Maine Board of Pesticides Control

Miscellaneous Pesticides Articles
July 2014

(identified by Google alerts or submitted by individuals)

Several Hundred Honey Bees And Bumblebees Died **4:09 PM** THU JUNE 19, 2014

Another Large Bee Die-off Attributed to Pesticides

By RACHAEL MCDONALD

Originally published on Thu June 19, 2014 1:26 pm

Several hundred honey bees and bumblebees died at a Eugene apartment complex Tuesday after trees on the property were sprayed with pesticides. The state is investigating.

Listen 0:58

The State Department of Agriculture (http://www.oregon.gov/ODA/PEST/Pages/index.aspx) found out about the bee deaths from a TV report and sent an investigator out Wednesday. Bruce Pokarney is with ODA:

One of hundreds of bees that died at an apartment complex in Eugene Tuesday. The ODA found out about the die-off from a report on KVAL
Credit KVAL

Pokarney: "What we've discovered is that a commercial pesticide operator had applied a pesticide, active ingredient Imidacloprid on 17 trees at the complex early Tuesday morning. Most of those trees if not all of them were Linden trees. Those are the same trees that were involved in bee death incidents last year in Oregon."

Last summer 50 thousand bumblebees were found dead in a parking lot in Wilsonville after pesticides were applied to Linden Trees. After that incident, state regulators required label statements on products containing chemicals that harm bees. The labels advise against spraying trees in full bloom and attracting pollinators, as in this case.

Pokarney says ODA will potentially pursue enforcement action against the company, Glass Tree Care. The company says it's cooperating with the investigation.

Portland Press Herald, June 24, 2014

Buzz about bees: New study claims widely used class of pesticides is killing them

Neonicotinoids work by affecting the central nervous system of insects, causing paralysis and death, according to the EPA.

BY **NORTH CAIRN** STAFF WRITER
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Many home-garden plants, promoted as "bee-friendly," are pretreated with a class of pesticides shown to harm and kill bees and other pollinating insects, according to several consumer groups that will release pesticide test results Wednesday at a press conference in Portland.

The Organic Consumers Association, Friends of the Earth, in conjunction with more than 20 beekeeping and organic gardening associations, including Maine Organic Farmers and Gardeners Association, will present the results of the study – said to be based on the largest data samples to date – at The Honey Exchange on Stevens Avenue. In addition, the organization will present homeowners and backyard gardeners with tips on how to reduce exposure to the pesticides – known as neonicotinoids – and to protect bees.

In this file photo, the queen bee is surrounded by the worker bees in one of The Honey Exchange's Portland hives. *Carl D. Walsh/Staff Photographer*

Neonicotinoids work by affecting the central nervous system of insects, causing paralysis and death, according to the U.S. Environmental Protection Agency.

They are systemic pesticides, meaning that they affect not just the surface of leaves of treated plants but also are absorbed through the entire system, penetrating even into the soil, said Erin Forbes, a master beekeeper from Portland. They are also persistent, lasting up to 15 years in soil, she said.

These pesticides have come under scrutiny in recent years due to the decline of honeybees, particularly from colony collapse disorder. Entomologists and biologists have been working to unravel the multiple factors involved in the disorder, which have decimated bee populations worldwide.

"Neonicotinoids "are absolutely a contributing factor to the increased decline of honeybee colonies in recent years," said Forbes. Most plants that are started in soil and transported from one state to another have neonicotinoids in the soil, she said.

Dozens of environmental and agricultural organization nationally and internationally are calling for action to address problems of neonicotinoids.

Last Friday, the White House issued a statement calling the decline of honeybees, native bees and other pollinators – including birds, bats and butterflies – a serious problem that "poses a significant challenge that needs to be addressed to ensure the sustainability of our food production systems, avoid additional economic impacts on the agricultural sector and protect the health of the environment."

President Obama called for several measures, including the establishment of a task force charged with developing a national strategy to improve pollinator health.

The White House stopped short of singling out neonicotinoid pesticides but included "pesticide exposure" as one of several factors in bee decline.

Pollinators contribute more than $24 billion to the U.S. economy annually – with honeybees accounting for more than $15 billion, according to the White House statement.

"Honeybees enable the production of at least 90 percent of commercially grown crops in North America," the statement said. Globally, animal pollinators enable the production of 87 of the leading 115 food crops, the White House estimated.

Portland Press Herald, June 24, 2014
http://www.pressherald.com/2014/06/24/buzz-about-bees-new-study-claims-widely-used-class-of-pesticides-is-killing-them/

Aliens in the Maine woods

Wednesday, June 18, 2014 at 10:52AM
Joe Rankin

Terrestrial invasive plants can wreak havoc with forests

By Joe Rankin

Forests for Maine's Future writer

Licensed forester Jeff Williams does the usual things foresters do: writes management plans, runs boundary lines, oversees harvests, lays out logging roads, marks trees. But more and more these days he's having to deal with invasive forest plants.


Pulling garlic mustard on Cutts Island (Photo: Maine Natural Areas Program)Williams, who owns Maine Forest Management in Hollis, said 30 to 40 percent of his time is spent helping his clients cope with the likes of glossy buckthorn and Japanese barberry, and that percentage goes up every year.

In fact, it's very seldom that he gets a job these days that doesn't involve invasive plant issues. Sometimes he even does herbicide applications (he has a master applicator's license) if a client can't find a contractor to do the work at an affordable price.

"As bad as it is now it's inevitable that it'll get worse," Williams said. "As it is now there are pockets where it's a real problem. In 50 years it'll be a huge problem for southern Maine and forests in Maine period."

Woodland invasive plants are also known as terrestrial or upland invasives. They haven't gotten the publicity that exotic insect pests or aquatic invasive plants have. Non-woodsy types who have heard of the emerald ash borer or recognize Eurasian milfoil on sight might give you a blank look at a mention of glossy buckthorn, black swallowwort or Asiatic bittersweet.

That's not unexpected, said Tom Doak, the executive director of the **Small Woodland Owners Association of Maine.**


Asiatic bittersweet (Photo: Tom Rawinski, USFS)Exotic insect pests prompt more attention because they kill trees outright and the damage is highly visible; the uncontrolled spread of invasive aquatic plant species threatens to imperil the state's thousands of lakes and ponds, said Doak.

In contrast, woodland invasives are more insidious: they're green in a landscape of green and growing things. "They don't generally kill the trees, but they occupy the land and prevent forest trees from growing," said Doak.

An invasive plant is generally defined as one that spreads quickly and crowds out other plants and trees. Most are exotics, immigrants from Asia, Africa or Europe. Many are sunlight lovers. They invade marshes (common reed), wetlands (purple loosestrife), grassy areas and roadsides (Japanese knotweed), and field-woodland edges (honeysuckle and autumn olive.)

Generally speaking, it's harder for an invasive plant to make it in a healthy forest with a full canopy. But some have no problem. There are vines, trees and shrubs already invading forests in Maine and others that will likely be here soon.


Common buckthorn (Photo: Maine Natural Areas Program)So, what species would a list of Maine's "most unwanted" woodland invasives include? The experts we talked to reeled these off:

Glossy buckthorn (*Frangula alnus*) and **common buckthorn** (*Rhamnus cathartica*) are fast-growing shrubs that form dense thickets in wetlands and woodlands. Their habit of leafing out before other plants and retaining their leaves late into the fall gives them an advantage and helps them shade out native species.

Japanese barberry (*Berberis japonicus*) is, as its name suggests, a spiny shrub originally from Asia. It grows three to six feet high. It forms dense thickets that can be impossible to bull your way through. The red berries are highly attractive to birds, which help spread it.

Asiatic bittersweet (C*elastrus orbiculata*) is the Boston strangler of invasives. This relative of American bittersweet grows fast and twines around trees as it reaches for sunlight, eventually smothering the host plant. It not only reproduces using attractive red berries, but also root suckers.

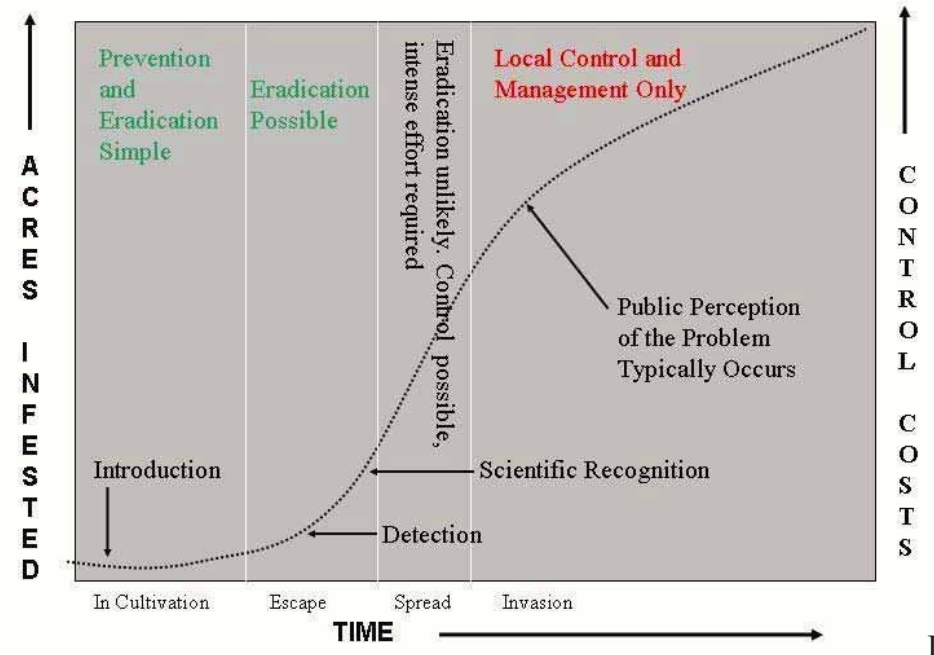

A forest of barberry at Laudholm Reserve in Wells (Photo: Maine Natural Areas Program)**Norway maple** (*Acer platanoides*) is an escapee from the nursery trade. This sugar maple look-alike, still planted as a shade and street tree, grows fast, even in shade, and forms dense colonies that can elbow aside native trees and shrubs.

Garlic mustard (*Alliaria petiiolata*) is a biennial herb that tolerates a wide spectrum of soils and growing conditions, and is especially aggressive in rich, moist upland forest soils, where it forms dense colonies of three-foot tall plants, shouldering aside all manner of native wildflowers and herbs.

Black swallowwort (*Cynanchum louiseae*) is a viney native of southwestern Europe that likes moist soils. It engulfs native plants and creates thickets.

Morrow honeysuckle (*Lonicera morrowii*) is a native of Asia that grows as high as 16 feet, forming dense thickets and shading out native plants. While it likes sunlit forest edges it will also invade mature forests.

You'll note some common themes in the descriptions. These invasive plants tend to be fast growing; they form dense thickets, a sort of non-compete strategy; they adapt to a wide range of habitats and they have shiny,



How the

invasive game usually plays out (Graphic: Tom Rawinski, USFS)brightly-colored fruit, a sure-fire reproductive strategy since birds ingest the fruit then excrete the seeds far and wide along with a little fertilizer.

Some of these plants were introduced accidentally, some on purpose, either as ornamentals or for erosion control. It wasn't until later, sometimes decades later, that the threat they posed was realized. However, even today, some, such as Norway maple and Japanese barberry, are staples of the nursery trade.

These forest invasives currently pose the greatest threat in southern Maine, say, south and west of Augusta. Not coincidentally that's where the most people live. In fact, these weedy plants tend to be closely associated with another weedy species: humans.

"The behavior of invasive plants follows human activity very closely. Humans are an edge species. Our yards are open. When we look to the woods we see the edge. Invasive plants thrive on the disturbance we humans create. We are bombarded by invasive plants that are basically just trying to heal the wounds that we inflict on the land," said Tom Rawinski, a botanist with the **U.S. Forest Service's Northeastern Area State and Private Forestry** program. He works on invasive plant issues throughout New England and New York.

Many invasive plants get their start at the edge where grasslands (read, lawns and fields) meet the

woods and Glossy buckthorn berries (Photo: Maine Natural Areas Program)sunlight is ample. For some it's just a beachhead to invade the forest. Sometimes we help them do it, by creating disturbance. A timber harvest is just such a disturbance.

Harvesting opens the canopy, flooding the forest floor with sunlight. If invasives like barberry or buckthorn are already present it's like throwing gasoline on a fire: they enjoy an explosion of growth.

"Many landowners come to us and ask for harvest oversight and don't realize they have invasives," said Williams. "We'll recommend that they treat the invasives first. And in some cases we won't administer the harvest if they're unwilling to so. In other situations, if they're young plants we'll do mechanical treatment in the spring when the soil is soft and you can pull them. In the worst situations we do recommend herbicide control."

Sometimes, humans create disturbances in other, more subtle, ways, by encouraging or favoring one species over another.

In southern New England burgeoning white-tailed deer populations have in some places wiped out native wildflowers and understory plants, essentially clearing the field for invaders like garlic mustard or Japanese stiltgrass, said Rawinski.

 On its way to Maine? Shade tolerant Japanese stilt grass. (Photo: Chuck Bargeron, University of Georgia, Bugwood.org) "The millions of acres of stiltgrass in the eastern U.S. is not the problem," he said. "The deer are the problem, because they've eaten all the natural competitors."

At **Laudholm Reserve in Wells** deer pressure resulted in an understory "where there is nothing but Japanese barberry. It's actually impenetrable. It's quite spectacular," said Ann Gibbs, the state horticulturist with the Maine Department of Agriculture, Conservation and Forestry and an expert on invasive plants.

One of the things that makes invasive plants so successful is that they're unpalatable to wildlife. Deer won't touch barberry. Ditto for black swallowwort.

While invasive plants can crowd out native species and alter an ecosystem beyond recognition in a few years, some pose an even more pernicious threat, to the very genome of related plants.

Asiatic bittersweet, for instance, hybridizes readily with American bittersweet. In parts of Massachusetts, said Rawinski, it's hard to find a pure American bittersweet anymore. It's a victim of "genetic swamping" by its Asian relative and saving it could require eliminating Asiatic bittersweet "for perhaps a mile, which is almost impossible," Rawinski said.

Invasive plants can be controlled, if detected early enough. If you suddenly wake up to discover you've got  Leaves of the shade tolerant, fast-spreading Norway maple. (Photo: Paul Wray, Iowa State University, Bugwood.org)acres and acres of glossy buckthorn in your forest, it's almost impossible to deal with, even with herbicides.

"Early detection and rapid response are the answers," said Gibbs. "Once you get something established in an area it's a major undertaking to control and very expensive. The best thing is to keep things out."

Think of your garden: if you wait until the weeds get established, reclaiming your cabbages and cucumbers may be just too much trouble, then you swap the tiller for the lawnmower.

 In the field: The Nature Conservancy's Nancy Sferra leads an invasive plants workshop. (Photo: Tom Rawinski, USFS)Rawinski remembers a sugarbush in Putney, Vermont. It had "majestic old sugar maples, but essentially the whole understory was glossy buckthorn. The task of running the lines from tree to tree or just negotiating that sugarbush would have been a nightmare. That's a situation where, with early detection, you could have contained it."

The **Maine Forest Service**, SWOAM, and conservation organizations such as **Maine Audubon**, **Maine Coast Heritage Trust** and **The Nature Conservancy** have been working to educate people about invasive plants by offering workshops and field tours. A couple of good places to start your own education — the **National Invasive Species Information Center** website and the **Maine Natural Areas Program** invasive plants website.

The control issue is complicated by the fact that there is such a broad spectrum of invasive species and those invasives don't recognize the property lines we think are so important.

"It's a landscape problem, not an individual property problem," said Williams, the forester from Hollis. "The biggest hurdle is educating landowners, even the person who has a one-acre lot adjoining a property that's managed, and trying to get everyone to work together.

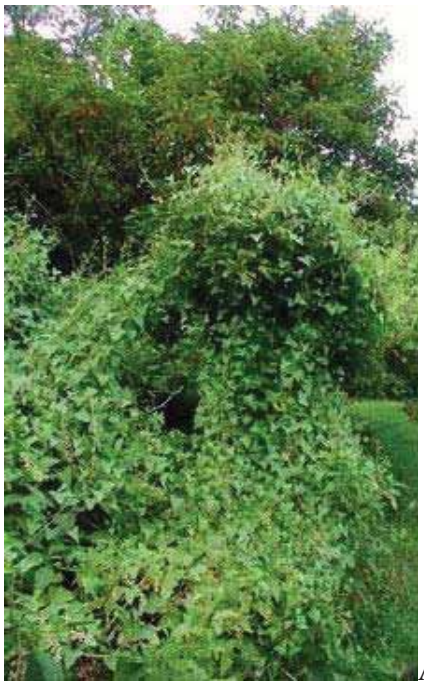
"We've had some luck going to neighbors and saying, 'are you willing to work with us and control these in a responsible manner?' And I've had landowners who are willing to pay for control of invasives on an abutting landowner's property just because they'll reap the benefit in the long term. And sometimes landowners will share the costs."

 Berries of Japanese barberry. (Photo: Maine Natural Areas Program)And when it comes to most of these species that's what we're talking about — control. Nobody is talking eradication. And, in fact not every plant can be controlled in every area. The scale

of the problem is just too immense. On the one side you have plants that seed and sucker with abandon to spread their genes and on the other you have humans with limited financial resources to fight them. In other words, we need to pick our battles, knowing we won't ever be able to declare "mission accomplished."

"The greatest challenge is to make sure our limited resources and limited energy are directed appropriately and strategically," said Rawinski. To protect a beautiful hardwood forest, perhaps, or a marsh or a wetland with endangered native plant species, a community forest, park or a wildlife refuge.

It's not a battle for the faint of heart. As Gibbs points out, "These problems didn't happen overnight and you can't take care of them overnight. If you want to be successful and control an invasive plant population you have to be persistent and in it for the long haul."


A plague in the mid-Atlantic states: mile a minute vine has barbs and came by its common name honestly. (Photo: Leslie J. Mehrhoff, University of Connecticut, Bugwood.org)And it's not as though the invaders we're fighting now are going to be the only ones. There are others on our doorstep or headed our way.

Among them is mile-a-minute vine (*Persicaria perfoliata*) — the common name gives you an idea of how fast it grows. It has barbs on the stem, is self-pollinating and a prolific seeder. It likes edges, but will grow in woods as well, where it climbs trees to get the sun it needs. Deer won't touch it.

Then there is Japanese stiltgrass (*Microstegium vimineum*), which now covers millions of acres in more than two dozen states. Stiltgrass is a prolific seeder and very tolerant of low light levels. Deer won't eat it, either. Stiltgrass is one of those stealth invasives. It looks like other native grasses. Even experts might not pick it out.

It's not the only one. Rawinski said Linden arrowwood (*Viburnum dilitatum*) an understory shrub that resembles our native arrowwood, is a common ornamental that can live in deep forest. "It's not on anyone's invasive plant list, but it probably should be," he said.

He feels the same about rusty willow (*Salix atrocinerea*), also known as large gray willow, which he's found in York and Cumberland counties. He calls it a "sneaky invader." First identified on Cape Cod a century ago, it's managed to spread widely in the northeast, choking the banks of ponds and lakes,

because it so closely resembles some native willows that even botanists don't give it a second glance.

Which brings us back to educating yourself about the trees, shrubs, wildflowers and vines in your woodlot or your community forest or the local park. Many people keep a list of birds they see on their property. Fewer have a plant list. But it's a good first step.

"You ought to learn to recognize the most common invasive plants, and then spend some time on your property," said Doak. "Don't always look up at the trees, look down as well. Get a sense of whether you have any of them and then learn whether you have a problem."

Joe Rankin writes forestry articles and keeps honeybees at his home in New Sharon.

BANGOR DAILY NEWS

Bee-killing pesticides found in 51 percent of "bee-friendly" plants in garden centers throughout U.S., Canada

Sam Hill | BDN

Phil Gaven, owner of the Honey Exchange and beekeeper, opens the top to one his hives to extract the queen bee for sale.
Buy Photo

By Danielle Walczak, BDN Staff
Posted June 25, 2014, at 6:16 p.m.

Fifty-one percent of plants sold at three major big-box stores across the U.S. and Canada contain a pesticide fatal to the pollinating insect, according to a new study by Friends of the Earth U.S., Pesticide Research Institute and SumOfUS. The study's results were released Wednesday at a press conference in Portland.

The Maine Organic Farmers and Gardeners Association assisted with conducting the pesticide sampling, the results of which were published in the report Gardeners Beware 2014. The report showed 36 of 71 garden plant samples purchased from top garden retailers — Home Depot, Wal-Mart and Lowes — in 18 cities in the U.S. and Canada contain neonicotinoid, or neonic, pesticides.

Neonic pesticides work systematically throughout the whole plant creating long-lasting prevalence in the plant and exposure of the pesticide to honeybees.

Several flowers in the study contained neonic levels lethal for bees, and researchers assumed comparable concentrations were also present in the flowers' pollen and nectar.

"The irony there is hard to ignore," MOFGA deputy director Heather Spalding said. "People are going out [and] growing these plants. There is an awareness of the decline in pollinator and bee populations. People think, '[I will] enrich my landscape with plants that will support the health of bees.' The very plants they are buying are filled with chemicals, killing bees."

According to the study, bee kills are a visible impact of systemic insecticides. It also states exposure to "levels of neonics that do not cause immediate bee death can still damage colonies." The immune system is affected, making bees more vulnerable to disease. Neonics affect the bees' ability to find food and return to the hive by impacting its learning and memory, as well as the bee's reproduction, reducing queen fertility and brood success.

"This class [of pesticides] is so widespread. It is taken up into every cell of the plant. It is there for life of the plant. It's not just applied. It's just there, working all the time, so there are many concerns of the harmful effects that it has — not only harmful to bees but other insects, butterflies and reptiles and birds," Spalding said.

Research director Lisa Archer, of the food and technology program at Friends of the Earth, said major producers should pay attention.

"Ultimately, this study is a snapshot of the market — it paints a picture. We really hope these companies will see this as a wake-up and see they need to take responsibility for the products on their shelf and take stand," she said. "There is no reason they shouldn't take action and begin urging suppliers to look for new alternatives."

Last year, the European Union banned three of the most widely used neonics based on other studies showing neonics can kill bees outright. BJ's Wholesale Club announced Wednesday it will require vendors to remove neonics from plants by the end of 2014.

"Clearly if these retailers can do that than the companies here can, too," said Archer, who cites thousands of grassroots campaigns and a petition signed by half a million people urging Lowes and Home Depot to stop selling neonics, as a driving force behind these changes.

The success of two-thirds of the food crops consumed by humans worldwide every day is reliant on pollinators such as bees.

These pollinators are in decline, according to the report.

"It's really a matter of basic decency and responsibility — being transparent with customers," Archer said.

Responsibility is what Peter Beckford of Rebel Hill Farm said he feels of the flowers he has been growing organically for the past 26 years on his Clifton farm. He focuses on plants native to Maine, at least from the eastern side of the Rocky Mountains.

"Everything we're growing is good for bees and pollinators," he said. "We're growing plants that the pollinators have a lot of use for because they are native plants."

Spalding and Archer suggest buying local, organic plants as an alternative to potentially neonics-ridden plants sold by major suppliers.

Despite Beckford's efforts to create a habitat for pollinators, the reality of food production is quite different, according to Meghan Gaven, owner of The Honey Exchange.

"I think a lot of people don't realize how we grow food. They don't realize that we take ten's of thousands, sometimes hundreds of thousands of hives and move them to a single crop," she said during a Wednesday press conference in front of her exchange on Stevens Avenue in Portland. "Over a million honeybee hives were moved to California to help with the almond bloom. Each hive has 50,000 bees in it. Because there are 750,000 acres devoted to almonds and when they're in bloom, you've got honeybees there. But when they're not in bloom, there's no point in having honeybees there because there's nothing for them to eat. So you have to move them there and then move them somewhere else," she said.

Pesticide use in agriculture is highly regulated, according to Tony Jabczak, Maine State Beekeeper at Maine's Department of Agriculture. Comparatively, homeowner use of pesticides is far more concentrated and left up to the consumer.

Jabczak said he thinks labeling and educating about pesticide use, versus a complete ban, can help combat the use of neonics. More specifically, it may spur more research regarding synergies, which occur when different types of pesticides are mixed together, significantly increasing the pesticide's toxicity.

"Plant material should be labeled, if nothing else, for consumer protection," Jabczak said. "We do a good job in training farmers, but the public has access to a lot of materials. You'd be surprised how little common sense is out there. Education is definitely a concern, as far as I'm concerned."

Jabczak cites the Varroa mite, introduced in the U.S. in 1985, for the decline in bee populations, which he said are rebounding. He said neonics is a complex issue that can be improved upon by more educated consumer choice and more research about synergies.

Last week, President Barack Obama announced a federal strategy to protect pollinators and called on the Environmental Protection Agency to assess the effect of pesticides, including neonics, on bees and other pollinators within 180 days.

Reps. Earl Blumenauer, D-Oregon, and John Conyers, D-Michigan, introduced the " Saving America's Pollinators Act" in 2013 and are seeking to suspend the use of neonics on bee-attracting plants.

The bill has bipartisan support and 68 co-sponsors.

BDN reporter Sam Hill contributed to this story.

http://www.centralmaine.com/2014/06/24/study-pesticides-making-bee-friendly-plants-bee-killers/

Kennebec Journal/Morning Sentinel, June 24, 2014

Study: Pesticides making 'bee-friendly' plants bee-killers

A press conference Wednesday in Portland will highlight the results of studies showing neonicotinoids, common in garden pesticides, are toxic to bees.

By North Cairn Portland Press Herald
ncairn@pressherald.com | 207-791-6325

A study by the environmental advocacy group Friends of the Earth to be released Wednesday in Portland shows that many home-garden plants, promoted as "bee-friendly," are pretreated with a class of pesticides that can kill bees and other pollinating insects.

Pesticide test results show that more than half of garden plants from major retailers in 18 cities in the U.S. and Canada, including Portland, contain neonicotinoids, commonly used garden pesticides that are toxic to bees and many other organisms, according to the study.

Erin MacGregor-Forbes, a master beekeeper, checks her hives in Portland. Many plants touted as "bee-friendly" are pretreated with a class of pesticides shown to harm and kill bees and other pollinating insects, according to a study to be released Wednesday at a press conference in Portland. Portland Press Herald photo by Derek Davis

Erin MacGregor-Forbes, a master beekeeper, touches a queen bee while checking her hives in Portland. Many plants touted as “bee-friendly” are pretreated with a class of pesticides shown to harm and kill bees and other pollinating insects, according to a study to be released Wednesday at a press conference in Portland. Portland Press Herald photo by Derek Davis

Chad Churchill, nursery manager at Highland Avenue Greenhouse & Farm Market in Scarborough can be seen through pac choi as he waters sugar snap peas in the greenhouse Tuesday. Portland Press Herald photo by Shawn Patrick Ouellette

The results will be made public by representatives of Friends of the Earth and Pesticide Research Institute, along with more than 20 consumer and environmental organizations and beekeeping and organic gardening associations, including Maine Organic Farmers and Gardeners Association. Of plant samples that tested positive for the pesticides, 40 percent contained two or more neonicotinoids.

The result of the widespread use of these pesticides means that many home gardens have likely become a source of harm for bees, the report concluded.

Test samples were gathered by environmental advocates from various organizations, beekeepers and researchers from various universities, said Tiffany Finck Haynes of Friends of the Earth. Testing of the samples was conducted by independent laboratories in each of the study cities in the U.S. and Canada, she said.

In addition, the damaging affects of these pesticides may be far more widespread than first thought, leaving a lasting imprint of injury to birds, mammals, humans and the soil, said a separate report released Tuesday in England, from the Bee Coalition, a collaboration of the main British environmental groups. The study, “Worldwide Integrated Assessment,” reviewed 800 studies from across the globe to create a worldwide profile of the impact of these pesticides on a wide range of invertabrate species in soil, vegetation, aquatic and marine habitats. The authors recommend a significant reduction global phasing out of the neonic pesticides.

GROWING CONCERNS

This week's reports join a growing body of studies linking neonics with colony collapse disorder, in which honeybees quit their hives for no apparent reason and do not return. A Harvard University study in May focused the collapse of honeybee colonies on neonicotinoids — insecticides that also function as nerve poisons and simulate the effects of nicotine. Scientists specifically examined low doses of two neonicotinoids — imidacloprid and clothianidin — to determine their effects on healthy bee hives over the course of a winter.

The results of the Harvard study supported the conclusion that even sub-lethal exposure to neonicotinoids is probably the main factor causing CCD to occur, the researchers wrote in their paper, published May 9 in the Bulletin of Insectology.

Neonicotinoid pesticides – also referred to as neonics – work by affecting the central nervous system of insects, causing paralysis and death, according to the U.S. Environmental Protection Agency.

The Friends of the Earth study – said to be based on the largest data samples to date – called on major retailers to stop selling plants containing neonics and urged consumers to buy only organic plants instead, to help deter the spread of problems associated with the use of such pesticides, including imidacloprid, a synthetic nicotine.

In addition, the sponsoring organizations are expected to offer homeowners and backyard gardeners tips on how to reduce exposure to the pesticides – known as neonicotinoids – and to protect bees.

Neonicotinoids are systemic pesticides, meaning that they affect not just the surface of leaves of treated plants but also are absorbed through the entire system, penetrating even into the soil, said Erin Forbes, a master beekeeper from Portland. They are persistent, too, lasting up to 15 years in soil, she said, describing neonicotinoids as "the most common class of pesticide mixtures in the world (and) the most common household pesticide." It is even found in many pet flea collars, Forbes said.

"They are absolutely a contributing factor to the increased decline of honeybee colonies in recent years," said Forbes. Most plants that are started in soil and transported from one state to another have neonicotinoids in the soil, she said. "Here in Maine that's the most common route of transmission."

Neonic pesticides have come under scrutiny in recent years due to the decline of honeybees, particularly from Colony Collapse Disorder. Entomologists and biologists have been working to unravel the multiple factors involved in the disorder, which have decimated bee populations worldwide. The problem has been particularly severe in Europe, where declines of more than 50 percent have been reported in some areas.

The mechanisms of the disorder and the factors in its steady spread remain unclear, but many possible causes have been suggested, including pesticides, particularly neonicotinoids; infections with certain mites; malnutrition; various pathogens; genetic factors; immune deficiencies; habitat loss; changing climate conditions and evolving beekeeping practices. Many scientists believe the disorder is erupting from a combination of these problems.

"There's a lot of factors in the mix," said Carol Cottrill of Rumford, president of the Maine State Beekeepers Association.

"Pesticides have always been part of the mix of things affecting pollinators," Cottrill said. "Neonicotinoids may be part of the problem but they aren't the whole problem."

Cottrill praised the new report, calling it "sane and sensible" in its focus on education of homeowners about how to avoid unwitting use of neonics and the need to check on the nursery stock they purchase. "You can buy plants that have not been pretreated," she said.

GREENHOUSES ON THE WATCH

David LeBlanc, general manager at Longfellow's Greenhouses in Manchester, said the nursery and garden center began using fewer chemical sprays several years ago, and one reason was concern about effects the sprays have on bees.

"We are probably 90 percent biological control," LeBlanc said. "We buy good bugs to go after our bad bugs. But if we have a problem that isn't controllable with our biological program, we have to go in sometimes and spray some material."

LeBlanc said some of the pesticides they use are neonicotinoids. He said there seems to be scientific disagreement about the cause of declining bee populations, and the evidence against neonicotinoids hasn't been clear enough to rule out using them.

At Highland Avenue Greenhouse in Scarborough, annual and perennial plants are free of neonicotinoids, because they are not grown in pretreated soil, said Christine Viscone, who co-owns the business with her husband Joe. Consumers concerned about pesticides in plants or soil would benefit from trying local garden centers and asking whether trees and shrubs, in particular – which often are imported from other states to Maine – are treated with neonicotinoids before they buy stock for their own gardens, she said.

"If we can educate people and let them make informed decisions, it will be helpful … better than a ban," Cottrill said. "If we ban something, I want to know what they're going to use to replace it. You've got to give (people) an alternative. My personal fear is that if they ban the neonics, what are they going to replace them with?"

A proposal to impose a temporary, two-year ban on the sale, distribution and use of neonicotinoid pesticides in Maine failed in the Legislature last year.

Concerns about the risks of neonicotinoids caused Wyman's of Maine, the nation's largest producer of wild blueberries, to opt out of use of the pesticides altogether on its 10,000 acres of berries, said Ed Flanagan, the company's president and CEO.

"We have never used them," Flanagan said. Beekeepers at Wyman's – one of whom, David Hackenberg, is credited as being among the early detectors of Colony Collapse Disorder in 2006 – expressed suspicions about the toxic effects of the pesticides, which weaken bees' immune systems. The decision was made early on to seek other alternatives, Flanagan said.

Last Friday, the White House issued a statement calling the decline of honeybees, native bees and other pollinators – including birds, bats and butterflies – a serious problem that "poses a significant challenge that needs to be addressed to ensure the sustainability of our food production systems, avoid additional economic impacts on the agricultural sector and protect the health of the environment."

President Barack Obama called for several measures, including the establishment of a task force charged with developing a national strategy to improve pollinator health.

The White House stopped short of singling out neonicotinoid pesticides but included "pesticide exposure" as one of several factors in bee decline.

Pollinators contribute more than $24 billion to the U.S. economy annually – with honeybees accounting for more than $15 billion, according to the White House statement.

“Honeybees enable the production of at least 90 percent of commercially grown crops in North America,” the statement said. Globally, animal pollinators enable the production of 87 of the leading 115 food crops, the White House estimated.

According to the Food and Agriculture Organization of the United Nations, shortages of bees in the U.S. have increased the cost to farmers renting them for pollination services by up to 20 percent.

Kennebec Journal writer Susan McMillan contributed to this story.

Bee Health 6:21 PM WED JUNE 25, 2014

Study: Pesticides in Nursery Plants Killing Bees

By PATTY WIGHT (/PEOPLE/PATTY-WIGHT)

(http://mediad.publicbroadcasting.net/p/mpbn/files/201406/6510010063_2cc839f323_m_0.jpg)

A honeybee comes in for a landing on the same flower occupied by a bumblebee.
Credit Martin LaBar

Bee-lovers who ply nurseries for welcoming plants may be bringing home more than just beautiful blossoms: A new study finds that as many as half of garden plants sold at top retailers contain neonicotinoid pesticides. "Neonics," as they're referred to, have been linked to recent declines in the honey bee population.

Listen 4:50

Patty Wight reports on the concern about nursery plants treated with pesticides that kill bees.

Now, some environmental and consumer groups want big retailers to stop supplying neonic-treated plants or require warning labels. But some gardening and bee experts say the evidence against using neonics is murky.

The report, called "Gardeners Beware," was spearheaded by Friends of the Earth US and the Pesticide Research Institute, and supported by other environmental and consumer organizations. The groups tested for pesticides in 71 plants purchased from large garden retailers across 18 cities, including Portland, says Charlotte Warren, spokesperson for the national Organic Consumers Association.

"The testing revealed that many home garden plants sold at Home Depot, Lowe's and Wal-Mart stores in the Portland area, have been pre-treated with pesticides shown to harm and kill bees," she said today at a press conference.

Master Maine Beekeeper Erin MacGregor-Forbes says plants treated with neonics retain the pesticide for their entire lives. "Neonicotinoids insecticides are systemic insecticides which are treated on the plant, absorbed into the plant, and then expressed through the pollen and nectar and the leaves of the plant," she said.

And many people who buy these plants, says Forbes, think they are bee friendly, when they may actually harm or kill them.

There's been worldwide concern over bee populations, which have declined by about a third since 2006, in a phenomenon called Colony Collapse Disorder. Though the decline has been attributed to a host of factors, Charlotte Warren says the report is part of a growing body of evidence that neonics play a major role.

Some big retailers are taking notice. BJ's Wholesale Club announced Wednesday they will require vendors to either stop supplying neonic-treated plants, or require warning labels.

Charlotte Warren says other retailers should follow BJ's lead. "We're here today to ask Home Depot, Lowe's and WalMart to do the same," she said.

"We don't want to hurt the environment - we hate spraying," says Tom Estabrook, vice president of Estabrooks Farm and Greenhouses in Yarmouth. He says there are conflicting studies on how much neonicotinoids harm bees. As the debate plays out, he says he'll follow state guidelines, which allow their use.

"Unfortunately, it's a part of our crop," he says. "We have to protect the investment that we've made. We have to make sure the plants are healthy for when they go home with you as a customer."

Estabrook isn't the only one who questions why neonicotinoids are so vilified. Maine State Apiarist Tony Jadzcak says neonicotinoids were developed to replace previous insecticides that were much more toxic.

"I mean, if we're going look at insecticides, maybe we out to look at all of them," Jadzcak says. "Because I think this class of insecticide is kind of taking a bad name, or getting too much bad publicity, compared to some of the other stuff that is commonly used."

Jadzcak says some neonicotinoids on their own are not that toxic. But they become significantly more so when mixed with certain fungicides. While Jadzcak supports better labeling for neonic-treated plants, he says asking big box retailers to end the pesticide's use could have unfortunate consequences.

"What materials will be put on those shelves in place of that?" he asks. "And my feeling on this is they're going to put some of the older chemistry materials back on the shelves, which we're currently trying to phase out for a variety of reasons."

Others point out that the focus on pesticides is too narrow, when bee population declines are due to a number of factors, including mites, viruses, habitat loss, and poor nutrition.

Master BeeKeeper Erin MacGregor-Forbes acknowledges the issue is complex. "But the neonicotinoids are the one that human beings can control," she says. "The problem is, the neonicotinoids are the one component actually earn somebody money, and that is the reason it's so difficult to fight."

One garden center says stopping their use may not be as difficult as it seems. Highland Avenue Greenhouse in Scarborough says they "grow naked" - meaning no pesticides. Co-owner Christine Viscone says it happened by accident - the greenhouse lost its pesiticide license when out-of-state credits didn't transfer to Maine.

"We decided, you know what? Instead of going back and taking the test again, we're going to implement what we've been learning for years in all of these pesticide credit seminars," she says. "They're teaching us about how to use biologicals."

Viscone says the change was surprisingly doable and it's in line with demand from eco-conscious customers. To what extent other greenhouses may need to change their pesticide policies will be decided in the near future. President Obama has asked the Environmental Protection Agency to assess the effect of pesticides like neonicotinoids on bees and other pollinators within the next six months.

TAGS: bee health (/term/bee-health) neonicotinoids (/term/neonicotinoids) MPBN (/term/mpbn)

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http://www.centralmaine.com/2014/06/25/study-plants-from-big-box-stores-in-augusta-contained-bee-harming-pesticides/

Kennebec Journal/Morning Sentinel, June 25, 2014

Study: Plants from big-box stores in Augusta contained bee-harming pesticides

Home Depot and Lowe's said they comply, or plan to comply, with federal regulations requiring proper labeling for plants that use the pesticides.

By Jesse Scardina Staff Writer
jscardina@centralmaine.com | @JesseScardina | 207-861-9239

On the shelf of a garden center in Fairfield Wednesday was a common pesticide brand that included imidacloprid.

Cathy Hebert, co-owner of Sunset Flowerland & Greenhouse, said she probably wouldn't have bought the pesticide if she'd known how harmful it is to bees.

Cathy Hebert, co-owner of Sunset Flowerland & Greenhouse said pesticides are important for greenhouses and large growers to produce crops of plants, and says the industry has responded to the need to inform consumers about potential harmful impacts. Staff photo by Jesse Scardina

Hebert, who operates 21 greenhouses at the Ridge Road business, said she is aware of pesticides that are considered harmful to bees, but she didn't know imadacloprid, the largest-selling pesticide in the country, was one of them.

“That’s one of the problems, (the pesticides) are coming in with this name,” Hebert said.

A nationwide study released this week said neonicotinoid, found in imadcloprid and other pesticides, kills honeybees as well as butterflies and birds. The study said that the pesticide is commonly found on plants sold at large retailers, such as Home Depot, Lowe’s and Walmart.

That study coincides with Environmental Protection Agency labeling standards that are beginning to take effect that more specifically say what’s in pesticides.

Representatives from Unity-based Maine Organic Gardener and Farmer’s Association bought four common landscaping plants from the two large home goods stores in Augusta — Home Depot and Lowe’s — as part of the study and found that three of them tested positive for neonicotinoid, or neonic, pesticides, deputy director Heather Spaulding said Wednesday.

Home Depot and Lowe’s both were quick to put out news releases Wednesday saying they comply, or plan to comply, with the federal regulations requiring that plants that use the pesticides are properly labeled.

“If giant purchasers of plants can demand that plants be neonic free, it can make an impact,” Spaulding said. “Applying systemic neonic pesticides is like taking antibiotics all the time to not get sick.”

The study, Gardeners Beware 2014, was led by the environmental advocacy group Friends of the Earth, and it focused on larger retailers because they make an impact on wholesale agricultural practices.

Neonic is a class of insecticides that can be harmful to honeybee colonies, and in 2013, the European Commission enacted a two-year ban on the use of the insecticide. One type of neonic insecticide commonly found in plants bought at the retail level is imidacloprid, the largest selling insecticide in the country, amounting over a billion dollars in sales in 2009, according to the Journal of Agricultural and Food Chemistry.

Yet, Hebert said, large-scale growing and greenhouse operations need some level of pesticide treatment.

“In a greenhouse environment, everything is protected, so insects can go rampant,” she said. “In a greenhouse environment, the bugs would go crazy. The plants would be destroyed.”

Hebert said that her family has owned and operated greenhouses since the 1950s as pesticide treatments have evolved with more research and studies.

“It’s becoming more aware,” she said. “We pay attention to the studies, and we try to do a lot more.”

Of the four different perennials MOFGA bought — two each from Home Depot and Lowe’s in Augusta — both flowers from Lowe’s tested positive for the insecticide, while one of the two from Home Depot tested positive.

Both national chains quickly issued statements Wednesday supporting the research and the recent Pollinator Health Task Force, established by the Obama administration to focus efforts on research aimed toward helping pollinating species such as bees recover from population declines.

Home Depot is taking steps to require live goods suppliers to label plants that have been treated with neonics by fourth quarter of 2014, according to Catherine Woodling, Home Depot’s corporate communications manager.

“We’re also glad to provide customers with alternative products for their insecticide needs and are actively working with our live goods suppliers to find alternative insecticides for protecting live goods and bees,” Woodling said in a statement.

Lowe's issued a statement stating it has been monitoring the latest science from various sources, including the U.S. Department of Agriculture and environmental groups. The statement also said the company expects all its vendors to abide by Environmental Protection Agency guidelines regarding insecticides.

In 2013, the EPA issued new labeling standards for neonic pesticides, including a Bee Advisory Box, that highlights the need to protect pollinators.

"This product can kill bees and other insect pollinators," the label states in bold, red lettering.

While those labeling changes are still months away from the marketplace in some instances, Spalding suggested that the best way for consumers to know what products have been used on plants is to simply ask.

"We encourage folks to talk to the garden center and be sure it's not in the soil," Spalding said.

Hebert said that not many customers ask for specific information such as the types of pesticides used; however, it's something they would answer.

"Not many people have approached us asking that, but we would talk to anyone about it," she said.

And while pollination will eventually limit a plants flowering ability, Hebert said bees play a vital role in vegetation and gardening.

"For seed production, you need the pollinators," she said. "How would I buy seeds if I don't have pollination?"

Jesse Scardina — 861-9239

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NEWS Posted June 24 | Updated June 24

Study: Pesticides making 'bee-friendly' plants bee-killers

A press conference Wednesday in Portland will highlight the results of studies showing neonicotinoids, common in garden pesticides, are toxic to bees.

BY NORTH CAIRN PORTLAND PRESS HERALD
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centralmaine.com
KENNEBEC JOURNAL | Morning Sentinel

Wednesday in Portland shows that many home-garden plants, promoted as "bee-friendly," are pretreated with a class of pesticides that can kill bees and other pollinating insects.

Pesticide test results show that more than half of garden plants from major retailers in 18 cities in the U.S. and Canada, including Portland, contain neonicotinoids, commonly used garden pesticides that are toxic to bees and many other organisms, according to the study.

ADDITIONAL IMAGES

Erin MacGregor-Forbes, a master beekeeper, checks her hives in Portland. Many plants touted as "bee-friendly" are pretreated with a class of pesticides shown to harm and kill bees and other pollinating insects, according to a study to be released Wednesday at a press conference in Portland. *Portland Press Herald photo by Derek Davis*

The results will be made public by representatives of Friends of the Earth and Pesticide Research Institute, along with more than 20 consumer and environmental organizations and beekeeping and organic gardening associations, including Maine Organic Farmers and Gardeners Association. Of plant samples that tested positive for the pesticides, 40 percent contained two or more neonicotinoids.

The result of the widespread use of these pesticides means that many home gardens have likely become a source of harm for bees, the report concluded.

Test samples were gathered by environmental advocates from various organizations, beekeepers and researchers from various universities, said Tiffany Finck Haynes of Friends of the Earth. Testing of the samples was conducted by independent laboratories in each of the study cities in the U.S. and Canada, she said.

In addition, the damaging affects of these pesticides may be far more widespread than first thought, leaving a lasting imprint of injury to birds, mammals, humans and the soil, said a separate report released Tuesday in England, from the Bee Coalition, a collaboration of the main British environmental groups. The study, "Worldwide Integrated

Daily Reporter
Greenfield, Indiana

Ore. Agriculture Department adopts new pesticide rule to protect bees

THE ASSOCIATED PRESS
First Posted: June 26, 2014 - 11:38 pm
Last Updated: June 26, 2014 - 11:40 pm

SALEM, Oregon — Alarmed by multiple incidents of bee deaths this summer, the Oregon Agriculture Department has temporarily restricted the use of pesticides containing two active ingredients that are dangerous to bees.

In a statement Thursday, the department said it's banning the use of products containing dinotefuran and imidacloprid on linden and similar trees.

The agency says the rule applies to all users, including professional applicators and homeowners.

After high profile bee deaths last year, the Agriculture Department ordered that pesticide labels be revised for 2014 to note that use of the ingredients was prohibited on trees that bees like. However, the agency says two recent bee death incidents — in Eugene and in Beaverton — involved the use of product with an older label, which just noted that the product is highly toxic to bees.

The agency says its temporary rule goes into effect immediately and will be enforced for six months while it completes its bee death investigation.

The Agriculture Department last week suspended the pesticide license of the tree care service responsible for spraying an insecticide blamed for killing 1,000 bees at a Eugene apartment complex.

News
From the Oregon Department of Agriculture

ODA issues new pesticide restrictions to protect pollinators

June 26, 2014... The Oregon Department of Agriculture is taking additional steps to protect bees and other pollinators from exposure to specific pesticide products following multiple incidents of bee deaths this summer. In adopting a temporary rule, ODA is prohibiting the use of pesticide products containing the active ingredients dinotefuran and imidacloprid on linden trees or other species of Tilia.

The rule applies to all users, including professional applicators and homeowners.

"Although we took significant steps last year to restrict the use of these pesticide products, we've seen more cases involving bumblebees attracted to blooming linden trees and pesticide applications," says ODA Director Katy Coba. "In order to protect our pollinators, we feel it's important to adopt additional restrictions."

Last year, based on high profile incidents of bee deaths, ODA adopted a required label statement on pesticide products containing imidacloprid and dinotefuran prohibiting the application of these products on linden trees and other Tilia species. For 2014, newly-labeled products distributed into Oregon are required to state the restriction. Products with pre-2014 labels are still in commerce and, prior to the temporary rule, could be used when plants were not in bloom. Two recent incidents of large bee deaths– one in Eugene, the other in Beaverton– involved the use of imidacloprid products with an older label, which alerts the user that the product is "highly toxic to bees exposed to direct treatment or residues." To address confusion or misunderstanding caused by having two different label statements, ODA is simply prohibiting the application of any product containing imidacloprid or dinotefuran on linden, basswood, and other trees of Tilia species.

Failure to comply with the new rule could result in license suspension or revocation as well as imposition of a civil penalty.

The temporary rule, which goes into effect immediately, will be enforced for 180 days and will protect pollinators while allowing ODA to complete its investigation of recent bee death incidents as well as determine any future regulatory actions.

ODA is contacting all pesticide license holders in Oregon regarding the new rule and will continue to provide outreach and education on pollinator protection. Additional information can be found on the ODA website at <http://www.oregon.gov/ODA/PEST/Pages/Pollinator.aspx>.

Media contact: Bruce Pokarney, (503) 986-4559

From: Nancy Oden [mailto:cleanearth@tds.net]
Sent: Tuesday, June 24, 2014 10:14 PM
To: Jennings, Henry
Subject: Insecticides put world food supplies at risk, say scientists | Environment | The Guardian

Henry - Please put this article in Board's packets after you print it out. These are SCIENTISTS saying this, not me. thanks. - Nancy Oden
http://www.theguardian.com/environment/2014/jun/24/insecticides-world-food-supplies-risk

Insecticides put world food supplies at risk, say scientists

Regulations on pesticides have failed to prevent poisoning of almost all habitats, international team of scientists concludes

Damian Carrington
The Guardian, Monday 23 June 2014

Jump to comments (570)

Farmers use helicopters to spray insecticide and fertilizer on wheat crops in Henan province, China. Photograph: TPG/Getty Images

The world's most widely used insecticides have contaminated the environment across the planet so pervasively that global food production is at risk, according to a comprehensive scientific assessment of the chemicals' impacts.

The researchers compare their impact with that reported in Silent Spring, the landmark 1962 book by Rachel Carson that revealed the decimation of birds and insects by the blanket use of DDT and other pesticides and led to the modern environmental movement.

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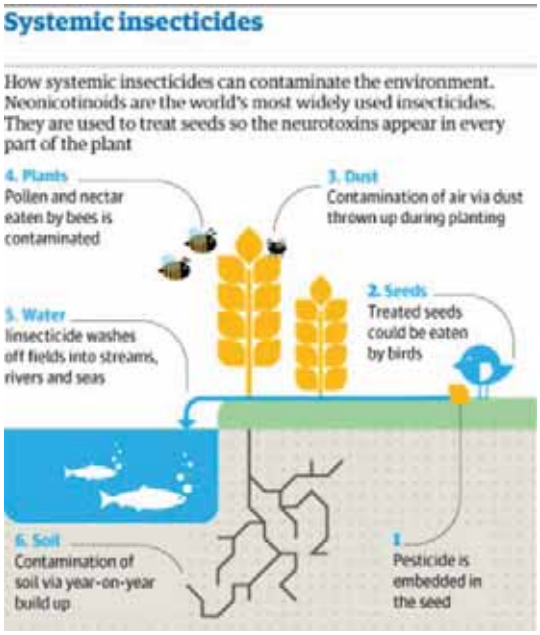
Do farmers really need bee-harming insecticides?
Syngenta says some farmers have no choice but to use banned neonicotinoids, which are linked to declining bee populations. But is it true that no alternatives exist? With your help, **Karl Mathiesen**

Billions of dollars' worth of the potent and long-lasting neurotoxins are sold every year but regulations have failed to prevent the poisoning of almost all habitats, the international team of scientists concluded in the most detailed study yet. As a result, they say, creatures essential to global food production – from bees to earthworms – are likely to be suffering grave harm and the chemicals must be phased out.

The new assessment analysed the risks associated with neonicotinoids, a class of insecticides on which farmers spend $2.6bn (£1.53bn) a year. Neonicotinoids are applied routinely rather than in response to pest attacks but the scientists highlight the "striking" lack of evidence that this leads to increased crop yields.

"The evidence is very clear. We are witnessing a threat to the productivity of our natural and farmed environment equivalent to that posed by organophosphates or DDT," said Jean-Marc Bonmatin, of the National Centre for Scientific Research (CNRS) in France, one of the 29 international researchers who conducted the four-year assessment. "Far from protecting food production, the use of neonicotinoid insecticides is threatening the very infrastructure which enables it." He said the chemicals imperilled food supplies by harming bees and other pollinators, which fertilise about three-quarters of the world's crops, and the organisms that create the healthy soils which the world's food requires in order to grow.



Systemic insecticides. Photograph: /Guim

Professor Dave Goulson, at the University of Sussex, another member of the team, said: "It is astonishing we have learned so little. After Silent Spring revealed the

investigates.

Syngenta seeks 'emergency' exemption to use banned insecticide on UK crops

Neonicotinoids are the new DDT killing the natural world

unfortunate side-effects of those chemicals, there was a big backlash. But we seem to have gone back to exactly what we were doing in the 1950s. It is just history repeating itself. The pervasive nature of these chemicals mean they are found everywhere now.

“If all our soils are toxic, that should really worry us, as soil is crucial to food production."

The assessment, published on Tuesday, cites the chemicals as a key factor in the decline of bees, alongside the loss of flower-rich habitats meadows and disease. The insecticides harm bees’ ability to navigate and learn, damage their immune systems and cut colony growth. In worms, which provide a critical role in aerating soil, exposure to the chemicals affects their ability to tunnel.

Dragonflies, which eat mosquitoes, and other creatures that live in water are also suffering, with some studies showing that ditchwater has become so contaminated it could be used directly as a lice-control pesticide.

The report warned that loss of insects may be linked to major declines in the birds that feed on them, though it also notes that eating just a few insecticide-treated seeds would kill birds directly.

One of the last living male dusky seaside sparrows is seen in this 1981 file photo while in captivity at Santa Fe Community College in Gainesville, Florida. DDT pesticide spraying since the 1940s contributed to the extinction of this species. Photograph: Nathan Benn/Corbis

“Overall, a compelling body of evidence has accumulated that clearly demonstrates that the wide-scale use of these persistent, water-soluble chemicals is having widespread, chronic impacts upon global biodiversity and is likely to be having major negative effects on ecosystem services such as pollination that are vital to food security,” the study concluded.

The report is being published as a special issue of the

peer-reviewed journal Environmental Science and Pollution Research and was funded by a charitable foundation run by the ethical bank Triodos.

The EU, opposed by the British government and the National Farmers Union, has already imposed a temporary three-year moratorium on the use of some neonicotinoids on some crops. This month US president Barack Obama ordered an urgent assessment of the impact of neonicotinoids on bees. But the insecticides are used all over the world on crops, as well as flea treatments in cats and dogs and to protect timber from termites.

However, the Crop Protection Association, which represents pesticide manufacturers, criticised the report. Nick von Westenholz, chief executive of the CPA, said: “It is a selective review of existing studies which highlighted worst-case scenarios, largely produced under laboratory conditions. As such, the publication does not represent a robust assessment of the safety of systemic pesticides under realistic conditions of use.”

Von Westenholz added: “Importantly, they have failed or neglected to look at the broad benefits provided by this technology and the fact that by maximising yields from land already under cultivation, more wild spaces are preserved for biodiversity. The crop protection industry takes its responsibility towards pollinators seriously. We recognise the vital role pollinators play in global food production.”

A Bulgarian beekeeper grabs dead bees during a demonstration in Sofia to call for a moratorium on the use of neonicotinoid pesticides in April. Photograph: Dimitar Dilkoff/AFP/Getty Images

The new report, called the Worldwide Integrated Assessment on Systemic Pesticides, analysed every peer-reviewed scientific paper on neonicotinoids and another insecticide called fipronil since they were first used in the mid-1990s. These chemicals are different from other pesticides because, instead of being sprayed

over crops, they are usually used to treat seeds. This means they are taken up by every part of the growing plant, including roots, leaves, pollen and nectar, providing multiple ways for other creatures to be exposed.

The scientists found that the use of the insecticides shows a “rapid increase” over the past decade and that the slow breakdown of the compounds and their ability to be washed off fields in water has led to “large-scale contamination”. The team states that current rules on use have failed to prevent dangerous levels building up in the environment.

Almost as concerning as what is known about neonicotinoids is what is not known, the researchers said. Most countries have no public data on the quantities or locations of the systemic pesticides being applied. The testing demanded by regulators to date has not determined the long-term effect of sub-lethal doses, nor has it assessed the impact of the combined impact of the cocktail of many pesticides encountered in most fields. The toxicity of neonicotinoids has only been established for very few of the species known to be exposed. For example, just four of the 25,000 known species of bee have been assessed. There is virtually no data on effects on reptiles or mammals.

From: Nancy Oden [mailto:cleanearth@tds.net]
Sent: Tuesday, June 24, 2014 10:17 PM
To: Jennings, Henry
Subject: Study further confirms link between autism and pesticide exposure | The Verge

Henry - Please also print out this article and put it Board's packets. No need to attach my name to either one of these articles.....hopefully they will look at them and maybe, perhaps, possibly, someday see the light - that is, that manmade chemical pesticides must be phased out because they're causing mass killing of earth's creatures. Thanks. - Nancy Oden
http://www.theverge.com/2014/6/23/5832142/study-further-confirms-link-between-autism-and-pesticide-exposure

http://www.theverge.com/2014/6/23/5832142/study-further-confirms-link-between-autism-and-pesticide-exposure

THE VERGE

Study further confirms link between autism and pesticide exposure

Living near farms and fields can put a fetus at risk

By Arielle Duhaime-Ross on June 23, 2014 12:01 am Email @ArielleDRoss 162**Comments**

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(Andrew Stawarz / Flickr)

Complications during pregnancy, viral infections, and genetic disorders have all been associated with autism. But for the past few years, an increasing number of researchers have started to focus their attention on another important risk factor: environmental pollutants. These neurotoxins, which include everything from pesticides, to mercury and diesel, are thought to alter brain development in fetuses. Now, a new study further confirms this link by showing that pregnant women who live within a mile of farms and fields where pesticides are employed see their risk of having a child with autism increase by 60 percent — and that risk actually doubles if the exposure occurs in the third trimester.

"Pesticides are one of the toxicants that appear to have the strongest association with autism," says Dan Rossignol, an autism expert at Jeff Bradstreet's International Child Development Resource Center in Florida who did not participate in the study, published today in *Environmental Health Perspectiv*es. These latest results, he says, "strengthen that association."

In the study, researchers linked data from the California Pesticide Use Report to the residential addresses of 970 children participating in the ongoing Childhood Autism Risks from Genes and Environment (CHARGE) study. This allowed the scientists to make connections between various developmental delays, and the types of chemicals that mothers may have been exposed to before conception, and during pregnancy. They also took note of prenatal vitamin intake, socio-economic status, and metabolic disorders during pregnancy to avoid interference by possible confounders.

the risk could go up "as much as threefold."

"Women who live within a mile of organophosphate or pyrethroids agricultural pesticide applications were more likely to have a child with autism spectrum than women living further away," said Janie Shelton, an epidemiologist at the University of California Davis and lead author of the study, in an email to *The Verge*. Currently, 1 in 68 American children have some form of autism spectrum disorder. But the risk could go up "as much as threefold" when women are exposed to organophosphates later in pregnancy, Shelton said. This means that scientists need to "investigate [these results] further, while taking preventive steps to decrease exposure to women during and just prior to conception."

For Rossignol, "the only type of study that would have been better" would have been a study "where women were followed before, during, after pregnancy — as well as their babies — to determine if, over time, those higher exposure to pesticides had a higher risk of autism." Richard Frye, an autism researcher at the University of Arkansas who was not involved in the study, agrees with Rossignol, and pointed out in an email that "there could be bias in the sample of patients because the participants volunteered for the study." This means that these participants are the kinds of people that that "seek medical care for their children" — which isn't necessarily representative of all parents. But overall, both scientists praised the study's design.

pregnant women should avoid contact with agricultural pesticides

Shelton and her team would like to continue the research — if they can get more funding. One of their goals is to find out if certain sub-groups are more vulnerable to pesticide exposure. But,

regardless of the outcome, Shelton thinks the message is clear: Pregnant women should avoid contact with agricultural pesticides.

"The neurotoxicity of many agricultural agents have been suspected from animal studies for sometime," Frye said, so "this information needs to be taken seriously for not only expecting women, but women who are planning to become pregnant." He thinks that taking steps to prevent autism and other developmental delays is "much better for society" than treating children "once they have been born with such abnormalities." But to do that, he said, we need to proactively educate mothers about the risks — and what they can do to fight back. "Simple things like proper nutrition and folate supplement [intake] is still suboptimal in some areas," but these are "simple factors that can have a large impact at preventing autism and developmental disorders."

- **Source** Environmental Health Perspectives

Mosquito spraying may have killed bees

Carcasses litter Wakefield school

By Yasmeen Abutaleb

| GLOBE CORRESPONDENT JULY 09, 2014

DAVID L RYAN/GLOBE STAFF

It is unclear what killed the insects, but several beekeepers across the state have experienced similar losses.

WAKEFIELD — Dead bumblebees littered the sidewalk in front of Wakefield's Saint Joseph School. Some were still dying, while others were found in clusters around trees and shrubs that decorated the front of the school.

One local homeowner reported seeing “hundreds if not thousands” of dead and dying bees over the weekend in an e-mail to the Pollinator Stewardship Council, a group that helps protect bees across the country.

While it is unclear what killed the insects, several beekeepers across the state have experienced similar losses — losing up to 10,000 bees at a time — which they have attributed to pesticide spraying.

At this time of year, communities often spray areas where mosquitoes breed to prevent the spread of mosquito-borne illnesses, such as West Nile virus and Eastern equine encephalitis. The pesticides typically contain toxic ingredients that kill bees and other insects and animals.

Saint Joseph has never sprayed pesticides on its plants or trees, said Alyne Flynn, a school administrator.

But the East Middlesex Mosquito Control Project, which oversees spraying in Wakefield, sprayed sumithrin on residential streets about 2 to 3 miles from Saint Joseph starting at 8:15 p.m. on three evenings last week, said David Henley, the group’s superintendent. The pesticide is also known by the brand name Anvil 10+10.

Henley said that mosquito control sprayed because trappings showed high numbers of mosquitoes, but the group has not identified disease-carrying insects.

Sumithrin is highly toxic to bees, specialists said, and it was sprayed when bees could still be out foraging for pollen. Bumblebees can travel up to 5 miles, so a traveling community could have become infected, leading to the rapid die-off, said Dr. Alex Lu, associate professor of environmental exposure biology at the Harvard School of Public Health.

“Sumithrin is not a good choice for mosquito control, especially in the area with dense population,” Lu said.

State health officials conduct aerial spraying of disease-carrying mosquitoes when they are most prevalent, which is typically in late July or August. The spraying has faced criticism from farmers and beekeepers who worry about the pesticide’s unintended victims.

Beekeepers across the country have also reported dramatic losses to pesticide control, Lu said, adding that bees are needed to pollinate nutritious foods such as apples, blueberries, and strawberries. Bees have been dying off in alarming numbers over the past several years, leaving the nation with too few hives.

“There have been mass bee deaths that have been unexplained,” said Kimberly Klibansky, a beekeeper in Rowley.

Klibansky and her husband, also a beekeeper, both lost whole hives in 2012, about 100,000 bees. “Farmers are going out to their fields and the bees are just gone,” she said. “There’s no evidence of dead bees at their hives.”

Lauren Mangarelli, an 8-year-old student at Saint Joseph, said she noticed many dead bees in the parking lot and in front of the school over the past couple of days.

“It’s kind of weird because I see them everywhere,” Mangarelli said. “It’s freaking me out. They’re everywhere, and we’re barefoot a lot, and I don’t want to step in them.”

Bee activists said local pesticide groups can work with farmers and beekeepers to protect both public health and bee populations by spraying pesticides only late at night when it is completely dark. Local governing bodies and the state can also allow some beekeepers to opt out of having areas near their hives sprayed, they said.

“Bees are the canary in the coal mine,” said Michele Colopy, program director of Pollinator Stewardship Council. “We understand that the public health concerns and protections will always trump concerns for non-target species, and beekeepers realize that, but there are ways we can work together to protect bees from mosquito spray.”

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***NATURE* | LETTER**

Declines in insectivorous birds are associated with high neonicotinoid concentrations

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Recent studies have shown that neonicotinoid insecticides have adverse effects on non-target invertebrate species[1, 2, 3, 4, 5, 6]. Invertebrates constitute a substantial part of the diet of many bird species during the breeding season and are indispensable for raising offspring[7]. We investigated the hypothesis that the most widely used neonicotinoid insecticide, imidacloprid, has a negative impact on insectivorous bird populations. Here we show that, in the Netherlands, local population trends were significantly more negative in areas with higher surface-water concentrations of imidacloprid. At imidacloprid concentrations of more than 20 nanograms per litre, bird populations tended to decline by 3.5 per cent on average annually. Additional analyses revealed that this spatial pattern of decline appeared only after the introduction of imidacloprid to the Netherlands, in the mid-1990s. We further show that the recent negative relationship remains after correcting for spatial differences in land-use changes that are known to affect bird populations in farmland. Our results suggest that the impact of neonicotinoids on the natural environment is even more substantial than has recently been reported and is reminiscent of the effects of persistent insecticides in the past. Future legislation should take into account the potential cascading effects of neonicotinoids on ecosystems.

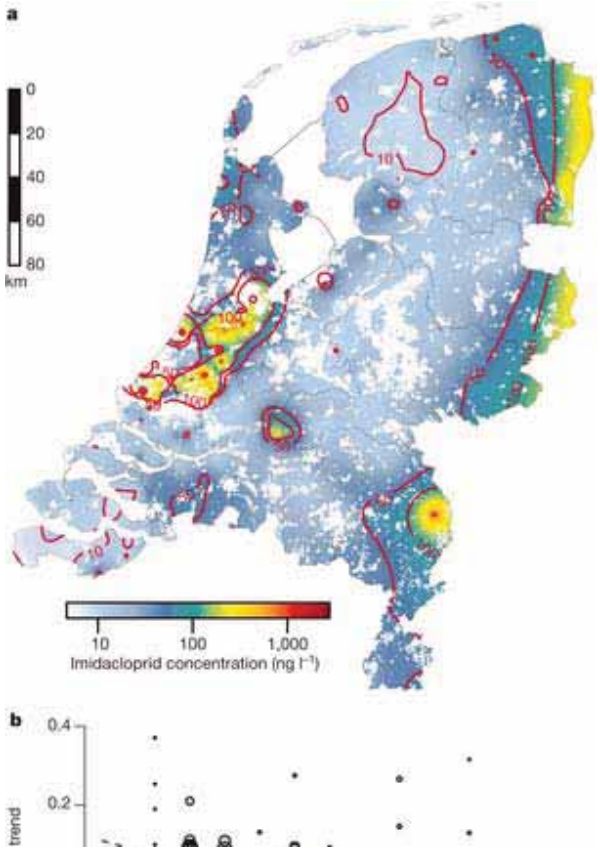
Main

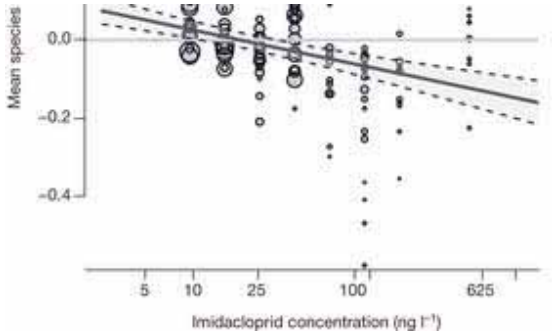
Although concerns have been raised about the direct effects of neonicotinoids on non-target vertebrate species[8], neonicotinoids are in general thought to be less harmful to mammals and birds than to insects. The main mode of action of neonicotinoids occurs through binding nicotinic acetylcholine receptors in the central nervous system of invertebrates[9], and neonicotinoids bind with substantially less affinity to these receptors in vertebrates[10]. This property has made neonicotinoids highly favoured agrochemicals worldwide over the past two decades[11]. In the Netherlands, imidacloprid was first administered by the Board for the Authorisation of Plant Protection Products and Biocides (Ctgb) in August 1994. Annual use increased rapidly from 668 kg in 1995 to 5,473 kg in 2000 and 6,332 kg in 2004 (ref. 12). Since 2003, imidacloprid has ranked consistently in the top three pesticides that exceed the environmental concentrations permitted by quality standards in the Netherlands[4, 13].

As neonicotinoids have relatively long half-lives in soil and are water soluble, they have the potential to accumulate in soils and to leach into surface water and ground water. Their systemic property (that is, their ability to spread through all of the tissues of the plants under treatment), together with their widespread use, indicates that many organisms in agricultural environments are likely to become exposed[8]. Indeed, studies have shown, both in experimental and in field conditions, that neonicotinoids may affect non-target invertebrate species across terrestrial and aquatic ecosystems[4, 5, 6]. The question remains, however, whether the effects are sufficiently severe to affect ecosystems through trophic interactions: that is, beyond the direct lethal and sublethal effects on individual species. In the past, the introduction of insecticides has caused prey-base collapses, which in turn affected avian populations[14, 15, 16], showing that pesticide-induced declines in invertebrate densities can cause food deprivation for birds. Thus, if natural insect communities are indeed affected by neonicotinoids to the extent of causing disruptions in the food chain, we may expect insectivorous bird species to be affected as well.

The present study takes advantage of two standardized, long-term, country-wide monitoring schemes in the Netherlands (see Methods)—the Dutch Common Breeding Bird Monitoring Scheme[17] and surface-water quality measurements[4]—to investigate the extent to which average concentrations of imidacloprid residues in the period 2003–2009 spatially correlate with bird population trends in the period 2003–2010. We selected 15 passerine species that are common in farmlands and depend on invertebrates during the breeding season (Extended Data Table 1 and Supplementary Methods). We interpolated concentrations of imidacloprid in surface water to bird monitoring plots (Extended Data Figs 1, 2, 3, Supplementary Data and Supplementary Methods) and examined how local bird trends correlate with these concentrations (Fig. 1).

Figure 1: Effect of imidacloprid on bird trends in the Netherlands.





a, Interpolated (universal kriging) mean logarithmic concentrations of imidacloprid in the Netherlands (2003–2009). **b**, Relationship between the average annual intrinsic rate of population increase over 15 passerine bird species and imidacloprid concentrations in Dutch surface water. Each point represents the average intrinsic rate of increase of a species over all plots in the same concentration class, whereas the size of the point is scaled proportionally to the number of species–plot combinations on which the calculated mean is based. Binning into classes was performed to reduce scatter noise and aid in visual interpretation. Actual analysis, and the depicted regression line, was performed on raw data (n = 1,459). The regression line is given by 0.1110 − 0.0374 (s.e.m. = 0.0066) × log[imidacloprid] (P < 0.0001). Dashed lines delineate the 95% confidence interval.

The average intrinsic rate of increase in local farmland bird populations was negatively affected by the concentration of imidacloprid (Fig. 1b, linear mixed effects regression (LMER): d.f. = 1,443, t = −5.64, P < 0.0001). At the separately tested individual species level, 14 out of 15 of the tested species had a negative response to interpolated imidacloprid concentrations, and 6 out of 15 had a significant negative response at the 95% confidence level after Bonferroni correction (Table 1 and Extended Data Fig. 4). Thus, higher concentrations of imidacloprid in surface water in the Netherlands are consistently associated with lower or negative population growth rates of passerine insectivorous bird populations. From our analysis, the imidacloprid concentration above which bird populations were in decline was 19.43 ± 0.03 ng l^{-1} (mean ± s.e.m.) (Fig. 1b). In areas with imidacloprid measurements above this concentration, bird populations declined by 3.5% on average annually.

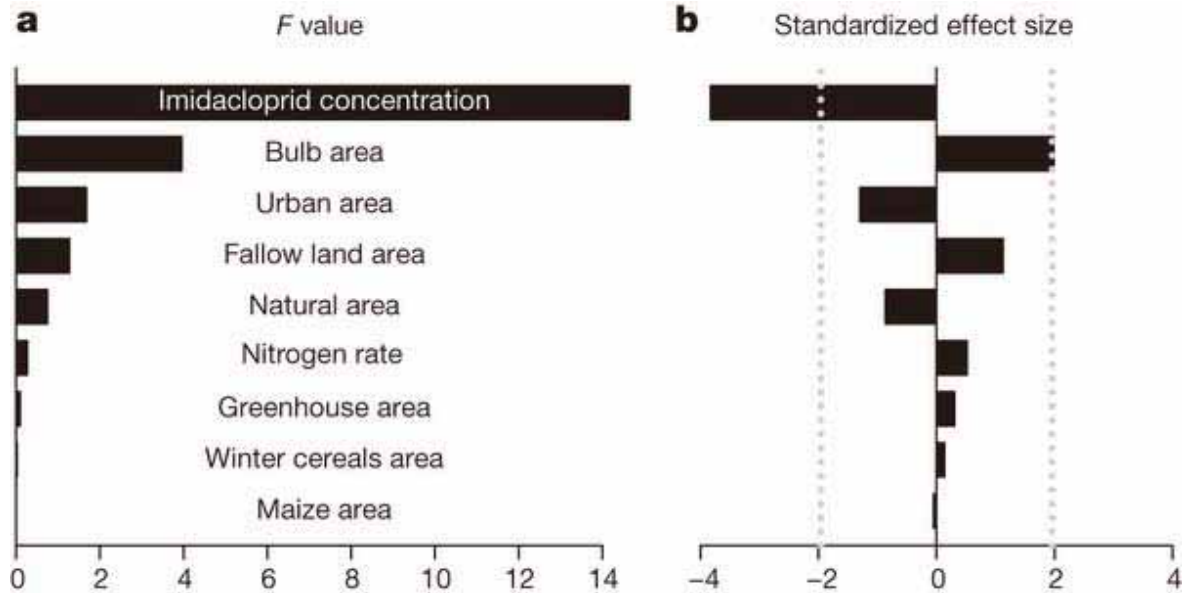
Table 1: Effect of imidacloprid on insectivorous bird species population trends

We checked whether two alternative explanations could have caused spurious correlations between imidacloprid concentrations and bird population trends over the period 2003–2010. First, it is possible that our results could simply reflect a spatial pattern of local farmland bird declines that started before the introduction of imidacloprid[18]. Therefore, we tested whether declines were present before the introduction of imidacloprid, in 1994. In contrast to the strongly negative relationship between imidacloprid concentration and bird population trends in 2003–2010 (Fig. 1b), the 2003–2009 imidacloprid concentrations were not significantly associated with bird trends in the period 1984–1995 (t = −1.43, P = 0.15 for $LMER_{<1995}$; t = −2.16, P = 0.031 for $LMER_{>2003}$; using plots only with trend data for both periods, d.f. = 365; see Extended Data Fig. 6 and Supplementary Methods). Overall, bird population trends in these two periods, paired by plot and species, were uncorrelated (r = −0.028, Pearson product moment test; t = −0.5455, d.f. = 379, P = 0.56). We can thus conclude that the spatial pattern observed does not reflect long-term ongoing local declines caused by other

factors. This finding suggests that imidacloprid is likely to have contributed to the declining population trend of the local birds.

Second, we tested whether spatial differences in land-use changes related to agricultural intensification confounded the effects of imidacloprid in our analyses. We performed multiple mixed effects regression analyses in which we included the local changes in land area use (urban area, natural area, and the production areas of maize, winter cereals and fallow land) and the amount of fertilizer applied (nitrogen in kg ha^{-1}) as fixed explanatory variables (see Supplementary Data), in addition to imidacloprid concentrations. These variables have been put forward frequently as causal factors related to farmland bird declines[19, 20, 21], although their major effect may have already occurred earlier in the twentieth century. As imidacloprid usage is likely to be related to horticulture and greenhouses[4], spatial changes in these variables may confound the effects of imidacloprid on bird trends. We therefore also incorporated changes in the area of greenhouses and the area of flower bulb production in our analysis. The results indicate that the concentration of imidacloprid and the changes in urban and natural areas were negatively correlated with local population trends, whereas the changes in the bulb and fallow land were positively correlated (Fig. 2). However, only imidacloprid and bulb area were significantly correlated with local trends (Extended Data Table 2).

Figure 2: Comparison of the effect of agricultural land-use changes and the effect of imidacloprid on bird population trends.



a, The marginal variance ratio (*F*) of each effect was estimated from a mixed effects model with all species data pooled. **b**, The standardized effect size (*t* value) for each covariate from the mixed effects model. The vertical dotted lines represent significance thresholds at $\alpha = 0.05$ (two-sided test). The imidacloprid concentrations and the proportional changes in bulb production areas were the only variables that had significant effects (LMER: d.f. = 1,349, $t = -3.825$, $P = 0.0001$ for imidacloprid; and $t = 1.989$, $P = 0.0468$ for bulbs).

So far, the suggested potential risks of neonicotinoids for birds have focused on the acute toxic effects caused by direct consumption[8]. Our results suggest another possibility: that is, that the depletion of insect food

resources has caused the observed relationships. Two lines of evidence seem to support this. First, 9 out of 15 species tested in the present study are exclusively insectivorous. All 15 species feed their young (almost) exclusively with invertebrates, and food demand is the highest in this period. Adult skylarks, tree sparrows, common starlings, yellowhammers, meadow pipits and mistle thrushes are also granivorous to some extent and may thus directly consume coated seed. However, meadow pipits and mistle thrushes forage on seeds only outside the breeding season, and for all 15 species the bulk of the diet during the breeding season consists of invertebrates[7]. Second, recent *in situ* research involving the same areas as the present study revealed strong declines in insect macrofauna, including species that have a larval stage in water, where imidacloprid concentrations were elevated[4]. These insects (particularly Diptera, Ephemeroptera, Odonata, Coleoptera and Hemiptera) are an important food source in the breeding season for the bird species that we investigated[7]. However, as our results are correlative, we cannot exclude other trophic or direct ways in which imidacloprid may have an effect on the bird population trends. Food resource depletion may not be the only or even the most important cause of decline. Other possible causes of decline include trophic accumulation of this neonicotinoid through consumption of contaminated invertebrates and, for the six partly granivorous species involved, sublethal or lethal effects through the ingestion of coated seeds[8]. The relative effect sizes of these pathways urgently need to be investigated.

Farmland birds have experienced tremendous population declines in Europe in the past three decades, with agricultural intensification as the primary causal factor[19, 20, 21, 22]. Among aspects of intensification, pesticides are known to be a major threat to farmland birds[15, 23, 24]. Neonicotinoids have recently replaced older intensively used insecticides such as carbamates, pyrethroids and organophosphates. After neonicotinoids were introduced to the Netherlands in the mid-1990s, their application was intensified, and the concentrations found in the environment frequently exceeded environmental standards, despite these concentrations being shown to have severe detrimental effects on several insect communities. Our results on the declines in bird populations suggest that neonicotinoids pose an even greater risk than has been anticipated. Cascading trophic effects deserve more attention in research on the ecosystem effects of this class of insecticides and must be taken into account in future legislation.

Methods

Data

We derived population trends for 15 insectivorous farmland passerine species (see Supplementary Data, Supplementary Methods and Extended Data Table 1 for the list of species) using long-term breeding bird data from the Dutch Common Breeding Bird Monitoring Scheme, a standardized[25, 26] monitoring scheme maintained and coordinated by Sovon, Dutch Centre for Field Ornithology, in collaboration with Statistics Netherlands[17]. The scheme has been running in the Netherlands since 1984. Data originating from these monitoring plots are generally considered to be adequately representative and reliable for population trend estimation[17, 18, 25, 27, 28]. The monitoring plots are well scattered throughout the Netherlands and range in size between 10 ha and 1,000 ha (Extended Data Fig. 2).

We used previously described information on imidacloprid concentrations in Dutch surface water[4]. This data set was collected by the Dutch waterboard authorities as part of the regular monitoring of surface-water

pesticide contamination[13] (see Supplementary Data for details). Imidacloprid concentration measurements throughout the Netherlands are available (Extended Data Fig. 1); hence, this data set is considered an adequate representation of the actual water contamination levels in the Netherlands. The geographical locations of the two monitoring programs do not generally spatially coincide. To combine the data sets, we interpolated imidacloprid concentrations from water quality measurement locations to bird monitoring plots (see Supplementary Data).

Statistical analysis

To assess the overall effects of expected concentrations on all species simultaneously, we used linear mixed effects models with species- and plot-specific population trends (intrinsic rates of increase or $log[\lambda]$) as the response, log[concentration of (interpolated) imidacloprid] as the fixed explanatory variable and species as a random factor. Additionally, we performed linear regressions of the population trends against the logarithm of the imidacloprid concentrations for each species separately using weighted least squares. The trends per plot were weighted by the mean species population size of the plot, to avoid the large influence of the demographic stochasticity of small populations. Population trends were calculated as the slope of log[territory counts] versus year of sampling (that is, a continuous trend) (see Supplementary Data). Regressions were performed using all monitoring plots located less than 5 km between the edge of a plot and an imidacloprid measurement location. This cut-off point of 5 km balanced the preferable proximity between bird and imidacloprid measurements with the amount of data retained in the analyses. However, regardless of how we varied the cut-off value between 1 and 25 km (that is, including between 7% and 99% of the bird monitoring plots, respectively), the effect size of imidacloprid on bird population trends remained strongly significantly negative (see Supplementary Methods and Extended Data Fig. 5). We examined potential confounding of the spatial imidacloprid concentrations with several different candidate explanatory variables that have been postulated as possible causes of farmland bird declines[19] and that are relevant to the Netherlands[17]. We used eight variables[12] that are potentially confounded with the introduction of imidacloprid: namely, proportional change in the area of maize, proportional change in winter cereal cropping area, proportional change in flower bulb area, change in the amount of fertilizer application (nitrogen in kg ha^{-1}), proportional change in greenhouse area, proportional change in urban area, proportional change in natural habitat area and proportional change in fallow land area (Supplementary Data). We compared the significance of all explanatory variables using a multiple mixed effects model (with species intercept as a random effect) paired with *F* tests based on single term deletions of the full model (Fig. 2a). In addition, we compared standardized effect sizes (coefficient/s.e.m.) between explanatory variables based on single species multiple linear regression models (Fig. 2b and Supplementary Methods).

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Download references

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Contributions

C.A.H. performed the statistical analysis. C.A.H., R.P.B.F., C.A.M.v.T., H.d.K. and E.J. wrote the manuscript.

Competing financial interests

The authors declare no competing financial interests.

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Extended data figures and tables

Extended Data Figures

1. Extended Data Figure 1: Distribution of the 555 imidacloprid measurement averages over the period 2003–2009, as used in the main analysis. (471 KB)
   The data are taken from refs 4 and 13.

2. Extended Data Figure 2: Distribution of the 354 bird monitoring plots in the Netherlands. (233 KB)
   The figure depicts the spatial distribution of bird monitoring plots from which local species-specific trends were calculated.

3. Extended Data Figure 3: Spatial and serial (yearly) autocorrelation of imidacloprid measurements. (89 KB)
   a, Semivariance (dots) and Matern variogram model (fitted line) used in the interpolation of the concentrations (nugget = 0.1901, sill = 1.6989, range = 13.2 km). **b**, Serial correlation (between years) of imidacloprid concentrations. Each value gives the number of pairs of measurements at each year lag that were used to calculate the coefficients. Serial correlations remain invariant with respect to temporal lag, indicating high temporal consistency in local imidacloprid concentrations.

4. Extended Data Figure 4: Population trends as a function of imidacloprid concentration per individual bird species. (444 KB)
   The red lines depict the weighted mean trend, also given as slope coefficients (β) and with corresponding P values.

5. Extended Data Figure 5: Robustness check for the effect of the cut-off value for the distance between bird monitoring plots and water measurement locations (varied between 1 and 25 km). (106 KB)
   The larger the cut-off distance, the more species–plot annual rates of increase are retained in the analysis subset of the total database of 3,947 records (**a**) but at the cost of increased noise in the response and a decrease in the effect of imidacloprid on the bird trends (**b**). However, in all cases, the effect of imidacloprid was significant and negative ($P < 0.0001$).

6. Extended Data Figure 6: Bird species trends before and after imidacloprid introduction. (125 KB)
   Comparison of the relationship of bird species trends in the periods 1984–1995 (**a**) and 2003–2010

(**b**) with the imidacloprid concentrations in 2003–2009, based on all plots monitored in both time periods. Each point in the scatter plot represents the average intrinsic rate of increase of a species over all plots in the same concentration class. Binning into classes was performed to reduce scatter noise and aid in visual interpretation. The actual analyses and the depicted significant regression line were based on raw data. The bird trends were significantly affected by the imidacloprid concentration in 2003–2010 (t = −2.16, d.f. = 365, P = 0.031) but were not significantly affected in the period before imidacloprid administration (t = −1.43, d.f. = 365, P = 0.15).

Extended Data Tables

1. Extended Data Table 1: Species information (381 KB)

2. Extended Data Table 2: Multiple mixed effects regression of population trends (pooled over 15 species, n = 1,926) (353 KB)

Supplementary information

PDF files

1. Supplementary Information (158 KB)
   This file contains Supplementary Data, Supplementary Methods and Supplementary References.

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Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure

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Summary

1. Insect pollination is a vital ecosystem service that maintains biodiversity and sustains agricultural crop yields. Social bees are essential insect pollinators, so it is concerning that their populations are in global decline.

2. Although pesticide exposure has been implicated as a possible cause for bee declines, we currently have a limited understanding of the risk these chemicals pose. Whilst environmental exposure to pesticides typically has non-lethal effects on individual bees, recent reports suggest that sublethal exposure can affect important behavioural traits such as foraging. However, at present, we know comparatively little about how natural foraging behaviour is impaired and the relative impacts of acute and chronic effects.

3. Using Radio-Frequency Identification (RFID) tagging technology, we examined how the day-to-day foraging patterns of bumblebees (*Bombus terrestris*) were affected when exposed to either a neonicotinoid (imidacloprid) and/or a pyrethroid (λ-cyhalothrin) independently and in combination over a four-week period. This is the first study to provide data on the impacts of combined and individual pesticide exposure on the temporal dynamics of foraging behaviour in the field over a prolonged period of time.

4. Our results show that neonicotinoid exposure has both acute and chronic effects on overall foraging activity. Whilst foragers from control colonies improved their pollen foraging performance as they gained experience, the performance of bees exposed to imidacloprid became worse: chronic behavioural impairment. We also found evidence, suggesting that pesticide exposure can change forager preferences for the flower types from which they collect pollen.

5. Our findings highlight the importance of considering prolonged exposure (which happens in the field) when assessing the risk that pesticides pose to bees. The effects of chronic pesticide exposure could have serious detrimental consequences for both colony survival and also the pollination services provided by these essential insect pollinators.

Key-words: bumble bee colony, crop pollination, imidacloprid, insect pollinator, lambda-cyhalothrin, neonicotinoid, pyrethroid

Introduction

Understanding and mitigating the causes of global insect pollinator declines has important consequences for food security and the global economy (Kremen & Ricketts 2000; Biesmeijer *et al.* 2006; Potts *et al.* 2010). Insect pollinators not only provide an essential ecosystem service for maintaining healthy and diverse wild plant populations (Ollerton, Winfree & Tarrant 2011), but also ensure effective pollination of *c.* 75% of agricultural crop species with an estimated global economic value of over $150 billion per annum (Gallai *et al.* 2009; Hein 2009). Social bees (honeybees, bumblebees and stingless bees) are key insect pollinators (Greenleaf & Kremen 2006; Winfree *et al.* 2007, 2008), so it is particularly worrying that populations have experienced significant declines in recent years (Oldroyd 2007; vanEngelsdorp *et al.* 2008; Goulson, Lye & Darvill 2008; Brown & Paxton 2009; Cameron *et al.* 2011; Burkle, Marlin & Knight 2013). Multiple factors have been implicated as causes of bee declines (Vanbergen *et al.* 2013) including habitat loss (e.g. Carvell *et al.* 2006; Kremen *et al.* 2007), pathogens and disease (e.g. Cox-Foster

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et al. 2007; Cameron *et al*. 2011; Meeus *et al*. 2011) and pesticides (e.g. Thompson 2001; Desneux, Decourtye & Delpuech 2007).

Although the risks posed by a 'pesticide exposure landscape' (Osborne 2012) have received a great deal of recent interest, we know comparatively little about the possible impacts that such chemicals may be having on individual bees, their colonies and populations (Godfray *et al*. 2014). As current agricultural practices rely heavily on pesticides to sustain high crop yields, insect pollinators can be exposed to multiple chemicals in the environment. Bees foraging on treated crops are exposed to pesticides both when they touch flowers (topical exposure) to extract nectar or pollen and when consuming these floral rewards (oral exposure). When these bees return to their nest, with pesticide residues on their cuticle and/or in the nectar or pollen they are carrying, other colony members (workers, males and the queen) and brood are also likely to be exposed. Indeed, recent studies report more than 30 different pesticides inside individual honeybee (*Apis mellifera*) colonies (Johnson *et al*. 2010; Mullin *et al*. 2010) and neonicotinoid residues in the nectar stores of bumblebee (*Bombus terrestris*) colonies placed in the field next to oilseed rape fields grown from untreated seed (Thompson *et al*. 2013). The level of pesticide to which bees are exposed depends on the amount applied to the target crop. Pesticide application guidelines are currently informed by a hazard quotient based on ecotoxicological tests assessing the lethal dosage (LD_{50}) for a range of indicator taxa (including *A. mellifera* as the only bee species). The objective is to provide application guidelines that kill target pests whilst avoiding lethal effects for essential insect pollinators, such as foraging bees. A growing criticism of this risk assessment procedure is that it does not consider potential sublethal effects (Thompson & Maus 2007), in spite of a growing body of evidence indicating that pesticide exposure, at levels found in treated crops, can lead to behavioural effects in bees (see Thompson 2003; Desneux, Decourtye & Delpuech 2007; Cresswell 2011; Blacquière *et al*. 2012; Gill, Ramos-Rodriguez & Raine 2012) and/or increase their susceptibility to parasites (Alaux *et al*. 2010; Vidau *et al*. 2011; Aufauvre *et al*. 2012; Pettis *et al*. 2012; Fauser-Misslin *et al*. 2014; but see Baron, Raine & Brown 2014).

Social bees rely on the cooperation of many individuals carrying out a multitude of tasks to ensure the colony functions efficiently. Foraging is a fundamental task because colony growth relies on a continuous food supply; therefore, any factors that impair foraging behaviour may have serious consequences for colony survival (Gill, Ramos-Rodriguez & Raine 2012; Bryden *et al*. 2013) and reproduction (Whitehorn *et al*. 2012). Laboratory and semi-field studies of honeybees indicate that exposure to field-realistic pesticide concentrations can cause neuronal inactivation (Palmer *et al*. 2013), affect motor function (Williamson *et al*. 2013), learning performance (e.g. Decourtye *et al*. 2004, 2005; Williamson & Wright 2013), communication (Eiri & Nieh 2012) and also impair homing ability and foraging behaviour (e.g. Yang *et al*. 2008; Mommaerts *et al*. 2010; Henry *et al*. 2012; Schneider *et al*. 2012; Fischer *et al*. 2014). However, the vast majority of studies to date have focused on the behavioural effects that follow acute exposure (i.e. within 48 hours), yet bees in the field are likely to be exposed to pesticide residues over extended periods of time (Garthwaite *et al*. 2012a,b). Therefore, it is important for us to increase our understanding of both the potential acute and chronic effects on individuals induced by prolonged exposure to field pesticide levels.

A recent study by Gill, Ramos-Rodriguez & Raine (2012) examined the effect of chronic exposure to two pesticides (a neonicotinoid and a pyrethroid) on bumblebee (*B. terrestris*) colonies. This study used pesticide exposure levels within the range found in the field, and bees were able to forage freely for pollen and nectar in the field. Using Radio-Frequency Identification (RFID) tagging technology, they collected detailed information on when individual foragers left and re-entered each colony and the amounts of pollen collected. These data showed that overall foraging performance was impaired after prolonged pesticide exposure (4 weeks) with knock-on effects for colony growth. Whilst this study was one of the first to quantify the impact of pesticides on natural foraging behaviour in insect pollinators, it did not report the temporal dynamics of behavioural impairment, nor did it discriminate between acute and chronic exposure effects. Such information is important because it (i) improves our understanding of how persistent sublethal pesticide exposure might affect the efficiency of beneficial pollinators; (ii) identifies whether subtle pesticide induced behavioural impairments might accumulate over time; and (iii) can be used to inform risk assessment protocols about the appropriate time period over which ecotoxicological testing should be conducted to detect sublethal effects and subsequently minimize the risks of pesticide exposure for foraging bees.

Here, we present a detailed analysis of the day-to-day foraging patterns of 259 *B. terrestris* foragers (Fig. 1) from 40 colonies over 28 days in the field. In this analysis, we examine how the temporal dynamics of foraging behaviour are affected following prolonged exposure to either a neonicotinoid (imidacloprid), a pyrethroid (λ-cyhalothrin), or the combination of both pesticides. Colonies were exposed to these two commonly used pesticides at levels approximating field exposure over a 4-week period (Gill, Ramos-Rodriguez & Raine 2012). Our results provide new insights, showing that prolonged pesticide exposure has both acute and chronic effects on fundamental aspects of forager behaviour and performance.

Materials and methods

EXPERIMENTAL SET-UP

The forty *B. terrestris* colonies used in the experiment each had a queen and an average of four workers (range = 0–10) at the start

Fig. 1. *Bombus terrestris* worker foraging on a Dahlia flower (photo: RJG).

of the experiment (day-0). These colony sizes reflect a realistic developmental stage of natural colonies when many agricultural crops come into flower in Europe (see Thompson 2001; Brittain & Potts 2011) and when the majority of pesticide treatments are applied (March to June: Garthwaite *et al*. 2012a,b). We used a split block design to control for variation in colony size. Before the experiment began, we ranked colonies by size according to the number of workers and pupae present, with the four highest ranked (largest) colonies being assigned to block 1, the next four highest ranked to block 2, and so on. Within each block, the four treatments [*n* colonies: control = 10; imidacloprid (*I*) = 10; λ-cyhalothrin (*LC*) = 10; imidacloprid and λ-cyhalothrin: mixed (*M*) = 10] were randomly assigned among the four colonies, and we confirmed there was no significant difference among treatments in colony size (Kruskal–Wallis: $H = 1{\cdot}79$, $P = 0{\cdot}62$).

Colonies were each housed in a two-chambered wooden nest box (28 × 16 × 11 cm). The rear chamber housed the nest (the 'brood chamber'), and a front chamber was used for pesticide exposure (the 'food chamber'). Colonies were kept at room temperature in a naturally lit laboratory throughout the experiment (although the brood chamber was covered when not being observed to mimic the darkness of a subterranean nest). Nest boxes were connected to the outside environment through an outlet tube leading to an exit hole in the laboratory window, allowing natural foraging behaviour. The laboratory is situated on the Royal Holloway University of London campus in Egham, Surrey (a 135 acre parkland site containing a diversity of wild and horticultural flowers), with further parkland areas, abundant privately owned gardens and some agricultural land adjacent to the campus within flight range of *B. terrestris*. Running the experiment from July onwards, however, minimized worker exposure to pesticides in the environment outside the laboratory as application to flowering crops visited by bees is low at this time of year (Garthwaite *et al*. 2012a,b), and the agricultural land within bumblebee flight range of campus did not contain a flowering crop during the experimental period.

Between the outlet tube and nest box were three sections of transparent Perspex tubing allowing us to observe the bees as they left or entered the nest box (setup described in Gill, Ramos-Rodriguez & Raine 2012). Between these three tube sections were two RFID readers that automatically monitored the passage of all tagged workers as they entered and left the colony. Two RFID readers were required per colony to establish whether the bee was entering or leaving the nest box, recording the tag (bee) ID number and exact time it passed underneath with at least 99% accuracy (Molet *et al*. 2008), with minimal disturbance to natural foraging patterns.

PESTICIDE TREATMENT

In the food chamber was a gravity feeder (used for the sucrose treatment) placed on a petri dish (90 mm diameter) lined with filter paper (used for the spray treatment). Bees did not have to collect sucrose solution from the feeder as they had free access to collect nectar from flowers in the field; nor did bees have to walk over the filter paper lining the petri dish as they had enough room to walk around the dish. Thus, all bees could choose to ignore the filter paper and sucrose solution feeder.

The feeder contained either a control sucrose solution (control and *LC* colonies) or 10 ppb imidacloprid sucrose solution (*I* and *M* colonies). This concentration falls within the range found in the pollen and nectar of flowering crops visited by bees (also see Gill, Ramos-Rodriguez & Raine 2012). During the experiment, the sucrose treatment was applied every 2 days (or 3 days over the weekends) between 13:00 and 14:00 ($n = 12$ feeder replenishments per colony during the 28-day period). We provided 10 mL of sucrose treatment per application in week 1, with a 2 mL increment at the start of each subsequent week (week 2 = 12 mL, week 3 = 14 mL and week 4 = 16 mL) to reflect an increase in colony demand as they developed. Before sucrose feeders were refilled, they were thoroughly rinsed and dried to remove any remaining residues.

The spray treatment was applied using a hand sprayer following the E.P.A. OPPTS 850:3030 application guidelines (http://www.regulations.gov/#!documentDetail;D = EPA-HQ-OPPT-2009-0154-0017). The filter paper received $0{\cdot}69 \pm 0{\cdot}05$ mL of either a control solution (control and *I* colonies) or a 37·5 ppm λ-cyhalothrin solution (*LC* and *M* colonies), the maximum label-guidance concentration for spray application to oilseed rape in the UK. Spray treatments were applied once at the start of each experimental week using a new piece of filter paper for each application. This follows label guidance for the minimum time period between re-applications of λ-cyhalothrin to crops (i.e. at least 7 days between spraying events and a maximum of four applications within the flowering season).

OBSERVATIONS AND MEASUREMENTS

Colony inspections, feeding and monitoring foraging performance

Colonies were inspected once per day from Monday to Saturday to assess the number of newly eclosed (callow) workers, the number of dead workers (removed and frozen) and queen condition. All dead workers and newly eclosed males ($n = 4$ males) were removed and frozen (−20 °C). The volume of sucrose solution we provided colonies was *c*. 50% of the sugar that would be typically brought back by foragers (assuming colonies having an average of 9–10 foragers and each foraging for 8 h day^{-1}: Peat & Goulson 2005; Raine & Chittka 2008; Gill, Ramos-Rodriguez & Raine 2012). Additionally, colonies were not provided with any pollen during the experiment. Therefore, bees had to collect all their pollen and *c*. 50% of their nectar (sugar) from real flowers in the field.

All workers present at the start of the experiment (precise age unknown) were individually tagged with RFID transponders glued

to the dorsal part of the thorax (for details see Supporting Information). Similarly, during the experiment, all newly eclosed workers were tagged within 1–3 days of eclosion (precise age known). In total, 854 workers were tagged, with each tag providing a unique (16-digit) code for unambiguous identification. We used separate sets of equipment (forceps and marking cages) to tag and handle the bees from each treatment to prevent artificial cross-contamination. Any workers that eclosed between day 26 and day 28 (n = 206) were not tagged, because they would be very unlikely to forage before the end of the experiment (Goulson 2010). Workers that lost their tag during the experiment (n = 19 bees: 2·2% of tagged individuals) were re-tagged with a new tag as soon as tag loss was observed. We classified a foraging bout as a period of at least five minutes between a worker leaving and re-entering a colony and specified that workers must perform at least four foraging bouts during the 28-day experiment to be considered a forager (see Gill, Ramos-Rodriguez & Raine 2012). We set this threshold to ensure that our analyses only included motivated foragers (excluding workers that only explored the tube or the vicinity outside the laboratory window).

Pollen foraging was observed for 1 h day^{-1} (5 days week^{-1}) for each colony. Observation periods were always two (*c.* 16:00) and 21 h (*c.* 10:00 the following day) after treatment application/renewal. We identified which individual workers brought back pollen loads by timing when they passed underneath the RFID readers (using a stopwatch synchronized with the RFID reader), and then matching this observed time with RFID records. We scored the amount of pollen in each forager's corbiculae (pollen baskets) as none (zero), small (score = 1), medium (score = 2) or large (score = 3) relative to the size of the worker. Scoring pollen loads using this method accounted for the fact that smaller workers are unable to carry as much pollen as larger workers because they have smaller corbiculae (Goulson *et al.* 2002; Spaethe & Weidenmüller 2002). In addition, we recorded the colour of all pollen loads collected using pollen identification cards (Kirk 2010) to help identify the source.

End of the experiment

Nest box entrances were closed after dark on day 28 and the colonies frozen. Window exits remained open for a further 18 h with each outlet tube connected to an individual bottle trap to catch any returning foragers. All tagged workers present in the frozen colonies were identified using their RFID tag, and all recently eclosed (untagged) workers were assumed to have developed in the colony in which they were found. Untagged workers (those that eclosed on, or after, day 26) were assumed to have eclosed on day 26 when analysing worker size. Worker size was assessed by measuring thorax width three times per bee using digital callipers and then averaging these values.

DATA ANALYSIS

Queen loss occurred in 14 colonies, either because the queen went outside and did not return (presumed to have got lost or died whilst out foraging) or the queen was found dead inside the nest box. In 11 colonies, queen loss occurred within the first 2 weeks (mean = day 6, range = day 2–day 9). We accounted for the effect of early queen loss by considering it as a potential explanatory factor in our statistical analyses, and previous analysis showed there was no effect of treatment on the loss of queens (Gill, Ramos-Rodriguez & Raine 2012). The remaining three colonies that experienced queen loss later in the experiment (mean = day 20, range = day 16–day 23) were pooled with queen-right colonies (n = 3 + 26 = 29 colonies). Two colonies did not survive the full 28 days (WB28 and WB32, both in the M treatment group) and were deemed to have failed (see Supporting information for details). Data from these two colonies were included in our analyses until the day they failed (from day 4, we included nine, and from day 9, we included eight M colonies).

When assessing both the daily number of foragers and the number of foraging bouts each forager performed, we included all foragers that had completed at least one foraging bout on that day. For analyses of foraging bout duration, we excluded the lower and upper extreme values for each treatment group (i.e. the shortest and the longest foraging bout) for each treatment group per day to normalize the data. For the analysis of successful pollen foraging bouts (in which pollen was observed in corbiculae), we included only those bouts in which a forager returned to the same colony it left (without visiting any other in between). The rationale for this was to match the bout duration from RFID records to the size of pollen load collected. However, when analysing the colour of pollen loads, we included all successful foraging bouts. Body size could only be obtained from individuals either present in colonies at the end of the experiment or found dead during the experiment.

The first step of the data analysis was to examine trends in foraging behaviour within each treatment group over the course of the experiment. To do this, we examined the relationship between specific foraging performance measures (number of foragers, number of foraging bouts and bout duration per day) and the time since the start of the experiment for all colonies within a treatment. Trends across treatment groups were then explored by comparing their respective regression slopes (β) from a linear regression. For analysis of pollen foraging, we calculated the mean size of pollen loads each forager collected per day. As pollen load size was scored on a four-point scale (0, 1, 2, and 3), we used a Spearman's rank correlation for each treatment. These analyses were carried out in MINITAB (v.13; State College, PA, USA).

To investigate potential differences over time among treatments, we carried out a linear mixed effects model (LMER function; R Core Development Team) with treatment (categorical), queen loss (categorical) and day (integer) as fixed factors, and block as a random factor. This analysis focused on weekly time points (week 1 = days 1–7; week 2 = days 8–14; week 3 = days 15–21; week 4 = days 22–28) because daily analysis of foraging behaviour is susceptible to natural stochastic variation in the timing of worker eclosion and forager death and/or losses outside the colony. For count data (number of foragers and foraging bouts), we used a Poisson distribution, with the P-value calculated from a Z-value. For pollen score data, we calculated the value as a proportion of the maximum possible load the forager could have collected. As the minimum load was 0 (no pollen) and maximum was 3 (large): we divided the average pollen load score by the range [=3] to give a proportional value. For our analysis of successful foraging bouts the minimum load considered in the analysis was small (score = 1) and the maximum load was large (score = 3). To obtain proportional values we subtracted 1 from each score, yielding adjusted values of 0 for small loads, 1 for medium loads and 2 for large loads. Taking these adjusted values, we then calculated the mean pollen load size per worker and divided this average value by the range [=2]. These proportional data were arcsine square-root transformed, and P-values from the LMER analysis were calculated from a t-value and associated degrees of freedom. Our analysis considered days to be nested within each week. For each day, we provided either a single value per colony (i.e. number of foragers), or a value per forager nested within colony (i.e. number of foraging bouts, foraging duration or pollen score per forager). To provide values for foraging bout duration, we took the average time across all foraging bouts completed by each forager per day, and to provide a load score, we calculated the mean score for all pollen loads brought back each day per forager.

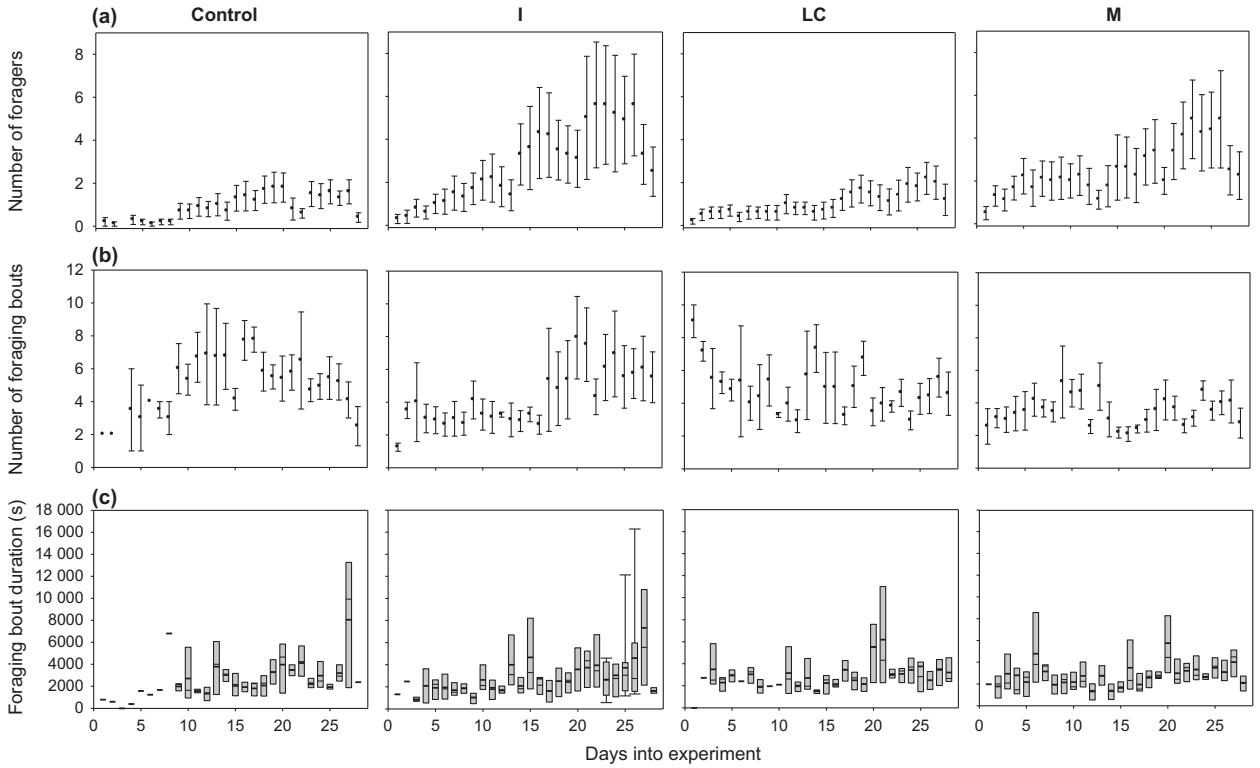

Fig. 2. Forager activity. Daily measure of foraging activity for all colonies in each treatment [left to right; control (n = 10); I = imidacloprid (n = 10); LC = λ-cyhalothrin (n = 10); M = mixed (days 1–3: n = 10; days 4–8: n = 9; days 9–28: n = 8)]. Row (a): mean (±SEM) number of foragers per colony per day. Row (b): mean (±SEM) number of foraging bouts carried out per colony (the value for each colony is the average number of foraging bouts carried out by foragers per day). Row (c): box and whisker plots (the thick and thin horizontal lines represent the mean and median values, the box indicates lower and upper quartiles, and whiskers represent 5% and 95% confidence limits) showing foraging bout duration (values per colony per day were obtained by taking the daily average duration of all foraging bouts carried out per forager, and averaging across all foragers).

Results

FORAGING ACTIVITY

Daily records of foraging activity (n = 259 foragers in total) showed a general increase in the average number of foragers per colony in all four treatments as the experiment progressed (linear regression: $F_{1,1074} = 64{\cdot}6$, $P < 0{\cdot}001$). We found that average colony size (defined as the cumulative number of workers eclosed minus those found dead) was positively correlated with the daily number of foragers as the experiment progressed (n = 40 colonies; linear regression: $F_{1,27} = 73{\cdot}4$, $P < 0{\cdot}001$; Fig. S1, Supporting information). The rate at which the number of foragers increased over time varied among treatments (Fig. 2a), with a greater rate of increase in I and M compared with LC and control colonies [linear regression with slopes (β): control: $\beta = 0{\cdot}053$, $F_{1,279} = 26{\cdot}6$, $P < 0{\cdot}001$; I: $\beta = 0{\cdot}180$, $F_{1,279} = 24{\cdot}9$, $P < 0{\cdot}001$; LC: $\beta = 0{\cdot}057$, $F_{1,279} = 25{\cdot}6$, $P < 0{\cdot}001$; M: $\beta = 0{\cdot}077$, $F_{1,234} = 11{\cdot}1$, $P = 0{\cdot}001$; Fig. S2a, Supporting information). There were already significantly higher numbers of foragers in I and M colonies compared with control colonies in week 1 of the experiment (LMER: $Z \geq 3{\cdot}44$, $P < 0{\cdot}001$; when excluding LC colonies), and these treatment differences remained significant for I and M colonies for the rest of the experiment ($Z \geq 2{\cdot}08$, $P \leq 0{\cdot}04$). In contrast, there was no significant difference between LC and control colonies in either weeks 2 or 3 ($Z \leq 1{\cdot}02$, $P \geq 0{\cdot}31$), but there was in week 4 ($Z = 2{\cdot}16$, $P = 0{\cdot}03$; see Table S1A for all analyses, Supporting information).

The number of foraging bouts carried out by foragers from control, LC and M colonies (Fig. 2b) remained relatively consistent throughout the experiment (linear regression: control: $\beta = -0{\cdot}0007$, $F_{1,110} = 0{\cdot}026$, $P = 0{\cdot}87$; LC: $\beta = -0{\cdot}004$, $F_{1,125} = 1{\cdot}50$, $P = 0{\cdot}22$; M: $\beta = 0{\cdot}0008$, $F_{1,155} = 0{\cdot}081$, $P = 0{\cdot}78$; Fig. S2b, Supporting information), whereas there was a steady increase in the daily number of foraging bouts carried out by I colonies (markedly from day 17; linear regression: $\beta = 0{\cdot}012$, $F_{1,157} = 10{\cdot}03$, $P < 0{\cdot}01$). There were no differences across all treatments in the daily number of foraging bouts performed during week 1 (LMER: $Z \leq 1{\cdot}57$, $P \geq 0{\cdot}12$). However, I foragers carried out significantly fewer foraging bouts than controls in week 2 (I: $Z = -6{\cdot}62$, $P < 0{\cdot}001$), LC foragers significantly fewer in weeks 2 and 4 ($Z \geq 2{\cdot}52$, $P \leq 0{\cdot}01$) and M foragers significantly fewer in weeks 2–4 ($Z \geq 3{\cdot}87$, $P < 0{\cdot}001$: Fig. 2b, Table S1B, Supporting information).

The average foraging bout duration increased significantly over time in all treatments (Fig. 2c; linear

regression: control: $\beta = 0{\cdot}014$, $F_{1,189} = 16{\cdot}4$, $P < 0{\cdot}001$; *I*: $\beta = 0{\cdot}013$, $F_{1,664} = 35{\cdot}1$, $P < 0{\cdot}001$; *LC*: $\beta = 0{\cdot}0050$, $F_{1,214} = 5{\cdot}3$, $P = 0{\cdot}02$; *M*: $\beta = 0{\cdot}0049$, $F_{1,155} = 7{\cdot}2$, $P < 0{\cdot}01$; Fig. S2c, Supporting information). When comparing across treatments we found that, on average, *LC* foragers carried out significantly longer foraging bouts than controls in week 1 ($t = 2{\cdot}99$, $P < 0{\cdot}01$) and *M* foragers carried out longer bouts than controls in weeks 1 and 4 ($t \geq 2{\cdot}37$, $P \leq 0{\cdot}02$: Table S1C, Supporting information).

POLLEN FORAGING

Due to the small size of colonies, we observed only 57 pollen foraging bouts during week 1 of which only two were performed by control foragers. This low control sample size meant that we could not compare pollen loads between treatments during week 1 due to lack of statistical power. The average size of pollen loads brought back by foragers (including foraging bouts with no pollen; Fig. 3a) showed no significant trend within treatment (Spearman's rank: control: 0·095, d.f. = 114, $P = 0{\cdot}31$; *I*: −0·089, d.f. = 349, $P = 0{\cdot}10$; *LC*: 0·068, d.f. = 118, $P = 0{\cdot}46$; *M*: −0·112, d.f. = 202, $P = 0{\cdot}11$). However, comparing among treatments, we found that *I* foragers collected less pollen than control foragers: although this difference was not quite significant in week 2 (LMER: $t = 1{\cdot}93$, $P = 0{\cdot}06$), *I* foragers brought back significantly less pollen in weeks 3 and 4 ($t \geq 4{\cdot}97$, $P < 0{\cdot}001$). Similarly, *M* foragers brought back significantly less pollen than controls in weeks 2–4 ($t \geq 2{\cdot}19$, $P \leq 0{\cdot}03$; Table S1D, Supporting information).

When examining the average size of pollen loads collected by 'successful' foragers (i.e. excluding all foraging bouts resulting in no pollen being brought back to the colony; Fig. 3b), we found no significant trend within treatment (Spearman's rank: control: 0·004, $P = 0{\cdot}98$; *I*: 0·15, $P = 0{\cdot}18$; *LC*: −0·20, $P = 0{\cdot}18$; *M*: 0·08, $P = 0{\cdot}54$). The only noteworthy difference among treatments was that *M* foragers brought back significantly smaller pollen loads than control foragers in week 2 (LMER: $t = 2{\cdot}64$, $P = 0{\cdot}01$; Table S1E, Supporting information). On average, successful foragers in control, *I* and *M* colonies took longer to collect pollen as the experiment progressed (linear regression: control: $\beta = 0{\cdot}016$, $F_{1,36} = 5{\cdot}4$, $P = 0{\cdot}03$; *I*: $\beta = 0{\cdot}013$, $F_{1,78} = 12{\cdot}4$, $P < 0{\cdot}01$; *M*: $\beta = 0{\cdot}008$, $F_{1,59} = 6{\cdot}1$, $P = 0{\cdot}02$; Fig. S3, Supporting information), with no change in mean bout duration in *LC* colonies

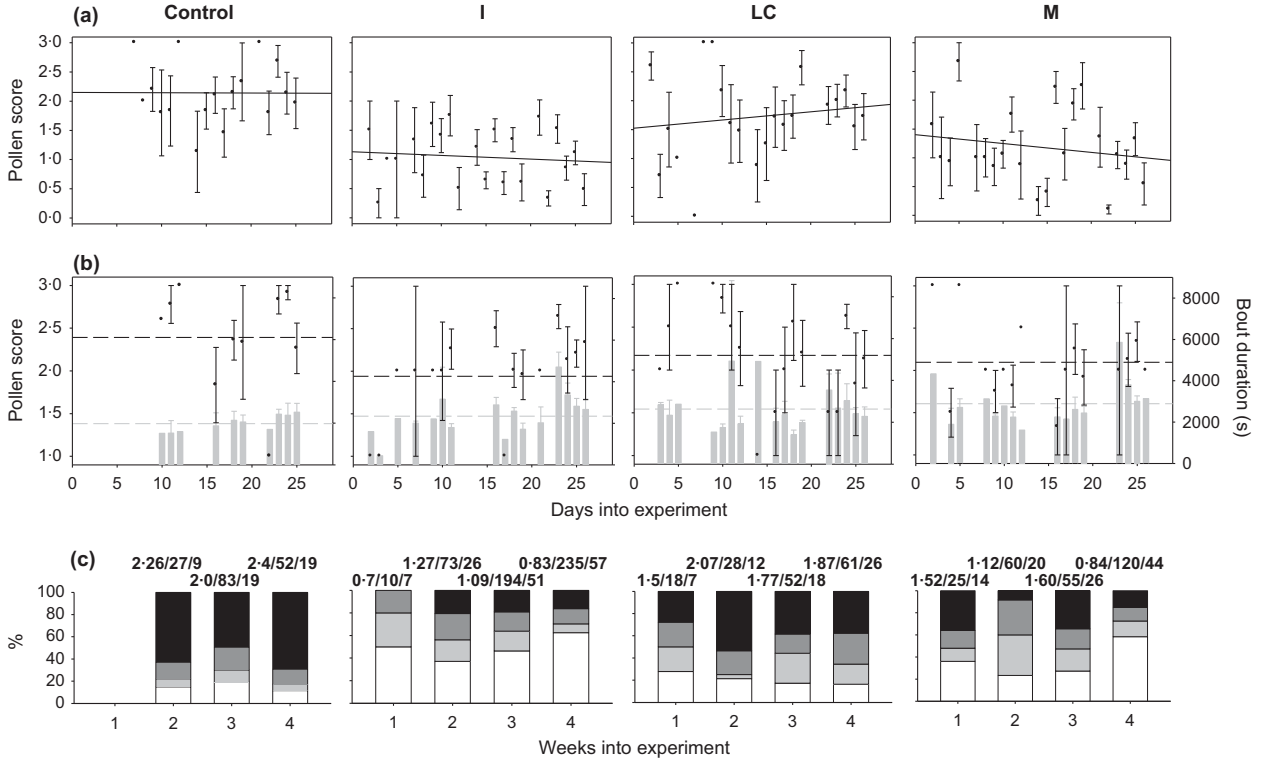

Fig. 3. Daily measures of pollen foraging performance by treatment. Row (a) Mean (±SEM) pollen load size brought back by all foragers per colony during foraging observations (total foragers/colonies: control = 30/7; *I* = 80/9; *LC* = 45/8; *M* = 75/7) with fitted regression line for comparison. Row (b) Mean (±SEM) pollen load size (scatter plot) brought back during successful pollen foraging bouts (i.e. all bouts from which bees returned with no pollen are excluded; total foraging bouts/foragers/colonies: control = 136/26/5; *I* = 243/66/8; *LC* = 129/40/6; *M* = 144/52/7). Columns represent mean (±SEM) duration of successful foraging bouts. Horizontal lines indicate mean pollen load size (black dashed line) and mean bout duration (grey dashed line) for all data points during the 4-week experiment. Rows (a) and (b): daily values for each colony were obtained by taking the mean score across all foraging bouts carried out by each forager, and then averaging across foragers. Row (c): The percentage of foraging bouts from which workers returned with either no pollen (score = zero; white), small pollen loads (score = 1; light grey), medium pollen loads (score = 2; dark grey) or large pollen loads (score = 3; black). Values above columns indicate the mean pollen score/total number of foraging bouts observed/number of foragers per week.

($\beta = 0{\cdot}00042$, $F_{1,44} = 0{\cdot}01$, $P = 0{\cdot}92$). Comparing bout durations across treatments, we found that *I* and *M* foragers did not differ from controls in either week 2 or 3 (*I*: $t \leq 1{\cdot}38$, $P \geq 0{\cdot}18$; *M*: $t \leq 1{\cdot}87$, $P \geq 0{\cdot}08$), but they took significantly longer to collect pollen in week 4 ($t \geq 2{\cdot}33$, $P \leq 0{\cdot}02$).

Compared with control, *I* and *M* foragers made 2·5 and 1·6 times more unsuccessful pollen foraging bouts in week 2, respectively, 2·4 and 1·4 times in week 3 and markedly increased to 5·4 and 5·1 more in week 4 (Fig. 3c; χ^2 test: week 2: $P = 0{\cdot}03$ and $P = 0{\cdot}36$; week 3: $P < 0{\cdot}001$ and $P = 0{\cdot}27$; week 4: $P < 0{\cdot}001$ and $P < 0{\cdot}001$; see Table S2A for all analyses, Supporting information). Furthermore, compared with control, *I* and *M* foragers brought back 3·1 and 7·6 times fewer large-sized pollen loads (score = 3) in week 2, 2·6 and 1·4 times fewer in week 3 and 4·3 and 4·6 times fewer in week 4 (Fig. 3c; χ^2 test: week 2: $P < 0{\cdot}001$ and $P < 0{\cdot}001$; week 3: $P < 0{\cdot}001$ and $P = 0{\cdot}09$; week 4: $P < 0{\cdot}001$ and $P < 0{\cdot}001$; see Table S2B for all analyses, Supporting information).

We identified 19 different colours of pollen from the 1093 loads we observed being brought into colonies during the experiment (Fig. S4, Table S3, Supporting information). Four of these colours represented 86% of all pollen loads, indicating that foragers were probably concentrating on four plant species (Fig. 4). These four colours were consistent with pollen load colours collected by honeybees from Dahlia spp. (DH), Himalayan Balsam *Impatiens glandulifera* (HB), Michaelmas Daisies *Aster* spp. (MD) and Oilseed rape *Brassica napus* (OSR). Whilst we cannot confirm unequivocally that the pollen originated from these species, we know that HB, DH and MD were flowering on the university campus. However, it is very unlikely that pollen was collected from OSR as it was not flowering during the experiment, thus we presume it came from another species that has a similar pollen colour. The percentage of foraging bouts returning with HB pollen was similar across all treatments (see Table S4A for details of analysis, Supporting information). In contrast, there was a striking difference in the preference for DH pollen across treatments: whilst it was only collected in 11% of the foraging bouts in both control and *LC* colonies, it was collected in 37% and 35% of foraging bouts in *I* and *M* colonies (control vs. *I*: $\chi^2 = 17{\cdot}1$, $P < 0{\cdot}001$; control vs. *M*: $\chi^2 = 13{\cdot}6$, $P < 0{\cdot}001$; Table S4B, Supporting information). Our results also suggest that *I*, *LC* and *M* colonies had a less strong preference for MD pollen compared with control colonies (Fig. 4; Table S4C, Supporting information), and whilst OSR pollen was hardly collected by control, *I* and *M* colonies (0–1·4% of foraging bouts), it was collected in 16% of *LC* colony pollen foraging bouts.

FORAGER AGE

Initially, we examined whether treatment affected the age at which workers first started foraging. Considering all workers that eclosed after the start of experiment, we found that the mean (±SEM) age to begin foraging across all treatments was $3{\cdot}8 \pm 0{\cdot}2$ days ($n = 214$ bees) after workers had been tagged (workers were tagged 1–3 days after eclosion) with no significant difference among treatments (mean ± SEM age in days: control = $3{\cdot}8 \pm 0{\cdot}4$; *I* = $3{\cdot}6 \pm 0{\cdot}3$; *LC* = $3{\cdot}6 \pm 0{\cdot}3$; *M* = $4{\cdot}2 \pm 0{\cdot}4$ LMER: $Z \leq 1{\cdot}32$, $P \geq 0{\cdot}19$). We then compared the total number of foraging bouts carried out by each worker and worker age when carrying out their last foraging bout to examine whether age was associated with level of foraging experience (considering only workers that eclosed after treatment started; $n = 212$ bees). We found a significant positive correlation between forager age and foraging experience either within each treatment (control: $\beta = 4{\cdot}8$, $F_{1,30} = 18{\cdot}0$, $P < 0{\cdot}001$; *I*: $\beta = 6{\cdot}0$, $F_{1,77} = 32{\cdot}6$, $P < 0{\cdot}001$; *LC*: $\beta = 2{\cdot}9$, $F_{1,39} = 14{\cdot}7$, $P < 0{\cdot}001$; *M*: $\beta = 2{\cdot}5$, $F_{1,65} = 18{\cdot}6$, $P < 0{\cdot}001$) or when analysing all foragers across treatments (linear regression: $F_{1,213} = 86{\cdot}7$, $P < 0{\cdot}001$). Subsequently, we examined whether the size of pollen load collected changed as foragers aged and whether this varied among treatments (Fig. 5). We found that older foragers from control colonies brought back significantly larger pollen loads (Spearman's rank coefficient: control: 0·19, d.f. = 112, $P = 0{\cdot}05$), whilst there was no change in load size with forager age in *LC* colonies (*LC*: 0·02, d.f. = 118, $P = 0{\cdot}81$). In contrast, we found a significant negative trend between pollen load size and forager age for both *I*

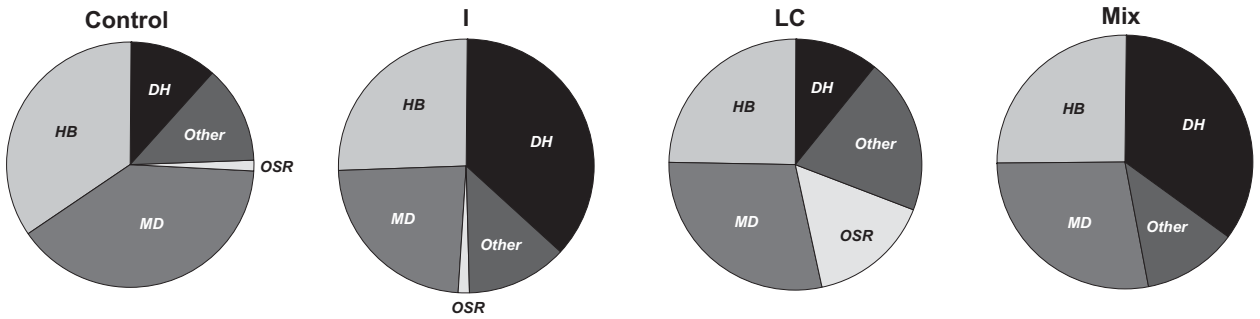

Fig. 4. Variation in pollen sources visited among treatment groups. Pie charts show the proportion of bouts in which bees visited each plant type based on pollen colour by treatment group: DH = Dahlia varieties; HB = Himalayan balsam; MD = Michaelmas daisies; OSR = Oilseed rape; Other = the 15 other identified pollen colours (see Supporting information for plant types representing 'Other').

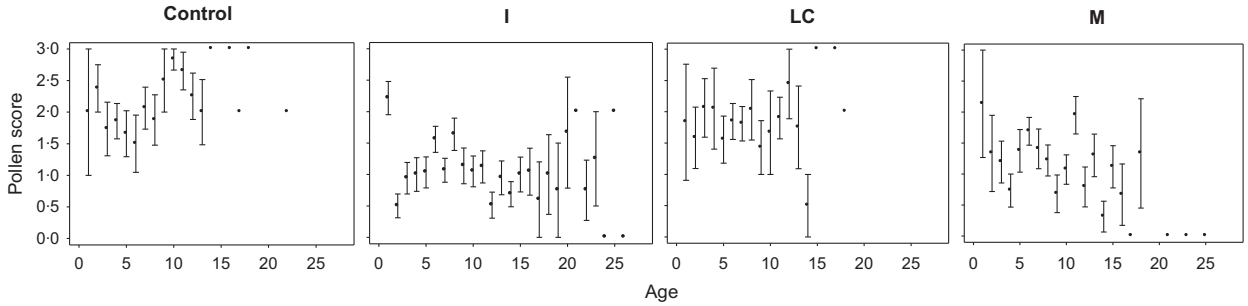

Fig. 5. Pollen foraging success as a function of forager age. Plots show the mean (±SEM) pollen score collected by foragers as they aged per treatment ['age' defined as the number of days after a worker had been Radio-Frequency Identification (RFID) tagged].

and *M* colonies (*I*: −0·12, d.f. = 343, $P = 0{\cdot}02$; *M* −0·16, d.f. = 200, $P = 0{\cdot}02$). We found a very similar pattern when we compared pollen load size with a measure of individual foraging experience (Fig. S5, Supporting information), defined by the number of days since each forager undertook its first foraging bout (Spearman's rank coefficient: control: 0·28, d.f. = 112, $P < 0{\cdot}01$; *I*: −0·09, d.f. = 343, $P = 0{\cdot}01$; *LC*: 0·046, d.f. = 118, $P = 0{\cdot}62$; *M*: −0·155, d.f. = 200, $P = 0{\cdot}03$).

WORKER SIZE

At the end of the experiment, the 40 colonies had produced 1060 workers [152 workers were present before the start ('pre-workers'), and 908 workers eclosed during the experiment ('eclosed workers')] of which we measured thorax widths for 808 individuals (67 pre-workers and 741 eclosed workers; the remaining 252 workers were either lost outside when foraging or were too decayed to measure accurately). We found no significant difference in worker body size between pre-workers and eclosed workers for control, *I* and *LC* colonies (control = 4·25 ± 0·12 vs. 4·10 ± 0·04 mm; *I* = 4·33 ± 0·11 vs. 4·23 ± 0·05 mm; *LC* = 4·42 ± 0·15 vs. 4·22 ± 0·04 mm; GLM: $F \leq 3{\cdot}31$, $P \geq 0{\cdot}07$), but in *M* colonies pre-workers were significantly larger than eclosed workers (mean ± SEM thorax width: 4·58 ± 0·09 vs. 4·16 ± 0·05 mm; GLM: d.f. = 1, $n_1 = 19$, $n_2 = 156$, $F = 8{\cdot}44$, $P < 0{\cdot}01$). There was no difference in the size of either pre-workers (LMER: $t \leq 0{\cdot}94$, $P \geq 0{\cdot}35$) or eclosed workers (LMER: $t \leq 1{\cdot}61$, $P \geq 0{\cdot}11$) among treatments. Intriguingly, however, eclosed worker size was more variable towards the end of the experiment compared with the start (coefficient of variation for workers that eclosed between days 1–7 vs. days 22–28: control = 0·130 vs. 0·182; *I* = 0·102 vs. 0·156; *LC* = 0·105 vs. 0·159; *M* = 0·124 vs. 0·152; also see Fig. S6, Supporting information).

We also examined whether the size of workers that became foragers varied as the experiment progressed (considering only eclosed workers; $n = 144$ foragers available to measure after the experiment). We found that average forager size increased as the experiment progressed in *I* and *LC* colonies (linear regression: *I*: $\beta = 0{\cdot}034$, $F_{1,32} = 4{\cdot}48$, $P = 0{\cdot}042$, *LC*: $\beta = 0{\cdot}021$, $F_{1,34} = 8{\cdot}42$, $P < 0{\cdot}01$). Forager size showed a positive (though not significant) trend over time in control colonies ($\beta = 0{\cdot}030$, $F_{1,43} = 3{\cdot}29$, $P = 0{\cdot}08$), with no clear trend for *M* colonies ($\beta = 0{\cdot}011$, $F_{1,31} = 0{\cdot}75$, $P = 0{\cdot}39$). There was also no significant difference when comparing forager size across all treatments per week [LMER: week 2: $t \leq 1{\cdot}23$, $P \geq 0{\cdot}23$; week 3: $t \leq 0{\cdot}89$, $P \geq 0{\cdot}38$; *NB* foragers that eclosed during weeks 1 and 4 could not be compared due to low sample size ($n = 20$ and 12 foragers available)].

Discussion

Our analyses provide valuable information about the acute and chronic effects of pesticide exposure on the temporal dynamics of bumblebee (*B. terrestris*) foraging in the field. Initial exposure to the neonicotinoid and pyrethroid pesticides (when colonies were at an early stage of development) had subtle, but detectable, effects on pollen foraging behaviour. However, prolonged exposure to these pesticides, particularly the neonicotinoid (imidacloprid), also resulted in significant chronic impairment of individual foraging performance.

We found that as colonies grew, the number of foragers per colony increased in all treatments; perhaps, an expected result given that colony growth increases both food demands (Pelletier & McNeil 2004; Lopez-Vaamonde *et al.* 2009) and the number of workers potentially available for foraging. However, colonies exposed to imidacloprid (*I* and *M*) had significantly higher numbers of foragers compared with control colonies in all 4 weeks of the experiment. A possible explanation could be that individual foragers were carrying out fewer foraging bouts, and subsequently colonies responded by recruiting more foragers to make up for this shortfall in food intake rate. Whilst *M* foragers did carry out significantly fewer foraging bouts than control foragers in weeks 2, 3 and 4, we found no such difference between *I* and control foragers throughout the whole experiment. These observations support the view that the increase in the number of workers going out to forage in *I* and *M* colonies during the early stages of this experiment is likely due to an acute effect of imidacloprid exposure on worker activity, rather than a colony response (increased worker recruitment). In other

words, it suggests that imidacloprid-exposed workers have a greater 'desire' to go out and forage. Imidacloprid is known to act as a neuronal partial agonist (Deglise, Grünewald & Gauthier 2002) that can acutely increase neuronal activity (Matsuda *et al*. 2001), which may explain why we observe increased forager activity (i.e. hyperactivity: Suchail, Guez & Belzunces 2001).

We also observed that the rate at which the number of foragers increased over time was greater in imidacloprid-exposed colonies (*I* and *M*) compared with control colonies, such that the number of foragers increasingly diverged from control levels during the experiment. Given that *I* and *M* colonies were similar in size to control colonies during the first two weeks and smaller during the latter 2 weeks (Gill, Ramos-Rodriguez & Raine 2012), this was not an effect due to differential colony size, showing that imidacloprid-treated colonies were allocating a higher proportion of workers to the task of foraging: an effect that became even more pronounced during the final 2 weeks of the experiment. Thus, in addition to the acute effect observed, prolonged exposure to imidacloprid appears to have a chronic effect on colony foraging activity. Given that the average age at which workers started foraging did not differ across treatments, this supports the view that imidacloprid-treated colonies were not selectively recruiting younger foragers, but recruiting a higher proportion of workers of all ages.

Our analysis also showed a decrease in pollen foraging efficiency of imidacloprid-exposed foragers (*I* and *M*), with their performance increasingly diverging away from that of control bees as the experiment progressed. On average, imidacloprid-exposed foragers brought back smaller pollen loads in week 4 than during the previous 3 weeks, suggesting this pesticide has a chronic effect on pollen foraging. Our findings support the hypothesis raised by Gill, Ramos-Rodriguez & Raine (2012), suggesting that increased forager recruitment and higher forager activity in imidacloprid-exposed colonies is a response to chronic impairment of individual pollen foraging ability.

The chronic effect on the size of pollen loads collected by foragers could be due to foraging performance of individual bees deteriorating with persistent pesticide exposure as adults, and/or that workers eclosing (and becoming foragers) later in the study were exposed for longer periods during brood development. In this study, we are unable to test the latter hypothesis because it was impossible to control the pesticide exposure for each individual (both during larval development or post-eclosion) or the age at which a forager first performed a foraging bout. However, our results provide support for the former hypothesis as prolonged adult pesticide exposure did significantly affect pollen foraging performance. In control colonies, foragers brought back larger pollen loads per bout as they got older, and more experienced (also see Fig. S5, Supporting information). These findings are consistent with a previous study showing that pollen collection rate increased with each subsequent bout for *B. terrestris* workers foraging on poppy flowers in a greenhouse (Raine & Chittka 2007a). However, our data go further to show longer-term individual improvement in pollen foraging efficiency over multiple days under field conditions. However, foragers exposed to pesticides did not show the same improvement in foraging performance. The pollen loads brought back by *LC* foragers did not increase in size as foragers got older, suggesting that prolonged exposure to λ-cyhalothrin may be preventing experience-dependent improvement in pollen foraging ability. Moreover, *I* and *M* foragers brought back smaller pollen loads as they gained experience, suggesting that exposure to imidacloprid results in deterioration of foraging performance with age and/or experience.

Analysing the colour of pollen loads collected by foragers (using pollen identification cards) revealed differences among treatments in the flowers visited. We found that imidacloprid-exposed colonies (*I* and *M*) had a significantly greater preference for Dahlia varieties, and a lower preference for Michaelmas Daisy and Himalayan Balsam than control foragers (Fig. 4). Whilst this study does not allow us to pinpoint the specific mechanism(s) underlying this differential preference, we suggest that imidacloprid could be affecting either individual forager's innate preference for specific flower types or colours (Raine & Chittka 2007b) and/or could be impairing their ability to find flowers, associate floral cues (as predictors of reward) or learn the motor skills required to handle specific flower types (Raine *et al*. 2006; Raine & Chittka 2008). For example, Dahlia varieties could be more abundant, easier to find and/or easier to extract pollen from than either Himalayan Balsam or Michaelmas Daisy. These hypotheses require further investigation, but previous research has reported that exposure to imidacloprid can affect bee learning performance (e.g. Decourtye *et al*. 2004; Williamson & Wright 2013) and flight ability and foraging behaviour (e.g. Yang *et al*. 2008; Mommaerts *et al*. 2010; Henry *et al*. 2012; Schneider *et al*. 2012; Fischer *et al*. 2014) all of which are important for successful foraging.

Whilst the size of workers was not affected by exposure to either imidacloprid or λ-cyhalothrin alone, the size of workers that eclosed in *M* colonies after the start of treatment with both pesticides were significantly smaller than workers present prior to pesticide application. These results suggest that multiple pesticide exposure can cause a decrease in the size of workers produced. In contrast, *B. terrestris* colonies chronically exposed to field-realistic levels of λ-cyhalothrin via spray-treated pollen, rather than walking across treated filter paper, show a significant reduction in worker body mass under *ad libitum* food conditions in the laboratory (Baron, Raine & Brown 2014). This suggests colony-level impacts of single pesticides could vary considerably depending on precise methods and profiles of exposure. Although we did not find an effect on the size of workers that became foragers during the 28 days of our experiment, the overall reduction in the size of workers eclosing in *M* colonies could eventually mean

smaller foragers being recruited later in the colony cycle. Large workers are more likely to forage, whereas smaller workers have a greater tendency to perform tasks within the nest (e.g. brood care Goulson *et al*. 2002; Jandt, Huang & Dornhaus 2009). This could in part be due to the fact that larger workers have greater visual acuity and antennal sensitivity which is important for foraging (Spaethe & Chittka 2003; Spaethe *et al*. 2007) and are able to carry much larger pollen loads per foraging trip (Goulson *et al*. 2002; Spaethe & Weidenmüller 2002). Taking this information together with our findings that *M* foragers carried out fewer foraging bouts and had chronically impaired pollen foraging ability, this suggests that multiple pesticide exposure can have a severe effect on the amount of pollen being brought into colonies after 3 or 4 weeks of exposure.

The acute and chronically impaired pollen foraging performance induced by neonicotinoid exposure shown in this study has implications for colony growth and survival. It is possible that colonies have sufficient redundancy in their worker force to be able to buffer the smaller acute effect of exposure that is either sporadic or lasts only a short time (i.e. 1–2 weeks) and/or if colonies are larger. But colonies are more likely to suffer significantly, and become more susceptible to colony failure, if exposure is persistent and/or colonies are smaller (Bryden *et al*. 2013). The increased number of foragers recruited in neonicotinoid-exposed colonies (*I* and *M*) seems to be a response to chronic impairment of the pollen foraging ability of individual bees, yet it is interesting that the rate at which forager numbers increased over time in *M* colonies was lower than for *I* colonies. A possible explanation for this is that *M* colonies were less able to recruit additional foragers compared with *I* colonies because of the additional effect(s) of λ-cyhalothrin exposure, such as a lower number of available workers due to increased mortality (Gill, Ramos-Rodriguez & Raine 2012).

In this experiment, we used early-stage colonies (containing an average of four workers) because this is the approximate size *B. terrestris* colonies are likely to be when a substantial amount of pesticides are applied to crops attractive to bees (Thompson 2001; Brittain & Potts 2011; Garthwaite *et al*. 2012a,b). Unlike perennial honeybee colonies, that overwinter as a colony and can start the spring with a work force of several thousand individuals, bumblebees have an annual life cycle in which newly produced gynes (unmated queens) emerge in the summer, mate and then hibernate alone overwinter. The following spring, these same queens must individually establish a new colony, requiring them to locate a suitable nest site, to produce and incubate at least their first batch of workers, and to forage extensively for nectar and pollen to feed themselves and their hungry offspring (Sladen 1912; Goulson 2010). In our experiment, we found that the queen from 11 of our 40 colonies went out to forage and subsequently did not return (even though workers were present in their colony nest box). In the earliest stages of nest searching and founding, queens will be flying around the landscape and are therefore likely to come into contact with pesticides when collecting nectar and pollen from treated crops. Such pesticide exposure could affect the queen's ability to return to the colony (e.g. Henry *et al*. 2012; Fischer *et al*. 2014), affect fecundity (e.g. Laycock *et al*. 2012; Elston, Thompson & Walters 2013) or impair nesting, brood rearing and/or foraging behaviour. Taking these possible impacts into consideration we might consider a lone queen performing this wide variety of tasks (without any workers to help) would be less able to buffer any detrimental effects of pesticide exposure with potentially serious consequences for future colony fitness (either through early queen loss or significant behavioural impairment).

Social bee colony (i.e. brood) development is reliant on a steady income of food from foraging workers. Pollen is the essential protein source required for brood development, in particular the rearing of gynes critical for the fitness of the colony (Sladen 1912; Free & Butler 1959). It is therefore concerning that we found a significant impact on pollen foraging performance. Indeed, just 2 weeks of imidacloprid exposure at a relatively early stage of colony development appears to be sufficient to significantly reduce the total number of gynes that were successfully reared by *B. terrestris* colonies 6 weeks later (Whitehorn *et al*. 2012). Our results provide a potential mechanism to explain these findings, and we also show that whilst imidacloprid exposure does not stop the flow of pollen into the colony, the rate at which it can be collected becomes reduced following a period of chronic exposure. Our findings also support the idea that even if colonies were able to continue recruiting foragers to compensate for impaired individual foraging efficiency, then other essential tasks may be affected. Therefore, it may not just be a lack of pollen but the knock-on effects to colony functioning as a whole, that cause reduced growth, survivorship and reproductive output in imidacloprid-exposed colonies (Gill, Ramos-Rodriguez & Raine 2012; Whitehorn *et al*. 2012; Bryden *et al*. 2013).

A concern for bees about the use of neonicotinoids is the systemic nature of their application, which means that pesticide residues are taken up by all tissues in treated plants including the nectar and pollen (Cresswell 2011; Blacquière *et al*. 2012). These residues can persist in the nectar and pollen for the entirety of the blooming period, meaning that bees are potentially exposed for long periods (likely >28 days of this study) during the year (Rortais *et al*. 2005; Halm *et al*. 2006). Moreover, neonicotinoid residues are known to be found in nearby non-agricultural plants (for example in field borders, Krupke *et al*. 2012) and have been found to persist in soils at high concentrations (see Goulson 2013). Therefore, to achieve a more complete understanding of the risk posed by specific pesticides, such as neonicotinoids, to bees (and other insect pollinators), it is imperative that we assess the exposure profile in the field. This does not simply mean measuring the concentration of pesticide to which bees are exposed at

a single time point (e.g. Mullin *et al.* 2010; Thompson *et al.* 2013), but understanding the likely frequency and duration of exposure in the field to single and multiple pesticides. Currently the honeybee is the only insect pollinator for which validated ecotoxicological testing protocols exist. Even for this species, higher tier semi-field and field studies are not designed to specifically assess potential sublethal chronic effects of plant protection products on individual bees (which could perhaps be revealed by monitoring activity patterns of individuals using RFID technology) and are unlikely to detect colony-level effects as monitoring periods during these studies are often relatively short. Pesticide regulatory bodies must consider the chronic effect of specific pesticides on foraging performance of bees (and other pollinators) not only as this is important for bee colony success, but also because it is likely have fundamental consequences for the essential pollination services they provide.

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Authors' contribution

RJG and NER designed and carried out the experiment and wrote the paper; RJG conducted data analysis; and NER conceived the project.

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Data S1. Supporting methods and results.
Fig. S1. Forager number as a function of effective colony size.
Fig. S2. Daily measures of foraging activity per colony by treatment.
Fig. S3. Daily mean duration of successful pollen foraging bouts conducted by a single forager.
Fig. S4. Weekly analysis (weeks 2, 3 and 4) of pollen collected by foragers from different plant types represented as proportions of all observed successful pollen foraging bouts.
Fig. S5. Relationship between pollen load size brought back by foragers and previous forager experience per treatment.
Fig. S6. Box and whisker plots showing thorax width of workers that were present before pesticide treatment(s) started (pre-workers), and workers that eclosed during weeks 1, 2, 3 and 4 of the experiment (eclosed workers).
Table S1. Weekly analyses: statistical outputs from a Linear Mixed Effects model (LMER) are comparisons of treatment- with control colonies ('intercept').
Table S2. Weekly analyses: statistical outputs from pairwise chi-square tests (χ^2) comparing control against each treatment (*I*, LC and *M*) groups in terms of the proportion of foraging bouts in which (A) no pollen (unsuccessful) or (B) large pollen loads (size = 3) were collected.
Table S3. Diversity and frequency of pollen types collected by foragers from each treatment group.
Table S4. Statistical outputs from chi-square tests (χ^2) showing comparisons between control and treatment (*I*, LC and *M*) colonies.

NEWS

Posted **12:01 AM**
Updated at **6:57 AM**

Maine towns on lookout for signs of mosquito-borne viruses

With positive test results for West Nile and EEE coming in from other states, some think this might be a rough year here.

BY **NORTH CAIRN** STAFF WRITER
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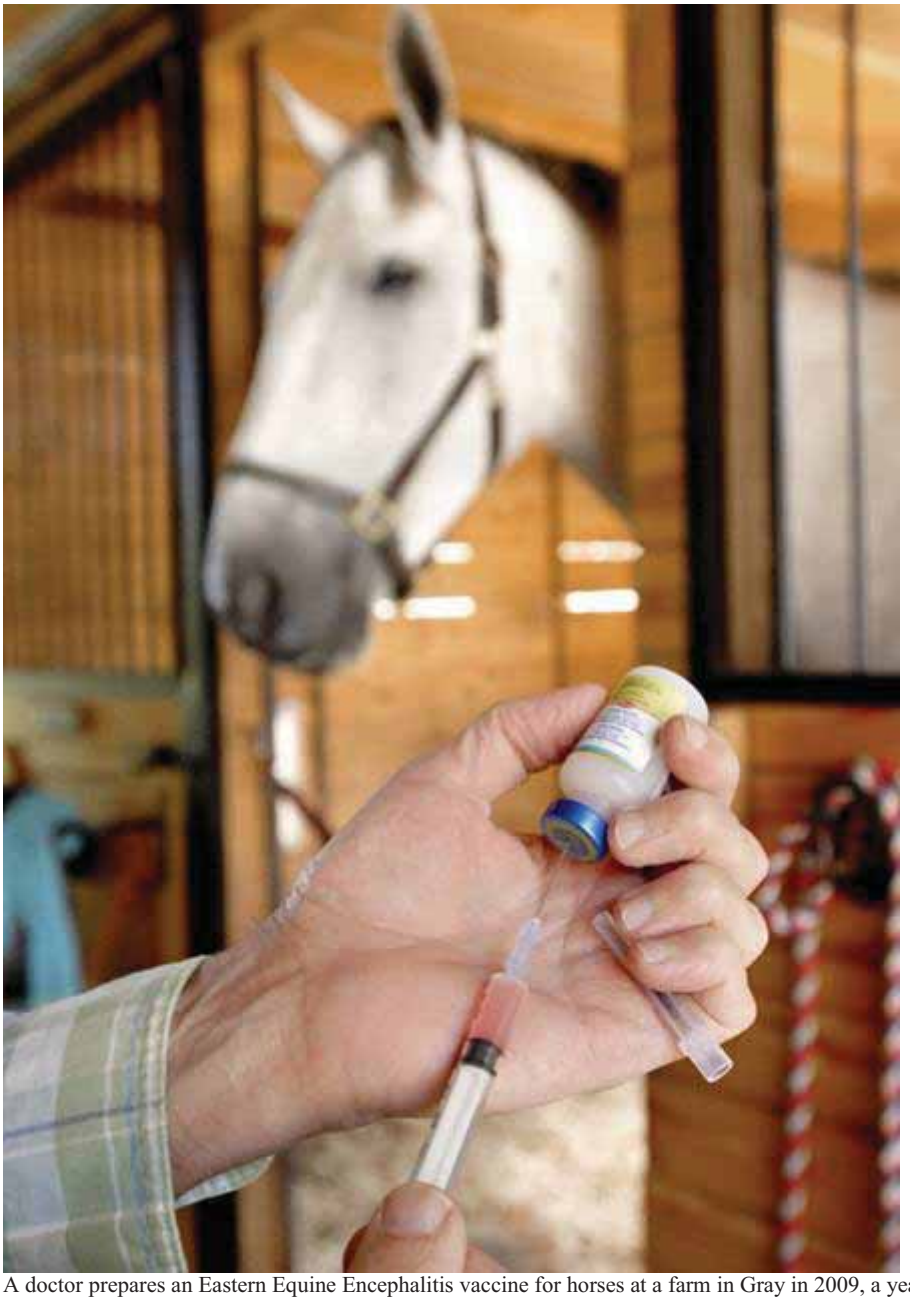
Prompted by recent positive test results for mosquito-borne diseases in other New England states, some Maine communities are gearing up for what some local officials fear might be a particularly nasty year for Eastern Equine Encephalitis and West Nile virus.

“We could be in for a bad year,” said Rob Yandow, town manager in York, where testing of mosquito pools was started June 1, about four to six weeks earlier than in many Maine towns and cities.

ADDITIONAL IMAGES



A doctor prepares an Eastern Equine Encephalitis vaccine for horses at a farm in Gray in 2009, a year when Maine experienced an unprecedented rate of EEE, which infected 19 animals. *2009 Press Herald file photo/John Patriquin*

In Maine, both viruses were first detected in 2001 in birds, and in 2012 – a particularly bad year for the viruses – the state reported the first human case of West Nile believed to be contracted here.

In New Hampshire, health officials this week identified two human cases of the mosquito-transmitted virus chikungunya in a couple who had been traveling in the Caribbean. Relatively rare and only recently detected in the U.S., this virus is seldom fatal but it can be debilitating, with symptoms that include headache, muscle pain, joint swelling and rash, according to the state's Department of Health and Human Services.

The earliest detection of EEE ever recorded in Vermont occurred June 17 at a mosquito test site in a county bordering Canada – surprising for being so early and so far north, health officials said.

High season for mosquito-borne diseases tends to run from July 15 through August, Maine health officials said. Some cases are still found in the fall before the first frost knocks down mosquito populations.

“We had a really bad year last year,” Yandow said. Tests of pools containing mosquito larvae in York last summer turned up more than two dozen positive results, the majority for EEE and about 25 percent for West Nile, he said.

“We had one mosquito pool that had both,” Yandow said.

Statewide, more than 25 testing sites are strategically located from southern Maine to Bangor, said Dr. Sheila Pinette, director of the Maine Center for Disease Control and Prevention. “We suspect that (EEE) is here already,” she said.

Over the course of the summer, she said, the state can test 1,000 pools. Test sites are chosen based on where livestock or wildlife, such as horses, deer and moose, have tested positive for viruses in the past.

In 2009, Maine experienced an unprecedented rate of EEE incidence with 19 animals and two mosquito pools testing positive for the virus. In fall 2008, a man vacationing in Cumberland County died of the disease, though it was never confirmed that he was infected while in Maine.

In 2013, Maine reported EEE in horses in Oxford and Somerset counties, as well as a horse, an emu, a pheasant and 26 mosquito pools in York County. Thirty pheasants in Lebanon died after being infected in 2012.

EEE is the more dangerous of the two viruses. It occurs in the eastern half of the nation, and causes disease in humans, horses and some bird species, according to the state CDC. Many people infected with EEE will experience no obvious symptoms. Those who do become ill may have symptoms ranging from mild-flu like illness to inflammation of the brain, coma and death. Among those developing severe cases of EEE, up to 33 percent die and most survivors suffer some brain damage, according to the federal Centers for Disease Control and Prevention.

West Nile occurs throughout the U.S., and has been prevalent in Southern states, particularly Texas, in recent years. It can cause disease in humans as well as birds and other mammals. Many

persons infected with West Nile virus will have no obvious symptoms. In those persons who do become ill, symptoms include headache, high fever, altered mental state, tremors, convulsions and rarely, paralysis. West Nile virus can also cause meningitis/encephalitis and be fatal.

“But no panic, no panic, no panic,” said Pinette. She emphasized that although the positive test result in Vermont was unusually early for that state, it reflected a consistent trend throughout New England over the past several years, with early detections found under very different conditions and habitats. In Maine, the first positive test in a mosquito pool generally is found at the end of July, she said.

Based on statewide and local history of the diseases in Maine and New England, Yandow issued what he now considers a routine precaution at the regular York Board of Selectmen’s meeting this week. The town began testing mosquito pools for EEE and West Nile virus about a month to six weeks early.

“We haven’t had a human case (ever),” Yandow said. “But it’s just a matter of time, I think.”

Other communities that have begun testing earlier than usual include Kittery, which with York is one of only two communities licensed by the state to use larvacide, a pesticide designed to kill mosquitoes before they reach their adult stage and begin to bite.

Mosquito-borne diseases are transmitted by infected insects through biting, which spreads the illnesses into human blood.

Lebanon, also in York County, is not testing or spraying at this point, said Cherry Lord, executive assistant to the city manager. “Typically, we don’t see it until mid- to late August,” she said.

Last year, Lebanon became the center of some public debate and controversy when officials authorized spraying near elementary schools as a preventive measure to protect children as young as 6 and through middle school. The spraying was deemed necessary, because the town’s two elementary schools are separated by a 100-foot-wide thicket of woods with a brook.

School officials in Lebanon were not available to comment Thursday on whether spraying might be considered this year, but the town does no testing. Only state test sites are set up there.

In nearby Sanford, testing has not yet begun, said City Manager Steven Buck. That community in the past has opted for a regional forum to promote public awareness, and in 2012 the school department sprayed around buildings and playing fields, he said.

Town and health officials emphasized the need for preventive measures in and around homes. People should avoid being outside at dawn or dusk, when the insects are especially active, and health officials recommend the proper use of insect repellents – synthetic or organic.

They advised residents to dispose of tin cans, plastic containers, ceramic pots or other water-holding containers. Leaf debris should be removed and brush trimmed to reduce mosquito-attracting habitat.

As the weather gets warmer, parents are advised to turn over plastic wading pools and wheelbarrows when they are not in use. It is recommended that birdbaths be kept clean – and empty when not in use.

Birds are also affected by the mosquito diseases. Health officials this year, as in the past, have asked that people report findings of three or more dead birds together in one location to the Maine CDC.

Parasites & Vectors IMPACT FACTOR 3.25

Research

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The effectiveness of permethrin-treated deer stations for control of the Lyme disease vector *Ixodes scapularis* on Cape Cod and the islands: a five-year experiment

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Abstract

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Background

The use of animal host-targeted pesticide application to control blacklegged ticks, which transmit the Lyme disease bacterium between wildlife hosts and humans, is receiving increased attention as an approach to Lyme disease risk management. Included among the attractive features of host-targeted approaches is the reduced need for broad-scale pesticide usage. In the eastern USA, one of the best-known of these approaches is the corn-baited "4-poster" deer feeding station, so named because of the four pesticide-treated rollers that surround the bait troughs. Wildlife visitors to these devices receive an automatic topical application of acaricide, which kills attached ticks before they can reproduce. We conducted a 5-year controlled experiment to estimate the effects of 4-poster stations on tick populations in southeastern Massachusetts, where the incidence of Lyme disease is among the highest in the USA.

Methods

We deployed a total of forty-two 4-posters among seven treatment sites and sampled for nymph and adult ticks at these sites and at seven untreated control sites during each year of the study. Study sites were distributed among Cape Cod, Martha's Vineyard, and Nantucket. The density of 4-poster deployment was lower than in previous 4-poster studies and resembled or possibly exceeded the levels of effort considered by county experts to be feasible for Lyme disease risk managers.

Results

Relative to controls, blacklegged tick abundance at treated sites was reduced by approximately 8.4%, which is considerably less than in previous 4-poster studies.

Conclusions

In addition to the longer duration and greater replication in our study compared to others, possible but still incomplete explanations for the smaller impact we observed include the lower density of 4-poster deployment as well as landscape and mammalian community characteristics that may complicate the ecological relationship between white-tailed deer and blacklegged tick populations.

Keywords: *Ixodes scapularis*; Tick; Permethrin; 4-poster; Feeding station; Host-targeted control; Lyme disease; *Borrelia burgdorferi*; *Odocoileus virginianus*; White-tailed deer; Blacklegged tick

Background

Blacklegged ticks (*Ixodes scapularis*) are the primary vector of Lyme disease between wildlife and human populations in eastern North America, so their abundance during periods of outdoor human activity is a key determinant of Lyme disease risk [1]. Methods to control this abundance are the focus of this study. Another key determinant, which we do not address, is the proportion of these ticks that are infected with the Lyme disease bacterium, *Borrelia burgdorferi.* The biology of this spirochete and the multi-host two year life cycle of blacklegged ticks have produced a highly complex ecological system that continues to challenge ecologists, public health experts, natural resource managers, integrated pest management (IPM) practitioners, and land use planners. Risk management solutions are in various stages of development, some of which require changes in land use practices or the use of biocontrol agents or pesticides that may be harmful to non-target organisms. However, because of the complexity of the Lyme disease ecological system [2] and the limitations and potentially negative impacts of sole reliance on

any single available method, it is likely that successful control strategies will require judicious application of an integrated approach consisting of multiple tactics. This necessitates knowledge about the efficacy of specific techniques in varying ecological settings.

White-tailed deer (*Odocoileus virginianus*) are important hosts for adult blacklegged ticks seeking bloodmeals, so their overabundance in the eastern US was historically assumed to be a significant determinant of Lyme disease risk [3]. Massachusetts, like other northeastern states, has seen dramatic increases in white-tailed deer populations. The Massachusetts Audubon Society estimates that fewer than 1000 white-tailed deer existed in the state in 1900; the current estimate is 90,000 (~4.5 km^{-2}) [4]. Extirpation of natural predators and increases in forage associated with forest clearing are considered the primary long-term drivers of deer overabundance, with restrictions on hunting in developed areas playing an increasingly important role (reviewed in [5]). However, there is little consensus on the feasibility or effectiveness of specific management techniques for deer population control [6]. Moreover, the mandates of private organizations and local, state, and federal managers of deer and their habitats frequently conflict in ways that complicate coordination [7]. These challenges are exacerbated by the considerable uncertainty about the impact of deer abundance on Lyme disease risk (reviewed in [2]).

As an alternative to direct population control of white-tailed deer, the use of deer-targeted pesticide application via "4-poster" feeding stations to control tick populations is now included among the risk management techniques being tested and in some cases implemented in areas of high Lyme disease incidence [8]. Because of their intended host specificity, 4-posters have the potential to reduce Lyme disease incidence as well as to reduce reliance on residential practices such as broad-spectrum acaricide application. To address the keen interest in quantifying 4-poster effectiveness, we conducted a 5-year controlled study of their effects on blacklegged tick populations on Cape Cod, Nantucket, and Martha's Vineyard, all of which are in coastal Massachusetts.

White-tailed deer frequently carry heavy burdens of adult stage blacklegged ticks seeking their final blood meal. However, blacklegged ticks have a complex life cycle involving multiple hosts (reviewed in [2]). After feeding to repletion, mated female ticks overwinter and deposit their eggs in the spring. On Cape Cod, deposited eggs typically hatch into larvae in late July and early August and then seek their first blood meal. If this search results in a blood meal from a host infected with the Lyme disease bacterium, *Borrelia burgdorferi,* and if transmission occurs, then the larva becomes infected. After feeding to repletion, larvae moult into nymphs. After overwintering, each nymph seeks a new host for what is typically the second blood meal in its life cycle. This second host is an additional opportunity for the tick to acquire the Lyme disease spirochete. In late summer, these nymphs moult into the adult stage and seek their final blood meal. All stages of feeding ticks are potentially affected by exposure of their hosts to 4-poster treatments, but this exposure is expected to be highest for adult ticks because of their relatively high abundance on large vertebrates. The impacts of 4-posters include direct mortality to larvae or nymphs attached to 4-poster visitors and reduced numbers of eggs due to reductions in adult populations. Our study was designed to estimate the magnitude of these effects by repeated sampling of nymph and adult ticks at 4-poster sites and untreated control sites.

Several previous studies have reported large reductions in tick abundance in areas treated with 4-posters relative to untreated control areas. Most notably, a coordinated six-year study in the northeastern US reported approximately 70% reduction in nymphs at the end of the study [9-13]. Only one of the five separately published studies contained independent within-site replication, so meta-analysis of the five sites became an important basis for inference about 4-poster effectiveness. Although the meta-analysis by Brei *et al*. [12] appears to have treated multiple samples from each site as statistically independent samples, the results at the northeast regional scale are compelling.

The northeast regional study deployed > 100 4-posters across its five study areas at a density of 4 to 5 stations km^{-2} (0.016 stations $acre^{-1}$). We were interested in estimating 4-poster efficacy for coastal southeastern Massachusetts and used a single controlled experiment with site replication. We expected our study to produce a geographically narrower but more statistically robust confirmation of the broader regional findings reported by Pound *et al*.[11]. In addition, we sought to refine design considerations for longer term deployment of 4-poster devices in southeastern Massachusetts. Given the rapid and dramatic effects seen in previous studies, we anticipated that 4-poster deployment at 1–2 stations km^{-2} (< 0.007 stations $acre^{-1}$), or approximately 40% of the density used in the northeast regional study, would produce measurable effects at a more feasible deployment density for area resource managers.

We conducted our 4-poster study in southeastern Massachusetts, where Lyme disease poses a serious health risk. Massachusetts ranks among the top 10 states in Lyme disease annual reporting to the US Centers for Disease Control [14]. In recent reporting, these top 10 states accounted for more than 93% of the total cases reported nationally over the 15 yr period documented in the report. Two counties in the region of southeastern Massachusetts where our study was conducted were among the top 10 counties nationally for average rate of Lyme disease incidence (reported cases) during the period 1997–2006 [14]. Our study was motivated by these factors and the need for environmentally sustainable management practices for reducing Lyme disease risk.

Methods

Deer 4-poster stations were activated in the fall of 2007 (mid August to mid November) and in spring (mid March to mid June) and fall of all subsequent years (2008–2011) at precisely the same locations each year (within 2 m of initial locations). Closure of stations during winter was partly the result of regulations prohibiting wildlife provisioning during the hunting season. At each site, multiple stations were distributed at approximately one station per 150 acres (1.65 stations km^{-2}), based in part on results from previous studies [15].

Selection of sites for this study was based on: 1) history of an active blacklegged tick population; 2) evidence of white tailed deer; 3) accessibility for maintenance and input of corn bait and permethrin; and 4) distance from residences (> 91 m). This resulted in seven treatment sites on Nantucket, Martha's Vineyard and Cape Cod (Figure 1). Comparable control sites (i.e., without 4-poster stations) were chosen based on location (> 1.6 km from treated sites), habitat, and presence of blacklegged ticks. This relatively low density of sites and of 4-poster stations within these sites (1–2 stations km^{-2}) was considered indicative of what tick control programs can realistically be expected to maintain in the study area.

Figure 1. Locations of treated sites (triangles) and controls (filled circles) in the 5-year study of 4-poster deer feeding station effects on blacklegged tick abundance. Treated sites had multiple 4-poster stations; all sites had multiple tick drag sampling locations. Site abbreviations are: SC = Shawme Crowell; BU = Burgess; BC = Bridge Creek; DP = Dennis Pond; SY = Syrjala; PH = Punk Horn; BN = Bell's Neck; JP = Jehu Pond; FM = Fulling Mill; CT = Cedar Tree Neck; SP = Sepiessa Point; CH = Chappaquiddick; LO = Loring Nature Center; and AP = Almanack Pond.

During periods of activation, each station was maintained weekly or biweekly, with corn added *ad libidum* and permethrin acaricide added to rollers at a rate of 7.5 ml per 50 lbs (23 kg) of corn consumed. Inputs to each station, including the amount of corn consumed monthly, the amount of permethrin added, the number of station visits, as well as any necessary replacements or repairs were recorded. Beginning in spring 2007 (before station deployment), nymph ticks were sampled at all treatment and control sites in May, June and July of each year using a cloth dragging procedure [16] whereby a 0.46 m^2 (50.8 × 90.4 cm) double-sided white flannel cloth was dragged along the ground at the edge of a trail or wooded road for 30 seconds at approximately one yard per second. This procedure was repeated along fixed transects in October and November of each year for collection of adult ticks. This resulted in a total of 9890 drags approximately evenly distributed

across sites (Table 1). Thus, each site was sampled 4–5 times between 1 May and 10 Nov of each year, for a total of approximately 24 sampling events (30 drags per visit per site for each site over the study period; see Table 1 for deviations). This is a relatively high sampling frequency and was intended to overcome under-sampling problems [17].

Table 1. Number of tick drag samples by treatment, site, and year for Cape Cod, Martha's Vineyard and Nantucket

For statistical analyses and prediction, we used log-linear negative binomial models with random effects (GLMM; generalized linear mixed effects models). Life stage, treatment, and time were treated as fixed effects. Each statistical formulation was fitted using either days or years elapsed since the beginning of the study. Each of the 42 transects in the study was assigned a unique ID and treated as a random effect. The random effects were modeled as effects on intercepts only and were included because of the expected correlation between repeated samples taken from each transect over the course of the study. This is intended to address microclimate or other unknown but persistent differences between sites. The negative binomial distribution was used because of the high variance to mean ratio in the data, as is common in tick sampling data due to patchy spatial distribution (see [17] for analysis of sampling implications). Because of the two-year semelparous life cycle, nymphs and adults sampled in a given year are predominantly descendents of nymphs and adults sampled two years earlier. Thus, the longest time series for a given population in our study is represented by samples from 2007, 2009 and 2011 (Figure 2). Our statistical analyses focused on these samples.

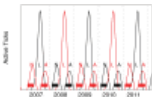

Figure 2. Diagram of sampling schedule (filled rectangles) superimposed on expected abundances of active ticks, *Ixodes scapularis*), which breeds only at the end of its two-year life cycle. Two overlapping populations are present at any given time and are represented here as different shades. N, L, and A denote periods of nymph, larval, and adult activity. Relative abundances are based on Figure eight in Ostfeld [2].

These log-linear models were used to evaluate statistical evidence for 4-poster treatment effects on nymphal and adult tick abundances and to estimate the size of these effects. Each of the candidate statistical models represented a specific hypothesized explanation of the data. Thus, the set included a 'no effects' model, a 'treatment only' model, a 'time only' model, a 'treatment + time' model, and a 'treatment × time' model. Evidence for 4-poster effects would be indicated by strong statistical support for models containing treatment effects. Support for a 'time only' model would indicate a regional change in tick abundance unrelated to 4-poster effects. Each model was fitted as a GLMM using the R implementation of AD Model Builder [18,19]. Support for each model was assessed using corrected Akaike Information Criteria (AICc; see Section 2.2 in [20]). AIC*c* weights were used to compute model-weighted predictions of tick density and unconditional standard errors for 95% confidence limits (eqn 6.12 in [20]). This so-called information-theoretic approach enables fuller extraction of the information contained in the data and allows evidence-based ranking of candidate models. When multiple models are supported (i.e., knowledge of the study system is uncertain), the final estimate of effect size (i.e., 4-poster effect) and its confidence limits incorporate the influence of all supported models. For this reason, the rejection of models via *p*-value cutoffs does not arise in our analysis.

We used Abbott's formula [21] to compute percent reduction of ticks relative to controls for comparison to other studies e.g., [13]. Specifically,

$$\mathrm{PctControl} = 100 \times \left(1 - \frac{E_{0,\mathrm{cntrl}} \times E_{t,\mathrm{trt}}}{E_{t,\mathrm{cntrl}} \times E_{0,\mathrm{trt}}}\right)$$

represents the effect of treatments between time $t = 0$ and $t = \mathrm{t}$, where E denotes mean abundance at control (cntrl) and treated (trt) sites predicted from the statistical models.

Pelage swab samples from white-tailed deer carcasses were collected prior to meat processing at hunter check-in stations on Chappaquiddick Island, Edgartown, MA, which we assume supports a closed deer population (no immigration or emigration). These samples were collected by wiping a cotton gauze pad on the neck, throat and chin area of each deer for thirty seconds. The samples were placed in amber glass vials and stored frozen. Samples were shipped on ice by overnight delivery to the Massachusetts Pesticide Analysis Laboratory for permethrin residue analysis using hexane extraction followed by gas chromatography with electron capture detection and mass spectrometry. Data from island hunters were used with these residue analyses to estimate the proportion of deer treated topically within the treatment zone.

Results

The model containing interactions between 4-poster treatment and time was the best fitting (based on log likelihood) and most parsimonious (based on AICc) of the models we used to analyze tick sampling data (Table 2). The interaction term in this model is interpreted as evidence that the treatments caused a stronger tick decline than was observed at the control sites. However, there was modest support in the data for two models without the interaction (i.e., $\Delta\mathrm{AIC}c < 2$; Table 2), leading to model selection uncertainty [20]. As a result of this uncertainty, we used AIC*c*-weighted model averaging to make predictions about treatment effects on tick abundance (Figure 3).Using Abbott's formula with the model-averaged estimates of treatment effects, our estimate of Pct Control was 8.4%, which is substantially lower than that reported for other studies. This estimate increases to 20% when only the interaction model is used by itself (rather than model-averaged estimates), but as already noted, inference based solely on this model is not supported by our data. Visual representation of aggregated drag counts (Figure 4) is consistent with the small effect detected in our statistical analyses.

Table 2. AIC statistics for models of tick treatment effects on tick drag sample abundances[1]

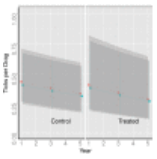

Figure 3. Predicted counts and 95% confidence limits (shaded areas) for blacklegged tick nymphs (red) and adults (blue) during 30-second drags on June 1 (nymphs) and October 1 (adults) during years 1, 3, and 5 of the study. Predictions are computed using AIC*c*-weighted averaging of all candidate log-linear generalized mixed effects models, but standard errors used for confidence intervals are based on fixed effect variance only.

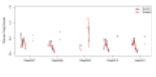

Figure 4. Graphical summary of tick drag data aggregated into means for 15-day intervals. Each point is the mean of all drags within the 15-day window for all 7 sites of the given treatment level. Average number of drags for each point is 330. See Methods section and Table 1 for additional details on distribution of sampling effort.

The rates of pesticide residue detections on pelage swabs (gauze pads; detection limit = 0.02 ug residue pad^{-1}) collected from harvested deer on Chappaquiddick

Island were 0.12, 0.69, 0.47, and 0.7 detections per deer for 2007, 2008, 2010, and 2011, respectively. The low number for 2007 and low rate of corn replenishment during station maintenance in that year suggest the possibility of a period of low deer usage during initial habitation to the station locations.

Discussion

We detected a relatively modest effect of 4-posters on blacklegged tick abundances in our coastal Massachusetts study area. Thus, our experiment supports previous findings that 4-posters reduce tick abundance, but the effect size we observed was smaller (Figure 3). Our study is the first to our knowledge that combines: 1) sampling over multiple generations and across multiple control and treatment replicates; 2) analysis of all nymph and adult tick data for a cohort population in a single count-based statistical model; and 3) detailed treatment of the repeated measures sampling design. The importance of these analytical considerations is described by Carroll *et al*. [9]. Although they dissected their analysis into separate comparisons between pairs of years, differences in effect size between our results, those of Carroll *et al*.[9] and other findings from the USDA Northeast Regional Study [11], are probably not due solely to differences in statistical methods or levels of replication. Uncertainty was also larger in our study compared with the meta-analytic results of Brei *et al*.[12], perhaps because we addressed model selection uncertainty and did not treat co-located stations or transects as statistically independent samples. Large deer home range size may reduce statistical independence of our study sites, but is considered less than 1.6 km in radius within seasons and possibly decreases in areas of high deer density (reviewed in [22]; also see [23]). Distances between our treated and control sites were always at least twice this radius, but note that any violation of the independence assumption would mean that our uncertainty estimate (i.e., the width of confidence limits in Figure 3) is too low and differences between our results and those of the USDA study may be even larger than what we have reported here. However, despite these potentially important analytical differences, we suspect that most of the difference between our results and those of others is due to the wider spacing of our 4-poster devices (1–2 stations km^{-2} vs. 4–5 stations km^{-2} in the USDA study). Other differences between studies may include deer densities and the operational periods for which the stations were maintained. Also, our study used permethrin as the acaricidal ingredient whereas the USDA study used amitraz. We are unaware of any known differences in effectiveness of these ingredients when used in 4-posters, but permethrin has been shown to be considerably more toxic than amitraz to several species of *Amblyomma* ticks [24,25].

In the region of our study, the Commonwealth of Massachusetts seeks to manage white-tailed deer abundances at a density of 6–8 deer mi^{-2} (2.8 – 3.1 deer km^{-2}), primarily through recreational hunting allowances [4]. However, significant variation in deer abundance likely exists among our three study areas (Cape Cod, Martha's Vineyard and Nantucket). Although our study was not designed to detect differences in 4-poster deer visitation among these areas, average annual corn consumption differed considerably based on rates of 4-poster replenishment (81, 182, and 326 kg $station^{-1}$ yr^{-1} for Cape Cod, Martha's Vineyard and Nantucket, respectively). Since station density was similar across sites, these consumption rates should be roughly indicative of deer density if relative corn consumption by non-target species is also similar across sites. Indeed, the State of Massachusetts estimates deer densities on Cape Cod to be much closer to its management goal than on the islands, where densities may be more than 15 deer km^{-2}[26].

Experimental exclusion of deer has been shown to affect the density of blacklegged ticks [27,28] (but see [29]), but the effects of these and other deer control experiments on human disease risk are not clear [2,30]. This is partly because deer are ineffective hosts of Lyme disease – Telford *et al*.[31] reported that only about 1% of ticks became infected after feeding on deer – and thus, as members of a larger host community, may contribute to a dilution effect on infection prevalence among questing ticks (demonstrated theoretically in [32]; empirical evidence for dilution in other disease systems is reviewed in [33]). If the role of deer in supporting tick populations is as large as commonly believed, successful management of tick abundance through technologies such as the 4-poster device could reduce the assumed need for deer eradication. However, the number of surviving, untreated deer that would be sufficient to support high tick abundance is difficult to estimate. The highest per capita deer treatment rate observed in our pelage residue samples from Chappaquiddick was 70%. Since the frequency distribution of ticks on deer is poorly known, it is possible that only a few untreated deer could weaken 4-poster effects. If such incomplete herd treatment does occur, social exclusion of subdominant individuals from feeding stations may also be important to consider (*personal communication*, M. Maquire, Cape Cod Cooperative Extension). These complexities, the existence of alternative tick hosts that might support tick abundance in the absence of deer or compensate for high mortality on treated deer, and the unknown degree to which these other hosts visit the 4-posters are all potentially important factors in the interpretation of tick abundance data such as ours.

Since there is no currently available pharmacological solution to Lyme disease, risk management focuses on reducing the likelihood of tick bites. The suite of management techniques includes modification of landscapes to reduce habitat suitability for ticks and their hosts, hunting programs to control deer populations, application of pesticides to the landscape, application of pesticides targeted to potential hosts (e.g., 4-posters) and increase of human awareness to modify behavior and promote personal protection practices. Some of these methods have been shown to affect the Lyme disease ecology (and presumably risk), but to varying degrees that depend on the ecological context, scale, and other details of the application. Landscape-scale experimental and observation programs that incorporate ecological and epidemiological approaches would help to identify those critical contextual details that should inform the balance of techniques. At that point, holistic and sustainable risk management strategies would be within reach.

Conclusions

The relatively modest effect of 4-posters on tick abundance in this five-year experiment, compared to larger effects seen in other studies, can possibly be explained by landscape characteristics, deer density and vertebrate host community composition in our study area, and the density of 4-poster stations we deployed. An important management implication is that the role of deer in the Lyme disease system may be more complicated than previously expected. It is important to weigh this possibility against concerns from the wildlife management community about the effects of wildlife provisioning and increased social contact between wildlife visitors at the 4-poster stations (e.g., wildlife disease transmission). This means that 4-posters deserve further study, experimental application, and refinement, but do not represent a low cost 'silver bullet' in the control of Lyme disease except perhaps under specific circumstances that remain to be identified. This is unsurprising given the complexity of the Lyme disease ecological system. 4-posters should be considered part of a broader suite of strategies, the most sustainable of which in the long term will embrace the strong linkages between ecological health and human disease risk and will support the differing mandates of environmental stewardship, wildlife management, and public health organizations.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors made substantial contributions to conception and design of this study and the interpretation of results. In addition, RK, BH and RH contributed to the review and oversight of field research. LD carried out and/or supervised the 4-poster station maintenance and tick and deer residue sampling. JG designed and performed the statistical analyses and modeling, prepared the manuscript and coordinated its review and revision by the participating authors, all of whom read and approved the final version.

Acknowledgments

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Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: a systematic literature review and meta-analyses

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Abstract

Demand for organic foods is partially driven by consumers' perceptions that they are more nutritious. However, scientific opinion is divided on whether there are significant nutritional differences between organic and non-organic foods, and two recent reviews have concluded that there are no differences. In the present study, we carried out meta-analyses based on 343 peer-reviewed publications that indicate statistically significant and meaningful differences in composition between organic and non-organic crops/crop-based foods. Most importantly, the concentrations of a range of antioxidants such as polyphenolics were found to be substantially higher in organic crops/crop-based foods, with those of phenolic acids, flavanones, stilbenes, flavones, flavonols and anthocyanins being an estimated 19 (95 % CI 5, 33) %, 69 (95 % CI 13, 125) %, 28 (95 % CI 12, 44) %, 26 (95 % CI 3, 48) %, 50 (95 % CI 28, 72) % and 51 (95 % CI 17, 86) % higher, respectively. Many of these compounds have previously been linked to a reduced risk of chronic diseases, including CVD and neurodegenerative diseases and certain cancers, in dietary intervention and epidemiological studies. Additionally, the frequency of occurrence of pesticide residues was found to be four times higher in conventional crops, which also contained significantly higher concentrations of the toxic metal Cd. Significant differences were also detected for some other (e.g. minerals and vitamins) compounds. There is evidence that higher antioxidant concentrations and lower Cd concentrations are linked to specific agronomic practices (e.g. non-use of mineral N and P fertilisers, respectively) prescribed in organic farming systems. In conclusion, organic crops, on average, have higher concentrations of antioxidants, lower concentrations of Cd and a lower incidence of pesticide residues than the non-organic comparators across regions and production seasons.

Key words: **Organic foods: Conventional foods: Composition differences: Antioxidants/(poly)phenolics**

Abbreviations: BS, basket study; CF, comparison of matched farms; EX, controlled field experiment; GRADE, Grading of Recommendations, Assessment, Development and Evaluation; MPD, mean percentage difference; MRL, maximum residue level; SMD, standardised mean difference.

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Increased public concerns about the negative environmental and health impacts of agrochemicals (pesticides, growth regulators and mineral fertilisers) used in crop production have been major drivers for the increase in consumer demand for organic foods over the last 20 years[1–3].

Organic crop production standards prohibit the use of synthetic chemical crop protection products and certain mineral fertilisers (all N, KCl and superphosphate) to reduce environmental impacts (nitrate (NO_3^-) leaching and P run-off and pesticide contamination of groundwater) and the risk of pesticide residues being present in crop plants[4]. Instead, they prescribe regular inputs of organic fertilisers (e.g. manure and composts), use of legume crops in rotation (to increase soil N levels), and application of preventative and non-chemical crop protection methods (e.g. the use of crop rotation, more resistant/tolerant varieties, mechanical and flame weeding, and biological disease and pest control products). However, organic standards permit the use of certain plant or microbial extract and/or mineral (e.g. Cu- and S-based) crop protection products[5,6].

As a result, organic and conventional crop production may differ significantly in crop rotation designs and fertilisation and crop protection protocols as well as in the type of crop varieties used[6–10]. Apart from minimising the risk of agrochemical residues being present in crops, the agronomic protocols used in organic farming systems may also affect mineral uptake patterns and metabolic processes in crop plants. Recent studies have shown that the switch from mineral to organic fertilisers results in significant differences in gene and protein expression patterns and, as a result, in secondary metabolite profiles; for example, approximately 10 % of proteins have been found to be either up- or down-regulated in response to contrasting fertiliser inputs in potato and wheat[10–15]. Also, a switch from pesticide-based conventional to organic crop protection protocols has been shown to have a significant, but more limited effect than fertilisation regimens, and there were some statistically significant interactions between fertilisation and crop protection protocols with respect to gene and protein expression pattern[10–15].

Over the last 20 years, a large number of scientific studies have compared the concentrations of nutritionally relevant minerals (e.g. Fe, Zn, Cu and Se), toxic metals (e.g. Cd and Pb), pesticide residues, macronutrients (e.g. proteins, fats and carbohydrates) and secondary metabolites (e.g. antioxidants, (poly)phenolics and vitamins) in crops from organic and conventional production systems (see the online supplementary material for a list of publications).

There is particular interest in antioxidant activity/concentrations, as there is strong scientific evidence for health benefits associated with increased consumption of crops rich in (poly)phenolics and other plant secondary metabolites with antioxidant activity (e.g. carotenoids and vitamins C and E)[16–18]. Most importantly, a substantial number of human dietary intervention studies have reported an increased dietary intake of antioxidant/(poly)phenolic-rich foods to protect against chronic diseases, including CVD, certain cancers (e.g. prostate cancer) and neurodegenerative diseases; a detailed description of the evidence has been given in recent reviews by Del Rio *et al.*[16] and Wahlqvist[17]. Also, these plant secondary metabolites are increasingly being recognised to contribute significantly to the health benefits associated with increased fruit, vegetable and whole grain consumption[16–18].

Several systematic literature reviews have recently analysed the available published information, using both qualitative and quantitative methods, with the aim of identifying the potential effects of organic and conventional production protocols on the nutritional quality of crops[19–21]. However, these systematic reviews (1) used different methodologies (e.g. weighted and unweighted meta-analyses) and inclusion criteria, (2) did not cover most of the large amount of information published in the last 4–5 years, (3) provided no structured assessment of the strength of the evidence presented, and (4) came to contrasting conclusions. As a result, there is still considerable controversy as to whether the use of organic production standards results in significant and consistent changes in the concentrations of potentially health-promoting (e.g. antioxidants, (poly)phenolics, vitamins and certain minerals) and potentially harmful (e.g. Cd and Pb) compounds in crops and crop-based foods[7,19–22]. However, there is increasing evidence and more widespread acceptance that the consumption of organic foods is likely to reduce exposure to pesticide residues[21,23,24].

There are major research synthesis challenges to assessing differences in crop composition resulting from farming practices. Most importantly, the studies available for meta-analyses (1) have used different experimental designs (e.g. replicated field experiments, farm surveys and retail surveys) and (2) have been carried out in countries/regions with contrasting agronomic and pedo-climatic background conditions (see the online supplementary material for a list of publications). This heterogeneity is likely to increase the amount of published data required to detect and understand variation in composition parameters resulting from the use of contrasting crop production methods. An additional problem is that many studies do not report measures of variation, which reduces the within-study power of unweighted analyses and the between-study power of weighted analyses. Weighted meta-analyses are widely regarded as the most appropriate statistical approach for comparing data sets from studies with variable experimental designs[25,26]. However, some studies have used unweighted analytical methods[19] to avoid the loss of information associated with conducting weighted meta-analyses on a subset of the available information.

Therefore, the main objectives of the present study were to (1) carry out a systematic literature review of studies focused on quantifying composition differences between organic and conventional crops, (2) conduct weighted and unweighted meta-analyses of the published data, (3) carry out sensitivity analyses focused on identifying to what extent meta-analysis results are affected by the inclusion criteria (e.g. using mean or individual data reported for different crop varieties or experimental years) and meta-analysis method (e.g. weighted *v.* unweighted), and (4) discuss meta-analysis results in the context of the current knowledge about the nutritional

impacts of compounds for which significant composition differences were detected.

The present study specifically focused on plant secondary metabolites (especially antioxidants/(poly)phenolics and vitamins), potentially harmful synthetic chemical pesticides, toxic metals (including Cd, As and Pb), NO_3^-, nitrite (NO_2^-), macronutrients (including proteins, amino acids, carbohydrates and reducing sugars) and minerals (including all plant macro- and micronutrients). Metabolites produced by micro-organisms on plants (e.g. mycotoxins) were not the subject of the present systematic literature review and meta-analyses.

Materials and methods

Literature search: inclusion criteria and search strategy

The literature search strategy and meta-analysis protocols used were based on those previously published by Brandt *et al.*[27], and flow diagrams of the protocols used are shown in Figs. 1 and 2. Relevant publications were identified through an initial search of the literature with Web of Knowledge using the following search terms: (1) organic* or ecologic* or biodynamic*; (2) conventional* or integrated; (3) names of ninety-eight relevant crops and foods (see online supplementary Table S1 for a full list). Publications in all languages, published in peer-reviewed journals, and reporting data on both desirable and undesirable composition parameters were considered relevant for inclusion in the meta-analyses. The search was restricted to the period between January 1992 (the year when legally binding organic farming regulations were first introduced in the European Union) and December 2011 (the year when the project ended) and provided 17 333 references. An additional 208 publications (published between 1977 and 2011) were found by (1) studying lists of references or (2) directly contacting the authors of the published papers and reviews identified in the initial literature search. The abstracts of all publications were then examined to determine whether they contained original data obtained by comparing composition parameters in organic and conventional plant foods. This led to the identification of 448 suitable publications. Of these, 105 papers were subsequently rejected, because reading of the full papers indicated that they did not report suitable data sets or contained the same data as other studies.

Data sets were deemed suitable if the mean concentrations of at least one mineral, macronutrient, secondary metabolite or NO_3^-/NO_2^- or the frequency of occurrence of pesticide residues in organic and conventional crops or crop-based foods were reported. Only four non-peer-reviewed papers with suitable data sets were identified but subsequently rejected, as the small number minimised any potential bias[28] from using peer review as a 'quality' selection criterion.

As a result, 343 peer-reviewed publications reporting crop composition data were selected for data extraction, of which

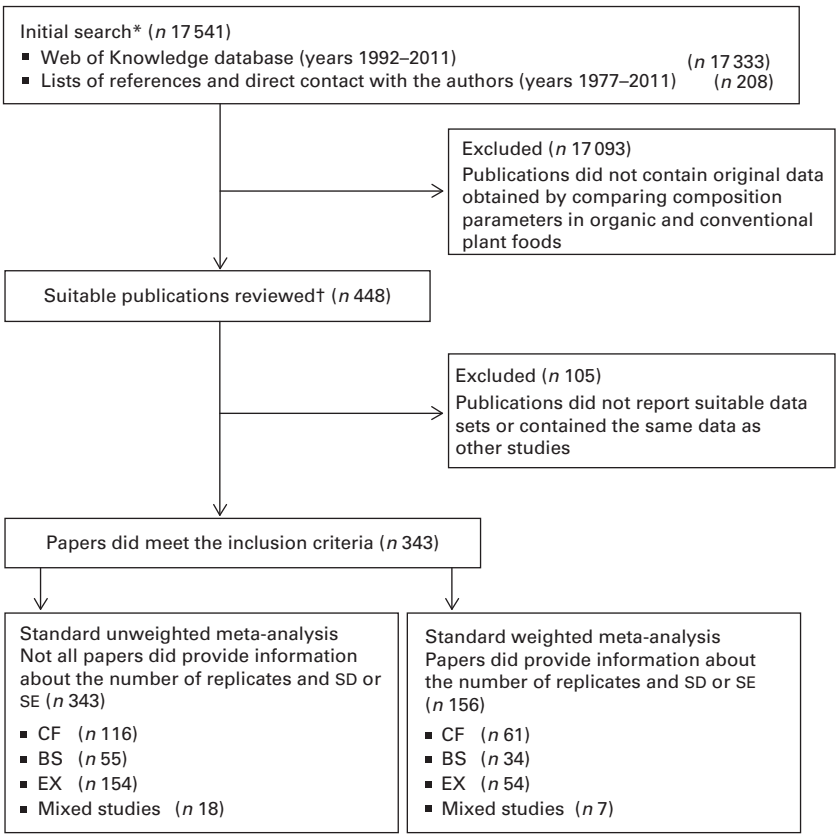

Fig. 1. Summary of the search and selection protocols used to identify papers included in the meta-analyses. *Review carried out by one reviewer; †Data extraction carried out by two reviewers. CF, comparison of matched farms; BS, basket studies; EX, controlled field experiments.

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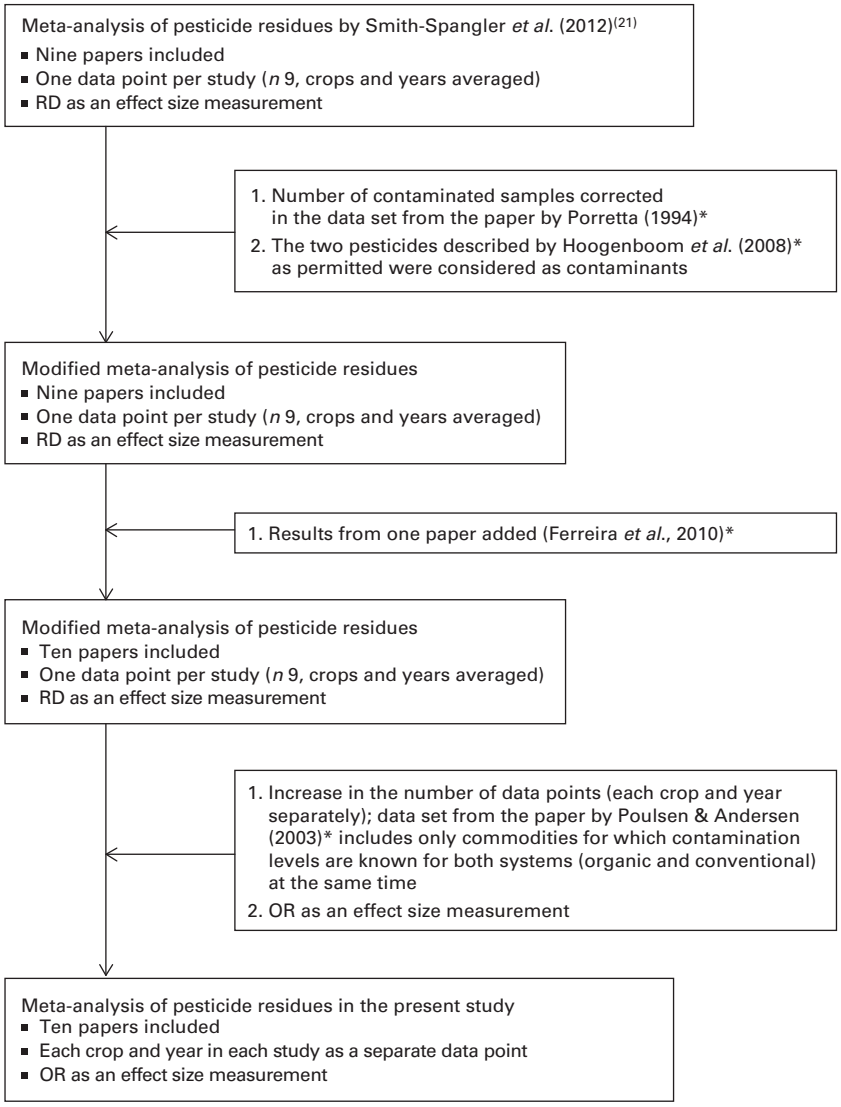

Fig. 2. Meta-analysis strategy used for the identification of data sets in the literature review. * References are summarised in Table S2 (available online). RD, risk difference.

156 references fulfilled the criteria for inclusion in the standard weighted meta-analysis and 343 fulfilled the criteria for inclusion in the standard unweighted meta-analysis. This represents a significantly greater evidence base than the three previous systematic reviews/meta-analyses of comparative crop composition data[19–21]. All publications included in these previous reviews (including studies published before 1992) were also used in the standard weighted meta-analysis carried out in the present study, except for a small number of papers that were found to report the same data as other publications that had already been included.

Data were extracted from three types of comparative studies: (1) comparisons of matched farms (CF), farm surveys in which samples were collected from organic and conventional farms in the same country or region; (2) basket studies (BS), retail product surveys in which organic and conventional products were collected in retail outlets; (3) controlled field experiments (EX) in which samples were collected from experimental plots managed according to organic or conventional farming standards/protocols. Data from all the three types of studies were deemed relevant for the meta-analyses if the authors stated that (1) organic farms included in farm surveys were using organic farming methods, (2) organic products collected in retail surveys were labelled as organic, and (3) organic plots used in EX were managed according to organic farming standards.

Several studies compared more than one organic or conventional system or treatment. For example, additional conventional systems/treatments were described as 'integrated,' 'low input,' 'low fertility' or 'extensive', and an additional organic system/treatment included in some studies was described as 'biodynamic'. Also, in some publications, organic or conventional systems with contrasting rotation designs (e.g. with or without cover crops) or fertilisation regimens (different types and levels of N inputs) were compared. In such cases, only the organic and conventional (non-organic) system identified

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by the authors as closest to the typical, contemporary organic/conventional farming system was used in the meta-analyses, as recommended by Brandt *et al.*[20]. Full references of the publications and a summary of descriptions of the studies included in the meta-analyses are given in Tables S2 and S4 (available online).

The database generated and used for the meta-analyses will be made freely available on the Newcastle University website (http://research.ncl.ac.uk/nefg/QOF) for use and scrutiny by others.

Data and information extraction and validation

Information and data were extracted from all the selected publications (see above) and compiled in a Microsoft Access database. A list of the information extracted from the publications and recorded in the database is given in Table S4 (available online).

Data reported as numerical values in the text or tables were copied directly into the database. Only data published in graphical form were enlarged, printed, measured (using a ruler) and then entered into the database as described previously[20].

Where data for multiple time points were reported, two approaches were used, depending on whether the analysed crop tissue was likely to be used as food/feed. For crops that are continuously harvested (e.g. tomato and cucumber), analytical data for mature/ripe products (e.g. fruits) collected at multiple time points during the season were averaged before being used in the standard meta-analyses; if analytical data for immature/unripe products were reported, they were not included in the mean. For crops (e.g. grape and cereals) in which products (e.g. fruits and grain) are harvested/analysed at different maturity stages, only analytical results for the mature product (that would have been used as food/feed) were used. In both the standard weighted and standard unweighted analyses, composition data reported for different cultivars/varieties and/or years/growing seasons in the same publication were averaged before being used in the meta-analyses.

Publications were assessed for eligibility and data were independently extracted from them by two reviewers. Data extracted by the two reviewers were then compared. Discrepancies were detected for approximately 2% of the data extracted, and in these cases, data extraction was repeated to correct mistakes. A list of the publications included in the meta-analyses is given in Table S2 (available online).

Study characteristics, summaries of the methods used for sensitivity analyses and ancillary information are given in Tables S2–S10 (available online). These include information on (1) the number of papers from different countries and publication years used in the meta-analyses (see online supplementary Figs. S1 and S2); (2) study type, location and crop/products assessed in different studies (see online supplementary Table S3); (3) the type of material/data extracted from the papers (see online supplementary Table S4); (4) data-handling methods/inclusion criteria and meta-analysis methods used in the sensitivity analyses (see online supplementary Table S5); (5) composition parameters included in the meta-analyses (see online supplementary Table S6); and (6) composition parameters for which meta-analyses were not possible ($n < 3$; see online supplementary Table S7).

Table S8 (available online) summarises basic statistics on the number of studies, individual comparisons, organic and conventional sample sizes, and comparisons showing statistically or numerically higher concentrations in organic or conventional crops for the composition parameters included in Figs. 3 and 4. Tables S9 and S10 (available online) summarise the numerical values for the mean percentage differences (MPD) and 95% CI calculated using the data included in the standard unweighted and weighted meta-analyses of composition parameters shown in Figs. 3 and 4, respectively (where MPD are shown as symbols).

Meta-analyses

A total of eight different meta-analyses were undertaken. The protocols used for the standard weighted and unweighted meta-analyses were based on the methodologies described by Palupi *et al.*[29] and Brandt *et al.*[20], respectively. In Fig. 3, the results obtained using standard random-effects meta-analysis weighted by inverse variance and a common random-effects variance component and unweighted meta-analysis of difference in means are shown. In addition, six sensitivity analyses were undertaken. Sensitivity analyses included (1) using data reported for each cultivar or variety of crops separately and/or (2) treating data reported for different years in the same publication as separate events in the weighted or unweighted meta-analyses (see online supplementary Table S5). The results of the sensitivity analyses are available on the Newcastle University website (http://research.ncl.ac.uk/nefg/QOF).

Effect sizes for all the weighted meta-analyses were based on standardised mean differences (SMD) as recommended for studies in which data obtained by measuring the same parameters on different scales are included in meta-analyses[25,26].

Both weighted and unweighted meta-analyses were carried out using the R statistical programming environment[30]. Weighted meta-analyses, with the SMD as the basic response variable, were conducted using standard methods and the open-source 'metafor' statistical package[31–34]. A detailed description of the methods and calculations used is given in the 'Additional Methods Description' section in the online supplementary material.

A positive SMD value indicates that the mean concentrations of the observed compound are greater in the organic food samples, while a negative SMD indicates that the mean concentrations are higher in the conventional food samples. The statistical significance of a reported effect size (i.e. SMD_{tot}) and CI were estimated based on standard methods[35] using 'metafor'[31]. The influence of potential moderators, such as crop/food type (fruits, vegetables, cereals, oil seeds and pulses, herbs and spices, and crop-based compound foods), was additionally tested using mixed-effect models[36] and subgroup analyses.

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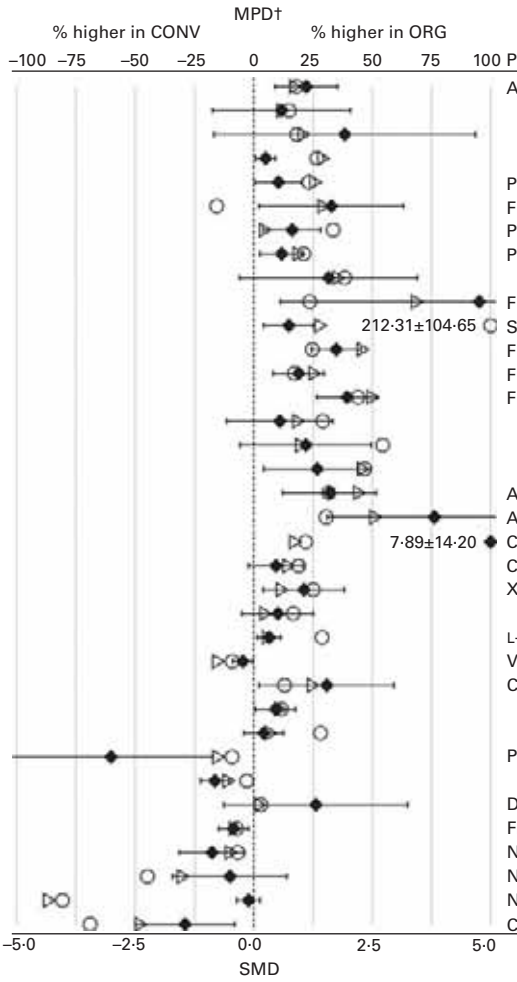


Parameters	Unweighted meta-analysis *n*	Ln ratio‡	*P**	Weighted meta-analysis *n*	*P**	Heterogeneity§
Antioxidant activity	160	4·74	<0·001	66	0·001	Yes (96 %)
FRAP	14	4·73	0·013	5	0·436	Yes (90 %)
ORAC	8	4·76	0·011	4	0·176	Yes (95 %)
TEAC	22	4·80	<0·001	7	0·030	Yes (26 %)
Phenolic compounds	129	4·74	<0·001	58	0·051	Yes (93 %)
Flavonoids (total)	20	4·54	0·282	8	0·039	Yes (89 %)
Phenolic acids (total)	9	4·85	0·008	3	0·012	Yes (86 %)
Phenolic acids‖¶	153	4·72	0·002	89	0·015	Yes (97 %)
Chlorogenic acid	24	4·84	0·009	14	0·103	Yes (98 %)
Flavanones‖¶	75	4·73	0·100	54	0·027	Yes (100 %)
Stilbenes	8	5·42	0·005	4	0·008	No (1 %)
Flavones and flavonols‖	194	4·78	<0·001	134	<0·001	Yes (97 %)
Flavones‖	27	4·72	0·063	23	0·001	Yes (85 %)
Flavonols‖¶	168	4·81	<0·001	111	<0·001	Yes (98 %)
Quercetin	23	4·79	0·028	17	0·341	Yes (95 %)
Rutin	12	4·93	0·013	9	0·127	Yes (96 %)
Kaempferol	14	4·90	0·006	13	0·023	Yes (94 %)
Anthocyanins (total)	20	4·82	<0·001	10	0·002	Yes (82 %)
Anthocyanins‖	53	4·79	0·004	22	0·001	Yes (99 %)
Carotenoids (total)	15	4·78	0·005	4	0·271	Yes (100 %)
Carotenoids‖¶	163	4·71	<0·001	82	0·126	Yes (98 %)
Xanthophylls¶	66	4·78	<0·001	33	0·018	Yes (97 %)
Lutein	21	4·74	0·019	13	0·203	Yes (90 %)
L-Ascorbic acid	65	4·73	0·005	30	0·018	Yes (76 %)
Vitamin E	25	4·56	0·300	15	0·046	No (0 %)
Carbohydrates (total)	60	4·71	0·003	16	0·037	Yes (98 %)
Carbohydrates‖¶	111	4·68	0·001	53	0·049	Yes (93 %)
Sugars (reducing)	20	4·78	0·034	3	0·360	No (0 %)
Protein (total)	87	4·53	<0·001	26	0·007	Yes (99 %)
Amino acids‖¶	332	4·58	0·001	117	<0·001	Yes (91 %)
DM¶	129	4·63	0·001	24	0·191	Yes (99 %)
Fibre	19	4·54	0·010	15	0·017	Yes (42 %)
N	88	4·55	0·001	35	0·015	Yes (97 %)
Nitrate¶	79	4·33	0·001	29	0·429	Yes (99 %)
Nitrite	15	4·17	<0·001	7	0·419	No (0 %)
Cd	62	4·25	<0·001	25	0·008	Yes (98 %)

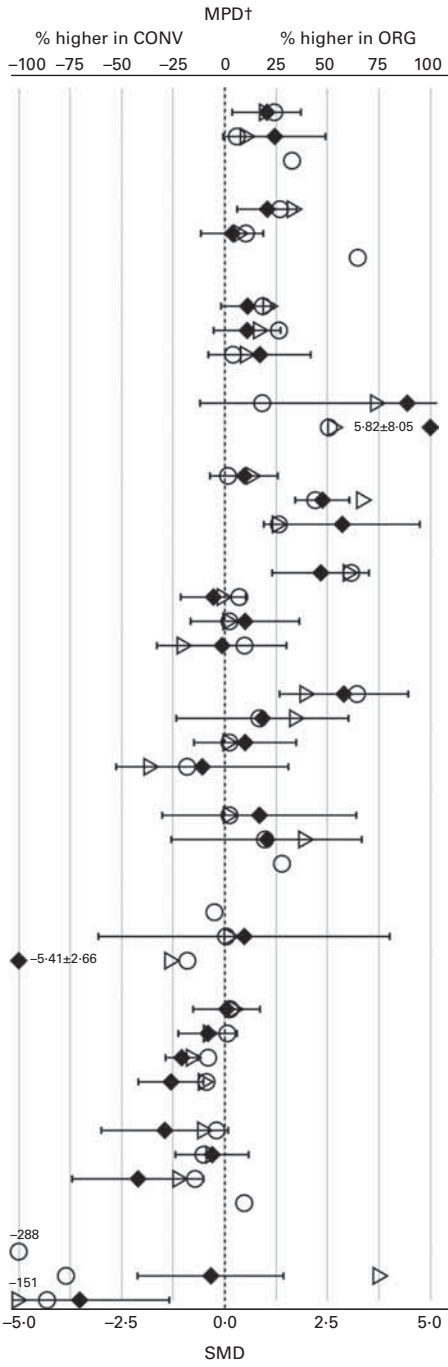
Fig. 3. Results of the standard unweighted and weighted meta-analyses for antioxidant activity, plant secondary metabolites with antioxidant activity, macronutrients, nitrogen compounds and cadmium (data reported for all crops and crop-based foods included in the same analysis). MPD, mean percentage difference; CONV, conventional food samples; ORG, organic food samples; *n*, number of data points included in the meta-analyses; FRAP, ferric reducing antioxidant potential; ORAC, oxygen radical absorbance capacity; TEAC, Trolox equivalent antioxidant capacity; SMD, standardised mean difference. Values are standardised mean differences, with 95 % confidence intervals represented by horizontal bars. * *P* value < 0·05 indicates a significant difference between ORG and CONV. † Numerical values for MPD and standard errors are given in Table S9 (available online). ‡ Ln ratio = Ln(ORG/CONV × 100 %). § Heterogeneity and the I^2 statistic. ‖ Data reported for different compounds within the same chemical group were included in the same meta-analyses. ¶ Outlying data points (where the MPD between ORG and CONV was more than fifty times greater than the mean value including the outliers) were removed. ○, MPD calculated using data included in the standard unweighted meta-analysis; ▷, MPD calculated using data included in the standard weighted meta-analysis; ◆, SMD.

We carried out tests of homogeneity (Q statistics and I^2 statistics) on all the summary effect sizes. Homogeneity was indicated if I^2 was less than 25 % and the P value for the Q statistics was greater than 0·010. Funnel plots, Egger tests of funnel plot asymmetry and fail-safe number tests were used to assess publication bias[37] (see online supplementary Table S13 for further information).

For the unweighted meta-analysis, the ratio of organic means:conventional means ($\bar{X}_O/\bar{X}_C$) expressed as a percentage was ln-transformed, and the values were used to determine whether the arithmetic average of the ln-transformed ratios was significantly greater than ln(100), using resampling[38]. The reported P values were derived from Fisher's one-sample randomisation test[39], and a $P<0{\cdot}05$ was considered statistically significant. For all composition parameters for which a statistically significant difference between organic and conventional food samples was detected in the standard weighted analysis (analysis 1), forest plots were constructed to show SMD and corresponding 95 % CI for individual studies and types of foods (see Fig. 4 and online supplementary Figs. S5–S41). In addition, the results of the standard unweighted analyses are shown in Figs. 3 and 4.

Table S12 (available online) summarises the results of the standard weighted and unweighted meta-analyses for all the composition parameters for which no analyses detected significant differences between organic and conventional products.

MPD were calculated for all parameters for which significant effects were detected by the standard unweighted and/or weighted meta-analysis protocols. This was done to facilitate value judgements regarding the biological importance of the relative effect magnitudes. A detailed description of the



Products‡	Unweighted meta-analysis *n*	Ln ratio§	Unweighted meta-analysis *P**	Weighted meta-analysis *n*	Weighted meta-analysis *P**
Antioxidant activity					
Fruits	93	4·79	<0·001	39	0·019
Vegetables	58	4·65	0·166	25	0·061
Other	5	4·89	0·030	–	–
Phenolic compounds (total)					
Fruits	58	4·74	0·006	30	0·008
Vegetables	61	4·69	0·003	25	0·638
Cereals	6	4·93	0·079	–	–
Phenolic acids‖¶					
Fruits	83	4·72	0·038	47	0·106
Vegetables	48	4·75	0·018	30	0·200
Cereals	21	4·63	0·266	12	0·189
Flavanones‖¶					
Fruits	59	4·68	0·257	40	0·086
Vegetables	16	4·90	0·017	14	0·156
Flavones and flavonols‖					
Fruits	87	4·69	0·108	47	0·272
Vegetables	98	4·85	<0·001	78	<0·001
Cereals	9	4·83	0·002	9	0·004
Carotenoids‖¶					
Fruits	36	4·97	<0·001	19	<0·001
Vegetables	101	4·64	0·149	39	0·523
Cereals	14	4·63	0·165	14	0·467
Compound food	12	4·65	0·410	10	0·938
Xanthophylls¶					
Fruits	20	5·04	<0·001	9	<0·001
Vegetables	26	4·73	0·056	5	0·394
Cereals	14	4·63	0·162	14	0·436
Compound food	6	4·48	0·171	5	0·615
Carbohydrates (total)					
Fruits	24	4·63	0·189	6	0·484
Vegetables	31	4·76	0·008	6	0·391
Cereals	4	4·79	0·319	–	–
Protein (total)					
Fruits	7	4·57	0·344	–	–
Vegetables	34	4·61	0·426	8	0·793
Cereals	43	4·45	<0·001	15	<0·001
Amino acids‖¶					
Fruits	38	4·61	0·478	18	0·907
Vegetables	152	4·61	0·234	18	0·295
Cereals	121	4·54	<0·001	63	<0·001
Compound food	21	4·52	<0·001	18	0·002
N					
Fruits	19	4·57	0·214	7	0·071
Vegetables	42	4·52	0·001	20	0·523
Cereals	14	4·48	0·001	7	0·011
Herbs and spices	12	4·69	0·005	–	–
Cd					
Fruits	4	3·78	0·066	–	–
Vegetables	34	4·36	0·045	10	0·719
Cereals	17	4·13	<0·001	8	0·002

Fig. 4. Results of the standard unweighted and weighted meta-analyses for different crop types/products for antioxidant activity, plant secondary metabolites with antioxidant activity, macronutrients, nitrogen and cadmium. MPD, mean percentage difference; CONV, conventional food samples; ORG, organic food samples; *n*, number of data points included in the meta-analyses; SMD, standardised mean difference. Values are standardised mean differences, with 95 % confidence intervals represented by horizontal bars. * *P* value $<0{\cdot}05$ indicates a significant difference between ORG and CONV. † Numerical values for MPD and standard errors are given in Table S10 (available online). ‡ For parameters for which $n \leq 3$ for specific crops/products, results obtained in the weighted meta-analyses are not shown. § Ln ratio = Ln(ORG/CONV × 100 %). ‖ Data reported for different compounds within the same chemical group were included in the same meta-analyses. ¶ Outlying data points (where the MPD between ORG and CONV was more than fifty times greater than the mean value including the outliers) were removed. ○, MPD calculated using data included in the standard unweighted meta-analysis; ▷, MPD calculated using data included in the standard weighted meta-analysis; ◆, SMD.

calculations is given in the 'Additional Methods Description' section in the online supplementary material.

We also calculated MPD using only data pairs included in the weighted meta-analyses to estimate the impact of excluding data for which no measures of variance were reported on the magnitude of difference. As the MPD can be expressed as '% higher' in conventional or organic crops, they provide estimates for the magnitude of composition differences that are easier to correlate with existing information on the potential health impacts of changing dietary intake levels for

individual or groups of compounds than the SMD values. The 95 % CI for MPD were estimated using a standard method[35].

For some composition parameters, individual effect sizes were more than fifty times greater than the pooled effect. This applied to one effect size each for phenolic acids, flavanones, flavones, flavonols, carbohydrates, DM and NO_3^-; four effect sizes for carotenoids and xanthophylls; eight effect sizes for amino acids; and forty-one effect sizes for volatile compounds. Such large differences can be considered biologically implausible, and these 'outlier' data pairs were therefore omitted from the final standard meta-analyses as shown in Figs. 3 and 4 and Tables S10 and S11 (available online).

Data reported for the frequency of occurrence of detectable pesticide residues (percentage of samples with detectable pesticide residues) in organic and conventional crops were compared using a weighted meta-analysis protocol based on the ln-transformed OR[40]. The formula used to calculate OR is given in the 'Additional Methods Description' section in the online supplementary material.

An overall assessment of the strength of evidence was made using an adaptation of the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) system[41].

Results

Analyses were based on data from publications reporting results from EX (154 papers), CF (116 papers), and BS (fifty-five papers) or results from more than one type of study (EX, CF and/or BS; eighteen papers) (see online supplementary Table S3).

Approximately 70 % of all the studies included in the meta-analyses were carried out in Europe, mainly in Italy, Spain, Poland, Sweden, the Czech Republic, Switzerland, Turkey, Denmark, Finland and Germany, with most of the remaining studies being carried out in the USA, Brazil, Canada and Japan (see online supplementary Table S3 and Fig. S2). Among the papers included in the meta-analyses, 174 reported comparison data for vegetables and a smaller number reported data for fruits and cereals (112 and sixty-one, respectively), while only thirty-seven reported data for other crops/crop-based food products (e.g. oil seeds and pulses, herbs and spices, and compound foods) (see online supplementary Table S3). Publications reported data for 907 different composition parameters, of which 182 were included in the meta-analyses (see online supplementary Tables S6 and S7).

Antioxidant activity

A large number of comparisons were available for antioxidant activity in organic and conventional crops (160 for the unweighted meta-analysis and sixty-six for the weighted meta-analysis), but the authors used a wide range of different methodologies. Both weighted and unweighted meta-analyses detected a significantly higher antioxidant activity in organic crops (Fig. 3) and the MPD was 17 (95 % CI 3, 32) % (Fig. 3).

When data reported for fruits and vegetables were analysed separately, a significant difference was detected for fruits, while only a trend towards a significant difference ($P=0{\cdot}06$) was observed for vegetables (Fig. 4), although there was no evidence of an interaction.

When data available for specific antioxidant activity assays were analysed, similar results were obtained for the Trolox equivalent antioxidant capacity assay with both the standard weighted and unweighted meta-analyses and for the ferric reducing antioxidant power and oxygen radical absorbance capacity assays with only the standard unweighted meta-analysis (Fig. 3).

Antioxidants/(poly)phenolics

The concentrations of secondary metabolites with antioxidant activity, including a wide range of nutritionally desirable (poly)phenolics, were also studied in a relatively large number of studies (see online supplementary Table S8).

For (poly)phenolics, the standard weighted meta-analysis detected significantly and substantially higher concentrations of total flavonoids, total phenolic acids, phenolic acids (where data reported for all individual phenolic acid compounds were included in the same analysis), flavanones, stilbenes, flavones, flavonols, kaempferol, total anthocyanins and anthocyanins in organic crops and/or processed foods made from organic crops. The unweighted meta-analysis yielded similar results, except for (1) total flavonoids, for which no significant difference was detected, and (2) flavanones and flavones, for which only trends towards higher concentrations in organic crops were detected (Fig. 3). The unweighted meta-analysis also detected significantly higher concentrations of chlorogenic acid (5-*O*-caffeoylquinic acid) in organic crops (Fig. 3). The MPD for most of the compounds were between 18 and 69 % for most of the above-mentioned antioxidant compounds (Fig. 3). Inclusion of data for which no measures of variance were reported in the calculation of MPD yielded similar values for phenolic compounds, phenolic acids, chlorogenic acid, flavones, quercetin, kaempferol and anthocyanins; higher values for phenolic acids (total), stilbenes and quercetin-3-rutinoside; and lower values for flavonoids, flavanones and flavonols (see Fig. 4 and online supplementary Table S9).

When data reported for phenolic compounds, phenolic acids and flavanones in fruits, vegetables, cereals and/or processed crop-based foods were analysed separately, significant differences were detected only for the concentrations of phenolic compounds and phenolic acids in fruits and a trend towards a significant difference ($P=0{\cdot}09$) was detected for the concentrations of flavanones in fruits (Fig. 4), although there was no evidence of an interaction. In contrast, when differences in the concentrations of flavones and flavonols were analysed separately for fruits, vegetables and cereals, significant differences were detected for vegetables and cereals, but not for fruits, with evidence of interactions (Fig. 4). For all other antioxidant/(poly)phenolic compounds, separate analyses for different crop types were not possible due to the unavailability of sufficient data.

Smaller, but statistically significant and biologically meaningful composition differences were also detected for a small number of carotenoids and vitamins. Both unweighted and

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weighted meta-analyses detected significantly higher concentrations of xanthophylls and L-ascorbic acid and significantly lower concentrations of vitamin E in organic crops. Higher concentrations of total carotenoids, carotenoids (where data reported for all individual phenolic acid compounds were included in the same analysis) and lutein were also detected by the unweighted meta-analysis (Fig. 3). The MPD were 17 (95% CI 0, 34)% for total carotenoids, 15 (95% CI −3, 32)% for carotenoids (where data reported for all individual carotenoid compounds were included in the same analysis), 12 (95% CI −4, 28)% for xanthophylls, 5 (95% CI −3, 13)% for lutein, 6 (95% CI −3, 15)% for vitamin C and −15 (95% CI −49, 19)% for vitamin E. Inclusion of data for which no measures of variance were reported in the calculation of MPD resulted in slightly higher values (see Fig. 4 and online supplementary Table S9).

When data reported for total carotenoids and xanthophylls in fruits, vegetables, cereals and processed crop-based compound foods were analysed separately, significantly higher concentrations in organic samples were detected only for fruits (Fig. 4), with evidence of interactions being detected for carotenoids, but not for xanthophylls.

The meta-analyses did not detect significant differences for a range of other secondary metabolites with antioxidant activity. These included some individual carotenoids (α-carotene, lycopene, β-cryptoxanthin and zeaxanthin), vitamins (α-tocopherol, γ-tocopherol, vitamin B and vitamin B_1), some specific phenolic acids (total hydroxycinnamic acids, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, 5-*O*-caffeoylquinic acid, ellagic acid, gallic acid and salicylic acid), some specific flavones and flavonols (apigenin, luteolin, myricetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, quercetin-3-*O*-glucoside and quercetin-3-*O*-malonyl glucoside) and some specific flavanones (naringenin and naringenin (*R*-enantiomer)).

Macronutrients, fibre and DM content

Both unweighted and weighted meta-analyses detected significantly higher concentrations of total carbohydrates and significantly lower concentrations of proteins, amino acids and fibre in organic crops/crop-based compound foods (Fig. 3). The unweighted meta-analysis also detected significantly higher concentrations of reducing sugars and DM in organic crops (Fig. 4). The MPD were 25 (95% CI 5, 45)% for total carbohydrates, 11 (95% CI 2, 20)% for carbohydrates (where data reported for all individual phenolic acid compounds were included in the same analysis), 7 (95% CI 4, 11)% for reducing sugars, −15 (95% CI −27, −3)% for proteins, −11 (95% CI −14, −8)% for amino acids, 2 (95% CI −1, 6)% for DM and −8 (95% CI −14, −2)% for fibre. Inclusion of data for which no measures of variance were reported in the calculation of MPD resulted in similar values for carbohydrates, proteins, DM and fibre; higher values for reducing sugars; and lower values for carbohydrates (total) and amino acids (see Fig. 4 and online supplementary Table S9).

When data reported for proteins and amino acids in vegetables, cereals and/or processed crop-based foods were analysed separately, significant differences were detected for cereals and processed crop-based foods, but not for vegetables (Fig. 4), although there was no evidence of an interaction. Also, when data reported for carbohydrates in vegetables, fruits and cereals were analysed separately, no significant effects could be detected in their concentrations (Fig. 4).

Toxic metals, nitrogen, nitrate, nitrite and pesticides

Both weighted and unweighted meta-analyses detected significantly lower concentrations of the toxic metal Cd and total N in organic crops, while lower concentrations of NO_3^- and NO_2^- in organic crops were detected only by the unweighted meta-analysis (Fig. 3). The MPD were −48 (95% CI −112, 16)% for Cd, −10 (95% CI −15, −4)% for N, −30 (95% CI −144, 84)% for NO_3^- and −87 (95% CI −225, 52)% for NO_2^- (Fig. 3).

Inclusion of data for which no measures of variance were reported in the calculation of MPD resulted in similar values for N, NO_3^-, NO_2^- and Cd (see Fig. 4 and online supplementary Table S9).

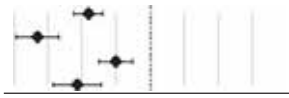
When data reported for N and Cd concentrations in fruits, vegetables and cereals were analysed separately, significant differences were detected for cereals, but not for vegetables and/or fruits (Fig. 4), although there was no evidence of an interaction.

For the toxic metals As and Pb, no significant differences could be detected in their concentrations between organic and conventional crops in the meta-analyses (see online supplementary Table S12).

The standard meta-analyses showed that the frequency of occurrence of detectable pesticide residues was four times higher in conventional crops (46 (95% CI 38, 55)%) than in organic crops (11 (95% CI 7, 14)%) (Fig. 5). Significantly higher frequencies of occurrence of pesticide residues in conventional crops were also detected when data reported for fruits, vegetables and processed crop-based foods were analysed separately (Fig. 5). Conventional fruits had a higher frequency (75 (95% CI 65, 85)%) of occurrence of pesticide residues than vegetables (32 (95% CI 22, 43)%) and crop-based compound foods (45 (95% CI 25, 65)%), while contamination rates were very similar in the different organic crop types. This resulted in significant differences in the OR for different crop types (Fig. 5).

Other minerals

For most of the minerals (including many plant marco- and micronutrients), the meta-analyses could not detect significant composition differences between organic and conventional crops (see online supplementary Table S12). However, for a small number of minerals, differences in composition were identified by both weighted and unweighted meta-analyses, which detected significantly lower concentrations of Cr and Sr (−59 (95% CI −147, 30)% and −26 (95% CI −45,



Products†	*n*	*P**	Percentage of positive samples: ORG Mean	ORG 95 % CI	CONV Mean	CONV 95 % CI
All	66	<0·001	10·55	6·74, 14·36	46·35	37·96, 54·75
Fruits	22	<0·001	11·45	4·92, 17·99	74·60	64·65, 84·55
Vegetables	36	<0·001	10·25	4·77, 15·73	31·95	21·72, 42·18
Compound foods‡	6	<0·001	12·59	1·52, 23·65	44·64	24·81, 64·48

Fig. 5. Results of the standard weighted meta-analysis comparing ln OR for the frequency of occurrence of pesticide residues (percentage of positive samples) in organic and conventional crops. A mixed-effect model with crop/product group as a moderator was used. OR, ln OR for each product group (◆); ORG, organic food samples; CONV, conventional food samples; *n*, number of data points included in the meta-analyses. Values are odds ratios, with 95 % confidence intervals represented by horizontal bars. * *P* value $< 0{\cdot}05$ indicates a significant difference between ORG and CONV. † Crops/product groups for which $n \leq 3$ were removed from the plots. ‡ Compound foods.

−6) %, respectively), but significantly higher concentrations of Mo and Rb (65 (95 % CI 26, 105) % and 82 (95 % CI 6, 157) %, respectively) in organic crops. Also, lower concentrations of Mn (−8 (95 % CI −13, −3) %) and higher concentrations of Ga and Mg in organic crops (57 (95 % CI −122, 8) % and 4 (95 % CI −5, 13) %, respectively) were detected only by the weighted meta-analysis, while slightly higher concentrations of Zn (5 (95 % CI −6, 15) %) in organic crops were only detected by the unweighted meta-analysis (see online supplementary Table S11). As differences for Zn and Mg were relatively small and as there is limited information about potential health impacts associated with changing intake levels of either mineral (Cr, Ga, Mo, Sr and Mo), more detailed results are provided only in the online supplementary material.

Effects of crop type/species/variety, study type and other sources of variation

Heterogeneity was extremely high ($I^2 > 75$ %) for most of the composition parameters, with I^2 ranging from 76 % for ascorbic acid to 100 % for carotenoids and DM (Fig. 3). The only exceptions were vitamin E, reducing sugars, fibre and NO_2^-, for which the small number of studies and/or high within-study variability limited the ability to distinguish heterogeneity between the effects.

Strong or moderate funnel plot asymmetry consistent with a publication bias was detected for approximately half of the parameters. However, it is not possible to definitively attribute discrepancies between large precise studies and small imprecise studies to publication bias, which remains strongly suspected rather than detected where asymmetry is severe (see Table 1 and online supplementary Table S13).

When meta-analysis results obtained from different study types (BS, CF and EX) were compared, similar results were obtained for most of the composition parameters included in Fig. 3 (see online supplementary Figs. S3 and S4). However, there was considerable variation between results obtained for different crop types, crop species, and/or studies carried out in countries with contrasting pedo-climatic and agronomic background conditions (see Fig. 4 and online supplementary Figs. S5–S41).

Non-weighted MPD were calculated to aid in the biological interpretation of effect size magnitude where either the weighted or unweighted meta-analysis had identified statistically significant results. For many parameters, MPD based on all the available data produced values very similar to those calculated using only data for which measures of variance were reported (= those used for the weighted meta-analysis; Fig. 3). However, for other parameters (flavonoids, total phenolic acids, flavanones, rutin, L-ascorbic acid, reducing sugars and Cd), inclusion criteria had a large effect on the MPD.

Also, when the calculated MPD were superimposed onto SMD (with 95 % CI) results at an appropriate scale (−100 to +100 for MPD and −5 to +5 for SMD), a reasonable match was observed, with MPD for most of the compounds being present within the 95 % CI for SMD (Fig. 3). However, for some parameters (Trolox equivalent antioxidant capacity, total phenolic acids, stilbenes, rutin, total carotenoids, L-ascorbic acid, vitamin E, reducing sugars, proteins, NO_3^-, NO_2^- and Cd), MPD were outside the 95 % CI of SMD, and therefore these should be seen as less reliable.

For the composition parameters included in Fig. 3, sensitivity analyses, which were based on different inclusion criteria and data-handling methods, yielded results broadly similar to those yielded by the standard weighted and unweighted meta-analyses.

The overall assessment of the strength of evidence using an adapted GRADE[41] approach highlighted uncertainties in the evidence base, but the overall strength of evidence was moderate or high for the majority of parameters for which significant differences were detected (see Table 1 and online supplementary Table S13).

Discussion

The results of meta-analyses of the extensive data set of 343 peer-reviewed publications indicated that organic crops and processed crop-based foods have a higher antioxidant activity and contain higher concentrations of a wide range of nutritionally desirable antioxidants/(poly)phenolics, but lower concentrations of the potentially harmful, toxic metal Cd. For plant secondary metabolites, this confirms the results of the meta-analyses carried out by Brandt *et al.*[20], which indicated that there are significant composition differences between organic and conventional crops for a range of nutritionally relevant compounds. However, it contradicts the results of the systematic reviews/meta-analyses by Dangour *et al.*[19] and Smith-Spangler *et al.*[21], which indicated that there are no significant composition differences between organic and conventional crops. The main reason for the inability of previous studies to detect composition differences was probably the

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Table 1. GRADE (Grading of Recommendations, Assessments, Development and Evaluation) assessment of the strength of evidence for standard weighted meta-analysis for parameters included in Fig. 3

(Standardised mean difference values (SMD) and 95 % confidence intervals)

Parameters	SMD	95 % CI	Effect magnitude*	Inconsistency†	Precision‡	Publication bias§	Overall reliability
Antioxidant activity	1·11	0·43, 1·79	Moderate	Medium	Poor	None	Moderate
FRAP	0·59	−0·89, 2·06	Moderate	Low	Poor	Medium	Moderate
ORAC	1·92	−0·86, 4·71	Large	Low	Poor	Strong	Low
TEAC	0·25	0·02, 0·48	Small	Medium	High	Medium	Good
Phenolic compounds (total)	0·52	0·00, 1·05	Small	Medium	Moderate	None	Moderate
Flavonoids (total)	1·64	0·09, 3·19	Large	Medium	Poor	Medium	Moderate
Phenolic acids (total)	0·81	0·18, 1·44	Small	Low	Moderate	Strong	Low
Phenolic acids‖	0·59	0·11, 1·07	Small	Medium	Moderate	None	Moderate
Chlorogenic acid	1·58	−0·32, 3·49	Large	High	Poor	Medium	Low
Flavanones‖	4·76	0·54, 8·98	Large	Medium	Moderate	None	Moderate
Stilbenes	0·74	0·19, 1·28	Small	Low	Moderate	Medium	Moderate
Flavones and flavonols	1·74	1·21, 2·28	Large	Medium	High	None	Good
Flavones	0·95	0·39, 1·51	Moderate	Medium	Moderate	None	Moderate
Flavonols‖	1·97	1·31, 2·64	Large	Medium	High	None	Good
Quercetin	0·55	−0·58, 1·69	Small	Low	Poor	Medium	Low
Rutin	1·10	−0·31, 2·50	Moderate	Medium	Poor	None	Low
Kaempferol	1·34	0·19, 2·50	Moderate	Low	Poor	None	Low
Anthocyanins (total)	1·60	0·59, 2·62	Large	Low	Moderate	Medium	Moderate
Anthocyanins	3·81	1·53, 6·09	Large	Medium	High	Medium	Moderate
Carotenoids (total)	7·98	−6·22, 22·18	Large	Medium	Poor	Strong	Low
Carotenoids‖	0·47	−0·13, 1·07	Small	Medium	Poor	None	Low
Xanthophylls‖	1·06	0·18, 1·94	Moderate	Medium	Poor	Medium	Low
Lutein	0·51	−0·27, 1·29	Small	Medium	Poor	Medium	Low
Ascorbic acid	0·33	0·06, 0·60	Small	Medium	Moderate	None	Moderate
Vitamin E	−0·23	−0·46, 0·00	Small	Low	Moderate	None	Moderate
Carbohydrates (total)	1·54	0·10, 2·99	Large	Low	Poor	Medium	Low
Carbohydrates‖	0·46	0·00, 0·91	Small	Medium	Moderate	None	Moderate
Sugars (reducing)	0·21	−0·23, 0·65	Small	Low	Moderate	None	Moderate
Protein (total)	−3·01	−5·18, −0·84	Large	Medium	Moderate	Medium	Moderate
Amino acids‖	−0·82	−1·14, −0·50	Small	Medium	High	Medium	Moderate
DM‖	1·31	−0·65, 3·28	Moderate	Medium	Poor	Medium	Low
Fibre	−0·42	−0·76, −0·07	Small	Low	Moderate	None	Moderate
N	−0·88	−1·59, −0·17	Moderate	Low	Moderate	Medium	Low
NO_3^-‖	−0·50	−1·73, 0·73	Small	Medium	Poor	Medium	Low
NO_2^-	−0·11	−0·38, 0·16	Small	Low	High	None	Moderate
Cd	−1·45	−2·52, −0·39	Moderate	Medium	Moderate	Medium	Moderate

FRAP, ferric reducing antioxidant potential; ORAC, oxygen radical absorbance capacity; TEAC, Trolox equivalent antioxidant capacity.

* Study quality was considered low because of high risks of bias and potential for confounding. However, we considered large effects to mitigate this *sensu* GRADE; large effects were defined as >20 %, moderate effects as 10–20 % and small as <10 %.

† Inconsistency was based on the measure of heterogeneity and the consistency of effect direction *sensu* GRADE.

‡ Precision was based on the width of the pooled effect CI and the extent of overlap in the substantive interpretation of effect magnitude *sensu* GRADE.

§ Publication bias was assessed using visual inspection of funnel plots, Egger tests, two fail-safe number tests, and trim and fill (see online supplementary Table S13). Overall publication bias was considered high when indicated by two or more methods, moderate when indicated by one method, and low when indicated by none of the methods. The overall quality of evidence was then assessed across domains as in standard GRADE appraisal.

‖ Outlying data pairs (where the mean percentage difference between the organic and conventional food samples was over fifty times higher than the mean value including outliers) were removed.

highly limited number of studies/data sets available or included in analyses by these authors, which would have decreased the statistical power of the meta-analyses.

In addition, most of the previous studies did not use weighted meta-analyses based on SMD. This approach is recommended when combining data from studies that measure the same parameter (e.g. the major phenolic compounds found in different crops), but use different scales[25,26,29]. In the study carried out by Dangour *et al.*[19], published data from (1) surveys in which the organic samples were produced to 'biodynamic-organic' standards and (2) field experiments investigating associations between organic and conventional production protocols and crop composition were not included in the meta-analyses. This would have further reduced the number of data sets and sensitivity of meta-analyses and contributed to the lack of significant composition differences being detected. In the meta-analyses carried out in the present study, 'biodynamic-organic' data sets were treated as organic, as biodynamic standards comply with the legal European Union organic farming standards. Data from comparative field experiments were also included, as controlled experimental studies are less affected by confounding factors (e.g. contrasting soil and climatic and agronomic background conditions between farms that supplied organic and conventional samples) than farm and retail surveys. The reason for excluding field experiments carried out in the study of Dangour *et al.*[19] is that in the field experiments the organic plots were not certified according to organic farming standards. In the meta-analyses carried out in the present study, field experiments investigating associations between organic and

conventional agronomic practices/protocols and crop composition were included, as the crop management practices rather than the certification process were assumed to affect crop performance and composition.

The finding of a four times higher frequency of occurrence of pesticide residues in conventional crops confirms the results of the study of Smith-Spangler *et al.*[21], in which a very similar set of studies (nine of the ten publications used in the present study) were used for analysis.

The potential (1) nutritional benefits of higher concentrations of antioxidant/(poly)phenolics in organic crops, (2) risks associated with potentially harmful pesticide residues, Cd, NO_3^- and NO_2^-, and (3) agronomic factors responsible for composition differences are discussed in more detail below.

Antioxidants/(poly)phenolics

Among the composition differences detected by the meta-analyses carried out in the present study, the higher antioxidant activity and higher concentrations of a wide range of antioxidants/(poly)phenolics found in organic crops/crop-based foods may indicate the greatest potential nutritional benefits. Based on the differences reported, results indicate that a switch from conventional to organic crop consumption would result in a 20–40 % (and for some compounds more than 60 %) increase in crop-based antioxidant/(poly)phenolic intake levels without a simultaneous increase in energy, which would be in line with the dietary recommendations[16,17]. This estimated magnitude of difference would be equivalent to the amount of antioxidants/(poly)phenolics present in one to two of the five portions of fruits and vegetables recommended to be consumed daily and would therefore be significant/meaningful in terms of human nutrition, if information linking these plant secondary metabolites to the health benefits associated with increased fruit, vegetable and whole grain consumption is confirmed[16–18].

However, it is important to point out that there is still a lack of knowledge about the potential human health impacts of increasing antioxidant/(poly)phenolic intake levels and switching to organic food consumption. For example, there are still gaps in the understanding of the (1) uptake, bioavailability and metabolism of (poly)phenolics after ingestion and (2) exact compounds/molecules and modes of action responsible for health benefits[16]. Also, it is important to consider that most of the human dietary intervention studies on associations between antioxidant/(poly)phenolic intake and health indicators were based on the comparison of standard diets with diets in which the amount of specific (poly)phenolic-rich foods (e.g. cocoa, red wine, tea/coffee, berries, citrus and nuts) was high[16,17].

There are, to our knowledge, only two human dietary intervention studies in which contrasting antioxidant/(poly)phenolic intake levels were generated by providing diets based on conventional and organic crops; both studies focused on assessing antioxidant status in humans and were inconclusive with respect to the identification of potential health impacts of organic food consumption[21,42,43]. However, there are several animal dietary intervention studies that have identified significant associations between organic feed consumption and animal growth and physiological (including immune and endocrine) parameters and/or biomarkers of health when compared with conventional feed consumption[44,45]. Among these studies, one recent factorial animal study has gone one step further and assessed associations between contrasting crop fertilisation and crop protection protocols used in conventional and organic farming systems and (1) the composition (including (poly)phenolic content) of crops/compound feeds made from crops and (2) the growth, physiological, immunological and hormonal parameters of rats that consumed these feeds[46]. With respect to composition differences, the study yielded results similar to those of the meta-analyses carried out in the present study. For example, rat feeds produced from organic crops had lower concentrations of proteins and Cd, but higher concentrations of polyphenols and the carotenoid lutein. The study also demonstrated that composition differences were mainly linked to contrasting fertilisation regimens (green and animal manures *v.* mineral fertiliser inputs). The consumption of feeds made from organic crops by the rats resulted in higher levels of body protein, body ash, leucocyte count, plasma glucose, leptin, insulin-like growth factor 1, corticosterone, and IgM, and spontaneous lymphocyte proliferation, but lower levels of plasma IgG, testosterone and mitogen-stimulated proliferation of lymphocytes[46]. Redundancy analysis identified total polyphenol concentrations in feeds as the strongest driver for the physiological/endocrinological parameters assessed in rats. This suggests that a switch from conventional to organic crop consumption may have impacts similar to those of an increase in the intake of foods with high antioxidant/(poly)phenolic contents. This hypothesis would merit further exploration in animal and human dietary intervention studies.

Many of the antioxidants, including (poly)phenolics, found in higher concentrations in organic crops are known to be produced by plants in response to abiotic (e.g. wounding and heat, water and nutrient stress) and biotic (pest attacks and disease) stress and form part of the plants' constitutive and inducible resistance mechanisms to pests and diseases[47–49]. Therefore, higher concentrations of (poly)phenolics in organic crops may be due to higher incidence/severity of pest and disease damage, causing enhanced (poly)phenolic production as part of the inducible plant resistance response. The differences in antioxidant concentrations between organic and conventional crops may therefore have been due to contrasting pest and disease damage and/or fertilisation intensity. However, there are, to our knowledge, no sound published data/evidence for a causal link between higher pest/disease incidence/severity and antioxidant/(poly)phenolic concentrations in organic crops. In contrast, there is increasing evidence that differences in fertilisation regimens between organic and conventional production systems (and, in particular, the non-use of high mineral N fertiliser inputs) are significant drivers for higher (poly)phenolic concentrations in organic crops[20,49–52]. For example, Sander & Heitefuss[50] reported that increasing mineral N fertilisation resulted in reduced concentrations of phenolic resistance compounds in wheat leaves and increased severity of foliar

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disease (powdery mildew). Similarly, a review by Rühmann *et al.*[51] describes the negative correlations between N fertilisation/supply-driven shoot growth and concentrations of phenylpropanoids and apple scab resistance in young leaves in apple trees[51]. In tomato, deficiency of both N and P was found to be linked to flavonol accumulation in plant tissues[52]. More recently, Almuayrifi[49] has demonstrated that the non-use of synthetic pesticides and fungicides has no effect on phenolic acid and flavonoid concentrations and profiles in wheat, but that the use of standard, conventional mineral (NPK) fertiliser regimens is associated with significantly lower phenolic acid and flavonoid concentrations in wheat leaves compared with organic wheat crops fertilised with green and animal manures only. The variability in relative differences in antioxidant/(poly)phenolic concentrations found between studies and crops may therefore at least partially be explained by variability in the fertilisation protocols in both the organic and non-organic systems compared. The finding in the present study that organic crops have significantly lower N, NO_3^- and NO_2^- concentrations would support the theory that differences in antioxidant/(poly)phenolic concentrations between organic and conventional crops are driven by contrasting N supply patterns. This view is supported by previous studies that have suggested that under high N availability, plants allocate carbohydrates from photosynthesis to primary metabolism and rapid growth while producing less amounts of secondary metabolites involved in defence[51].

However, additional research is required to gain a more detailed understanding of the relative contribution of fertilisation and crop protection regimens and disease and pest prevalence/severity to the expression of constitutive and inducible resistance mechanisms in different organically managed crop plants[50].

Cadmium and pesticide residues

Cd is a highly toxic metal and one of the only three toxic metal contaminants (the other two being Pb and Hg) for which the European Commission has set maximum residue levels (MRL) in foods[53]. Cd accumulates in the human body (especially in the liver and kidneys) and therefore dietary Cd intake levels should be kept as low as possible[53]. The on average 48% lower Cd concentrations found in organic crops/crop-based foods in the meta-analyses carried out in the present study are therefore desirable, although the exact health benefits associated with reducing Cd intake levels via a switch to organic food consumption are difficult to estimate. Similar to the results of the present study, a recent literature review by Smith-Spangler *et al.*[21] has also reported that of the seventy-seven comparative data sets (extracted from fifteen publications), twenty-one indicated significantly lower and only one significantly higher Cd concentrations in organic foods. Differences in Cd contamination levels between organic and conventional winter wheat have recently been shown to be mainly linked to differences in fertilisation regimens (especially the high mineral P inputs used in conventional farming systems), although contrasting rotation designs also contributed to differences in Cd concentrations between organic and conventional wheat[7]. A range of other soil (e.g. pH) and agronomic (e.g. liming) factors are known to affect Cd concentrations in crops[54], and these may explain the variability in results between individual comparative studies, crop species and crop types (see Fig. 4 and online supplementary Figs. S4 and S22).

The present study demonstrated that the prohibition of synthetic chemical pesticide use under organic farming standards results in a more than 4-fold reduction in the number of crop samples with detectable pesticide residues. This supports previous studies that have concluded that organic food consumption can reduce exposure to pesticide residues[21–23]. The considerably higher frequency of occurrence of detectable residues in conventional fruits (75%) than in vegetables (32%) may indicate higher levels of crop protection inputs being used in fruit crops, but could also have been due to the use of more persistent chemicals, different sprayer technologies used and/or pesticide applications being made closer to harvest. The finding of detectable pesticide residues in a proportion (about 11%) of organic crop samples may have been due to cross-contamination from neighbouring conventional fields, the continued presence of very persistent pesticides (e.g. organochlorine compounds) in fields or perennial crop tissues from past conventional management, and/or accidental or fraudulent use of prohibited pesticides in organic farms.

Pesticide residues that are below the MRL set by the European Commission[55,56] are considered by regulators not to pose risk to consumers or the environment, as they are significantly lower than concentrations for which negative health or environmental impacts can be detected in the regulatory pesticide safety testing carried out as part of the pesticide approval process[55]. However, a significant number of crop samples included in the regulatory European Food Safety Authority pesticide residue monitoring in Europe are still found to contain pesticide residues above the MRL[57]. For example, in recent European Food Safety Authority surveys, pesticide residues above the MRL have been found in 6·2% of spinach, 3·8% of oat, 3·4% of peach, 3·0% of orange, 2·9% of strawberry and lettuce, 2·8% of table grape and 2·7% of apple samples analysed[57]. There is still scientific controversy about the safety of some currently permitted pesticides (e.g. organophosphorus compounds) even at levels below the MRL and complex mixtures of pesticides, as additive/synergistic effects of pesticide mixtures have been documented and safety testing of pesticide mixtures is currently not required as part of the regulatory pesticide approval process[58–60]. Similar to Cd, the lower risk of exposure to pesticide residues can be considered desirable, but potential health benefits associated with reducing pesticide exposure via a switch to organic food consumption are impossible to estimate.

It should be pointed out that (1) there are only eleven studies in which the frequencies of occurrence of pesticide residues were compared, (2) eight of these studies focused on only one crop species, (3) no comparative studies for cereals, oilseeds and pulses were identified in the literature review, and (4) the data available did not allow scientifically

robust comparisons of the concentrations of pesticides. Therefore, it is important to carry out further studies to improve our understanding of differences in the frequency of occurrence and concentrations of pesticide residues between organic and conventional crops.

Proteins, amino acids, nitrogen and nitrate/nitrite

The concentrations of proteins, amino acids and N (which are known to be positively correlated in plants) were found to be lower in organic crops, and this is consistent with the results of previous studies that have linked lower protein concentrations to lower N inputs and N availability in organic crop production systems[61,62]. The nutritional significance/relevance of slightly lower protein and amino acid concentrations in organic crops to human health is likely to be low, as European and North American diets typically provide sufficient or even excessive amounts of proteins and essential amino acids. Also, while some studies concluded that protein content in most European and North American diets is too high and that this contributes to the increasing incidence of diabetes and obesity[63], other studies reported that increasing protein intake levels may be a strategy to prevent obesity[64]. Therefore, the lower protein and amino acid concentrations found in organic foods are unlikely to have a significant nutritional or health impact.

The higher NO_3^- and NO_2^- concentrations in conventional crops are also thought to be linked to high mineral N inputs, as both NO_3^- and NO_2^- are known to accumulate in plants under high-mineral N input regimens[65]. The higher NO_2^- concentrations in conventional crops/crop-based foods are nutritionally undesirable, as they have been described to be risk factors for stomach cancer and methaemoglobinaemia in humans[65]. However, while increasing dietary NO_2^- intake levels is widely considered to be potentially harmful for human health, there is still controversy about the potential health impacts of crop-based dietary NO_3^- intake[65–67].

Effects of crop type/species/variety, study type and other sources of variation

One of the main challenges to interpreting comparisons of organic and inorganic food production systems is the high heterogeneity arising from combinations of (1) crops, crop types and/or crop-based foods, (2) countries, and/or (3) pedo-climatic and agronomic background conditions. As has been mentioned in previous reviews[19–21], pooling diverse information was necessary, because for most of the composition parameters, the number of published studies available was not sufficient to carry out separate meta-analyses for specific countries/regions and crop types and species. Consequently, heterogeneity was extremely high ($I^2 > 75\%$) for most of the composition parameters for which significant differences were detected.

For many composition parameters, the method of synthesis did not have large effects on results, in terms of both statistical significance and the magnitude of relative difference between organic and conventional crops. This indicates that there is now a sufficiently large body of published information to identify differences that are relatively consistent across study types, crops, and pedo-climatic and agronomic environments. Therefore, for these parameters, future studies should focus on increasing our understanding of the underlying agronomic, pedo-climatic and crop genetic factors responsible for composition differences between organic and conventional crops.

For other composition parameters (e.g. ferric reducing antioxidant power, oxygen radical absorbance capacity, Trolox equivalent antioxidant capacity, and levels of flavonoids, stilbenes, total carotenoids, L-ascorbic acid, proteins, NO_2^- and Cd), differences in methods had a large impact in terms of both significant effects being detected and/or estimates of the magnitude of difference based on MPD and SMD. For these compounds, additional high-quality studies (that report measures of variance) are required to increase the power of weighted meta-analyses.

Overall assessment of the strength of evidence for antioxidant/(poly)phenolic parameters indicated high or moderate reliability for thirteen of the nineteen parameters and moderate reliability for Cd. This supports the conclusion that future research would likely be confirmatory.

In contrast to previous literature reviews[19–21], the larger number of studies now available allowed separate meta-analyses to be carried out for different crop types (e.g. fruits, vegetables and cereals), but only for a limited number of composition parameters. This demonstrates that there is variation between crop types with respect to (1) whether the production system has a significant effect and/or (2) the magnitude of difference between organic and conventional crops, although sample sizes remain insufficient to detect interactions between crop types in many cases.

The present study also identified variation between studies (1) carried out in countries with different pedo-climatic conditions and agronomic protocols (e.g. rotation designs, irrigated or non-irrigated crop production, and level and type of animal manures used) and/or (2) focused on different crop species. This is not surprising as both genetic and environmental/agronomic factors are known to affect the concentrations of N, NO_3^-, NO_2^-, proteins, sugars, antioxidants/(poly)phenolics, Cd and pesticides in crops[7,9–12,20,47–52,62]. However, due to the lack of detailed information on agronomic and pedo-climatic background conditions in most of the available literature, it is currently not possible to quantify the relative contribution of genetic and environmental/agronomic sources of variation.

The unweighted MPD were calculated to provide an estimate of the magnitude of difference that is meaningful when considering nutritional/health impacts of changes in crop composition. However, care should be taken when interpreting MPD values, as they do not take variability in the precision of individual studies into account[25] and provide less precise estimates of effect than weighted estimates.

However, there is now evidence from a large number of quality studies that consistently show that organic production systems result in crops/crop-based compound foods with higher concentrations of antioxidants/(poly)phenolics and lower concentrations of Cd and pesticide residues compared

with conventional production systems. There is little uncertainty surrounding this overall result, but further research is required to quantify more accurately the relative impacts of (1) crop types, species, and varieties/cultivars/hybrids and (2) agronomic and pedo-climatic background conditions on the relative difference between organic and conventional crop composition.

The need for use of standardised protocols for comparative food composition studies

The present study identified deficiencies in a large proportion of the published studies. These included a lack of standardised measurements and a lack of reporting (and, in particular, the non-reporting of measures of variability and/or replication) for many composition parameters, and there was evidence of duplicate or selective reporting of data collected in experiments, which may lead to publication bias. Particularly, there is a lack of studies comparing pesticide residue levels in organic and conventional crops, and there has been very little effort taken to re-analyse and then publish available comparative data from food surveillance surveys (e.g. the regular pesticide residue and food composition surveys carried out by the European Food Safety Authority and national agencies in Europe and elsewhere). Also, in many studies, there was a lack of detailed information on (1) the geographical origin of samples in retail surveys and (2) agronomic (e.g. rotation, fertilisation, tillage and irrigation regimens), pedo-climatic and crop genetic backgrounds (in farm surveys and field experiments), which would allow potential sources of variation to be investigated.

Not all studies included in the meta-analyses used certified reference materials as a quality assurance measure for the accuracy of estimates of concentrations of compounds in crops. This is unlikely to have affected the estimates of relative differences between organic and conventional crops, as the same extraction and analytical methods were used for organic and conventional samples in all the studies included in the meta-analyses in the present study. However, data from studies that did not use reference materials are less reliable when used to estimate the concentrations of nutritionally relevant compounds in crops and total dietary intake levels of such compounds in crop-based foods.

Therefore, it is important to develop guidelines for studies comparing the impacts of agronomic practices on crop/food composition to minimise heterogeneity and/or allow agronomic, environmental and crop genetic drivers to be used as covariates in analyses.

The need for dietary intervention/cohort studies to identify health impacts

A recent review by Smith-Spangler *et al.*[21] has analysed the results of fourteen studies in which the effects of organic and conventional food (both crop and livestock product) consumption on clinical outcomes (e.g. allergic symptoms and *Campylobacter* infections) and health markers (e.g. serum lipid and vitamin concentrations) were studied. However, they concluded that the currently available data do not allow clear trends with respect to health markers and outcomes to be identified. Therefore, there is an urgent need for well-controlled human intervention and/or cohort studies to identify/quantify potential human health impacts of organic *v.* conventional food consumption.

Diet composition may have an effect on the relative impact of switching from conventional to organic food consumption, and this should be considered in the design of such studies. For example, the relative impact of switching from conventional to organic food consumption could be expected to be smaller for diets with high amounts of (poly)phenolic-rich foods.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114514001366

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The authors' contributions are as follows: M. B. (an animal and food scientist) designed the database, carried out many of the meta-analyses and contributed to the writing of the manuscript; D. S.-T. (a nutritionist) carried out a major part of the literature search and extraction and contributed to the writing of the manuscript; N. V. (a crop scientist) contributed to the literature search (especially for perennial and Mediterranean crops) and the preparation of the manuscript; C. S. (a human nutritionist) contributed to the design of the study, the discussion of potential health impacts of composition differences and the critical review of the manuscript;

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R. S. (an environmental modeller and data analyser) helped to design the literature search and database storage and helped to design and provided guidance for the meta-analyses used; G. B. S. (a research synthesis methodologist specialising in meta-analytical approaches) contributed to and provided advice on the additional analyses carried out in response to referees' recommendations; C. B. (an agronomist specialising on organic production systems) helped with the literature review (especially with respect to studies carried out in North and South America) and the preparation/review of the manuscript; B. B. (an agricultural microbiologist) contributed to the literature search, the critical review of the manuscript and the discussion related to the mechanisms for higher antioxidant concentrations in organic crops; E. M. (a plant pathologist) helped with the literature search and the critical review of the manuscript, in particular, with respect to interactions between antioxidant concentrations and crop resistance; C. G. (a plant pathologist/crop agronomist) helped with the literature search (especially with respect to Mediterranean crops) and the critical review of the manuscript; J. G.-O. (a human nutritionist) contributed to the literature review and the discussion of potential health impacts of composition differences identified in the meta-analyses; E. R. (a human nutritionist) helped with the literature review and the critical revision of the manuscript, especially with respect to human intervention studies focused on the health impacts of organic food consumption; K. S.-S. (an animal nutritionist/physiologists) contributed to the literature review and the critical revision of the manuscript, especially with respect to animal dietary intervention studies focused on the physiological and health impacts of organic feed consumption; R. T. (a human nutritionist) helped with the literature review and the critical revision of the manuscript, especially with respect to studies carried out in Scandinavian countries; D. J. (an agronomist specialising on organic production systems) contributed to the literature review (especially with respect to studies carried out in Eastern and Central European countries) and the preparation/review of the manuscript; U. N. (head of Europe's largest organic farming institutes) helped with the literature review (especially with respect to studies linking mineral nutrient supply and antioxidant concentrations in crops) and the critical review of the manuscript; P. N. (a plant pathologist/crop agronomist) contributed to the interpretation of data and the critical review of the manuscript; C. L. (an agronomist specialising on agricultural production system design and the study of interactions between agronomic practices, and food quality and safety) had primary responsibility for the design of the study, the management of the research project and the preparation of the manuscript.

Conflict of interest: the senior author of the paper, C. L., owns farm land in Germany that is managed according to conventional farming standards and a smallholding in Greece that is managed according to organic farming standards.

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Killer Fungus Could Threaten World Food Supply

by **Rebecca Rupp**

In John Christopher's sci-fi tale *No Blade of Grass* (http://www.amazon.com/No-Blade-Grass-John-Christopher/dp/0380003198), a virus wipes out all the world's grasses—the entire family Poaceae, which includes some 10,000 different species, among them wheat, barley, oats, rye, millet, rice, sorghum, and sugarcane.

The result, of course, was disastrous. There was worldwide famine (presumably also obliterating grazing animals and the pandas; bamboo is a grass). Starving people formed bands of marauders. In England—where the story is set—farms savvy enough to have concentrated on such non-grass crops as cabbages and potatoes turned themselves into armed fortresses and shot desperate people who tried to breach their stockades.

'Let Them Eat Cake'

It's not all that outlandish a scenario. Wheat, the staple crop that provides 20 percent of the world's calories, is the major component of the staff of life: bread. For much of history, the bulk of the human diet has consisted of bread—and when people are deprived of it, all hell breaks loose. The French and Russian Revolutions both began with bread riots. The Romans attempted to placate an unhappy populace with "bread and circuses." And the Bolsheviks won a lot of popular support by promising "peace, land, and bread." Marie Antoinette's famously insensitive "Let them eat cake" (http://www.nytimes.com/2001/09/04/books/a-resolute-biographer-and-a-kinder-gentler-antoinette.html) in the face of France's crippling bread shortages is generally said to have set the monarchy on the road to the guillotine.

To be fair, she almost certainly never said it—the story has been attributed to any number of rich and reputedly clueless figures, among them a Chinese emperor whom, upon being told his starving subjects lacked rice, supposedly replied "Let them eat meat!" However, no matter who said what, it's clear that lousy wheat harvests and lack of available bread played no small part in the French crown's demise.

(http://theplate.nationalgeographic.com/files/2014/07/7993639861_a85c7d3e60_z.jpg)

Photograph by Lina Nagano

Cultural Implications of Bread

An indication of the importance of bread is its widespread cultural significance. Almost every region has its characteristic bread—among them New England anadama bread, Irish soda bread, Indian naan and chapatis, Middle Eastern pita bread, French baguettes and croissants, and German pumpernickel. (This last is possibly the world's best-named bread; the word translates as "devil's fart.")

The sharing of bread traditionally signals hospitality (an act performed continually and duplicitously in *Game of Thrones* (http://insidetv.ew.com/2013/12/01/game-of-thrones-red-wedding-2/)). The medieval designations of "lord" and "lady" derive from the Old English for, respectively, "bread eater" and "loaf kneader," reflecting the central role of bread in the European household. Downton Abbey's aristocratic Lord and Lady Grantham—nibblers of cucumber sandwiches—have their roots in bread. Special breads play a role in any number of holidays and celebrations, from Passover's matzo to Good Friday's hot cross buns.

To supply the demand for bread (http://www.washingtonpost.com/wp-dyn/content/article/2008/04/04/AR2008040403937.html)—and buns, crackers, cookies, cupcakes, muffins, ice cream cones, and dozens of different kinds of pasta—the world produces nearly 700 million tons of wheat a year. In the United States, we've got over 60 million acres planted in wheat.

So what if something goes wrong?

(http://theplate.nationalgeographic.com/files/2014/07/5843671196_cf4de5091e_b.jpg)
A healthy wheat field in Kenya was affected by Ug99 fungus. Photograph by Petr Kosina/CIMMYT

SciFi Thriller Could be a Real-Life Nightmare

Wheat's nemesis is a fungus commonly known as wheat stem rust, the latest permutation of which is known as Ug99, first identified in Uganda and formally named in 1999. If Ug99 turns into a pandemic, we're in trouble. Just 10 percent of wheat is resistant to it; the other 90 percent of the world crop, a sitting duck, would flop over and rot within weeks of infection. Ug99 has the potential to make the horrific Irish Potato Famine look like a Sunday School picnic.

Nobelist Norman Borlaug (http://www.nobelprize.org/nobel_prizes/peace/laureates/1970/borlaug-bio.html), the father of the 20th-century Green Revolution, was able to fend off wheat stem rust beginning in the 1940s by crossing commercial rust-sensitive wheat varieties with rust-resistant strains. The problem with rust resistance, though, is that it's inevitably short-lived. On average, a single rust-resistant gene lasts only three to four years before rust finds a way to weasel around it.

Rust is a resilient organism with a large and relentlessly evolving genome. Balked of one means of nabbing its victim, rust will cunningly bide its time and come up with another mode of attack. In this sense, it's much like influenza virus, which killed 50 million people in the pandemic of 1918 and whose constantly shifting genetic makeup forces us to get new flu shots year after year. When it comes to persistence, there's nothing like a microorganism.

(http://theplate.nationalgeographic.com/files/2014/07/5242099029_1513a715a6_b.jpg)
Agustín Aguilar, CIMMYT greenhouse and laboratory assistant, at work in the greenhouse that houses transgenic wheat at CIMMYT's El Batán, Mexico headquarters. Photograph by Xochiquetzal Fonseca/CIMMYT

GMOs Could Offer a Fix

More effective rust resistance comes from a technique called "pyramiding" in which multiple resistance genes are loaded onto a single strain of wheat. As was true of the Three Musketeers, there's strength in numbers: multiply-resistant wheat strains can sometimes fight off rust for decades. Traditional breeding strategies are effective here, but are painfully slow, sometimes taking fifteen years or more to produce a rust-resistant variety. Better and faster is genetic engineering in which an entire string of rust-resistant genes—many plucked from tough wild grasses—is pieced together and inserted as a block into a wheat chromosome. Public opposition to GMOs, however, has slowed or blocked the development of such rust-resistant wheats—and that could come back to bite us. If Ug99 gets off the ground, we're going to need a lot of help, and fast.

Rust isn't a problem that's going to go away—and even the best and brightest of rust-resistant strains won't remain so forever. As Norman Borlaug presciently said: "Rust never sleeps." It's something to think long and hard about.

Especially if we hope to keep on eating our daily bread.

This story is part of* National Geographic*'s special eight-month Future of Food (http://food.nationalgeographic.com/)series.

Grens, Kerry. “Putting Up Resistance.” *The Scientist*, June 2014, pp 34-39.
See Stem rust of wheat (http://www.apsnet.org/edcenter/intropp/lessons/fungi/Basidiomycetes/Pages/StemRust.aspx) from the American Phytopathological Society.
Oppenneer, Betsy. *Celebration Breads: Recipes, Tales, and Traditions*. Simon & Schuster, 2010.

Trackbacks

1. *Killer Fungus Could Threaten World Food Supply | The Inquisitive Eater (http://inquisitiveeater.com/2014/07/10/killer-fungus-could-threaten-world-food-supply/)* says:
   July 10, 2014 at 3:02 pm
   (http://theplate.nationalgeographic.com/2014/07/10/killer-fungus-could-threaten-world-food-supply/#comment-547)

   […] To read the full article please visit The Plate. […]

   (/2014/07/10/killer-fungus-could-threaten-world-food-supply/?replytocom=547#respond)
2. *Morsels For The Mind – 18/07/2014 › Six Incredible Things Before Breakfast (http://www.scilogs.com/six_incredible_things_before_breakfast/morsels-for-the-mind-18072014/)* says:
   July 19, 2014 at 10:09 pm
   (http://theplate.nationalgeographic.com/2014/07/10/killer-fungus-could-threaten-world-food-supply/#comment-565)

   […] Rust never sleeps. Rebecca Rupp on the rust fungus that threatens our food supply. […]

   (/2014/07/10/killer-fungus-could-threaten-world-food-supply/?replytocom=565#respond)

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In our rapidly changing, globalized world, we all need to understand how food has made us who we are today and how it shapes our future. Starting with the May issue of *National Geographic* magazine and continuing on through 2014, National Geographic explores our complex relationship with what we eat and where our food comes from.

About Rebecca Rupp

Rebecca Rupp has a Ph.D. in cell biology and biochemistry, and is the author of more than 200 articles for national magazines and nearly two dozen books, both for children and adults. Her most recent book, *How Carrots Won the Trojan War*—an overview of the history and science of garden vegetables—won the GWA Gold Award as Best Garden Book of 2012. She lives in northern Vermont and attempts to be open-minded about everything except centipedes and lima beans.

Food by the Numbers


(http://www.nationalgeographic.com/foodbynumbers/)

Interested in food? Check out our "Food by the Numbers" (http://www.nationalgeographic.com/foodbynumbers/) video infographics.

From: Nancy Oden [mailto:cleanearth@tds.net]
Sent: Monday, July 21, 2014 12:50 PM
To: Jennings, Henry
Subject: The EPA Dithers While a Popular Pesticide Threatens Ecosystems | Mother Jones

Henry - Please print out this article (link below) and put it in the Board's packets for next meeting. Thank you. I don't know how much more actual, genuine (non-industry) science it will take to get the Board to ban Neonicotinoid pesticides.

We and the world know they're killing bees (and other helpful insects, too, of course) while the Board fiddles with what new pesticides they're going to approve this month. There is much that can be said about their inaction on pesticides toxic not only to bees, but us, too - - but I will not do so at this time.

Please just see that they get this article. I hope you've given them the others I've sent from time to time. Do any Board members even comment on any of them, ever? You can include this email, too, if you think it might help them come to some good decisions!

Thank you..... - Nancy Oden, Clean Earth Farms, Jonesboro, Maine

http://www.motherjones.com/tom-philpott/2014/07/silent-spring-eternal-epa-dithers-while-popular-pesticide-threatens-ecosystems

The EPA Dithers While a Popular Pesticide Threatens Ecosystems

—By ***Tom Philpott*** *(/authors/tom-philpott)* | Fri Jul. 18, 2014 4:18 PM EDT

Ah, summer—the season when trillions of corn and soybean plants tower horizon-to-horizon in the Midwest. All told, US farmers planted more than 170 million acres (http://www.nass.usda.gov/Newsroom/2014/06_30_2014.asp) in these two crops this year—a combined landmass roughly equal in size to the state of Texas (http://www.statemaster.com/graph/geo_lan_acr_tot-geography-land-acreage-total). That's great news for the companies that turn corn and soy into livestock feed, sweeteners, and food additives; but not so great for honeybees, wild pollinating insects like bumblebees, and birds.

That's because these crops—along with other major ones like alfalfa and sunflower—are widely treated with pesticides called neonicotinoids. Made by European chemical giants Bayer and Syngenta, these chemicals generate a staggering $2.6 billion in annual revenue (http://www.rollcall.com/news/pesticide_ban_is_just_one_piece_of_honeybee_puzzle-233624-1.html) worldwide—and have come under heavy suspicion as a trigger of colony collapse disorder and other, less visible, ecological calamities.

Last year, the European Union imposed a two-year ban on the chemicals (http://www.motherjones.com/tom-philpott/2013/05/eu-ban-bee-harming-pesticides-puts-pressure-us-epa), pending more study of their effects on pollinators. The US Environmental Protection Agency—which originally approved the products through a highly dubious process I laid out here—has stood by (http://www.epa.gov/pesticides/about/intheworks/ccd-european-ban.html) these ubiquitous pesticides.

Meanwhile, damning research piles up.

• In a study (http://onlinelibrary.wiley.com/doi/10.1111/1365-2435.12292/full) (press release here (http://www.sciencedaily.com/releases/2014/07/140709140308.htm)) that came out in early July and was published in the peer-reviewed journal *Functional Ecology*, UK researchers outfitted bumblebees with radio-frequency identification tags, dosed some of them with levels of neonics equal to what they might find in a treated field, and set them outside to observe their foraging behavior. The results suggest that the pesticides impair bees' learning ability: Bees from untreated colonies improved their pollen-collecting ability as they learned to forage, while their neonic-exposed counterparts saw their pollen collection dwindle with time. The takeaway is similar to that of another bumblebee study (http://www.nature.com/nature/journal/v491/n7422/full/nature11585.html#affil-auth) (my summary here (http://www.motherjones.com/tom-philpott/2012/10/yet-another-study-links-bayer-pesticide-bee)), this one by a different set of UK researchers and published by *Nature* in 2012. Bad foraging makes bee colonies more vulnerable to a host of threats that confront them: loss of habitat, parasitic mites, and viruses.

• Another study (http://www.nature.com/nature/journal/vaop/ncurrent/full/nature13531.html#affil-auth), also released in July, adds weight to the concern that neonics aren't just harming insects, but also birds (http://www.motherjones.com/tom-philpott/2013/03/not-just-bees-bayers-pesticide-may-harm-birds-too). In this one, published in *Nature* and well-summarized by *National Geographic* (http://news.nationalgeographic.com/news/2014/07/140709-birds-insects-pesticides-insecticides-neonicotinoids-silent-spring/), scientists looked at neonic concentrations in water and bird populations over time on Dutch farmland. They found that in the areas with relatively high concentrations of a common neonic called imidacloprid, bird populations "tended to decline by 3.5 per cent on average annually." The evidence is circumstantial—they proved that neonics are correlated with, but not the cause of, bird declines. But the case is pretty damning: The declines began in the mid-'90s, when neonics were introduced; and the correlation with neonic concentrations held up when the researchers controlled for other factors that could cause bird decline, like changes in crop type and amount of fertilizer used. The authors conclude that neonics may have "cascading effects" on ecosystems—by poisoning insects en masse, they harm the other species that feed on them, including birds. In that way, neonics are reminiscent of the "persistent insecticides in the past"—a reference to the harsh, now-banned chemicals like DDT that Rachel Carson thundered against in her seminal 1962 book *Silent Spring.*

> In the areas with relatively high concentrations of a common neonic, bird populations "tended to decline by 3.5 per cent on average annually."

• *The Silent* Spring analogy got a depressing boost earlier in the summer when a group of European scientists called the Task Force on Systemic Pesticides released a comprehensive analysis of the recent science on neonics' ecosystem effects. Their conclusion, published in the peer-reviewed *Environmental Science and Pollution Research* (https://www.motherjones.com/files/iucnneonics.pdf): "Population-level impacts have been demonstrated to be likely at observed environmental concentrations in the field for insect pollinators, soil invertebrates and aquatic invertebrates." Translation: the stuff is likely not just killing bees, but also earthworms and water bugs like dragonflies. "The evidence is very clear. We are witnessing a threat to the productivity of our natural and farmed environment equivalent to that posed by organophosphates or DDT," Jean-Marc Bonmatin, of the National Center for Scientific Research (CNRS) in France and one of the 29 international researchers who conducted the four-year assessment, told *The Guardian* (http://www.theguardian.com/environment/2014/jun/24/insecticides-world-food-supplies-risk). "Far from protecting food production, the use of neonicotinoid insecticides is threatening the very infrastructure which enables it."

I asked the Environmental Protection Agency for comment on its neonic stance amid such withering criticism. "The EPA will continue monitoring the open literature and other data sources for further developments on this issue," the agency replied in a statement. Meanwhile, it is painstakingly reviewing its approval (http://www2.epa.gov/pollinator-protection/schedule-review-neonicotinoid-pesticides) of each of the major neonicitinoid products, the first of which won't be completed until 2016-'17.

Lay Off the Almond Milk, You Ignorant Hipsters (/tom-philpott/2014/07/lay-off-almond-milk-ignorant-hipsters)

That Antioxidant You're Taking Is Snake Oil (/tom-philpott/2014/07/everything-we-know-about-antioxidants-and-vitamins-wrong)

WRP#EKIDSRWW (/authors/tom-philpott) *Food and Ag Correspondent*

Tom Philpott is the food and ag correspondent for *Mother Jones*. For more of his stories, click here (http://www.motherjones.com/authors/tom-philpott). To follow him on Twitter, click here (http://twitter.com/#!/tomphilpott). RSS (/RSS/AUTHORS/116126) | TWITTER (HTTP://TWITTER.COM/TOMPHILPOTT)

(/authors/tom-philpott)

Boston deploys goats against poison ivy in Hyde Park

By Faiz Siddiqui | GLOBE CORRESPONDENT JULY 23, 2014

The leader, Cole, ambled up to the metal fence, and with a wide stare and what looked like a grin, began to survey his new domain.

One by one, the others followed Cole through the gate: Chester, a fellow LaMancha goat with a paintbrush-like black tail; Dalia, an Alpine with perky white ears; and Christopher, another Alpine with a long gray beard that conjured up the image of a wise man.

CONTINUE READING BELOW ▼

It was not long before all of Boston's newest contract employees had disappeared among the

tall trees and brush in Hyde Park's West Street urban wild. Their task: Help to clear 2 acres overrun with poison ivy, buckthorn, Asiatic bittersweet, Japanese knotweed, and other invasive species growing on Parks and Recreation Department property.

Best of all for the cloven-hoofed friends, these menaces are lip-smacking delicacies.

"It's not only cute, but it makes really good sense," said 27-year-old Jessica Muscaro, the project coordinator for the Hyde Park Green Team.

Department officials said it was the first time Boston has sought the help of goats for a city project. Officials say the hairy, four-legged weed whackers represent a fast, clean, and efficient way to clear the area for green space without using herbicides or loud and polluting machinery.

The four goats will live in the urban wild for eight weeks, protected by a solar-powered electric fence. People are encouraged to look, but not touch, as the poison ivy oils may stick to the goats' coats, even though it does not harm their digestive tracts.

CONTINUE READING BELOW ▼

Teenagers from the Hyde Park Green Team will provide water and food to supplement the goats' diet , then begin pruning trees and building trails once the area cleared.

"Goats are an ecofriendly way to regulate overgrowth and manage pests and weeds, while giving nutrients back to the earth," Mayor Martin J. Walsh said in a statement.

Tony Barrows, who has lived in Hyde Park for 27 years, remembered taking his young daughter to the site, alongside the Neponset River, in the 1990s. That was before the trees had been choked by the Asiatic bittersweet and the trails covered by other invasive plants.

"I'd like to see some development along the river bank where people can jog, or just sit down, read a book," he said.

DAVID L RYAN/GLOBE STAFF

The goats will live on site at the West Street Urban Wild for eight weeks.

The $2,800 to rent the goats is being covered by grants provided to the Southwest Boston Community Development Corporation. James Cormier, owner of the Goatscaping Co. in Plympton, is providing the animals, which range from 120 to 170 pounds.

“It would be way more time-consuming for the city to come in and start chop, chop, chopping away,” he said.

The community development corporation’s assistant director, Pat Alvarez, said she came up with the idea to use goats after hearing about other cities using animals to make way for urban greenspace. Goats, sheep, llamas, and wild burros have cleared brush at O’Hare International Airport in Chicago. In Washington, D.C., goats helped clear the Congressional Cemetery in 2013.

Alvarez fondly remembered being chased around her yard by a “mean” billy goat as a child.

The four goats deployed in Boston were similarly mischievous Wednesday.

One began to chew on a Goatscaping sign dug into the dirt, prompting Cormier to yell, “Hey, don’t eat the sign!” Another, Dalia, attempted to climb a tree before giving up.

But they soon got down to business. Christopher's beard flapped against his chin as he chomped on a leafy bush. Dalia's long ears wiggled as she chewed a dense shrub to the stem. Within an hour, small sections of foliage had been cleared.

"They're quiet, unlike machinery," Alvarez said. "We also think they're going to be great ambassadors for the urban wild. Plus, they're just fun."

Faiz Siddiqui can be reached at faiz.siddiqui@globe.com.