grew each year, with an overall 30 per cent increase during the period, similar to the increases in companies located anywhere in the Greater Toronto Area (36%) and across Ontario (32%) [53].

Repeat Survey findings

Response rates across the six years of interest (2003-2008) ranged from 58% (in 2005) to 50% (in 2007). Explicit oversamples of those with lawns occurred for the 2005 (n = 355) and 2006 (n = 179) gardening seasons, resulting in an overall sample of 4,901 respondents.

As can be seen in Table 3, over half of households (55.6%) reported having or caring for a lawn, with greater proportions in the oversampled garden seasons (> 60%) in keeping with intentional selection in the oversamples (e.g. in 2005, 541/1085 or 49.9% in regular sample and 100% in oversample). The proportion hiring

Table 2 Relevant indicators of Toronto pesticide policy roll-out and program implementation.

Domain	Indicator	Gardening Season				
		2004	2005	2006	2007	2008
Enforcement/legal	Bylaw complaint investigations	1672	1118	294	74	127*
	Warning letters issued	NA	6	28	6	0
	Convictions	NA	3	0	1	3
Education & Outreach	Advertisements placed	353	503	335	850**	850
	Website - Pages of content	50	197	230	No info	No info
	Traffic/month	4,754	7,999	12,000		
	General information materials (postcard, brochure, pamphlet)	40,000	20,356	92, 949	89,250	> 75,000
	Technical manual	435	892	482	45	25
	Plastic "pesticide" free lawn signs	3000	1646	300	Discontinued	NA
	Telephone Inquiries	709	588	434	174	318
	Presentations at events	53	74	74	32	20
	Go Natural retail participation	NA	122 stores 83,343 materials	122 stores 98,000 materials	113 stores 53,334 materials	145 30,627 materials
	Fact sheets on natural gardening	NA	NA	> 2500	> 2500	> 2500

*Several lawn care companies introduced a new lower-risk pesticide in 2008, which triggered an increase in complaints, though the pesticide was compliant with the bylaw.

** In 2007 and 2008, TPH initiated an extensive month-long radio ad campaign, which significantly increased the number of ads.

NA = Not applicable

Table 3 Respondent* and household^ characteristics, by gardening season (n, %) (Rapid Risk Factor Surveillance Survey, Toronto)

Characteristics	Gardening Season						Totals (N = 4901)
	2003 (N = 608)	2004 (N = 609)	2005‡ (N = 1440)	2006‡ (N = 777)	2007 (N = 620)	2008 (N = 847)	
Gender* (weighted counts, wgtd %)	(n = 608)	(n = 607)	(n = 1453)	(n = 795)	(n = 614)	(n = 825)	
Women	316 (52.0%)	353 (58.2%)	795 (54.7%)	459 (57.7%)	344 (56.0%)	457 (55.3%)	2723 (55.6%)
Men	292 (48.0%)	253 (41.8%)	658 (45.3%)	336 (42.3%)	270 (44.0%)	368 (44.7%)	2178 (44.4%)
Education* (wgtd counts, wgtd %)	(n = 608)	(n = 607)	(n = 1453)	(n = 795)	(n = 614)	(n = 825)	
< High school	62 (10.2%)	82 (13.5%)	130 (9.0%)	77 (9.7%)	45 (7.3%)	63 (7.7%)	459 (9.4%)
High school	118 (19.5%)	111 (18.2%)	269 (18.5%)	138 (17.4%)	130 (21.2%)	182 (22.1%)	947 (19.3%)
Some post-2ndy	58 (9.6%)	60 (9.9%)	124 (8.6%)	76 (9.6%)	40 (6.5%)	75 (9.1%)	434 (8.9%)
Completed post-2ndy	361 (59.4%)	347 (57.2%)	904 (62.2%)	496 (62.3%)	394 (64.1%)	494 (59.8%)	2995 (61.1%)
Missing, don't know, refused	9 (1.4%)	7 (1.2%)	26 (1.7%)	8 (1.0%)	5 (0.9%)	11 (1.3%)	66 (1.3%)
Income^†							
< low income cutoff	122 (20.1%)	127 (20.9%)	201 (14.0%)	121 (15.6%)	91 (14.7%)	137 (16.2%)	799 (16.3%)
≥ > low income cutoff	357 (58.7%)	344 (56.5%)	842 (58.5%)	433 (55.7%)	336 (54.2%)	460 (54.3%)	2772 (56.6%)
Missing, don't know, refused	129 (21.2%)	138 (22.7%)	397 (27.8%)	223 (28.7%)	193 (31.1%)	250 (29.5%)	1330 (27.1%)
Municipality^							
East York	36 (5.9%)	33 (5.4%)	98 (6.8%)	60 (7.7%)	40 (6.5%)	64 (7.6%)	331 (6.8%)
Etobicoke	98 (16.1%)	76 (12.5%)	190 (13.2%)	98 (12.6%)	86 (13.9%)	142 (16.8%)	690 (14.1%)
North York	157 (25.8%)	155 (25.5%)	340 (23.6%)	172 (22.1%)	151 (24.4%)	189 (22.3%)	1164 (23.8%)
Old City of Toronto	168 (27.6%)	183 (30.0%)	388 (26.9%)	215 (27.7%)	174 (28.1%)	241 (28.5%)	1369 (27.9%)
Scarborough	116 (19.1%)	118 (19.4%)	333 (23.1%)	175 (22.5%)	133 (21.5%)	168 (19.8%)	1043 (21.3%)
York	29 (4.8%)	32 (5.3%)	70 (4.9%)	48 (6.2%)	27 (4.4%)	34 (4.0%)	240 (4.9%)
Other	1 (0.2%)	1 (0.2%)	2 (0.1%)	1 (0.1%)	3 (0.5%)	0 (0.0%)	8 (0.2%)
Missing, don't know, refused	3 (0.5%)	11 (1.8%)	19 (1.3%)	8 (1.0%)	6 (1.0%)	9 (1.1%)	56 (1.1%)
Had a lawn^							
Yes	333 (54.8%)	289 (47.5%)	896 (62.2%)	479 (61.7%)	308 (49.7%)	421 (49.7%)	2726 (55.6%)
No	265 (43.6%)	312 (51.2%)	529 (36.7%)	294 (37.8%)	308 (49.7%)	421 (49.7%)	2129 (43.4%)
Missing, don't know, refused	10 (1.6%)	8 (1.3%)	15 (1.0%)	4 (0.5%)	4 (0.6%)	4 (0.6%)	46 (0.9%)
Hired a lawn care company^ [among those with lawns]							
Yes	81 (24.3%)	77 (26.6%)	193 (21.5%)	97 (20.3%)	72 (23.4%)	98 (23.3%)	618 (22.7%)
No	248 (74.5%)	202 (69.9%)	693 (77.3%)	376 (78.5%)	232 (75.3%)	316 (75.1%)	2067 (75.8%)
Missing, don't know, refused	4 (1.2%)	10 (3.5%)	10 (1.1%)	6 (1.3%)	4 (1.3%)	7 (1.7%)	41 (1.5%)

‡ Explicit oversamples of those with lawns occurred for the 2005 (n = 355) and 2006 (n = 179) gardening seasons

†The cut-offs are based on income before taxes for a 2 person household in a community size of 500,00 and over. Since the cut-off for a 2 person household in a community size of 500,000 and

over is 27,601, a value within an income category ($20,000 to $29,999), all respondents within this category were designated below the LICO. All respondents within income categories > 30,000 were designated above the LICO.

lawn care companies remained relatively consistent over time (maximum 26.6% of those with lawns in 2004, minimum 20.3% in 2007, back up to 23.3% in 2008), agreeing with the business data and contrary to the fears of some lawn care spokespeople.

Across gardening seasons, the (weighted) proportion of respondents indicating awareness of the Toronto pesticide bylaw increased from 50.6% (2005) to 69.2% (2008) (Table 4). Among those with lawns, reported use of pesticides on their lawn decreased, both by a company they hired (14.7% in 2003 to 4.5% in 2008) or by a household member (24.6% in 2003 to 11.2% in 2008) with no overlap of 95% confidence intervals (Table 5). Respondent awareness of the Natural Lawn Care Campaign among those with lawns showed little change (36.8% in 2005 to 37.8% in 2008), but use of natural lawn care practices by a company they hired (4.8% in 2003 to 11.9% in 2008) or a household member (45.3% in 2004 to 66.3% in 2008) did increase (confidence intervals also non-overlapping).

Among households that indicated that they had reduced their pesticide use (data not in tables), the primary reason given was for health or environmental reasons (33.7% average), followed closely by the pesticide bylaw (23.9% average) and that simply their lawn did not require pesticides (16.3% average) [53]. While the pesticide bylaw was not the most influential factor, an upward trend (+5.1%) of citing the bylaw as the motivation was seen between gardening seasons 2006 and 2007. Among households that indicated they had changed towards increasing their use of natural lawn care methods, the primary influence was health or environmental reasons (42% average). The pesticide bylaw was cited as the reason by 20% of respondents. Among those never having used natural lawn care methods, the largest group (48.3%) reported not having much knowledge of natural lawn care practices or methods.

In bivariate analyses (Table 6), awareness of the pesticide bylaw and the Natural Lawn Care Campaign were moderately associated (Odds Ratio (OR) > 2 most seasons) so either one or the other had to be used in multivariable models. Dependent variables showed variation by respondent gender and education, and household income and location, so were included in multivariable logistic models (table 6). Male respondents were generally more aware of the pesticide bylaw (OR 1.2) and less aware of the Natural Lawn

Campaign (OR 0.8). Though less likely to use natural lawn care methods, their households were more likely to apply pesticides. In contrast, among respondents with less than high school education who were also more aware of the pesticide bylaw, their households less commonly applied pesticides or used natural lawn care methods more often. Low income households were less aware of the Natural Lawn Care Campaign and applied these methods less commonly. Across gardening seasons, trends towards greater awareness of the pesticide bylaw and less application of pesticides remained, along with greater use of natural lawn care methods (but not awareness). The same pesticide application trends can be observed graphically (Figure 3), among the regular sample (without oversample, hence slightly different OR from table).

Discussion

Municipal Implementation

We have described an innovative approach to designing and implementing a new policy involving regulation of outdoor use of pesticides for non-essential purposes. The policy gestation over several years, the substantial efforts to engage in dialogue and debate with the public and relevant stakeholders, the mobilization of adequate TPH staff resources and the widespread dissemination of information, both about the bylaw and alternatives, reached Toronto residents. By 2008, over two-thirds of respondents reported being aware of the bylaw.

On the enforcement front, residents were active in lodging complaints, but few actual prosecutions were needed. The specialized training for health inspectors and strong enforcement presence increased awareness and likely compliance. At the same time, the complexity of governance around pesticide use, with responsibilities

Table 4 Respondent* & household^ lawn care awareness and practices, City of Toronto, 2003-2009 All households (n, %)

Lawn Care Awareness & Practices	Gardening Season						Totals (n = 4901)
	2003 (n = 608)	2004 (n = 609)	2005 (n = 1440)	2006 (n = 777)	2007 (n = 620)	2008 (n = 847)	
Aware of pesticide bylaw*†(wgtd counts, wgtd %)	NA	NA	(n = 1452)	(n = 794)	(n = 614)	(n = 825)	(n = 3684)
Yes			734 (50.6%)	520 (65.4%)	415 (67.8%)	570 (69.2%)	2239 (60.8%)
No			133 (9.2%)	80 (10.1%)	48 (7.8%)	75 (9.1%)	335 (9.1%)
Missing, Don't Know, Refused			585 (40.3%)	195 (24.5%)	151 (24.6%)	179 (21.8%)	1110 (30.1%)

† Question pestby_1 from Pesticide Awareness Module in RRFSS. Some communities have bylaws that limit the outdoor use of pesticides, some are thinking about it and others do not. Do you think that "Name of Health Unit inserted here" currently has a bylaw that limits the outdoor use of pesticides?

Table 5 Respondent* & household^ lawn care awareness and practices, City of Toronto, 2003-2009 Only households with lawns (n, %, 95% CI for key practices)

Lawn Care Awareness & Practices	Gardening Season						Totals (n = 2726)
	2003 (n = 333)	2004 (n = 289)	2005 (n = 896)	2006 (n = 479)	2007 (n = 308)	2008 (n = 421)	
Lawn care company applied pesticides^ †							
Yes	49 (14.7%, 10.9 - 18.5)	40 (13.8%)	69 (7.7%)	18 (3.8%)	9 (2.9%)	19 (4.5%, 2.5 - 6.5)	204 (7.5%)
No	18 (5.4%)	27 (9.3%)	86 (9.6%)	69 (14.4%)	52 (16.9%)	66 (15.7%)	318 (11.7%)
Missing & Not Applicable	266 (79.9%)	222 (76.8%)	741 (82.7%)	392 (81.8%)	247 (80.2%)	336 (79.8%)	2204 (80.9%)
Household member applied pesticides^‡							
Yes	82 (24.6%, 20.0 - 29.3)	58 (20.1%)	145 (16.2%)	83 (17.3%)	43 (14.0%)	47 (11.2%, 8.1 - 14.2)	458 (16.8%)
No	229 (68.8%)	205 (70.9%)	708 (79.0%)	375 (78.3%)	248 (80.5%)	350 (83.1%)	2115 (77.6%)
Missing & Not Applicable	22 (6.6%)	26 (9.0%)	43 (4.8%)	21 (4.4%)	17 (5.5%)	24 (5.7%)	153 (5.6%)
Aware of Natural Lawn Care Campaign*§(wgtd counts, wgtd%)	NA	NA	(n = 977)	(n = 531)	(n = 344)	(n = 456)	(n = 2308)
Yes			359 (36.8%)	210 (39.4%)	133 (38.8%)	172 (37.8%)	875 (37.9%)
No			473 (48.4%)	300 (56.5%)	197 (57.3%)	269 (59.1%)	1239 (53.7%)
Missing, Don't Know, Refused			145 (14.9%)	22 (4.1%)	14 (3.9%)	14 (3.1%)	195 (8.4%)
Lawn care company used natural lawn care methods^°							
Yes	16 (4.8%, 2.5 - 7.1)	29 (10.0%)	67 (7.5%)	49 (10.2%)	32 (10.4%)	50 (11.9%, 8.8 - 15.0)	243 (8.9%)
No	23 (6.9%)	21 (7.3%)	46 (5.1%)	19 (4.0%)	13 (4.2%)	17 (4.0%)	139 (5.1%)
Missing & Not Applicable	294 (88.3%)	239 (82.7%)	783 (87.4%)	411 (85.8%)	263 (85.4%)	354 (84.1%)	2344 (86.0%)
Household member used natural lawn care methods^¶	NA						
Yes		131 (45.3%, 39.6 - 51.1)	518 (57.8%)	290 (60.5%)	196 (63.6%)	279 (66.3%, 61.7 - 70.8)	1414 (51.9%)
No		130 (45.0%)	333 (37.2%)	163 (34.0%)	97 (31.5%)	109 (25.9%)	832 (30.5%)
Missing & Not Applicable		28 (9.7%)	45 (5.0%)	26 (5.4%)	15 (4.9%)	33 (7.8%)	480 (17.6%)

† Question pbl_3 from Pesticides and Lawns Module in RRFSS. Did the lawn care company use any pesticides on your lawn to kill weeds or insects?

‡ Question pbl_8 from Pesticides and Lawns Module in RRFSS. There are also many commercial pesticides available off the shelf, such as Roundup, Killex and Weed and Feed, for HOME AND GARDEN use. Now some questions about these types of pesticides.

If pbl_2 = 1 (if hired/paid a lawn care company)Besides the services provided by the lawn care company, so far this year*, have you or someone else in YOUR HOUSEHOLD used pesticides on your LAWN to get rid of weeds or insects?

If pbl_2 = 5 (if not hired/paid a lawn care company]So far this year*, have YOU or someone else in YOUR HOUSEHOLD used pesticides on your LAWN to get rid of weeds or insects?

§ Question ng_1 from Pesticide Campaigns Module in RRFSS. Have you seen or heard ANYTHING about the Naturally Green Campaign in your community? The campaign includes lawn signs, brochures, and ads on the radio which encourage people to avoid pesticides and try pesticide free methods.

° Question pbl_7 from Pesticides and Lawns Module in RRFSS. [PBL_6 = No]Did they (the lawn care company) use any natural lawn care/pesticide-free methods?/ [PBL_6 = Yes] And did they use it?

¶ Question pbl_10 from Pesticides and Lawns Module in RRFSS.

If pbl_2 = 1 (if hired/paid a lawn care company) Besides the services provided by the lawn care company, have YOU or someone else in YOUR household used pesticide-free methods such as hand weeding or used products such as corn gluten on your lawn?

If pbl_2 = 5 (If not hired/paid a lawn care company), So far this year*, have YOU or someone else in YOUR household used pesticide-free methods such as hand weeding or used products such as corn gluten on your lawn?

Table 6 Logistic regression models of variables associated with respondent awareness or household practice outcomes, weighted with individual level variable weight (Odds Ratio, [Standard Error], significant coefficients bolded)

Independent variables	Dependent variable					
	Respondent aware of pesticide bylaw (n = 1804)	Lawn care company applied pesticides (n = 375)	Household member applied pesticides (n = 1863)	Respondent aware of Natural Lawn Care Campaign (n = 1804)	Lawn care company used natural lawn care methods (n = 196)	Household member used natural lawn care methods (n = 1283)
Gender (Woman referent)						
Man	**1.21 [0.17]**	**1.67 [0.25]**	**1.71 [0.12]**	**0.85 [0.11]**	0.77 [0.37]	**0.75 [0.12]**
Education (completed post-2ndy referent)						
< high school	**1.52**	0.75 [0.53]	**0.72 [0.27]**	1.02 [0.24]	0.34 [1.06]	**0.52 [0.27]**
highHigh school	**[0.39]**	0.98 [0.35]	**0.77 [0.17]**	**0.74 [0.15]**	1.16 [0.48]	0.89 [0.16]
> high school	1.01 [0.23]	**1.52 [0.46]**	1.04 [0.22]	0.94 [0.20]	0.38 [0.73]	0.97 [0.24]
	1.03 [0.29]					
Household income (≥ LICO referent)						
< low income cutoff	1.12 [0.24]	**0.59 [0.38]**	0.80 [0.20]	**0.72 [0.16]**	**0.26 [0.62]**	**0.51 [0.19]**
Municipality (East York referent)						
Etobicoke	0.65 [0.36]	1.30 [0.60]	**1.37 [0.30]**	**1.31 [0.23]**	1.07 [0.79]	1.15 [0.28]
North York	**0.60**	**1.65 [0.58]**	**1.85 [0.28]**	0.94 [0.22]	1.67 [0.76]	**0.60 [0.26]**
Old City of Toronto	**[0.34]**	0.79 [0.62]	0.94 [0.29]	1.14 [0.21]	**2.26 [0.80]**	1.12 [0.26]
Scarborough	0.78 [0.34]	**2.48 [0.58]**	1.92 [0.28]	0.94 [0.22]	**6.23 [0.78]**	0.90 [0.26]
York	1.15[0.36]	1.17 [1.32]	1.15 [0.38]	**1.75 [0.30]**	1.12 [1.25]	1.20 [0.36]
Other	**0.42 [0.45]** 0.18 [1.40]	NU	3.51e-05 [393.7]	1.02 [1.35]	NU	NU
Gardening period (referent year)	(2005)	(2003)	(2003)	(2005)	(2005)	(2005)
2004	NA	**0.49 [0.44]**	0.97 [0.23]	NA	NA	NA
2005	NA	**0.21 [0.42]**	**0.51 [0.21]**	NA	NA	NA
2005 Oversample	1.13 [0.29]	**0.43 [0.46]**	0.81 [0.22]	**0.77 [0.20]**	**1.60 [0.52]**	**1.41 [0.19]**
2006	1.08 [0.24]	**0.08 [0.53]**	**0.59 [0.24]**	1.04 [0.16]	**3.99 [0.62]**	**1.30 [0.19]**
2006 Oversample	**1.81 [0.48]**	0.02 [1.09]	0.71 [0.29]	**0.52 [0.28]**	1.44 [0.70]	**2.29 [0.26]**
2007	**1.53 [0.27]**	**0.06 [0.53]**	**0.53 [0.24]**	**0.81 [0.16]**	**2.31 [0.56]**	**2.22 [0.20]**
2008	**1.40 [0.23]**	**0.10 [0.46]**	**0.47 [0.23]**	**0.68 [0.15]**	**5.18 [0.58]**	**2.01 [0.19]**
Others						
Pesticide bylaw awareness	NU	NU	NU	**2.53 [0.18]**	NU	NU
Natural Lawn Care Campaign lawn care campaign awareness	**2.55 [0.18]**	NU	NU	NU	**1.66 [0.36]**	**1.79 [0.13]**

NA indicates not available in gardening season

NU indicates variable not used for the model because not appropriate as variable to be dependent variable, not included as wanted to include all seasons, or co-variation required selection between two awareness variable.

at the national (registration), provincial (classification as to uses and sale) and municipal levels, left an opening for the unsuccessful court challenge and created additional user confusion.

Overall costs for city taxpayers were reasonable: in the most intense launch year about 450K/2.5 million residents or CDN$0.20 per resident per year, within the lower range of the expenditure ratios reported in the

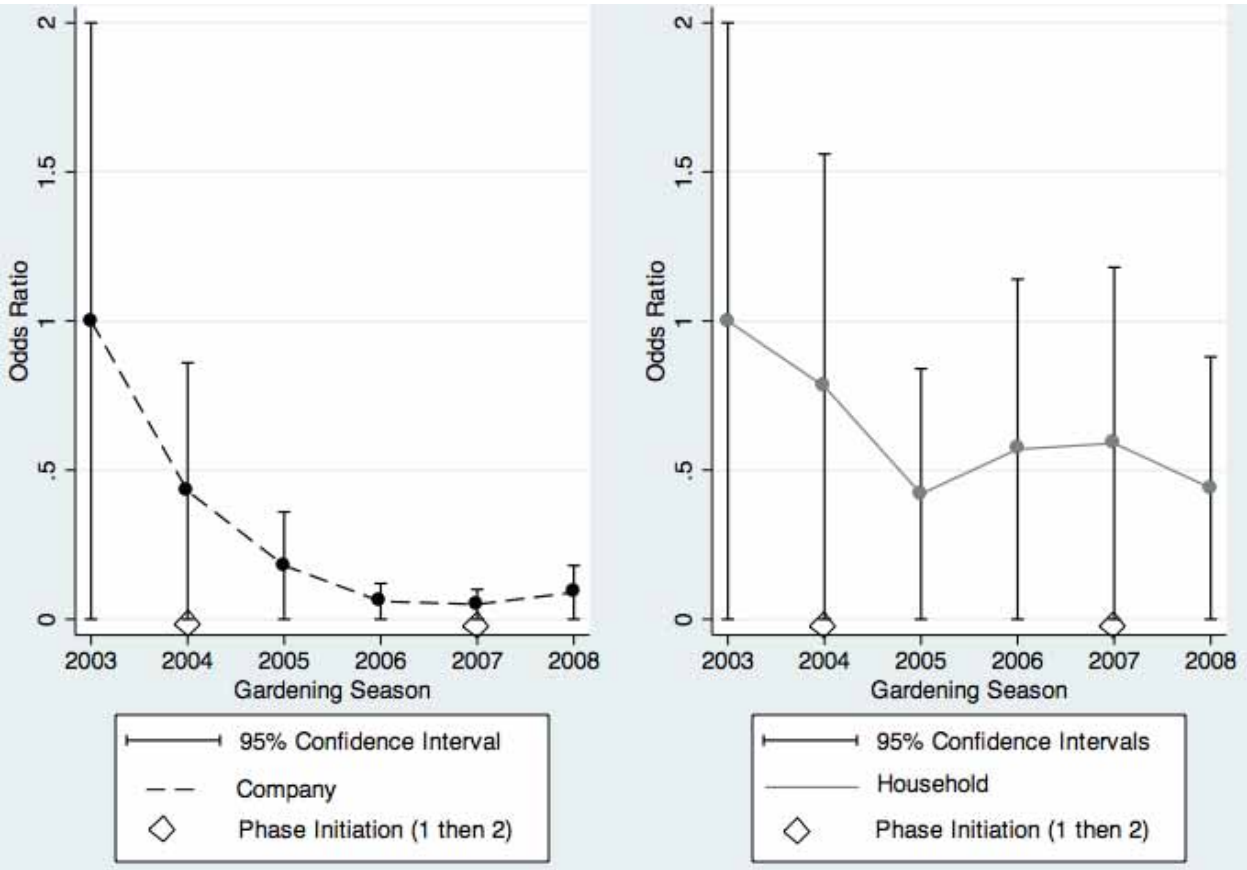

Figure 3 Adjusted* odds ratios (95% confidence intervals (CI)) for reported *application of pesticides* by lawn care company or by household member 2003-2008, without oversamples^. *Adjusted for respondent gender & education, household income & location. ^ Hence OR are slightly different from those in table 4

review by the Canadian Centre for Pollution Prevention [21]. These were substantially lower than environmental tobacco smoke bylaw implementation and enforcement costs, where persistent conflict was greater [55,56].

Resident practice changes

In a city where households already appeared to use pesticides less than other jurisdictions (only 15% company and 25% household application versus over 50% of households applying pesticides in other Canadian jurisdictions (Ipsos Reid, 2001, unpublished), significant further reductions in pesticide application were achieved. Comparing these falls in use is difficult given the different metrics used in the limited grey literature e.g. the drop in household contracted company applications can be framed as a modest absolute difference of 10% (14.7-4.5) or as a very large proportionate drop of 69% (10.2/14.7). Similarly for householder application, an absolute difference of 13% (24.6-11.2) or a proportionate decrease of 54% (13.4/24.6). The proportionate decreases would be comparable to the largest changes observed in the Canadian Centre for Pollution Prevention's review [21] including two other municipalities with bylaws - the very small community of Hudson, Quebec and the city of Halifax, Nova Scotia. Note that context can also influence such reductions, as comfort with pesticide use differed between rural and urban areas of Utah [57]. The absolute differences we observed would be more comparable to those achieved in Seattle and Chesapeake Bay through education and outreach alone.

However framed, we may ask why reported use did not approach zero, despite use of the suggested multiple channels in risk communication [58]. First, the bylaw permitted uses of some pesticides, which may be among those reported. Second, the continued availability of non-permitted products at stores may have led many homeowners to believe they were allowed to use them. Licensing of products is a federal mandate and the actual sales process a provincial one. Third, associations of the "perfect lawn" in suburban areas with higher socioeconomic status [14], the more status-conscious

nature of a large urban centre, and the link to "men's" work in outdoor yard care are all strong North American cultural characteristics. Finally, Toronto's multi-cultural nature may have made it harder to reach the wide variety of communities who have different uses of outdoor space and whose perceptions of pesticides are influenced more by cultural practices than external information [54].

Increases in use of natural lawn care methods were not universal. This may be because alternatives to pesticides require different approaches and may not be as immediately effective. As in much health promotion, uptake of positive behaviours can be easier than relinquishing of negative ones. Corresponding changes among hired lawn care companies were modest (7.1%) perhaps attributable to the substantial investment such companies have in existing technology, and the difficulties in switching to different suppliers and techniques.

Challenges in Evaluation

Systematic evaluation of the effectiveness of pesticide use reduction efforts including bylaws poses particularly prominent challenges. Feasible, external, independent indicators for measuring changes in pesticide use and contamination over time are limited. For example, pesticide sales (and use) data remain unavailable especially at a municipal level, except within companies. Environmental testing was conducted prior to bylaw implementation but not funded long-term. It was later funded by the provincial government (to successfully assess effectiveness of its own province-wide ban) [59].

We relied primarily on self-reported householder data on practices. Illegal activities, including pesticide use restricted under a bylaw, are generally under-reported on surveys [49]. However, householder data for all but the 2008 gardening season were collected prior to the time when residential users were subject to penalties under the bylaw, and much of the observed change occurred prior to that season. Desirability bias may also lead to over-estimation of changes in actual practices. Although this bias may have occurred with the shift in public perceptions towards use of pesticides on lawns being more socially inappropriate, such a shift would itself be a positive consequence of education and outreach efforts. There was also potential for recall bias, given that during the late fall and winter months respondents were being asked to report on practices that occurred a number of months previously. Being infrequent, pesticide applications may be more salient than other outdoor tasks, so some misclassification may be expected. However, this should be mitigated by the fact that such misclassification would not be differential across gardening seasons, as the same recall challenge would have occurred in 2003 as 2008. Other factors may reduce under-reporting, including the fact that the questions were asked as part of a longer survey with questions about many health-related topics, and that the surveys are anonymous and conducted over the telephone with an independent survey organization. On the other hand, that fact that some households reported pesticide use by companies they hired in later years indicates a risk of "over-reporting" as householders may not have been clear on permitted products or what was used.

Sampling via random digit dialling is increasingly posing challenges to representativeness with the increased use of cell phones. Further, telephone surveys face recruitment challenges as telephone advertising increases. Those achieved by RRFSS (> 50%) are on the high end, partly due to the extensive call-back procedures. Finally, conducting surveys only in English in a multi-cultural city may cause difficulties for less acculturated newcomers, though the costs of including multiple language interviewers in such surveys would be substantial and earlier ISR/RRFSS methodological work had found that the number of potential respondents 'non-functional' in English was much lower that the proportion for whom it was not their first language.

We were able to use a referent or comparison municipality in an interim examination of evaluation results for a report to City Council, showing less change in reported pesticide use observed in the community with no bylaw and smaller investment in public education [60]. However, the Toronto policy development process was extremely high profile, with widespread media attention throughout Ontario, making it highly unlikely that another community, its environmental groups and the public would be without influence from the Toronto experience. Further, the comparison municipality did not continue repeat surveys to provide data across all years as would be needed for a more rigorous comparison.

Despite these potential caveats associated with our findings, the existence of repeated measures data prior to, during and post- bylaw and education implementation is a real strength. Further, corroboration by TPH staff observations during engagements with lawn care companies, store owners and community groups, that the intent and messages of the bylaw and education program were being understood and that stakeholders were actively seeking information on pest and weed control with methods other than pesticides, assists interpretation of the repeat survey data findings. We can understand this as an *adequacy* evaluation, one that primarily seeks to assess coverage [61]. As in many environmental health interventions this one has face validity i.e. that less pesticide application will likely result in less environmental contamination and human pesticide exposure.

Hence the assessment could be focused on program implementation and reductions in reported use, in a way that was highly relevant to the stakeholders involved, particularly the political representatives on the BOH and City Council [62].

Directions

Other Canadian municipalities have followed the lead of Hudson, Halifax and Toronto; by February 2010, an estimated 154 municipalities in seven provinces had pesticide bylaws [63]. Municipal experiences with bylaw implementation were also important drivers for provincial legislation, prompting bans in Quebec, Ontario, Prince Edward Island, Nova Scotia and New Brunswick. The Toronto bylaw paved the way for broad acceptance by the public of stronger pesticide control legislation in Ontario even if it meant that pesticide products were no longer available for their personal use. It served to influence the "next step" in the evolution of public thinking about the use of pesticides. As part of a wider effort to reduce use of hazardous substances in the province, the Ontario-wide cosmetic pesticide ban, enacted on April 22, 2009, was more comprehensive in scope. It banned the sale of many common pesticides, limiting current exemptions to pesticide use, tightly restricting remaining uses and imposing larger fines and penalties, including imprisonment [64]. Building on the long policy development and implementation work of Toronto, Ontario's was an efficient regulatory process, one that other states, provinces or countries could emulate [65]. Many US states, however, have responded to the jurisdictional complexity with "pesticide pre-emption laws" [66] thereby removing the right of municipalities to pass ordinances on pesticide use.

Conclusions

As part of environmental policy implementation, we would encourage parallel efforts to evaluate impacts. In keeping with the growing emphasis on effectiveness [67] and public accountability [68], funding should be included for indicators in different relevant domains. Comparable reporting of both absolute as well as relative changes and controlling for relevant covariates would also be helpful. The same way that toxic substance release inventories in the US [69] and in Canada [70] have paved the way for our understanding of waste releases, we would urge the development of pesticides sales databases, as a key ingredient in tracking intentional chemical inputs to humans and our ecosystems. Coupled with implementation of better exposure incident information systems [46], they should facilitate more explicit evaluations of the impacts of environmental policies and programs.

List of Abbreviations

BOH: (Toronto) Board of Health; LICO: Low Income Cut-Off; MOH: Medical Officer of Health (for Toronto); OR: Odds Ratio; RRFSS: Rapid Risk Factor Surveillance System; TPH: Toronto Public Health.

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Authors' contributions

MC, LV, RW, CM, DC and MB participated in conception, design, data collection, analysis, interpretation, writing, and revisions. JL and SW participated in analysis, interpretation, writing and revisions. All authors have read and critically reviewed the manuscript, accept responsibility for its contents, and agree that the final paper is ready for submission. They have given permission to DC to submit on behalf of the authors.

Competing interests

Several authors are either current (LV, RW, CM, MC) or former (MB) employees of Toronto Public Health. SW, JL and DC each had short term contracts for their work on this project with TPH. We certify that our freedom to design, conduct, interpret and publish the research is not compromised by this relationship or the funding noted below.

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Residential Exposure to Pesticide During Childhood and Childhood Cancers: A Meta-Analysis

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abstract

CONTEXT: There is an increasing concern about chronic low-level pesticide exposure during childhood and its influence on childhood cancers.

OBJECTIVE: In this meta-analysis, we aimed to examine associations between residential childhood pesticide exposures and childhood cancers.

DATA SOURCES: We searched all observational studies published in PubMed before February 2014 and reviewed reference sections of articles derived from searches.

STUDY SELECTION: The literature search yielded 277 studies that met inclusion criteria.

DATA EXTRACTION: Sixteen studies were included in the meta-analysis. We calculated effect sizes and 95% confidence intervals (CIs) by using a random effect model with inverse variance weights.

RESULTS: We found that childhood exposure to indoor but not outdoor residential insecticides was associated with a significant increase in risk of childhood leukemia (odds ratio [OR] = 1.47; 95% CI, 1.26–1.72; I^2 = 30%) and childhood lymphomas (OR = 1.43; 95% CI, 1.15–1.78; I^2 = 0%). A significant increase in risk of leukemia was also associated with herbicide exposure (OR = 1.26; 95% CI, 1.10–1.44; I^2 = 0%). Also observed was a positive but not statistically significant association between childhood home pesticide or herbicide exposure and childhood brain tumors.

LIMITATIONS: The small number of studies included in the analysis represents a major limitation of the current analysis.

CONCLUSIONS: Results from this meta-analysis indicated that children exposed to indoor insecticides would have a higher risk of childhood hematopoietic cancers. Additional research is needed to confirm the association between residential indoor pesticide exposures and childhood cancers. Meanwhile, preventive measures should be considered to reduce children's exposure to pesticides at home.

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Dr Chen participated in the study conception, design, identification of studies, data collection, study selection, data extraction, data analysis and interpretation, and drafting and revision of the article; Ms Chang participated in data collection, study selection, data analysis, and revision of the article; Dr Tao participated in data collection, study selection, and data analysis; Dr Lu participated in the study conception, design, identification of studies, data collection, study selection, data extraction, analysis, and interpretation, and critical revision of the article; and all authors approved the final manuscript as submitted.

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Although pesticides are essential for eradication of pests in agriculture and for public health, they are toxic chemicals and can affect children's health in a variety of settings, such as at home, in parks and gardens, and on school grounds. Children greatly increase their chances of pesticide exposure when they play on pesticide-treated surfaces such as a floor or lawn and then put their hands into their mouths. It is known that households with children commonly use and store pesticide products.[1–3] The use of pesticides at child care facilities,[4] on athletic fields,[5] and on school grounds[6] could all present potential exposures and health hazards to children.

Because children's immune systems are still developing, they may provide less protection than adult immune systems. To be specific, their enzymatic and metabolic systems may be less able to detoxify and excrete pesticides than those of adults. Therefore, they are more vulnerable to pesticides. Epidemiologic studies also support the idea that pesticide exposure can have greater impact on children's health than on adults' health.[7,8] Children exposed to pesticides at home or at school have experienced acute toxic effects on their respiratory, gastrointestinal, nervous, and endocrine systems, as well as other serious medical outcomes.[6,9,10] Concern about the health effects of low-level exposure to pesticides in children has been increasing in recent years, generating a substantial number of epidemiologic studies demonstrating associations between pesticide exposures and childhood cancers.[11–16] However, most of these studies focused on parental occupational exposure or agricultural exposure, not exposure in the home. We found a few systematic reviews examining the association between residential pesticide exposure and childhood cancers. But the association was not elucidated in these reviews, because authors included parental occupational exposure data or studies investigating multiple risk factors that increase chance findings through multiple statistical testing.[12–14]

The aim of our study was to perform a systematic review of the currently available epidemiologic evidence to estimate the relationship between residential (or nonoccupational and nonagricultural) childhood pesticide exposure and childhood cancers. We sought to provide scientific evidence for preventive actions and for making legislative decisions.

METHODS

Data Source and Study Selection

We conducted a literature search in PubMed for articles published before February 2014. We used combinations of the following keywords to identify relevant articles: [residential, urban, indoor, house, home, household, domestic or school] AND [pesticide, insecticide, herbicide, fungicide, organochlorine or organophosphorus] AND [children, childhood, youth, teenager, adolescent, toddler, infant, neonate, prenatal or postnatal] AND [cancer, tumor, malignancy, neoplasm, neuroblastoma, lymphoma, leukemia, sarcoma, astrocytoma, glioma, craniopharyngioma, ependymoma, rhabdomyosarcoma or retinoblastoma]. The search was limited to human studies and written in English. All abstracts were screened to determine their suitability for review.

We included original epidemiologic studies reporting on nonoccupational pesticide exposure and children's health. We used the following criteria to exclude articles from the meta-analysis. We excluded those not reporting original results (eg, review articles, ecologic studies, or case reports); toxicological studies; studies conducted in occupational settings, on hazardous waste sites, on farms, or in proximity to agricultural pesticides; studies involving only adults or children with Down syndrome or without reporting children's health outcomes; studies with only pesticides in general (no specific pesticide groups) or studies with a list of chemicals including pesticides; studies without specific windows of exposure; or duplicate studies that included subjects already included in a more complete or more recent study examining a greater number of subjects.

Two authors of this article (M.C. and C.L.) independently retrieved and screened all the titles and abstracts of studies according to the predetermined selection criteria. We also manually screened references in the selected articles for additional relevant studies. The full texts of the studies with potential eligibility were obtained and assessed independently by the 2 authors (M.C. and C.L.) for final inclusion. Any discrepancies were resolved by consensus.

Data Extraction

From each eligible study, 2 authors (M.C. and C.C.) extracted information about the study design, location, study period, study population and control characteristics, exposure assessment method, outcomes, and key findings. The same 2 authors independently extracted and tabulated the most relevant estimators, namely odds ratios (ORs) and 95% confidence intervals (CIs). ORs and CIs are 2 commonly used estimators in most meta-analyses dealing with health risks associated with environmental chemical exposures.[12,13,15,17–21] The results were compared and consensus was obtained before the meta-analysis.

After classification of the studies, the data were subgrouped and calculated by pesticide categories, exposure locations, and type of cancer in the following stratified meta-analyses:

- Pesticide category and exposure locations:
  - Indoor pesticide exposure
    - Indoor insecticide exposure

- Outdoor pesticide exposure
  - Herbicide exposure
  - Outdoor insecticide exposure
- Cancer types: acute leukemia, leukemia, lymphoma, hematopoietic cancers (leukemia and lymphoma), childhood brain tumor, and all childhood cancers (including neuroblastoma, Wilms tumor, and soft tissue sarcoma)

We analyzed data from professional home treatment (ie, the work done by licensed pest control professionals) by performing a meta-analysis on data with professional home treatment together with parental home treatment or by using data for professional home treatments alone (if number of studies was ≥2). We calculated dose effect by performing a separate meta-analysis on data of the highest frequency of pesticide uses.

Data Analysis

We performed the meta-analysis by using the Comprehensive Meta Analysis version 2 (Biostat, Inc, Englewood, NJ) in accordance with Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines.[22] The random effects model was used in this analysis. The random effects summary of ORs and 95% CIs was estimated to provide an indicator of the overall strength of association between childhood pesticide exposure and childhood cancers. These associations are illustrated in the forest plots. In the plots, the CI for each study is represented by a horizontal line and the estimate of summary OR by a box square. The box area is proportional to the weight, which is the inverse of the variance of the effect estimate from each individual study in the meta-analysis. The diamond and broken vertical line for type of cancer represent the subtotal summary estimate, with CI indicated by its width. The null hypothesis is 1 and is represented by the central vertical dashed line from top to bottom of the plot. All statistical tests were 2 sided, and a P value of $<.05$ was considered statistically significant.

Assessment of Heterogeneity

Because the current review includes a limited number of studies, and the conventional statistical approach to evaluating heterogeneity using a χ^2 test (Cochran's Q) has low power when there are few studies,[23] we used the I^2 statistic to quantify the amount of variation in results across studies that is due to heterogeneity. I^2 can be interpreted as a measure of the percentage of the total variation that cannot be explained by chance.[23] An I^2 value of 25%, 50%, or 75% can be taken to mean low, moderate, or high degrees of heterogeneity.[23] A value of 0% indicates no observed heterogeneity, and estimations from either the fixed effects model or random effects model would be the same. The P values for heterogeneity are based on the Q statistic.

Publication Bias

Publication bias was tested with funnel plots and Egger's test.[24] The funnel plot was made by the natural logarithm of the estimate of ORs versus the SE from all included individual studies in a meta-analysis. We tested funnel plot asymmetry, which can result from unpublished small studies without statistically significant effects, by using the linear regression method.[24]

Sensitivity Analysis

To measure the robustness and determine whether some of the factors (or possible biases) have a major effect on the results of this meta-analysis, we conducted several sensitivity analyses by

- Removing the study with highest weight
- Removing the studies reporting extreme ORs (the highest and the lowest)
- Removing hospital-based studies (or performing a meta-analysis including only population-based studies)
- Removing extended exposure windows or ill-defined pesticide categories

RESULTS

Study Identification and Characteristics

Figure 1 describes this study's identification, screening, and selection process. From the initial 277 articles identified from PubMed search, 239 were excluded based on their titles or abstracts, and 17 were excluded based on the full text. We excluded 3 other studies from the analysis. One had a duplicated population, another had a study population located in a region with high agricultural pesticide use, and a third had insufficient data to permit the calculation.[25–27] No additional articles were identified from the references cited in the included articles. A total of 16 articles met the full inclusion criteria and were eventually included in the meta-analysis.[28–43]

The characteristics of the studies used in the meta-analysis are shown in Table 1. All 16 studies are case-controlled studies published between 1993 and 2012. The participation rates for most studies ranged between 65% and 96% for case groups and between 61% and 99% for control groups. The sample sizes ranged from 45[32] to 1184 cases,[38] and the upper age limits of case groups were between 9 and 19 years. Among these studies, 10 focused on hematopoietic malignancies, 5 on childhood brain tumor (CBT), and 2 on Wilms tumor and neuroblastoma. Four other studies reported data on >1 malignancy.[36–38,41]

The current meta-analysis was run separately for the 2 windows of exposure: before and after birth to diagnosis, and after birth to diagnosis. Because the outcomes from either window of exposure were similar (as shown in Supplemental Table 3), the

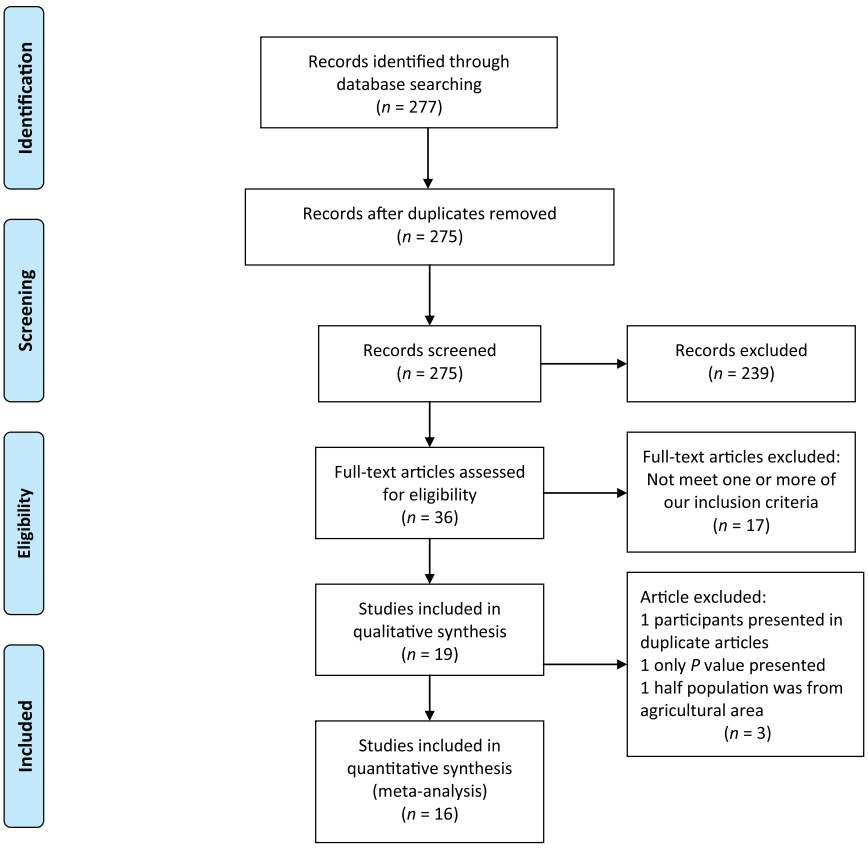

FIGURE 1

PRISMA 2009 Flow Diagram. (Reprinted with permission from Moher D, Liberati A, Tetzlaff J, Altman DG; The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med. 2009;6(6):e1000097)

following results and discussion focus on the window from prenatal and after birth until diagnosis.

Publication Bias

We examined the main findings from all studies and included them in an inverse funnel plot of log-transformed odds ratio versus SE. Although we were limited by the small number of studies included, we saw no clear trend of publication bias (or asymmetry) from visual inspection of the plot, with Egger's test *P* values at .92, .10, and .14 for indoor pesticides, herbicides, and outdoor pesticide exposures, respectively.

Study Synthesis

Table 2 summarizes the results of the subgroup meta-analyses and the assessment of heterogeneity. The results of 13 studies on home pesticide exposure, grouped by types of childhood cancer and listed by years of publication, are shown in Fig 2. Exposure to indoor insecticides during childhood was associated with a significant increase in risk of childhood leukemia (OR = 1.47; 95% CI, 1.26–1.72; I^2 = 30%) and childhood lymphomas (OR = 1.43; 95% CI, 1.15–1.78; I^2 = 0%).

Additional subgroup analysis combining studies on acute leukemia (AL) yielded elevated risks for exposure to both home pesticides (OR = 1.55; 95% CI, 1.38–1.75) and indoor insecticides (OR = 1.59; 95% CI, 1.39–1.81) with significantly lower heterogeneities (I^2 of 0%). When we combined studies on leukemia and lymphoma, we observed a statistically significant association between childhood hematopoietic malignancies and home pesticide exposure during childhood (11 out of 12 data were from indoor insecticides). There was low heterogeneity (OR = 1.46; 95% CI, 1.32–1.60; $I^2 \leq 5\%$). A positive but not statistically significant association between home pesticide exposure during childhood and CBT was observed (OR = 1.22; 95% CI, 0.83–1.81; I^2 = 23%) and this association decreased after data were combined with those for professional home treatment (OR = 1.11; 95% CI, 0.87–1.42; I^2 = 5%).

We conducted sensitivity analysis on the results to test whether these results were influenced by 1 or 2 studies (Supplemental Table 3). Sensitivity analysis conducted by removing highest weights, excluding extreme ORs, or deleting hospital and friends controls did not change the associations between home pesticide (or indoor insecticide) exposure and childhood AL, leukemia, lymphoma, and childhood hematopoietic malignancies (shown in Supplemental Table 3), and statistical significance remained. Heterogeneities were significantly lower (most I^2 were 0%) after extreme ORs were removed in the sensitivity analyses. When we replaced the indoor pesticide data of Ma et al[37] with insecticide data in the rerun meta-analysis, the result was very similar. This finding was consistent with the statement by those authors that "there was a considerable overlap between the definition as well as the results between indoor pesticides and insecticides."

Subgroup analysis on dose and multiple-agent effect yielded a statistically significant higher risk for childhood leukemia (OR = 1.92; 95% CI, 1.27–2.89) and hematopoietic malignancies (OR = 2.04; 95% CI, 1.40–2.97). However, when the studies on professional home treatment were grouped together, the seemingly significant increase in risk for childhood leukemia became not statistically significant.

TABLE 1 Overview of the Case-Controlled Studies Included in the Meta-Analysis

Study	Sample Size (case/control)	Age (y)	Study Population, Location, and Period	Exposure Assessment	Cases	Controls
Davis et al (1993), USA	45/85	≤10	Patients in Missouri, diagnosed 1985–1989	Maternal phone interview	CBT	Noncancer friends or other cancer matched with age and gender
Leiss et al (1995), USA	252/222	<15	Patients in Denver, 1976–1983	Parental interview	CBT, Leu, Lym, STS	Noncancer population matched by gender, age, region
Pogoda et al (1997), USA	224/218	≤19	Patients from West Coast, 1984–1991	Maternal phone interview	CBT	Noncancer population matched by gender, age, region
Infante-Rivard et al (1999), Canada	491/491	≤9	Patients from metropolitan Montreal, diagnosed 1980–1993	Parental phone interview	ALL	Noncancer population matched by age, gender, region
Meinet et al (2000), Germany	1184,234, 940/2588	≤15	Patients from West Germany, diagnosed 1992–1994	Mail and parental phone interview	Leu, NHL	Noncancer population matched by gender, age, region
Buckley et al (2000), USA	268/268	≤20	Patients in US, 1986–1990	Maternal phone interview	NHL	Noncancer population matched by age, gender, and race
Daniel et al (2001), USA	390/296	<19	Hospital patients in US and Canada, 1992–1994	Parental phone interview	Neuroblastoma	Noncancer population matched by age, region
Ma et al (2002), USA	162/162	≤14	Hospital patients in northern California, 1995–1999	Maternal in-home personal interview	ALL, Leu	Noncancer population matched by gender, age, mother's race, region
Menegaux et al (2006), France	280/288	<15	Hospital patients in France, diagnosed 1995–1999	Maternal personal interview	AL	Hospital noncancer children matched by age, gender, hospital, race
Rudant et al (2007), France	1060/1681	<15	Patients in France, diagnosed 2003–2004	Maternal phone interview	AL, HL, NHL	Noncancer population matched by age, gender
Urayama et al (2007), USA	294/369	<15	Patients from northern and central California, diagnosed since 1995	In-home interviews with caretaker	ALL	Noncancer children matched by age, gender, Hispanic status, maternal race, region
Cooney et al (2007), USA	523/517	<16	Patients in US and Canada, 1999–2002	Maternal phone interview	Wilms tumor	Noncancer children matched by age and region
Nielsen et al (2010), USA	201/285	≤10	Patients in US west coast, 1984–1991	Maternal in-person interview	CBT	Noncancer children matched by age and gender
Bailey et al (2011), Australia	388/870	<15	Patients in Australia, 2003–2007	Parental questionnaires and phone interviews	ALL	Noncancer population matched by gender, age, region
Ding et al (2012), China	176/180	≤14	Hospital patients in Shanghais China, 2010–2011	Maternal in-person interview and children's urine collections	ALL	Noncancer hospital children matched by gender and age
Greenop et al (2013), Australia	288/917	≤14	Patients in Australia, 2005–2010	Maternal in-person interview	CBT	Noncancer population matched by gender, age, and region

ALL, acute lymphoblastic leukemia; HL, Hodgkin lymphoma; Leu, leukemia; Lym, lymphoma; NHL, non-Hodgkin lymphoma; STS, soft tissue sarcoma.

TABLE 2 Meta-Analysis Using Random Effects Model for the Relationship Between Childhood Cancer and Exposure to Residential Pesticides During Childhood

Subgroup	Study *N*	Summary		Heterogeneity	
		OR	95% CI	*P*	I^2
Indoor pesticides[a,b]					
(A) AL	6	1.59	1.40–1.80	.839	0
Add professional home treatment	7	1.55	1.38–1.75	.794	0
Indoor insecticides	5	1.59	1.39–1.81	.725	0
(B) Leukemia	8	1.48	1.29–1.70	.267	20
Add professional home treatment	9	1.46	1.29–1.65	.327	13
Dose and multiple agents effects[c]	3	1.92	1.27–2.89	.959	0
Professional treatment only	3	2.04*	1.05–3.95	.061	64
Indoor insecticides	7	1.47	1.26–1.72	.197	30
(C) Lymphoma	4	1.43	1.15–1.78	.578	0
Indoor insecticides	4	1.43	1.15–1.78	.578	0
(D) Hematopoietic cancers	12	1.47	1.33–1.62	.457	0
Add professional home treatment	13	1.46	1.32–1.60	.513	0
Indoor insecticides	11	1.46	1.31–1.63	.388	5
Dose and multiple agents effect[c]	4	2.04	1.40–2.97	.894	0
(E) CBTs[d,e,f]	4	1.22	0.83–1.81	.275	23
Add professional home treatment	5	1.11	0.87–1.42	.380	5
(F) All cancers[f,g]	20	1.40	1.28–1.52	.390	5
Outdoor pesticide[a,b]					
(A) Leukemia	6	1.15	0.95–1.38	.190	33
Herbicide	5	1.26	1.10–1.44	.762	0
Yard insecticides[h]	3	1.11	0.60–2.05	.002	84
(B) Lymphoma	4	0.86	0.62–1.19	.131	47
Herbicide	3	1.52*	1.02–2.27	.090	58
Yard insecticides[i]	2	1.12	0.78–1.59	.314	2
(C) Hematopoietic cancers	10	1.04	0.88–1.23	.086	41
Herbicide	8	1.33	1.16–1.52	.350	10
Yard insecticides	5	1.09	0.75–1.58	.007	71
(D) CBTs	3	0.95	0.47–1.89	.012	77
Herbicide	2	1.98	0.94–4.14	.409	0
Yard insecticides[j]	2	1.29	0.86–1.92	.548	0
(E) All cancers[g]	16	1.10	0.93–1.32	.001	62
Herbicide	12	1.35	1.16–1.55	.221	23
Yard insecticides[k]	8	1.14	0.89–1.45	.028	55

*The summary ORs became not statistically significant in the sensitivity analysis when we removed ill-defined herbicide or highest weight or extreme ORs. Study *N:* number of studies included. Hematopoietic cancers include leukemia and lymphoma. All cancers include neuroblastoma and Wilms tumor and soft tissue sarcomas in outdoor pesticides. Study results with case numbers <3 are not included in the summary.

[a] In the study[35] where insecticides against different types of nuisance were reported, data with the highest OR were used.

[b] In the studies where results of different exposure windows in the same study were reported, the windows away from birth were used.

[c] The data of >10 per year were used in the study,[38] and the data of >5 per year were used in the study.[37]

[d] When both cancer-free controls and cancer controls were reported, cancer-free controls were used.

[e] The crude OR and 95% CI were calculated based on the data in the article.[42]

[f] Where >1 home pesticide usage was reported, home pesticides for nuisance pests were used.

[g] In the study[30] where the results were essentially the same during pregnancy and during childhood, the data reported from pregnancy through childhood were treated as during childhood.

[h] Includes studies[35,39,41] and ORs associated with yard pesticides were replaced by yard insecticides in studies.[35,39]

[i] Includes 2 data from the study.[41]

[j] Includes 2 studies.[32,40]

[k] In addition to all yard insecticides in each subgroup, an additional study[30] was included and ORs associated with yard pesticides were replaced by yard insecticides.

Part of the reason could be the small number of studies included.

Combining all studies reporting childhood cancers (including neuroblastoma[31] and Wilms tumor[30]) with childhood home pesticide exposure yielded a meta-rate summary OR of 1.40 (95% CI, 1.28–1.52) with a low degree of heterogeneity (I^2 of 5%). Therefore, the results show that there is a statistically significant risk of childhood cancers associated with exposures to home pesticides, especially indoor insecticides, during childhood.

Outdoor pesticides include outdoor insecticides, herbicides, and fungicides. Table 2 and Fig 3 show the cancer risks from exposure to residential herbicides during childhood. A statistically significant association between childhood leukemia and exposure to herbicides (OR = 1.26; 95% CI, 1.10–1.44, I^2 = 0%) was observed, and the sensitivity analysis confirmed the robustness of this association. The greatest risk estimates were observed in the association between childhood exposure to herbicides and the risk of leukemia. The observed association with increase in risk of childhood lymphoma became not statistically significant during the sensitivity analyses. No association appeared between herbicide exposure and CBT. When studies on all types of childhood cancers were combined, including neuroblastoma[31] and Wilms tumor,[30] a statistically significant association with residential herbicide exposure was observed (OR = 1.35; 95% CI, 1.16–1.55; I^2 = 23%). We did not find any statistically significant association between exposure to outdoor pesticides or outdoor insecticides and any types of childhood cancers (Fig 4). Because only a few studies were available on exposure to residential fungicides and childhood cancers, we did not include exposure to fungicides in the current analysis.

DISCUSSION

In this meta-analysis, we examined 16 epidemiologic studies on the possible association between residential pesticide exposure during childhood and childhood cancers. Overall, the results suggest that cancer risks are related to the type of pesticide and where it was used. Exposure to residential indoor insecticides but not outdoor insecticides during childhood was significantly associated with an

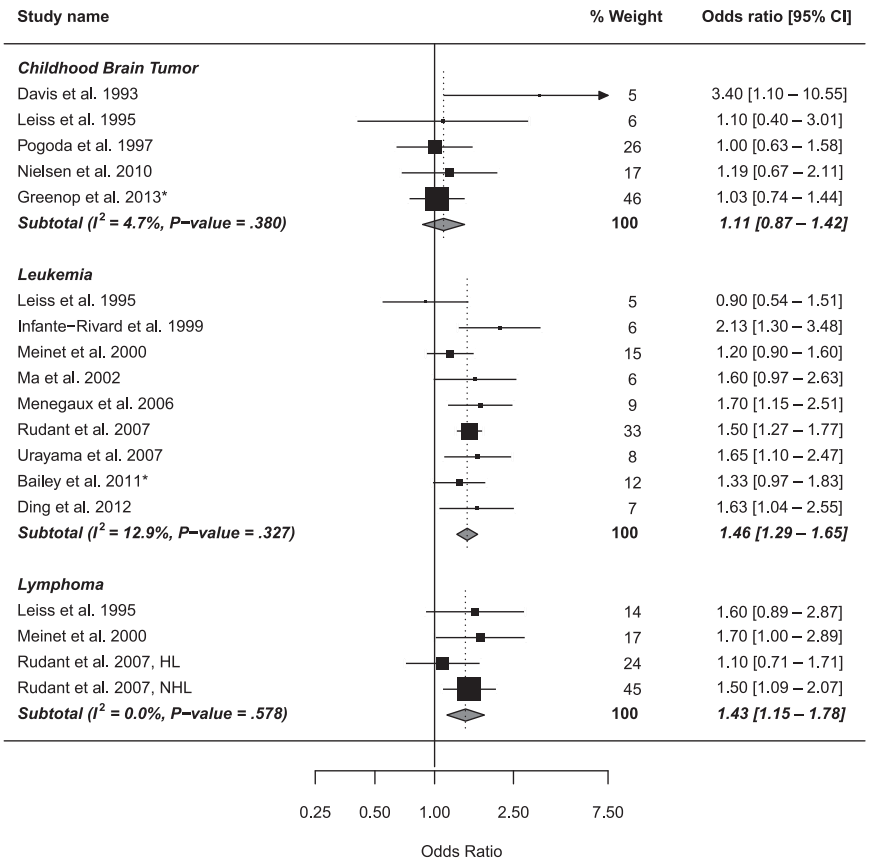

FIGURE 2
Meta-analysis of the association between childhood cancers and exposure to home pesticides during childhood. *Professional home treatments.

increasing risk of childhood cancers including leukemia, AL, and lymphoma but not CBT. Among the 5 studies reporting CBT outcomes in the analyses, 4 studies did not provide specific exposure locations, although the applications were probably indoors. This ambiguity about where pesticides were used could dilute the true effects of residential pesticides and therefore result in the association toward the null. Similarly, the fact that adding professional home treatment in hematopoietic cancers and CBT lowers the summary ORs could also result from the ambiguity of exposure location. The greatest risk estimates were observed in the association between childhood exposure to indoor insecticides and the risk of AL. The risk of childhood hematopoietic malignancies increased with the frequency of use. These observations provide additional support to the positive exposure–response relationship between indoor insecticide use and the increased risk of childhood hematopoietic malignancies.

We did not observe any significant childhood cancer risk associated with exposure to outdoor pesticides. However, when we looked into the different categories of outdoor pesticides, we found that exposure to herbicides was associated with a slightly higher risk of childhood cancers in general, which include leukemia, lymphoma, and CBT, although statistical significance appeared only in association with leukemia. No significant association between outdoor insecticides and childhood cancers was observed. This result emphasizes how important it is to specify the type and location of the pesticide when analyzing pesticide exposure and childhood cancer. Because of the small number of studies included in the current meta-analysis, more studies are needed to confirm these associations.

Results from the current analysis are in agreement with the main findings of 2 previously published studies on residential pesticide exposure and childhood leukemia.[13,14] Both observed significant associations between insecticide exposure and childhood leukemia. Although these results were based on a small number of studies, the consistency of the main findings suggests that there probably is a higher risk of childhood leukemia with indoor insecticide exposure during childhood. We have observed a slightly elevated risk of childhood leukemia associated with exposure to herbicides, with no evidence of heterogeneity. This finding is also consistent with that reported by Van Maele-Fabry et al[14] but not by Turner et al,[13] and both reported a high degree of heterogeneity (I^2 of 61% and 72%, respectively). Neither our study nor the study of Turner et al[13] observed any association between childhood leukemia and exposure to outdoor insecticides during childhood. Like Van Maele-Fabry et al,[14] we also did not observe any association between childhood leukemia and outdoor pesticide exposure.

We also found a positive association between childhood lymphoma and indoor insecticide exposure. Furthermore, the overall childhood cancer risk is elevated with childhood home pesticide exposure. There was a third study reporting that pesticide use at home or in the garden was statistically associated with the elevated risk of lymphoma, leukemia, and CBT.[20] However, Vinson et al[20] did not provide information on specific categories of pesticides or locations of use in their analysis; most of their study results were related to occupational exposure. Therefore, we

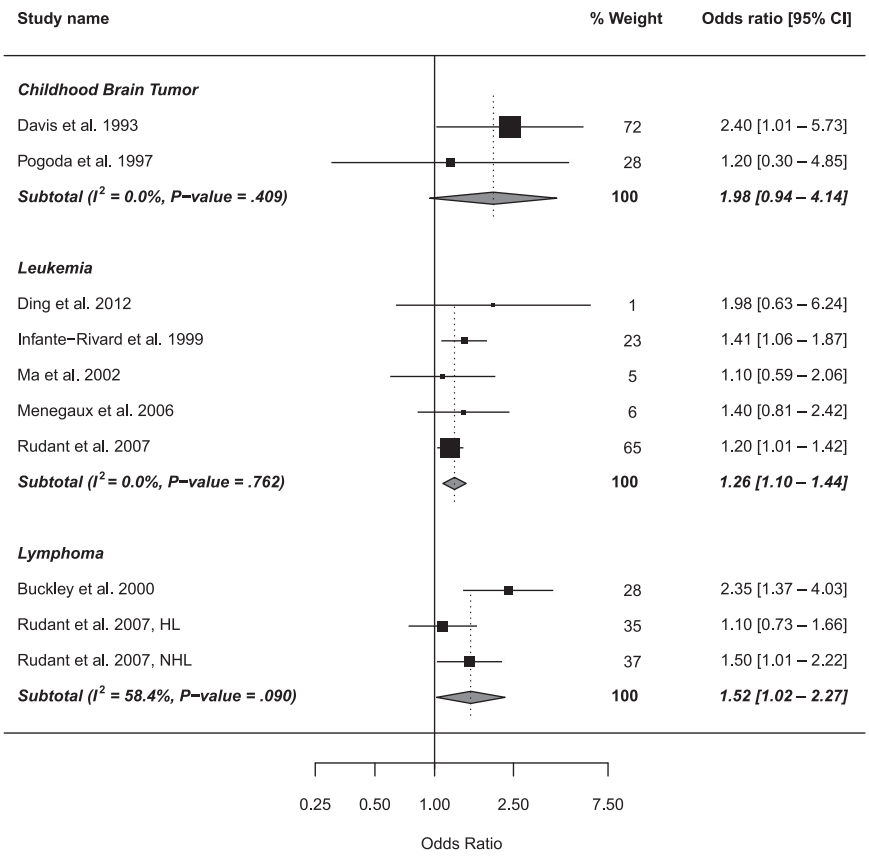

FIGURE 3
Meta-analysis of the association between childhood cancers and exposure to residential herbicides during childhood.

could not directly compare our results with those reported by Vinson et al.[20]

Although most of our findings are consistent with those of the earlier meta-analyses, there are some differences. One main difference is that several studies included in the previous 2 meta-analyses were excluded from the current analysis. These were studies that either were conducted in occupational settings, involved only adults, reported only pesticides in general (not specifying pesticide groups), or included other chemicals with pesticides. Therefore, we eliminate the effects from these studies in the summary ORs.

Although previous meta-analyses took into account exposure locations and pesticide categories when performing stratification analysis, Van Maele-Fabry et al[14] reported indoor and outdoor exposures but gave no information about pesticide category. Stratification analyses based on categories of pesticide exposure were run in the study by Van Maele-Fabry et al,[14] but no analysis was done on the exposure location for each category of pesticide; therefore, the true risk factors could be diluted. There were also no results from sensitivity analyses provided by Van Maele-Fabry et al.[14]

Unlike Van Maele-Fabry et al's[14] report and our observation, Turner et al[13] reported a statistically significant positive association between childhood leukemia and exposure to residential outdoor pesticides but not outdoor insecticides nor herbicides. However, these results were inconsistent with each other because outdoor pesticides were most likely to be outdoor insecticides or herbicides.

In the current meta-analysis, we divided studies into 3 subgroups based on the pesticide use pattern, such as indoor pesticides and insecticides, outdoor pesticides and herbicides, and outdoor pesticides and insecticides. We used a random effects model to estimate the summary ORs for each subgroup. In the home pesticide (mostly indoor insecticides) category, although some subgroup analyses were conducted on only a limited number of studies (<5), the observed heterogeneity was low ($I^2 \leq 13\%$) in these analyses. We also pooled studies to increase the accuracy of estimated summary ORs for hematopoietic malignancy and all cancers, and we observed zero or low levels of heterogeneity. Similarly, there was no observed heterogeneity in the herbicide category, including estimated summary ORs for hematopoietic malignancy and all cancers. These results of zero or low heterogeneity for indoor pesticides and herbicide exposure indicated the consistency of studies included and suggest that combining data is appropriate. However, the heterogeneity for outdoor pesticide or outdoor insecticide exposure was high. Because these studies included in the current meta-analysis differed in study design, study population, and the exposure and timing of exposure, the heterogeneity of the associations should be interpreted with caution.

Overall, our study has shown that childhood cancer risks are related to the type of pesticide use and its application locations during childhood. Childhood exposure to residential indoor insecticides was associated with an increasing risk of childhood cancers but not outdoor insecticides.

Although meta-analysis is a useful tool to assess causal relationships by combining results from different studies, outcomes can be constrained

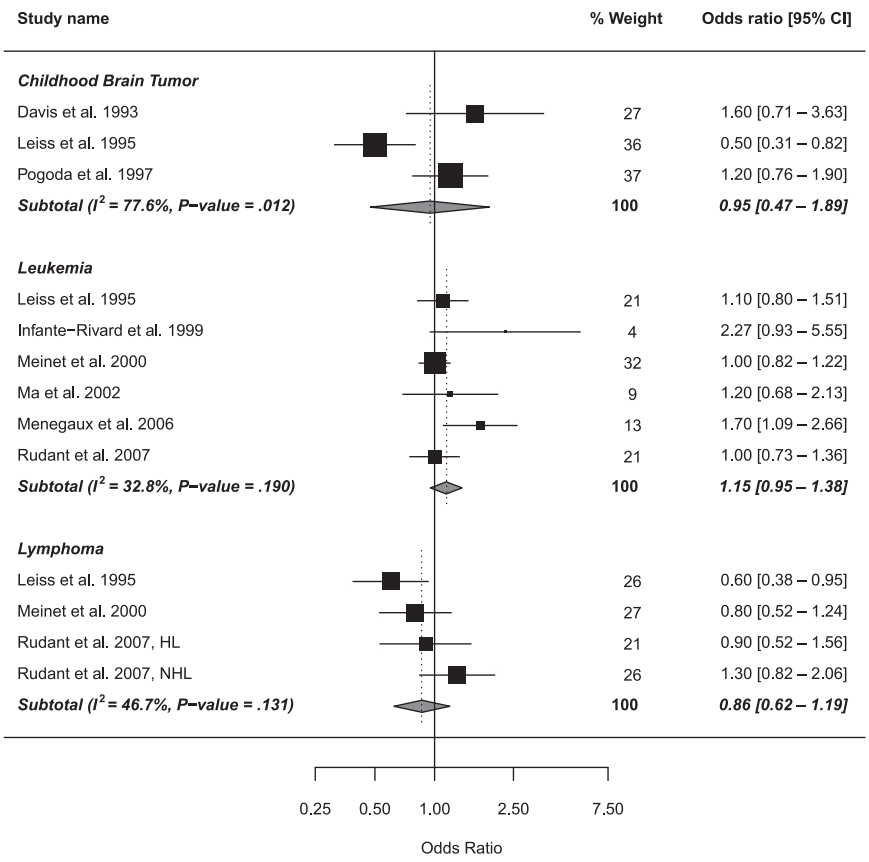

FIGURE 4
Meta-analysis of the association between childhood cancers and exposure to residential outdoor pesticides during childhood.

by the limitations of the original studies. In the current analysis, the small number of studies is a major limitation. Very few studies have assessed pesticide exposures and childhood cancers. In addition, other limitations such as selection bias, recall bias, misclassification, and publication bias might limit the applicability of the findings to the general population. To deal with the potential selection bias associated with hospital or friend controls, we performed a sensitivity analysis by excluding Davis et al[32] and Menegaux et al[39] from each pesticide category to reinforce the associations.

To reduce recall bias and misclassification, the studies we included used several strategies to reduce confounding factors and biases, such as restriction of entry to study of subjects with confounding factors, matching controls to have equal distribution of confounders, using standardized questionnaires, identical interviewing procedures for both cases and controls, and adjustment of the results. Publication bias refers to the fact that studies with less significant findings may be less publishable than those with positive outcomes; therefore, they would be unavailable for meta-analyses. For example, one of the studies from the current analysis stated that "neither residential use of insecticides nor use of pesticides in the garden was found to be significantly more frequent in any group of cases with solid tumors compared with controls, therefore no quantitative data were provided."[38] Although the results from the current meta-analysis do not seem to be significantly influenced by publication bias, this bias cannot be completely excluded. Note that when Van Maele-Fabry et al[14] assessed the impact of exclusion of nonpublished data and studies in languages other than English, they found that rerunning the meta-analysis and including nonpublished and non–English-language studies did not substantially modify the results.

A positive exposure–response relationship between residential indoor insecticide use and occurrence of childhood cancers was observed in the current study. Some studies have also shown that maternal pesticide exposure during pregnancy was associated with childhood cancers.[35,37,39] Although current data do not establish the most critical exposure period for the occurrence of childhood cancers, their development is probably multifactorial and probably includes gene–environment interactions.[11,44–46] Some studies assert a possible association between pesticide exposure with genetic predisposition and defined subtypes of childhood cancers.[26,42,43] Additional studies are needed to examine the potential mechanisms by which childhood exposure to pesticides could lead to the development of childhood cancers.

CONCLUSIONS

The current meta-analysis has revealed positive associations between exposure to home pesticides and childhood cancers, with the strongest association observed between indoor insecticide exposure and acute childhood leukemia. Although epidemiologic research is limited in identifying the association between the adverse health outcomes in young children and pesticide uses in residential areas, the findings from the present meta-analysis and those previously published have consistently demonstrated

associations between pesticide exposure and childhood cancers. While the research community is working toward a better understanding of the causality of pesticides in various childhood diseases, more and more pesticides are being used in farming, in landscape maintenance, and in the home. Therefore, public health policies should be developed to minimize childhood exposure to pesticides in the home. States and local authorities can establish programs, such as integrated pest management, to minimize residential pesticide uses, especially indoor uses.[47,48] In the meantime, parents, school and daycare teachers, and health care providers can learn about common pesticide types and labeling information and can stay aware of the short- and long-term effects of these chemicals.[49,50] Every effort should be made to limit children's exposure to pesticides.

ABBREVIATIONS

AL: acute leukemia
CBT: childhood brain tumor
CI: confidence interval
OR: odds ratio

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Residential Exposure to Pesticide During Childhood and Childhood Cancers: A Meta-Analysis

Mei Chen, Chi-Hsuan Chang, Lin Tao and Chensheng Lu

Pediatrics; originally published online September 14, 2015;
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Study confirms bats' value in combating crop pests

15 September 2015, by Tim Crosby

Josiah Maine, a former graduate student at Southern Illinois University Carbondale, examines a young corn plant in preparation for an experiment. Maine set out to further research the findings of his master's thesis adviser, SIU Assistant Professor of zoology Justin Boyles, who in 2011 published work suggesting bats might prevent significant crop losses each year. Their research, published in the *Proceedings of the National Academy of Sciences*, found that bats play a significant role in combating crop pests, saving up to $1 billion a year in crop damages. Credit: Russell Bailey

It's dusk in the countryside, and in the fading golden light a small, winged shape takes to the air over a local cornfield. Darting this way and that, the creature executes maneuvers far beyond what any modern fighter jet could manage.

The bat is on the hunt for insects, and according to new research, farmers have a billion reasons to be grateful for it.

Research recently written by former graduate student Josiah Maine and his adviser at Southern Illinois University Carbondale shows that bats play a significant role in combating crop pests, saving up to $1 billion a year in crop damages. The research article, titled "Bats initiate vital interactions in corn," is published in *Proceedings of the National Academy of Sciences*.

Maine set out to further research the findings of his master's thesis adviser, SIU Assistant Professor of zoology Justin Boyles, who in 2011 published work suggesting bats might save famers billions of dollars in crop losses each year

"Bats are voracious predators of insects, including many crop pest species," said Maine, who recently graduated from SIU with a Master of Science degree in zoology. "My hope was that it would give us a much better idea of the ecological and economic impact of bats in agriculture."

Using funding from the ecology group Bat Conservation International, Maine designed an experiment in corn fields near Horseshoe Lake in Southern Illinois over the 2013 and 2014 growing seasons that would test how corn fared with and without bats picking off various pests.

To test the theories Maine built "exclosures" – netted structures aimed at keeping bats outside of them and away from the corn. The exclosures were large, each enclosing more than 4,200 square feet and measuring 23 feet high. Maine constructed the exclosures using steel poles, cables and netting.

Keeping the bats out meant pests, such as the corn earworm, were largely free to wreak havoc on the corn crops. But simply keeping bats out would not tell the whole story, as other creatures, such as birds, also were kept out by the structures and away from the pests.

"This meant that the exclosures had to be removed daily so birds could forage normally," Maine said. "I arranged the netting so it could be slid on the cables to one end like a shower curtain. This way I could open the exclosures each day and close them each night," before the bats took to the air.

In all, Maine built six exclosures, each of which was

paired with a control plot where bats could forage as they normally would. This arrangement allowed him to directly compare pest abundance and crop damage between the exclosure and control plots.

"The main pest in my system was the corn earworm , a moth whose larvae cause billions of dollars' worth of damage to corn, cotton, tomatoes, and many other crops," Maine said. "The larvae feed on corn ears, causing direct damage to yield, but they also can introduce an avenue for infection of the corn ear by fungi, which produce compounds that are toxic to humans and livestock.

"Bats are known to feed on this moth, but it has been unclear whether they consume enough of the moths to suppress larval populations and damage to crops," Maine said.

After analyzing the results, Maine said he found nearly 60 percent more earworm larvae inside the exclosures – protected from the hungry bats – than in the unprotected control areas. He also found more than 50 percent more corn kernel damage per ear in the corn inside the exclosures. Further, he found the damaging fungal growth associated with pests was significantly higher on the corn inside the exclosures, and that the toxins produced by the fungus were much more concentrated in the corn inside the exclosures.

Bats also provide a natural solution to pests, which in turn provide additional value to agriculture by suppressing toxic fungi and reducing necessity for costly insecticides, Maine said.

Working under Boyles' guidance, Maine said the results of his experiments supported his adviser's earlier research and further quantified it.

"My study provides strong support for the idea that bats provide valuable services to society," he said. "By consuming crop pests, bats have tremendous ecological impacts in crop fields. Based on the difference in crop damage I observed, I estimated that bats provide a service to corn farmers worth about $1 billion globally. Bats likely provide additional value to agriculture by suppressing toxic fungi and reducing necessity for costly insecticides."

Boyles, who is extensively involved in bat research all over the country, also hailed the study and its implications.

"It highlights the importance of maintaining a healthy and high-functioning ecosystem," Boyles said. "Bats are much maligned, but deserve protection if for no other reason than the ecosystem services they provide to humans."

Boyles credited Maine with working hard to find answers.

"He is an outstanding student and would have been a superstar at any university," Boyles said. "Josiah's productivity far and away exceeds his peers."

More information: "Bats initiate vital agroecological interactions in corn." *PNAS* 2015 ; published ahead of print September 14, 2015, DOI: 10.1073/pnas.1505413112

Provided by Southern Illinois University

John Schieszer, MA

September 18, 2015

Pesticide Exposure Tied to Diabetes Risk

Exposure to pesticides is associated with a 61% increased risk for developing diabetes, results of a new meta-analysis suggest.

Pesticide exposure may be associated with a higher risk for diabetes.

The data, which were presented at EASD 2015, the annual meeting of the European Association for the Study of Diabetes, also indicate that different types of pesticides may pose a greater risk than others.

How diabetes develops is considered to be an interplay between genetic and environmental factors. Emerging evidence suggests that environmental contaminants, including pesticides, may play an important role in the pathogenesis of diabetes.

In this study, the researchers performed a systematic review and meta-analysis of observational studies that assessed the association between exposure to pesticides and diabetes. The association between exposure to any pesticide and all types of diabetes was examined. In addition, the researchers conducted a separate analysis for studies that looked only at type 2 diabetes.

"We were not surprised by our findings but, in most of the cases, similar findings are inflated, biased or simply not true. However, we were surprised by the consistency of the results in different subgroup and sensitivity analyses where the relative risk remains high," said study co-author Evangelos Evangelou, MSc, PhD, from the University of Ioannina School of Medicine in Greece and the Imperial College London in the United Kingdom.

The researchers identified 21 studies assessing the association between pesticides and diabetes, covering 66,714 individuals. There were a total of 5,066 cases and 61,648 controls.

Most of the studies did not report the specific diabetes type examined, and in almost all of the studies analyzed, pesticide exposure was determined by blood or urine biomarker analysis.

Results revealed that exposure to any type of pesticide was associated with a 61% increased risk for any type of diabetes. In the 12 studies analyzing only type 2 diabetes, the risk was increased by 64% for those exposed to pesticides.

For individual pesticides, increased risk was identified in association with exposure to chlordane, oxychlordane, trans-nonachlor, DDT, DDE, dieldrin, heptachlor and HCB.

"Organic contaminants may play a role in the pathogenesis of diabetes. Of course, diabetes has a clear genetic background and also age and obesity are strong risk factors. Exposure to pesticides or possible interactions with other known genetic or environmental risk factors may play a role in the risk of type 2 diabetes," Evangelou told *Endocrinology Advisor*.

"It is of interest how they (pesticides) are continuously released from fat stores to the blood and reach critical organs."

Subgroup analyses did not reveal any differences in the risk estimates based on the type of studies or the measurement of the exposure. However, the researchers found that by analyzing each pesticide separately, some pesticides appeared to be more likely to contribute to the development of diabetes compared with others.

The results need to be interpreted with caution due to the observational nature of the data, the researchers noted. Currently, they are performing additional analyses of the data and doing a further meta-analysis of pesticide exposure in relation to other outcomes, including neurological outcomes and several cancers.

"We should always keep in mind that the current evidence derives from observational studies where recall and other biases may inflate the observed effect sizes," said Evangelou.

"Our findings are supported by mechanistic studies and are scientifically interesting regarding the mechanisms underlying the linkage of pesticides and their mode of action with type 2 diabetes."

Evangelou said most of the examined pesticides in the current analyses were banned a few decades ago in Western countries, and their levels are decreasing compared with earlier years. However, Evangelou and colleagues believe this an important area of research due to the worldwide epidemic of diabetes.

Further studies are warranted to better elucidate the association between pesticide exposure and the development of diabetes, Evangelou.

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BANGOR DAILY NEWS (http://bangordailynews.com/)

Pesticide disposal program aims to help farmers, homeowners (http://bangordailynews.com/2015/09/20/living/pesticide-disposal-program-aims-to-help-farmers-homeowners/)

By Abigail Curtis (http://bangordailynews.com/author/abigail-curtis/), BDN Staff
Posted Sept. 20, 2015, at 7:16 a.m.

Got pesticides and are unsure of what to do with them? A state program aims to help, by allowing Mainers to dispose of banned or unusable pesticides next month at sites in Presque Isle, Bangor, Augusta and Portland.

According to the Maine Department of Agriculture, Conservation and Forestry's Board of Pesticides Control, it's not unusual for homeowners and farmers to sometimes discover old, unusable or obsolete pesticides in the shed, garage or cellar.

Products lauded as marvels in their day, like DDT and compounds of arsenic, mercury or lead, are now banned because of the risks they pose to human health, wildlife or the environment. In other cases, still-legal pesticides can freeze or get damp and solidify, which renders them unusable. But disposing of these substances can be hard. It's not allowed to just take them to the transfer station and people who want to do the right thing by disposing of them in an environmentally sound manner can often be discouraged to learn that this type of disposal can be very expensive, according to the website for the Maine Board of Pesticides Control (https://www.google.com/url?q=http://www.maine.gov/dacf/php/pesticides/public/obsolete_pesticide_collection.shtml&sa=D&usg=AFQjCNEvuJckhj85No3rUD0YBNG4edofYQ).

That's why state officials make it possible each October to do the free pesticide drop-off at locations around the state.

"It's important for the protection of public, wildlife and environmental health that these products are dealt with properly and not thrown in the trash or down the drain, where they can contaminate land and water resources, including drinking water," Commissioner Walt Whitcomb of the Maine Department of Agriculture, Conservation and Forestry said recently in a media release.

Through the program, the collected chemicals will be taken to out-of-state disposal facilities that are licensed by the Environmental Protection Agency. There, they will be incinerated or reprocessed, according to the Maine Board of Pesticides Control.

The collection program is jointly sponsored by the Board of Pesticides Control and the Maine Department of Environmental Protection, and is funded by pesticide product registration fees. According to the pesticide control board, the program has kept more than 90 tons of pesticides out of the waste stream since it began in 1982.

Participants must register by Saturday, Sept. 26, as drop-ins are not allowed. To register or to find more information about the program, please call 287-2731 or visit the website www.maine.gov/dacf/php/pesticides (https://www.google.com/url?q=http://www.maine.gov/dacf/php/pesticides&sa=D&usg=AFQjCNFLAkCePOmqi2C3I7cvXmsv2fcnng) for details.

National Business SEPTEMBER 19, 2015

Maine blueberry harvest slightly below average in 2015

1 of 3

FILE - In this Friday, July 27, 2012 file photo, wild blueberries are ready for harvesting at the Dolham Farm in Warren, Maine. Maine's blueberry season was mediocre in 2015. University of Maine scientists said the cold spring and dry summer conspired to knock the harvest down from 104 million pounds last year to about 85 pounds this year. **Robert F. Bukaty, File** - AP Photo

BY PATRICK WHITTLE
Associated Press

PORTLAND, MAINE — The volume of Maine's

wild blueberry crop has increased dramatically in the last several decades, but this season's harvest looks to have been slightly behind recent trends.

The 2015 harvest is likely less than 90 million pounds, and possibly as low as 85 million, University of Maine horticulture professor David Yarborough said. Recent years have yielded about 90 million pounds per year, and the 2014 total was 104 million, which was the second highest total on record.

Several factors contributed to this summer's middling year, including cold spring weather that wasn't optimal for bees to pollinate the blueberries, Yarborough said. The berries also suffered from die-off during the winter months and a dry summer, he said.

"No disaster, no big crop, but the consensus I can say here is average to below average," Yarborough said. "Sometimes too many great years in a row aren't too great, either — it pushes price down and you have oversupply."

The blueberry crop remains much larger than it was in decades past. The crop was about 20 million pounds per year in the 1970s and 50 or 60 million pounds per year 10 years ago, Yarborough said.

The summer blueberry crop is a vital, $250 million piece of Maine's economy, agriculture officials say. Nearly all of the blueberries are frozen and most are used as a food ingredient, according to a University of Maine report.

The wild "low bush" blueberries are only harvested commercially in Maine and eastern Canada. The 2015 wild blueberry harvest is finished for the year in Maine and is wrapping up in Atlantic Canada.

Ed Flanagan, chief executive officer of blueberry producer Jasper Wyman and Son, said he wasn't alarmed by the slight drop in harvest this year.

"There's going to be enough blueberries for the market," Flanagan said. "It's not normal to get record crops in a row."

MORE NATIONAL BUSINESS

Comments

Portland citizens’ group proposes broad pesticide ban

www.pressherald.com/2015/10/07/portland-citizens-group-proposes-strong-pesticide-ban/

By Kelley Bouchard Staff Writer | @KelleyBouchard | 207-791-6328

A group of Portland residents has proposed what would be the strongest municipal restriction on the sale and use of controversial outdoor bug and weed killers in Maine, and city councilors will begin discussing the plan Wednesday.

A residents’ group called Portland Protectors submitted the three-page ordinance proposal in August, hoping to prod city officials to address what they see as a mounting threat to humans, animals, beneficial insects and the environment as a whole, including Casco Bay.

Today's Poll

Banning pesticides

- Would you support a ban prohibiting the use of pesticides on private lawns in your town?
    - Yes
    - No

The proposal goes beyond weed and bug killers to include synthetic lawn fertilizers, which some believe contribute to the acidification of Maine's coastal waters and harm plant and animal species. Shutterstock photo
Search photos available for purchase: Photo Store →

Meanwhile, as Portland officials review the citizen proposal, city officials across the Fore River in South Portland are developing a proposal to restrict pesticides that they hope to present publicly in November.

A major concern among pesticide opponents is glyphosate, the active ingredient in Monsanto's broad-spectrum herbicide, Roundup. While the U.S. Environmental Protection Agency says it's "safe" when used correctly, the World Health Organization's International Agency for Research on Cancer in March classified it as "probably carcinogenic."

The Portland pesticide proposal goes beyond weed and bug killers to include synthetic lawn fertilizers, which some believe run into nearby sewers and streams, contribute to the acidification of Maine's coastal waters and harm the plant and animal species that live there.

"This proposal pushes the council into action," said Avery Yale Kamila, a founder of Portland Protectors.

The City Council's sustainability committee is set to take up the proposal at 5:30 p.m. Wednesday in Room 209 of City Hall.

The proposal would prohibit the sale and use of synthetic fertilizers and pesticides, other than those allowed in organic farming or classified by the EPA as exempted materials, such as cedar oil and sodium lauryl sulfate.

The proposal also would require Portland to establish an ongoing community education campaign and would force commercial pesticide applicators to be certified in organic land care management, in addition

to state-required licensing and training.

It contains no blanket exemptions, but would allow the council to grant seven-day emergency waivers to address public health or environmental threats, such as mosquitoes and other infestations, with the least-toxic material available.

“The presence of weeds, vegetative overgrowth and common fungal diseases encountered in the usual course of landscape management shall not constitute an emergency,” according to the proposal.

Twenty-five Maine communities, including Ogunquit, Brunswick, Rockland, Wells, Lebanon and Waterboro, have pesticide-control ordinances that ban or regulate the type or method of pesticides used in municipal, agricultural and forestry applications, and near drinking-water supplies.

Ogunquit is the only town to extend its ordinance broadly to include all private property owners, but it’s not an outright ban. It allows restricted pesticides to be used to kill noxious or invasive plants, such as poison ivy, and to address health and safety threats, such as disease-carrying insects.

Takoma Park, Maryland, was the first U.S. city to ban the use of “cosmetic lawn pesticides” in 2013. Some provinces and hundreds of municipalities across Canada have taken similar steps, along with anti-pesticide measures in France, Germany and the Netherlands.

In Maine, the use of various synthetic, natural and organic pesticides is overseen by the Board of Pesticides Control.

Deven Morrill, a supervisor at Lucas Tree Experts, is chairman of the board. Lucas provides a variety of commercial and residential landscaping services, including tree trimming, lawn care and mosquito control.

Morrill said many Maine companies already practice integrated pest management, which aims to use the least amount of the least toxic or environmentally hazardous materials to achieve the best results in landscaping.

“We offer both organic and conventional programs and we’re happy to do either,” Morrill said. “We applaud citizens groups for taking on this subject. It can be an emotional topic for some people, but it can be an educational opportunity as well. It allows us to explain what we do and why we do it.”

Morrill said any pesticide regulation must weigh the benefits and the costs of restricting use. Hindering the ability to combat the emerald ash borer or the Asian longhorned beetle, for instance, could devastate Maine’s forest industries, he said.

Portland city staffers are reviewing the citizen-proposed ban and are expected to issue recommendations in the coming weeks, said Councilor David Marshall, chairman of the sustainability committee.

However, it probably will be several months before some form of the proposal goes before the full council, especially with council elections coming in November.

“A lot of these things can be tough to implement,” Marshall said. “At this point it’s very preliminary. There will be plenty of opportunity for the public to comment.”

South Portland city staffers have been working on a pesticide ordinance for a few months and expect to present a draft to the City Council in November. They’re talking with proponents of a ban and various people who would be affected by it, including business owners and applicators, said Julie Rosenbach, South Portland’s sustainability coordinator.

“It’s extremely complicated, but it makes it very interesting,” Rosenbach said.

“I want a very strong ordinance, and part of that is going to be making it a very feasible ordinance.”

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SCIENTIFIC REPORTS

OPEN

Neonicotinoid pesticides severely affect honey bee queens

Geoffrey R. Williams[1,2], Aline Troxler[1,2], Gina Retschnig[1,2], Kaspar Roth[1,2], Orlando Yañez[1,2], Dave Shutler[3], Peter Neumann[1,2,4] & Laurent Gauthier[2]

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Queen health is crucial to colony survival of social bees. Recently, queen failure has been proposed to be a major driver of managed honey bee colony losses, yet few data exist concerning effects of environmental stressors on queens. Here we demonstrate for the first time that exposure to field-realistic concentrations of neonicotinoid pesticides during development can severely affect queens of western honey bees (*Apis mellifera*). In pesticide-exposed queens, reproductive anatomy (ovaries) and physiology (spermathecal-stored sperm quality and quantity), rather than flight behaviour, were compromised and likely corresponded to reduced queen success (alive and producing worker offspring). This study highlights the detriments of neonicotinoids to queens of environmentally and economically important social bees, and further strengthens the need for stringent risk assessments to safeguard biodiversity and ecosystem services that are vulnerable to these substances.

Bees are vital to global biodiversity and food security through their pollination of plants, including several key crops[1,2]. Overwhelming evidence now suggests that numerous wild and managed bee populations are in decline, likely because of multiple simultaneous pressures including invasive parasites, changes to climate, and changing land use[3,4]. This has led to concerns over human food security and maintenance of biodiversity. The neonicotinoid class of chemical pesticides has recently received considerable attention because of potential risks it poses to ecosystem functioning and services[5]. Ubiquitously used for management of harmful insects in the last decade, these systemic chemicals persist in the environment, thereby promoting their contact with non-target organisms such as pollinating bees[6].

Alarmingly, exposure to field-realistic concentrations of neonicotinoids impairs productivity of important social bee pollinators[7–9] that have, among females, reproductive division of labour between workers and queens. A plethora of literature has demonstrated lethal and sub-lethal effects of neonicotinoid pesticides on social bees in the field and laboratory. These examinations have focused largely on workers (females chiefly responsible for essential colony housekeeping and foraging duties rather than reproduction; their production of haploid offspring is primarily regulated by queen pheromones and other colony conditions[10,11]), and to a lesser extent overall colony function[7,12,13]. The role of queens (primary reproductive females that can produce diploid offspring) in social bee colony survival is indispensable, and relies heavily on *a priori* successful development and successful mating flights that trigger profound molecular, physiological, and behavioural changes[10,14]. Previous investigations have observed that bumble bee colonies exposed to neonicotinoids produced fewer gynes (future queens)[9,15] and that honey bee colonies replaced queens more frequently[8]; however, mechanisms responsible for these observations have not been identified. This is remarkable considering anecdotal reports of 'poor quality queens' (*i.e.* queen failure) of an important pollinating species, the western honey bee (*Apis mellifera*; hereafter honey bee), throughout the northern hemisphere[16].

In this study, we hypothesised that exposure to field-realistic concentrations of neonicotinoid pesticides would significantly reduce honey bee queen performance due to possible changes in behaviour, and reproductive anatomy and physiology. To test this, we exposed developing honey bee queens to

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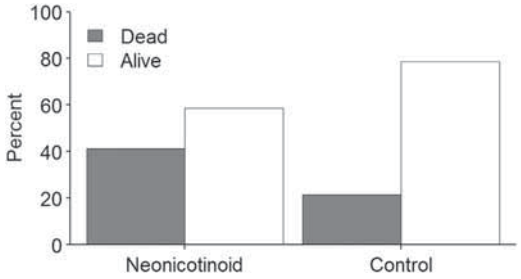

Figure 1. Queen survival after 4 weeks. Percent honey bee queens that were alive after 4 weeks. No significant difference was observed between treatments. $^{*}P \leq 0.1$, $^{**}P \leq 0.05$, $^{***}P \leq 0.01$ (comparison with Controls).

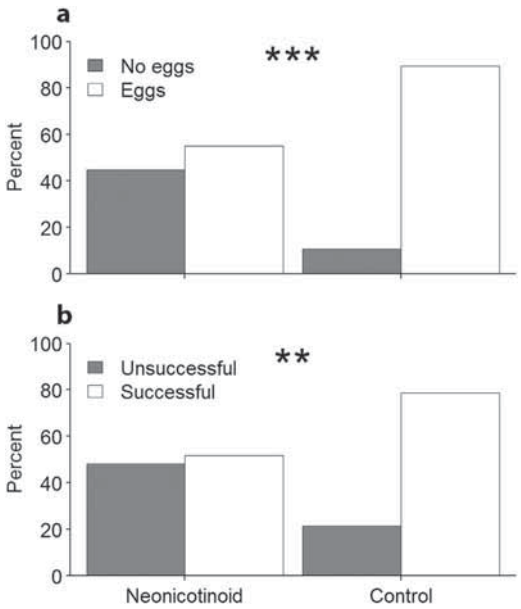

Figure 2. Queen oviposition and survival after 4 weeks. (**a**) Percent of honey bee queens that oviposited (*i.e.* laid worker eggs). (**b**) Percent of honey bee queens that were alive and had produced diploid offspring by the end of the experiment (= Successful). Significant differences between treatments denoted by $^{*}P \leq 0.1$, $^{**}P \leq 0.05$, $^{***}P \leq 0.01$.

environmentally-relevant concentrations of the common neonicotinoid pesticides thiamethoxam and clothianidin. Both pesticides are widely applied in global agro-ecosystems[17] and are accessible to pollinators such as social bees[18], but are currently subjected to two years of restricted use in the European Union because of concerns over their safety[19]. Upon eclosion, queens were allowed to sexually mature. Flight behaviour was observed daily for 14 days, whereas production of worker offspring was observed weekly for 4 weeks. Surviving queens were sacrificed to examine their reproductive systems.

Results

Queen rearing success. No significant difference between treatments was observed for queen rearing success (*i.e.* grafting to emergence) (contingency table $\chi_1^2 = 0.3$, $P = 0.61$). Success was $38.1 \pm 9.5\%$ and $44.0 \pm 14.6\%$ in the controls and neonicotinoids, respectively (mean ± standard error).

Mating nucleus colony observations. After four weeks post queen emergence, 25% fewer neonicotinoid queens were alive compared to controls (contingency table $\chi_1^2 = 2.6$, $P = 0.11$; Fig. 1). Regardless of whether they survived to four weeks, 38% fewer neonicotinoid queens produced workers compared to controls (contingency table $\chi_1^2 = 8.2$, $P = 0.004$; Fig. 2a). Even within our abbreviated observation interval, a significant 34% reduction in success (*i.e.* alive and producing worker offspring) was observed among neonicotinoid-exposed queens compared to controls (contingency table $\chi_1^2 = 4.5$, $P = 0.03$; Fig. 2b).

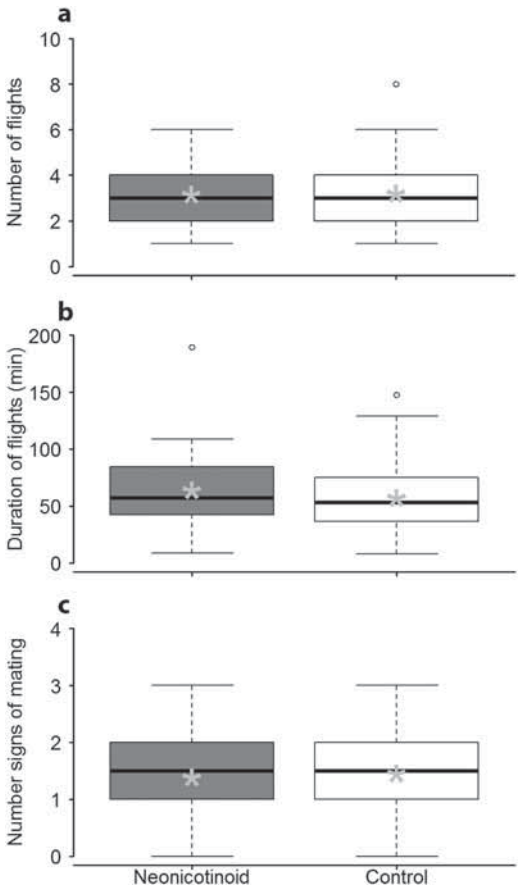

Figure 3. Queen flight over a 4-week interval. (**a**) Number of flights by honey bee queens. **(b)** Total duration of flights by honey bee queens (**c**) Number of signs of mating. Boxplots show inter- quartile range (box), median (black line within interquartile range), means (grey asterisk), data range (dashed vertical lines), and outliers (open dots). No significant difference was observed between treatments for any measure. $^{*}P \leq 0.1$, $^{**}P \leq 0.05$, $^{***}P \leq 0.01$ (comparison with Controls).

Figure 4. A marked queen returning to the entrance of a baby mating nucleus hive during experimental observations; arrow denotes mating sign (remnants of a male's everted endophallus protruding from the queen's vagina[20]).

No difference between treatments was observed for any measured queen flight parameter; both sets of queens undertook similar numbers (Kruskal Wallis $\chi_1^2 = 0.1$, $P = 0.99$; Fig. 3a) and durations (mixed model with queen as a random factor, $F_{1,174} = 0.2$, $P = 0.67$; Fig. 3b) of flights, and had comparable signs of mating (*i.e.* remnants of a male's everted endophallus inserted into the opening of the returning queen's reproductive tract[20]) (contingency table $\chi_1^2 = 1.9$, $P = 0.17$; Fig. 3c, Fig. 4).

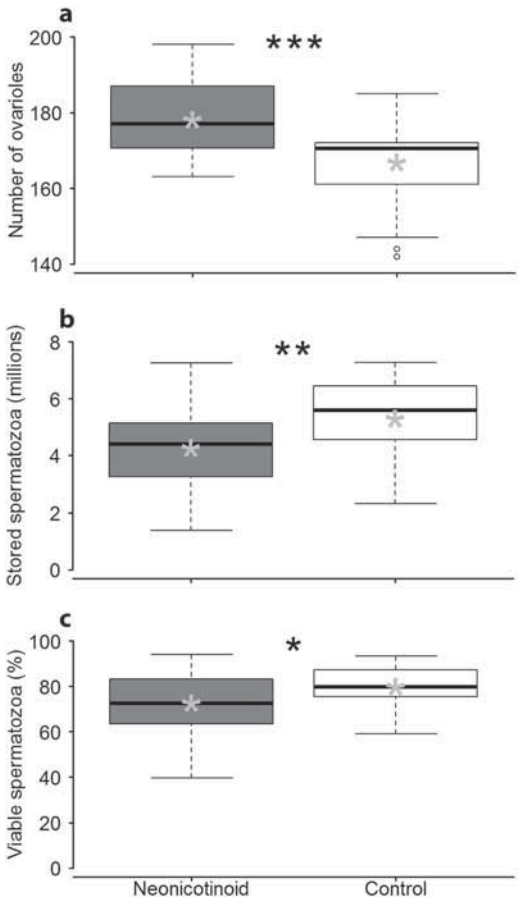

Figure 5. Queen anatomy and physiology after 4 weeks. (**a**) Ovary size, represented by number of ovarioles, of honey bee queens. (**b**) Number of spermatozoa stored in spermathecae of honey bee queens. (**c**) Percent viable spermatozoa stored in spermathecae of honey bee queens. Boxplots show inter- quartile range (box), median (black line within interquartile range), means (grey asterisk), data range (dashed vertical lines), and outliers (open dots). $^{*}P \leq 0.1$, $^{**}P \leq 0.05$, $^{***}P \leq 0.01$ (comparison with Controls).

Queen dissections and laboratory measurements. For queens surviving the four-week observation period, ovary sizes of those exposed to neonicotinoids were 6.8% larger compared to controls (ANOVA $F_{1,35} = 9.0$, $P = 0.005$; Fig. 5a). Neonicotinoid queens had 20% fewer stored spermatozoa ($F_{1,35} = 4.8$, $P = 0.03$; Fig. 5b) and a 9% lower proportion of living versus dead sperm ($F_{1,35} = 3.3$, $P = 0.08$; Fig. 5c). For the queens that survived and produced worker offspring ($N = 37$), there were no significant correlations among emergence mass, ovariole number, sperm number, or sperm vitality (all $|r| < 0.26$, all $P > 0.13$). Similarly, no significant differences were observed when queens were separated by treatment (controls: max $|r| = 0.24$, minimum $P = 0.29$ ($N = 22$), neonicotinoids: max $|r| = 0.21$, minimum $P = 0.44$ ($N = 15$)).

Discussion

The results demonstrate for the first time possible mechanisms by which exposure to field-realistic concentrations of neonicotinoid pesticides during development can significantly affect queens of a social bee. Increased rates of honey bee queen failure have been reported in recent years[21]. Even within our abbreviated observation interval, we observed significant effects of neonicotinoids on honey bee queen anatomy and physiology, but not behaviour that resulted in reduced success (*i.e.* dead queens or living ones not producing worker offspring). Additionally, we found no significant effect on queen rearing success (proportion of emerged queens) between the treatments, suggesting that there were no lethal effects of pesticide during this stage of queen development. Because honey bees are haplodiploid, wherein males typically result from unfertilised eggs and females (*i.e.* workers or queens) develop from fertilised ones, production of workers confirms successful queen mating[10]. Honey bee queens seldom start to oviposit beyond 3 weeks of emerging[20], so absence of developing workers in a colony during our 4-week observation period most likely suggests that a queen did not mate or was for some other reason unable to lay fertilised eggs[10].

Honey bee queens are highly polyandrous, and normally embark on a series of mating flights within 14 days of emerging from their cells during which they should be fertilised with a sufficient number of spermatozoa to last their lifetime; they rarely leave the colony once they start ovipositing[10]. Our study suggests that queen flights were not influenced by neonicotinoid exposure because similar frequencies and lengths were observed compared to controls. This was unexpected because neonicotinoids can negatively affect worker bee flight behaviour[7,12]. It is possible that our study investigating queen flights cannot be directly compared to these studies due to differences among investigations regarding female caste (queen versus worker), model species (honey bee versus bumble bee), experimental treatment (neonicotinoids thiamethoxam and clothianidin vs. the neonicotinoid imidacloprid and the pyrethroid λ-cyhalothrin), experimental method (visual observations vs. radio-frequency identification tagging), treatment exposure (colony versus individual), or task measured (mating versus foraging).

Longevity of honey bee queens depends largely on proper development to sexual maturity and appropriate behavioural, anatomical, and physiological changes that occur following successful mating[10,14]. Therefore, negative effects on delicate queen reproductive systems that result in abnormal physiology or anatomy, or that impair storage of spermatozoa or oviposition, could result in costly queen replacement by the colony[10]. Surprisingly, we observed ovariole hyperplasia in neonicotinoid-exposed queens compared to controls. Increased ovary size suggests that neonicotinoids can affect development of queen reproductive system; it is unclear how hyperplasia observed here may influence egg production and fertilisation, or may correspond to other anatomical or physiological changes. Furthermore, we observed a significant reduction in the number and quality of stored spermatozoa within queen spermathecae. It is possible that neonicotinoids, due to neuronal hyper-excitation[22], cause dysfunction of queen physiology and anatomy responsible for transporting and storing newly-received drone spermatozoa during mating. Proper storage of adequate quantities of spermatozoa is crucial to queen survival because a queen is quickly replaced by a colony after depletion of healthy spermatozoa[10].

Poor queen health is considered an important cause of honey bee colony mortality in North America and Europe[16,23], yet few data can explain these observations over such broad regions[24,25]. Considering the widespread use of neonicotinoids in developed countries, our study suggests that these substances are, at least partially, responsible for harming queens and causing population declines of social bee species. Failure of queens exposed to neonicotinoids during development to successfully lay fertilised eggs that subsequently develop into workers or queens is worrisome; both castes are vital to colony survival, particularly when emergency queen replacement is needed. This is especially important for wild social bees that cannot rely on human intervention to mitigate effects of queen failure or colony mortality.

Current regulatory requirements for evaluating safety of pesticides to bees fail to directly address effects on reproduction[26]. This is troubling given the key importance of queens to colony survival and their frailty in adjusting to environmental conditions. Our findings highlight the apparent vulnerability of queen anatomy and physiology to common neonicotinoid pesticides, and demonstrate the need for future studies to identify appropriate measures of queen stress response, including vitellogenin expression[27]. They additionally highlight the general lack of knowledge concerning both lethal and sub-lethal effects of these substances on queen bees, and the importance of proper evaluation of pesticide safety to insect reproduction, particularly for environmentally and economically important social bee species.

Methods

Apiary setup. The study was performed in Bern, Switzerland, during May-September 2013 using *A. mellifera carnica* honey bees. Six sister queen experimental colonies were established in early May; each contained typical quantities of adults, immatures, and food (honey and beebread) for the season. Colonies were randomly assigned to either neonicotinoid or control treatments, with each group represented equally.

Pesticide treatment. Treatments were administered via pollen supplements that were prepared from bee-collected pollen and honey (3:1 by mass, respectively) obtained from non-intensive agricultural areas of Switzerland. Supplements for the neonicotinoid treatment were additionally spiked with 4 ppb thiamethoxam and 1 ppb clothiandin (both Sigma-Aldrich) to represent environmentally relevant concentrations observed in pollen of treated crops[28,29]. These amounts were confirmed (4.16 and 0.96 ppb for thiamethoxam and clothianidin, respectively) by the French National Centre for Scientific Research using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Colonies were each outfitted with a pollen trap prior to administering treatments. This promotes pollen supplement consumption by removing bee-collected pollen from returning foragers. Each colony received 100 g pollen supplement every day for 36 days to ensure that colonies contained young bees exposed to the neonicotinoids during queen rearing; supplements were well-received, but never completely consumed during each feeding period.

Queen rearing. Queens were produced in experimental colonies using standard honey bee queen-rearing techniques[30]. Briefly, original sister queens were removed from colonies 27 days post initial exposure to create queenless cell-builder nuclei, each composed of 2 food frames and 1 kg brood nest workers. One-day old larvae from each colony were grafted into artificial queen cells and subsequently placed in respective cell-building nuclei overnight. Contents of each cell-building nucleus, including

Figure 6. Experimental baby mating nuclei; each nucleus was equipped with a modified entrance consisting of a flat plastic flask and apiculture queen-excluding screen to observe exiting and returning queens without disrupting workers.

artificial queen cells, were returned to their original experimental mother colony the following day to ensure proper queen development; colonies continued to receive pollen supplements until after queen cell-capping. Prior to emergence, queens were transferred to cages supplied with a food paste (1 part honey: 3 parts powdered sugar by mass) that were maintained in the laboratory in complete darkness at 34.5 °C and 60% humidity[31]. Queen cells were observed every 6 hours starting 11 days post-grafting. Emerged queens were visually inspected, numbered on the dorsal thoracic plate using queen marking numbers, and re-caged with five attendant workers from her mother colony during the expected period of queen emergence (~1 day). Subsequently, each queen was placed in a mating nucleus hive (APIDEA Vertriebs) with 300 g apiculture candy (Südzucker) and 100 g brood nest workers from her original mother colony, and confined for 3 days in darkness at 12 °C to promote colony formation prior to placement outdoors. In total, 29 neonicotinoid and 28 control queens were employed for the ensuing performance measures.

Mating nucleus colony observations. Entrances of mating nucleus colonies were observed daily from 11.00–17.00 for 14 days, the typical period of queen flight[10]. Each colony was equipped with an observation landing board constructed using a flat plastic flask (ThermoFisher Scientific) and apiculture queen-excluding screen to document exiting and returning queens without disrupting workers (Fig. 6). Flights by queens were defined as periods away from colonies, including observations on landing boards. After the initial 14-day entrance observation period, presence of queens and developing workers was assessed weekly for an additional 14 days by visually inspecting all frames of each mating nucleus.

Queen dissections and laboratory measurements. Queens surviving the 4-week mating nucleus observation and assessment period (16 and 22 neonicotinoid and control queens, respectively) were removed from their colonies and anaesthetised using carbon dioxide to allow for inspection of their reproductive anatomy. Spermathecae and ovaries were removed and placed in Kiev buffer[32] or PBS buffer supplemented with 2% paraformaldehyde, respectively. Numbers of spermatozoa stored in each spermatheca were calculated using a Thoma haemocytometer (ThermoFisher Scientific) using compound microscopy (Model BX41, Olympus)[33]. Viability of spermatozoa in spermathecae was determined using a laboratory kit (Live/Dead® Viability Kit, Life Technologies), wherein a 50-μl aliquot suspension of the spermathecal content was dyed using SYBR-14 and propidium iodide to view 10 fields of view of living and dead spermatozoa, respectively, using fluorescent compound microscopy (Model BX41, Olympus). Number of ovarioles per ovary was determined by real-time counting under stereo microscopy (Model SZX10, Olympus) using a fine needle[34].

Statistics. Statistical software was used to perform analyses (SAS 9.3; SAS Institute) and to create figures (R 2.15.3; The R Foundation for Statistical Computing). Comparison of numbers of queens from each treatment that were both alive and producing workers (successful) versus either dead or not producing workers (unsuccessful) was done with contingency table analyses, as were comparisons of numbers of queens alive versus dead, and numbers of queens producing workers versus not. For some flight comparisons, most queens appeared multiple times in the data; to account for pseudoreplication, queen was a random factor in mixed models. For reproductive parameters, non-parametric Kruskal-Wallis tests were used when data were not normally distributed, whereas ANOVAs were used when data were normally distributed[35,36]. Correlation analyses (Pearson and Spearman gave qualitatively similar results) were used to evaluate associations among emergence mass, ovariole number, number of spermatozoa, and sperm vitality.

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Author Contributions

G.R.W., P.N. and L.G. conceived the study and designed the experiment; G.R.W., A.T., G.R., K.R., O.Y. and L.G. carried out the experiment; D.S. analysed the data; G.R.W. prepared the manuscript; G.R., D.S., P.N. and L.G. edited the manuscript.

Additional Information

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