Maine Board of Pesticides Control

Miscellaneous Pesticides Articles
June 2015

(identified by Google alerts or submitted by individuals)

Chamberlain, Anne

From: Fish, Gary
Sent: Wednesday, May 06, 2015 9:48 AM
To: AF-Pesticides
Subject: FW: US gives farmers approval to spray crops from drones **FYI**

FYI…

3. US gives farmers approval to spray crops from drones

Associated Press
Scott Smith
May 5, 2015

A drone large enough to carry tanks of fertilizers and pesticides has won rare approval from federal authorities to spray crops in the United States, officials said Tuesday.

The drone, called the RMAX, is a remotely piloted helicopter that weighs 207 pounds (94 kilograms), said Steve Markofski, a spokesman for Yamaha Corp. U.S.A., which developed the aircraft.

Smaller drones weighing a few pounds had already been approved for limited use to take pictures that help farmers identify unhealthy crops. The RMAX is the first time a drone big enough to carry a payload has been approved, Markofski said.

The drone already has been used elsewhere, including by rice farmers in Japan. The FAA approved it for the U.S. on Friday.

"I certainly understand their cautious approach," Markofski said. "It's a daunting task given our airspace is complicated."

The drone is best suited for precision spraying on California's rolling vineyards and places that are hard to reach from the ground or with larger, piloted planes, said Ken Giles, professor of biological and agricultural engineering at the University of California, Davis. Giles tested the drone in California to see if it could be used here.

"A vehicle like this gives you a way to get in and get out and get that treatment done," Giles said.

Brian Wynne, president and CEO of the Association for Unmanned Vehicle Systems International, said in a statement that the approval highlights other potential uses.

"The FAA is taking an important step forward to helping more industries in the U.S. realize the benefits (drone) technology has to offer," he said.

To view this story at its original source, follow this link: https://www.yahoo.com/tech/s/us-gives-farmers-approval-spray-crops-drones-005109741.html

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"No other human occupation opens so wide a field for the profitable & agreeable combination of labor with cultivated thought as agriculture." Abraham Lincoln

Please be safe this season--visit www.agrisafe.org for helpful safety information.

Colo. tries to clamp down on pesticide use on pot

Trevor Hughes, USA TODAY *8:06 a.m. EDT May 12, 2015*

A Colorado courtroom dispute has the potential to dramatically alter how marijuana is grown across the state. Health inspectors are trying to clamp down on pesticide use in pot production. VPC

DENVER — A dry courtroom dispute unfolding here has the potential to dramatically alter how marijuana is grown across Colorado, as health inspectors try clamp down on pesticide use by pot growers.

The court fight is over whether Denver health officials and state agriculture inspectors have the right to quarantine and test marijuana they believe has been improperly contaminated with certain pesticides.

Marijuana store Organic Greens is asking a city judge to lift one of those quarantines and allow it to sell 15-20 pounds of marijuana its owner admits was treated with a fungicide called Eagle 20. He says the chemical is widely used within the industry and by other farmers to fight powdery mildew, and that it poses little risk to consumers.

(Photo: Trevor Hughes, USA TODAY)

USA TODAY

Pot for pets? Some dog lovers say cannabis eases pain

(http://www.usatoday.com/story/news/nation/2015/05/11/cannabis-pet-treats/27006099/)

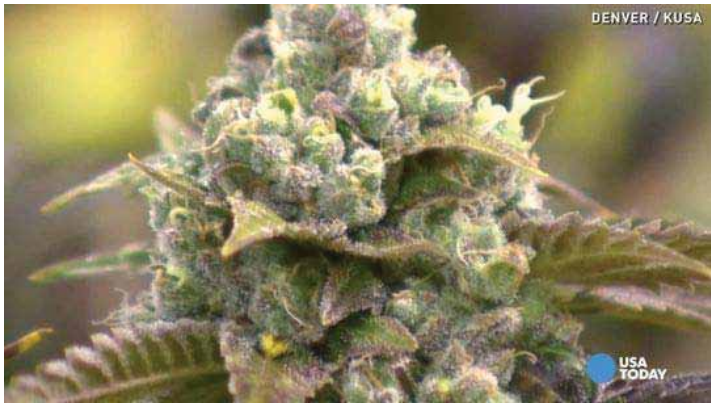
USA TODAY

Nevada lawmakers take Colorado marijuana tour

(http://www.usatoday.com/story/news/nation/2015/04/25/nevada-lawmakers-take-colorado-marijuana-tour/26387241/)

Marijuana in Colorado wholesales for about $2,500 a pound. Colorado in 2014 legalized recreational marijuana sales under a licensing system that was intended to ensure legal pot was grown safely and cleanly.

Denver and state officials — noting it's misleading for the company to call itself organic — says Organic Greens is violating state and federal law by using a chemical not approved for marijuana. Virtually no pesticides have been approved for use on marijuana, which means the state could seize any pot plants testing positive for Eagle 20.



Denver city officials have temporarily barred the sale of approximately 60,000 marijuana plants. Why? The plants might have been contaminated by unapproved pesticides.

The city has already placed "holds" on tens of thousands of plants worth millions of dollars from multiple growers as it awaits test results.

Denver officials say sick children could be harmed if they inhale or ingest marijuana treated with Eagle 20, a charge disputed by Organic Greens owner Andrew Boyens.

"Everything we produce is safe," Boyens said on the stand Monday afternoon.

Marijuana plants grow inside a special container designed for organic cultivation. GrowSpace Storage is marketing the pods as a way for marijuana farmers to raise their cannabis without the need for pesticides by isolating the plants inside sealed containers impervious to outside air and contamination. *(Photo: Trevor Hughes, USA TODAY)*

Under questioning from city and state attorneys, Boyens and a toxicologist working on his behalf both acknowledged that Eagle 20 is not specifically approved for use on marijuana, which its maker, Dow Chemical, independently confirmed.

Under Colorado's legal marijuana system, licensed growers may use only approved pesticides on their plants. Otherwise, city officials say, no one truly knows what the risks are.

"The science has not been done," said Marley Bardowski, a lawyer and enforcement expert with the Denver City Attorney's Office. "The bottom line is that the testing hasn't been done. The research hasn't been done."

Citing the ongoing case, city and state health and agriculture officials declined to comment on whether they plan to continue placing "holds" on marijuana plants suspected of being contaminated with unapproved pesticides.

Marijuana industry experts say the pesticide problem is a huge new stumbling block for pot growers trying to stay legal.

USA TODAY

Cannabis oil use now legal in Tennessee

(http://www.usatoday.com/story/news/nation/2015/05/05/governor-signs-cannabis-oil-bill/26907707/)

"We're really stuck," said Mike Elliot of the pro-legalization Marijuana Industry Group.

A court ruling in their favor could embolden health officials to even more aggressively inspect for pesticide use. State regulators have repeatedly delayed rollout of a program to test all consumer marijuana for pesticide contamination, and Denver health inspectors appear to have stepped into that vacuum.

Under state law, all licensed marijuana growers are supposed to keep a log of what pesticides were applied to their plants, how much, and when. Boyens testified that inspectors couldn't find his log because his employees accidentally spilled coffee on it and then threw it away. City health inspectors say his marijuana tested positive for trace amounts of at least three other pesticides.

USA TODAY

Freeman: Marijuana 'I'll eat it, drink it, smoke it, snort it'

(http://www.usatoday.com/videos/news/nation/2015/05/11/27114293/)

Colorado's legal marijuana marketplace is being closely watched by lawmakers around the world as they consider whether to relax their prohibitions on a widely used but otherwise entirely unregulated product.

Monday's hearing focused largely on testimony from defense toxicologists, who argued the amounts being used on marijuana poses little danger to users. City and state officials repeatedly countered by pointing out Eagle 20 hasn't been approved for use on marijuana at any level.

The hearing before Denver District Court Judge John Madden continues Tuesday.

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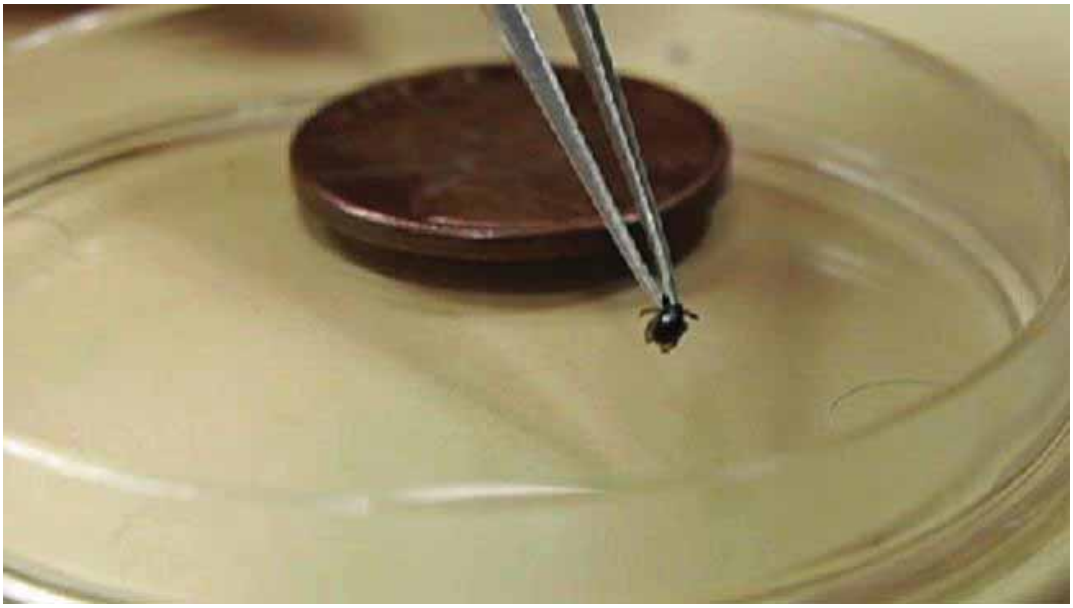
http://www.necn.com/news/new-england/Despite-Spread-of-Lyme-Disease-Massachusetts-Dedicates-No-Money-to-Prevention-303294451.html

Despite Spread of Lyme Disease, Massachusetts Dedicates No Money to Prevention

Ticks and Lyme have become one of the region's most commonly reported infectious diseases

By Beth Daley and New England Center for Investigative Reporting



The predawn rumble of pesticide-spraying trucks is a rite of spring in almost 200 Massachusetts communities. Some $11 million is spent in the state each year controlling and counting the pests and educating residents about how to avoid contracting mosquito-borne diseases such as West Nile virus.

Yet no state funds are dedicated to tick-borne diseases, one of which, Lyme, infects at least 5,500 residents a year in Massachusetts and likely many more. Residents may notice that gap even

more this spring: The winter's deep snow probably insulated ticks from low temperatures and heavy winter mortality, say some entomologists.

Ticks and Lyme have spread across Massachusetts in the past 40 years to become one of the region's most commonly reported infectious diseases, yet the state's public health priorities have not kept pace. Two years ago, a special state Lyme commission suggested a modest investment of less than $300,000 for a public education program, yet no money has been set aside, and the commission's other specific recommendations – from promoting more awareness in the medical community to better disease surveillance – have not been adopted.

"The state needs to step up to the plate,'' said Larry Dapsis, deer-tick project coordinator and entomologist for Barnstable County, which funds the state's only county tick-education program. Tracking the West Nile virus in mosquitoes that can make humans sick can be like "looking for a needle in a haystack,'' Dapsis said. "For ticks we look at the landscape and, well, it's scary."

There are at least six tick-borne diseases in Massachusetts, and experts expect more soon: The Lone Star tick, which can transmit several pathogens and spark a bizarre allergy to red meat, took up residence on the Massachusetts mainland last year in Sandy Neck Beach Park in West Barnstable. A new human tick-borne disease – borrelia miyamotoi – was reported in the Northeast in 2013 and is being found in people in Massachusetts: In 2014, Cape Cod Hospital had 26 cases.

"When we do surveillance on mosquitoes, we are also trying to communicate risk to people and tell them how to avoid contracting the disease; that is missing with ticks,'' said Chris Horton, superintendent of the Berkshire County Mosquito Control Project in Pittsfield, one of the state's 11 regional mosquito control districts. Berkshire County has the highest incidence of anaplasmosis, a tick-borne illness that can cause fever, chills and confusion, with 41 cases per 100,000 residents in 2013. In Hampshire and Worcester counties, it is less than 2 cases per 100,000 residents, some of the lowest in the state.

Few argue against mosquito control in Massachusetts. Started out in part because of the nuisance factor, it has evolved to try to limit disease threats to the public. And, many argue, it works: Usually less than 30 people a year are reported to contract West Nile and even fewer get Eastern Equine Encephalitis.

State Representative Carolyn Dykema, a Holliston Democrat, filed a bill this year for the third time to expand the authority of the mosquito districts to include ticks, but few tick experts expect it to gain traction.

"(Ticks) are very different to control,'' compared with mosquitoes, Horton said. For example, mosquitoes are airborne at specific times of day at which they can be targeted with pesticides, he said, and larvaeside can be sprayed in standing water where they breed. Ticks are not very mobile, can be found throughout landscapes, and populations can dramatically vary even within short distances.

State officials receive about $40,000 in federal dollars a year to help support Lyme disease surveillance and education, and officials say other duties around Lyme are not covered by any specific state line item but is part of the department's general funding.

Largely, state officials say they are focusing on educating residents to protect themselves with tick checks, covering up when outside, and using tick-killing sprays on footwear and outerwear.

"We consider the outreach we do critically important – and that has not stopped,'' said Katie Brown, the state's public health veterinarian. A state epidemiologist, she said, is now developing multimedia tick education presentations that schools can borrow, and officials created public service videos last year that are available for local boards of health and to the public.

Northeast states, in general, don't spend much to prevent tick-borne diseases, although Maine voters in November approved $8 million for a lab that will test ticks and conduct other research at the University of Maine in Orono by 2017.

In Massachusetts, an $111,000 Community Innovation Challenge grant last year subsidized testing costs of ticks to track what pathogens and parasites were being found in 32 communities. Findings of the testing at the Laboratory of Medical Zoology at the University of Massachusetts Amherst included: the discovery of Lone Star ticks in counties in which they had previously not been recorded (they are considered resident in only Barnstable County); that ticks harboring more than one pathogen that can cause illness in humans are present throughout the state; and that those most frequently bit by ticks appear to be children and older people.

While Stephen M. Rich, the laboratory director and other tick experts were hoping the testing would continue to be funded, the state canceled the entire grant program this year. The lab still tests ticks for a $50 fee for residents who mail them in, and Rich is still working to grow the program. He said that to have a robust tick surveillance and prevention program, all a town would need to do is devote $1,000 to $3,000 of their state funding.

"To establish a disease surveillance and prevention system, like the one we have for mosquitoes, will require enabling legislation that allows towns to subsidize this service that Massachusetts residents want," Rich said in an email.

A controversial illness

Lyme Disease is one of the most vexing public health issues in Massachusetts. First discovered in a group of children in Lyme, Conn. in the mid-1970s, it has spread throughout every community in Massachusetts and much of the Northeast.

Deer ticks – often no bigger than the size of a poppy seed – become more active as the weather heats up, latching onto pets and people as they pass through forested areas and tall grasses. As the parasites feed on blood, they can pass pathogens to people that sicken them, the most common of which is Lyme.

Early symptoms of Lyme can include a skin rash that looks like a bullseye, headache, fatigue, and fever. If caught early, a month or less of antibiotics cures most cases, but if the infection is left untreated, it can spread to the joints, heart and nervous system, causing such symptoms as facial paralysis, arthritis and tingling sensations, and in very rare cases, death.

There were 5,665 confirmed and probable cases of Lyme in 2013, the last year of available data, but federal officials say the number of cases is underreported. Two years ago, the U.S. Centers for Disease Control and Prevention, using a new way of measuring Lyme disease diagnoses, said cases of Lyme were likely 10 times more common than previous national counts, affecting possibly 300,000 people a year in the U.S., the bulk in the Northeast. By that measure, the number of Lyme cases in Massachusetts would be about 50,000.

Communities trying to fill the gap

In the absence of any dedicated state program or funding, communities, individuals, Lyme patients and health associations are, themselves, attempting to educate residents.

The first Central Massachusetts Lyme Conference was held in Worcester in March. The Massachusetts Association of Public Health Nurses is holding a daylong Brewster seminar on Lyme and other tick-borne disease on April 16. In North Andover, the public health nurse is developing prevention materials to be placed in the library and other public places. In Medfield, residents are discussing whether to spray for ticks on the perimeter of two playing fields.

Such patchwork attempts, however, have not yet appeared to result in any reduction of tick-borne disease statewide, according to statistics.

“It really comes down to a budget item,’’ said Chris Kaldy, chair of the Medfield Lyme Disease Study Committee. The community works hard at tick education, that includes providing tick check cards for first- and third-graders to bring home. Kaldy would like to do more, but, she said, “We don’t have the money to do mailings (and) other ways to get the word out.”

Meanwhile, some communities have added a controversial prevention effort: Deer kills. Because a deer can harbor hundreds of ticks, some studies and experiments show killing deer can reduce tick-borne diseases in people. Dover, Sudbury and other communities have allowed bow-and-arrow hunting on some town lands for several years; Westborough began allowing it two years ago.

The Environmental Bond bill passed last year required the state to develop a plan to safely and humanely cull deer where their numbers have risen too high, such as in the Blue Hills Reservation outside Boston.

“We have a real threat to public health,’’ said Sen. Brian A. Joyce, a Milton Democrat, who proposed the language in the bond bill.

A spokesman for the state Department of Conservation and Recreation said state officials are developing recommendations for the Blue Hills herd and will seek the public's input before any formal plan is adopted.

But others, including some academics, say it is not clear fewer deer will translate into fewer cases of Lyme, in part because ticks get transported on so many other animals.

One of the only points of agreement for most people involved in the Lyme – and deer – debate is that people should personally protect themselves. Experts also warn that while pest-control companies are increasingly offering tick control, such as spraying yard perimeters, they need to beware of claims, especially of all-natural products.

While Nootkatone, a bio-active natural component of Alaskan yellow cedar oil, has been shown to kill ticks in high numbers, it is currently quite expensive to produce, according to Tom Mather, a University of Rhode Island professor and tick expert who runs www.tickencounter.org, a website dedicated to tick prevention. Many pest control companies offer non-yellow cedar products that do not work well against ticks, said Mather, who has tested some of the products' main ingredients. It turns out that red cedar is just not the same as yellow cedar.

"We found no tick-killing effect using two different red cedar products. People need to be warned. There are so many formulations of botanical oils but so few have actually been tested against ticks," Mather said.

And it may be an important year to work on tick protection. According to Jim Dill, a pest management specialist at the University of Maine, the extreme cold probably didn't kill many ticks this winter because "most of them were three feet under…warm and well-insulated" by the snow, he said.

Beth Daley is a reporter at the New England Center for Investigative Reporting, an independent, nonprofit news center based at Boston University and WGBH News. She can be reached bdaley@bu.edu. Follow her on Twitter at @bethbdaley.

MENU

Colony Loss 2014 – 2015: Preliminary Results

MAY 13, 2015 • BLOG

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Note: *This is a preliminary analysis. Sample sizes and estimates are likely to change. A more detailed final report is being prepared for publication in a peer-reviewed journal at a later date.*

The Bee Informed Partnership (http://beeinformed.org), in collaboration with the Apiary Inspectors of America (AIA) and the United States Department of Agriculture (USDA), is releasing preliminary results for the ninth annual national survey of honey bee colony losses. For the 2014/2015 winter season, a preliminary 6,128 beekeepers in the United States provided valid responses. Collectively, these beekeepers managed 398,247 colonies in October 2014, representing about 14.5% of the country's estimated 2.74 million managed honey bee colonies[1].

About two-thirds of the respondents (67.2%) experienced winter colony loss rates greater than the average self-reported acceptable winter mortality rate of 18.7%. Preliminary results estimate that a total of 23.1% of the colonies managed in the Unites States were lost over the 2014/2015 winter. This would represent a decrease in losses of 0.6% compared to the previous 2013/2014 winter, which had reported a total loss estimated at 23.7%. This is the second year in a row the reported colony loss rate was notably lower than the

9-year average total loss of 28.7% (see Figure 1).

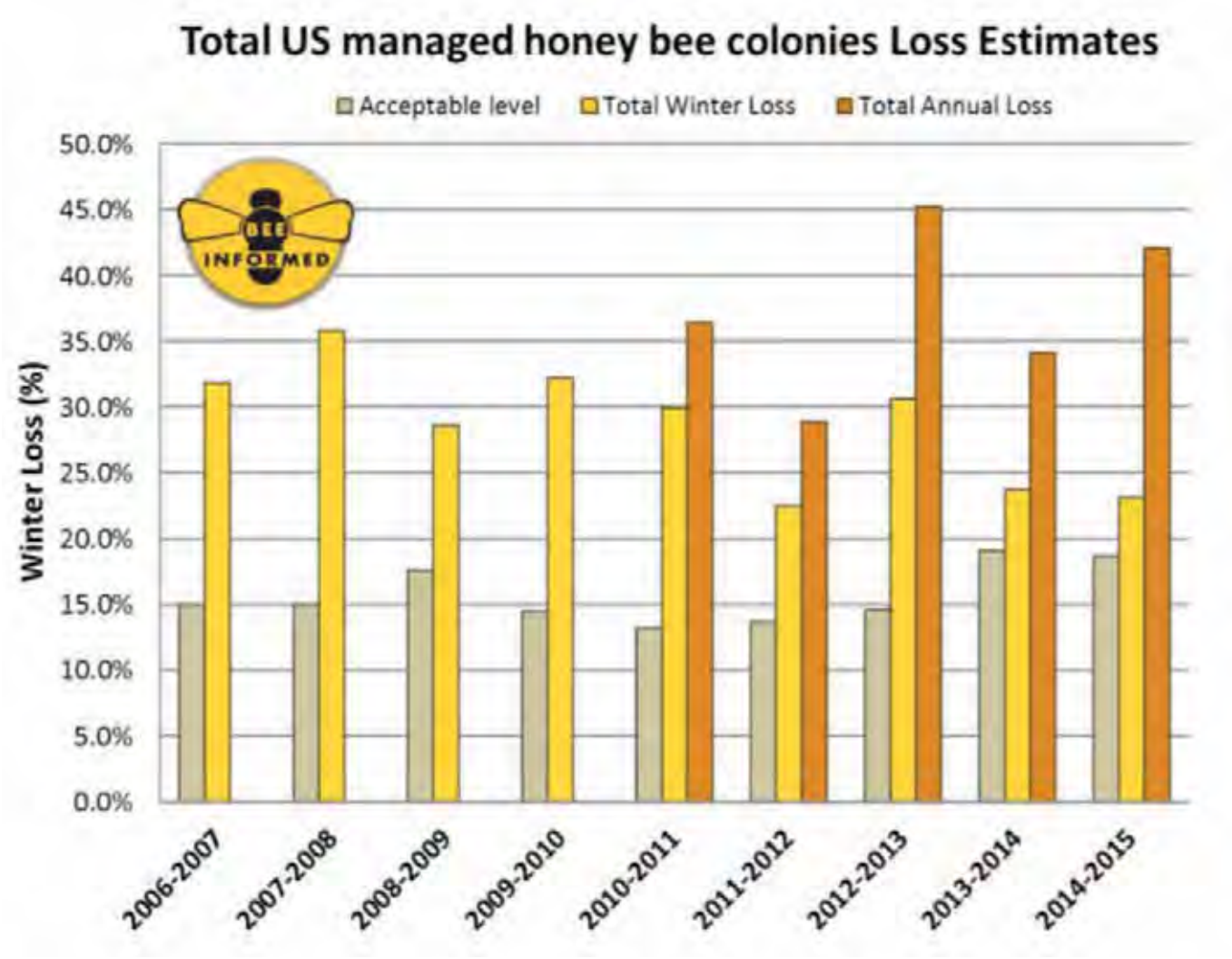

Figure 1: Summary of the total colony losses overwinter (October 1 – April 1) and over the year (April 1 – April 1) of managed honey bee colonies in the United States. The acceptable range is the average percentage of acceptable colony losses declared by the survey participants in each of the nine years of the survey. Winter and Annual losses are calculated based on different respondent pools.

Beekeepers do not only lose colonies in the winter but also throughout the summer, sometimes at significant levels. To quantify this claim of non-winter colony mortality of surveyed beekeepers, we have included summer and annual colony losses since 2010/2011. In the summer of 2014 (April – October), colony losses surpassed winter losses at 27.4% (totalsummer loss). This compares to summer losses of 19.8% in 2013. Importantly, commercial beekeepers appear to consistently lose greater numbers of colonies over the summer months than over the winter months, whereas the opposite seems true for smaller-scale beekeepers. Responding beekeepers reported losing 42.1% of the total number of colonies managed over the last year (total annual loss, between April 2014 and April 2015). This represents the second highest annual loss recorded to date.

As in previous years, colony losses were not consistent across the country, with annual losses exceeding 60% in several states, while Hawaii reported the lowest total annual colony loss of ~14% (see Figure 2).

Figure 2: Total annual loss (%) 2014-2015 by state. Respondents who managed colonies in more than one state had all of their colonies counted in each state in which they reported managing colonies. Data for states with fewer than five respondents are withheld.

This survey was conducted by the Bee Informed Partnership, which receives a majority of its funding from the National Institute of Food and Agriculture, USDA (award number: 2011-67007-20017).

[1] Based on NASS 2015 figures

[2] Previous survey results found a total colony loss in the winters of 24% in the winter of 2013/2014, 30% in 2012/2013, 22% in 2011/2012, 30% in 2010/2011, 32% in 2009/2010, 29% in 2008/2009, 36% in 2007/2008, and 32% in 2006/2007 (see reference list).

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Written By: The Bee Informed Team

has written 33 post in this blog.

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Federal government announces plan to bolster honeybee, butterfly populations

Published May 19, 2015 | Associated Press

WASHINGTON – The federal government hopes to reverse America's declining honeybee and monarch butterfly populations by making more federal land bee-friendly, spending more money on research and considering the use of less pesticides.

Scientists say bees -- crucial to pollinate many crops -- have been hurt by a combination of declining nutrition, mites, disease, and pesticides. The federal plan is an "all hands on deck" strategy that calls on everyone from federal bureaucrats to citizens to do what they can to save bees, which provide more than $15 billion in value to the U.S. economy, according to White House science adviser John Holdren.

"Pollinators are struggling," Holdren said in a blog post, citing a new federal survey that found beekeepers lost more than 40 percent of their colonies last year, although they later recovered by dividing surviving hives. He also said the number of monarch butterflies that spend the winter in Mexico's forests is down by 90 percent or more over the past two decades, so the U.S. government is working with Mexico to expand monarch habitat in the southern part of that country.

The plan calls for restoring 7 million acres of bee habitat in the next five years. Numerous federal agencies will have to find ways to grow plants on federal lands that are more varied and better for bees to eat because scientists have worried that large land tracts that grow only one crop have hurt bee nutrition.

The plan is not just for the Department of Interior, which has vast areas of land under its control. Agencies that wouldn't normally be thought of, such as Housing and Urban Development and the Department of Transportation, will have to include bee-friendly landscaping on their properties and in grant-making.

That part of the bee plan got praise from scientists who study bees.

"Here, we can do a lot for bees, and other pollinators," University of Maryland entomology professor Dennis vanEnglesdorp, who led the federal bee study that found last year's large loss. "This I think is something to get excited and hopeful about. There is really only one hope for bees and it's to make sure they spend a good part of the year in safe healthy environments. The apparent scarcity of these areas is what's worrying. This could change that."

University of Montana bee expert Jerry Bromenshenk said the effort shows the federal government finally recognizes that land use is key with bees.

"From my perspective, it's a wake-up call," Bromenshenk wrote in an email. "Pollinators need safe havens, with adequate quantities of high-quality resources for food and habitat, relatively free from toxic chemicals, and that includes pollutants as well as pesticides and other agricultural chemicals."

The administration proposes spending $82.5 million on honeybee research in the upcoming budget year, up $34 million from now.

The Environmental Protection Agency will step up studies into the safety of widely used neonicotinoid pesticides, which have been temporarily banned in Europe. It will not approve new types of uses of the pesticides until more study is done, if then, the report said.

"They are not taking bold enough action; there's a recognition that there is a crisis," said Lori Ann Burd, environmental health director for the advocacy group Center for Biological Diversity. She said the bees cannot wait, comparing more studies on neonicotinoids to going to a second and third mechanic when you've been told the brakes are shot.

The report talks of a fine line between the need for pesticides to help agriculture and the harm they can do to bees and other pollinators.

Lessening "the effects of pesticides on bees is a priority for the federal government, as both bee pollination and insect control are essential to the success of agriculture," the report said.

Print Close

URL

http://www.foxnews.com/science/2015/05/19/federal-government-announces-plan-to-bolster-honeybee-butterfly-populations/

http://www.csmonitor.com/Science/2015/0519/What-s-in-Obama-s-plan-to-reverse-honey-bee-and-butterfly-decline-video

The CHRISTIAN SCIENCE MONITOR

May 19, 2015

What's in Obama's plan to reverse honey bee and butterfly decline (+video)

President Barack Obama has announced a plan to increase the dwindling population of honey bees and monarch butterflies by making federal land more suitable to the unsung workers that support American agriculture.

The Obama administration has a multi-pronged approach: planting more diverse vegetation on millions of acres of federal land, allocating $82.5 million of federal funds for research, and pushing a reduction in the use of pesticides.

However, some scientists say that these measures aren't enough to save the bees, or the US farm economy.

Recommended: Are you scientifically literate? Take our quiz

The plan begins with the creation of a Pollinator Health Task Force, which is expected to include representatives from 14 different federal departments who will create a strategy to improve the quality of pollinator habitats.

Through these measures, the Obama administration hopes to combat Colony Collapse Disorder, an as-yet unexplained syndrome that causes entire colonies of bees to die, leaving its queen bee, honey, and immature bees behind. Bee populations are also weakened by malnutrition, which is caused by a lack of agricultural diversity on lands that grow only one crop, and by exposure to pesticides.

Meanwhile, monarch butterflies face a similar problem as the milkweed, their natural food source, has declined as a result of farming practices.

“Pollinators are struggling,” John P. Holdren, director of the Office of Science and Technology Policy, wrote in a White House blog post. “Last year, beekeepers reported losing about 40% of honey bee colonies, threatening the viability of their livelihoods and the essential pollination services their bees provide to agriculture.”

Holdren also estimates that, through pollination, bees provide $15 billion of service to the US economy. Honey bees and monarch butterflies are two of the most productive pollinating species, a vital service to agriculture. In 2013, agriculture and agriculture-related industries contributed $789 billion to the US gross domestic product (GDP), a 4.7-percent share.

Many in the environmental community appreciate that the president has taken up an issue many would dismiss as inconsequential.

"Here, we can do a lot for bees, and other pollinators," University of Maryland entomology professor Dennis van Englesdorp, who led the federal bee study that outlined the scale of last year's loss, told the Associated Press. "This I think is something to get excited and hopeful about. There is really only one hope for bees and it's to make sure they spend a good part of the year in safe healthy environments. The apparent scarcity of these areas is what's worrying. This could change that."

Others think the administration should be pushing harder on agricultural producers to grow diverse crops and discontinue pesticide use, rather than putting the onus on the federal government.

“If you don’t change farming and you don’t change pesticide use, you’re not going to make substantial changes in the health of pollinators,” Simon Fraser University biology professor Mark Winston told the Washington Post.

Mr. Obama has begun a symbolic effort to save the bees in his own back yard, signing off on a beehive and a pollinator's’ garden on the White House’s South Lawn. And when May Berenbaum, the National Medal of Science winner thanked Obama for caring about bees, he shook her hand and said “I *do* care about bees — and we’re going to fix them!”

POLLINATORS:

White House lays out ambitious plan to save bees

Tiffany Stecker, E&E reporter

Greenwire: Tuesday, May 19, 2015

The White House released its comprehensive strategy to stem the steep decline in pollinators today, the start of what's likely to become a growing debate in the federal government and Congress.

The goals are ambitious: limit honeybee overwintering losses to 15 percent within 10 years; boost monarch butterfly numbers to 225 million in the insect's winter habitat in Mexico, a roughly fourfold increase from the current population; and restore and enhance 7 million acres of land for pollinators over the next five years through federal actions and public-private partnerships.

To do this, federal agencies must boost research on environmental stressors to bees and butterflies; expand pollinator acreage in the Conservation Reserve Program (CRP), which pays landowners not to farm on large tracts of land; provide seed mixes that offer plenty of blooms with good-quality pollen; and improve outreach, especially between beekeepers and farmers, according to the White House Task Force on Pollinator Health, which is headed by the Agriculture Department and U.S. EPA.

"The President has emphasized the need for an 'all hands on deck' approach to promoting pollinator health, including engagement of citizens and communities and the forging of public-private partnerships," John Holdren, assistant to the president for science and technology and director of the White House Office of Science and Technology Policy, wrote in a **blog post**.

Beekeepers, agriculture organizations, the pesticide industry and environmentalists have been waiting for the report for nearly a year, since President Obama released his memorandum directing federal resources toward research and other actions to stave off a pollinator decline (***Greenwire***, June 20, 2014).

Pollinators are struggling, Holdren said. A recent USDA report found beekeepers had lost more than 40 percent of their honeybee colonies last year. Despite a recent uptick, monarch butterfly populations have also suffered dramatic losses of around 90 percent (***E&ENews PM***, Jan. 27).

Scientists say the drop in pollinators is tied to a combination of the loss of forage, poor-quality pollen, diseases, and parasites like the Varroa mite and pesticide exposure.

The strategy also calls on Congress to approve the $82 million dedicated to pollinators in Obama's fiscal 2016 budget, the bulk of which will go to USDA's research arms and the agency that administers CRP. The request is $34 million over fiscal 2015 enacted levels.

"I would say that's a down payment," Tom van Arsdall, a spokesman for the Pollinator Partnership, said on the $82 million.

About $20 billion in crops depend on pollinators for production. Beekeepers in particular have been struggling to maintain viable colonies in the last decade, Darren Cox, president of the American Honey Producers Association, said in a statement.

"As an industry we have managed pests, pathogens and other bee health challenges successfully for decades, including the varroa mite. But significant habitat loss and increasing pesticide pressures are combining with those stressors to make for an all-too-formidable opponent, even for the mighty and long resilient honey bee," Cox said.

The report fell short of addressing environmental groups' calls for restricting neonicotinoids, pesticides that absorb into a plant and can present themselves in pollen. A total of 128 groups signed a **letter** in March asking EPA to tighten regulations on seed treatments for neonicotinoids and speed up the timeline for reviewing the chemicals.

"The agency outlined it may consider restrictions on a broad range of foliar use products, but did not outline restrictions for pesticide coated seeds -- one of the largest uses of bee-harming pesticides," Tiffany Finck-Haynes, food futures campaigner with Friends of the Earth, said in an email. The report also doesn't address pesticide impacts on native bees, she added.

The task force report repeated EPA's position that it would review the neonicotinoids imidacloprid, thiamethoxam, clothianidin and dinotefuran between now and 2017. The agency said it will propose a ban on spraying pesticides that kill bees on contact during the bloom period. EPA is also continuing to revise its study of neonicotinoid benefits on soybeans and complete similar assessments for other crops. The soybean assessment released last October, which found that neonicotinoid seed treatments offer little to no benefit to soybean producers, was criticized by the pesticide industry and was a central discussion point in a recent House Agriculture Committee hearing (***E&E Daily***, May 14).

EPA is also considering using state pollinator protection plans, which are designed to improve communication between beekeepers and farmers on the use of pesticides, as a mitigation strategy as it relates to legally binding pesticide label instructions.

These plans are supported by the pesticide industry and are met with skepticism from beekeepers.

"We've seen some great successes from the states that have already done this as a way to really encourage local stakeholder involvement and conversation," said Jeff Donald, a spokesman with Bayer CropScience. Bayer develops treatments for the Varroa mite, as well as neonicotinoids.

But beekeepers still question the overall effectiveness of the plans, Cox said.

"We very much have concerns on the reliance of state pollination protection plans," he added.

Additional reports on forage and pollinator nutrition, the effects of the Varroa mite, and crop production are expected to be released this week.

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MAJ RUNDLÖF

A study in Sweden monitored how bees respond to neonicotinoids in the wild.

POLLINATORS

Bee studies stir up pesticide debate

The threat that neonicotinoids pose to bees becomes clearer.

BY DANIEL CRESSEY

The case for restricting a controversial family of insecticides is growing. Two studies published on 22 April in *Nature*[1,2] address outstanding questions about the threat that the chemicals pose to bees, and come as regulators around the world gear up for a fresh debate on pesticide restrictions.

Many bee populations are in steep decline, with multiple causes identified, including parasites and the loss of food sources. Also blamed are neonicotinoids, a widely used class of insecticides that are often applied to seeds, and find their way into the pollen and nectar of plants. The use on seeds of three — clothianidin, imidacloprid and thiamethoxam — is temporarily banned in the European Union because of concern that they might harm pollinators; the ban is up for review in December. In the United States, there are no such restrictions, but the US Environmental Protection Agency said on 2 April that it was "unlikely" to approve new outdoor neonicotinoid-pesticide uses without new bee data.

So far, the data are mixed. Many studies that link the poor health of bee colonies to the pesticides have been criticized, for example for not using realistic doses. Some defenders of the chemicals have argued that if neonicotinoids are harmful, bees will learn to avoid treated plants.

Geraldine Wright, an insect neuroethologist at Newcastle University, UK, and her colleagues investigated this aspect. They confined honeybees (*Apis mellifera*) and bumblebees (*Bombus terrestris*) to boxes and gave them a choice between plain nectar and nectar laced with imidacloprid, thiamethoxam or clothianidin. The researchers found that the bees showed no preference for the plain nectar. In fact, the insects were more likely to choose the nectar containing imidacloprid or thiamethoxam[1], although it is not clear whether the preference would occur in the wild.

Wright's team also analysed the response of the bees' taste neurons to neonicotinoids, and found that they reacted the same regardless of concentration — indicating that the bees cannot taste the pesticides and that the preference is caused by some other mechanism. Other studies have shown that neonicotinoids activate receptors in bee brains linked to memory and learning.

In contrast to Wright and colleagues' work, the second paper[2] looked at honeybees and wild bees, including bumblebees, in the field. Maj Rundlöf, an ecologist at Lund University in Sweden, and her colleagues analysed eight fields of oilseed rape sown with seeds treated with clothianidin and eight fields sown with untreated seeds across southern Sweden.

Honeybees did not respond differently in the treated and untreated fields. But the researchers found that wild-bee density in treated fields was around half that in untreated fields. Nests of solitary bees and bumblebee-colony growth were also reduced in treated fields. "I'm worried about the effects on wild bees," says Rundlöf.

She suggests that honeybees have larger colony sizes, which could sustain higher losses of foraging bees before showing overall health effects. But that suggests another problem. "Honeybees are the model organism that is used in toxicity testing for pesticides," she says. If they are not representative of bees in general, it could explain why more studies have not detected negative effects.

Dave Goulson, a bee researcher at the University of Sussex in Brighton, UK, also suspects that honeybees are more resilient than wild bees to neonicotinoids. Rundlöf's paper is "probably the best field study done so far", he says, and avoids many previous problems, such as contaminated controls. "Any reasonable person would have to accept this is a real effect," he adds.

The debate is heating up. In March, Goulson reanalysed[3] data from a 2013 study by the UK Food and Environment Research Agency (see go.nature.com/w9jlti), which had concluded that neonicotinoid pesticides do not harm bees: Goulson found that they do. In the same month, work from the United States found[4] that the probable harm from exposure to imidacloprid in seed-treated crops was "negligible" in honeybees, and last year a study[5] done in Canada reached a similar conclusion for clothianidin on oilseed rape.

Christopher Connolly, who studies human and bee neuroscience at the University of Dundee, UK, and has published work[6] showing that neonicotinoids interfere with neuron function in bumblebees, says that he was already convinced that the pesticides are bad for bees. Now, "the questions need to move to a different level", to elucidate the mechanisms. ■

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LETTER

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Bees prefer foods containing neonicotinoid pesticides

Sébastien C. Kessler[1]*, Erin Jo Tiedeken[2]*, Kerry L. Simcock[1], Sophie Derveau[3], Jessica Mitchell[4], Samantha Softley[1], Jane C. Stout[2] & Geraldine A. Wright[1]

The impact of neonicotinoid insecticides on insect pollinators is highly controversial. Sublethal concentrations alter the behaviour of social bees and reduce survival of entire colonies[1–3]. However, critics argue that the reported negative effects only arise from neonicotinoid concentrations that are greater than those found in the nectar and pollen of pesticide-treated plants[4]. Furthermore, it has been suggested that bees could choose to forage on other available flowers and hence avoid or dilute exposure[4,5]. Here, using a two-choice feeding assay, we show that the honeybee, *Apis mellifera*, and the buff-tailed bumblebee, *Bombus terrestris*, do not avoid nectar-relevant concentrations of three of the most commonly used neonicotinoids, imidacloprid (IMD), thiamethoxam (TMX), and clothianidin (CLO), in food. Moreover, bees of both species prefer to eat more of sucrose solutions laced with IMD or TMX than sucrose alone. Stimulation with IMD, TMX and CLO neither elicited spiking responses from gustatory neurons in the bees' mouthparts, nor inhibited the responses of sucrose-sensitive neurons. Our data indicate that bees cannot taste neonicotinoids and are not repelled by them. Instead, bees preferred solutions containing IMD or TMX, even though the consumption of these pesticides caused them to eat less food overall. This work shows that bees cannot control their exposure to neonicotinoids in food and implies that treating flowering crops with IMD and TMX presents a sizeable hazard to foraging bees.

Determining the impacts of pesticides on pollinators is important to resolve for the future of world food security. Pollinating insects like bees increase the yields of human crops, but in doing so, are inadvertently exposed to pesticides in floral nectar and pollen[6,7]. Several studies have concluded that bees exposed to sublethal doses of neonicotinoid pesticides in food have difficulty learning floral traits, feeding, navigating and foraging[2,3,8–11], and have impaired motor function[12]. These changes in behaviour often lead to colony failure[2,3]. This body of work has galvanized public concern over bee welfare, and in 2013, led to a two-year ban on the use of the three most common neonicotinoids (IMD, TMX, CLO) on flowering crops by the European Union. The agricultural importance of these pesticides has motivated agrochemical producers and government scientists to challenge this ban. Critics of laboratory-based experiments contend that such studies use food laced with neonicotinoid concentrations that exceed the levels found in nectar and pollen[13], or give bees no choice of food solutions[4,5]. They propose that free-living bees and other insect pollinators could choose to avoid the nectar and pollen of pesticide-treated crops[4] if pollinators are repelled by neonicotinoids[14,15], and if alternative sources were provided such as field margins in agricultural settings.

These arguments require that pollinators are able to detect neonicotinoids in food in order to avoid exposure. We tested whether bees avoid sucrose solutions (that is, nectar) containing neonicotinoids using a two-choice test designed to identify the bumblebee's gustatory detection thresholds for nectar toxins[16]. Individual foraging-age worker bumblebees or cohorts of 25 forager honeybees were housed in plastic boxes for 24 h and given access to two types of food tubes: one containing sucrose solution and one containing sucrose solution laced with a specific concentration of the IMD, TMX or CLO. The concentrations used included values in the range reported from nectar and pollen (0.5–150 nM, Extended Data Table 1). Neither bumblebees nor honeybees avoided concentrations found within the naturally occurring range (Fig. 1a, b), even though high concentrations of TMX and CLO reduced their survival (Extended Data Fig. 1). We also tested whether these pesticides inhibited the honeybee's feeding reflex (proboscis extension) or caused honeybees to retract the proboscis once extended[17]. None of the sucrose solutions containing IMD, TMX or CLO affected proboscis extension or retraction (Extended Data Fig. 2).

Unexpectedly, we observed that both bumblebees and honeybees showed a preference for solutions containing IMD or TMX over sucrose alone (Fig. 1, Extended Data Tables 2, 3). Concentrations of IMD and TMX proximate to those found in nectar (1–10 nM, Extended Data Table 1) were most attractive to bumblebees (Fig. 1a), whereas honeybees preferred to consume IMD and TMX across a broader range of concentrations (Fig. 1b). The 'attractive' effect of IMD also depended on bee age: newly emerged adult worker bumblebees and honeybees largely avoided 1–10 nM IMD (Extended Data Fig. 3a). In addition, the presence of neonicotinoids influenced the total amount of food consumed from both tubes during 24 h (Fig. 1c, d). Bumblebees fed with IMD or CLO consumed less total food on average than those fed TMX or the sucrose control (Fig. 1c, Extended Data Table 2); this effect has also been observed by others[11,15]. In contrast, the total food consumption of forager honeybees was reduced only when bees fed from solutions containing 100 nM or 1 μM TMX or CLO (Fig. 2d, Extended Data Table 2). Thus, even in treatments where bees ate considerably less food in 24 h, they still preferred to consume solutions containing IMD over sucrose alone. Bumblebees also consumed 1.5–10-fold more of the neonicotinoid-laced food than honeybees and were, therefore, exposed to higher pesticide doses (Extended Data Table 4).

Insects detect nutrients and toxins in food via gustatory neurons in hair-like sensilla on the proboscis (mouthparts)[18]. Toxic, non-nutritious compounds elicit spikes in 'bitter'-sensing neurons[19,20], but can also be detected via suppression of the responses of sugar-sensing neurons[21,22]. Previous research has established that gustatory neurons located in sensilla on the honeybee's mouthparts are more sensitive to toxins in food[17] than its antennae[21] or tarsi[23]. If bees have mechanisms for detecting neonicotinoids, sensilla on the mouthparts should respond to these substances in the same way they respond to other toxins[17]. To test this, we recorded from gustatory neurons in sensilla on the galea (part of the proboscis) of bumblebees and honeybees using the tip recording technique (Fig. 2a, b). Stimulation with IMD, TMX or CLO in water did not elicit spikes from any of the neurons in the galeal sensilla of either bumblebees (Fig. 2c) or honeybees (Fig. 2d), whereas

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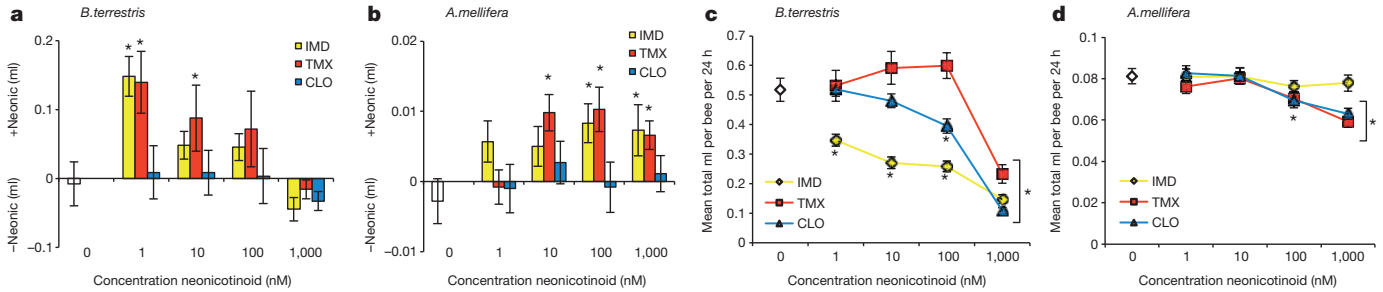

Figure 1 | Foraging-age bees prefer to eat food containing neonicotinoids. **a**, **b**, Bumblebees (**a**) and honeybees (**b**) given a choice of sucrose or sucrose containing a neonicotinoid pesticide chose to eat solutions containing IMD and TMX (Extended Data Table 2, bumblebees: generalized linear model (GLM): $\chi_2^2 = 12.1$, $P = 0.002$; honeybees: GLM, $\chi_2^2 = 11.1$, $P = 0.004$). Data represent the mean difference in the amount consumed over 24 h; positive values indicate a preference for solutions containing neonicotinoids. White bars indicate the sucrose control. Asterisks indicate $P \leq 0.002$ (Bonferroni-adjusted critical value) for one-sample *t*-tests against the '0' value (indicating no preference, see Extended Data Table 3). Sample sizes: bumblebees: IMD: 1 nM = 57, 10 nM = 66, 100 nM = 65, 1 μM = 66; TMX: 1 nM = 38, 10 nM = 39, 100 nM = 36, 1 μM = 40; CLO: 1 nM = 57, 10 nM = 59, 100 nM = 48, 1 μM = 62. Honeybees: $n = 40$ cohorts of 25 bees per treatment. Experiments were replicated with individuals taken from over 20 different bumblebee colonies and 4 honeybee colonies. **c**, The total amount of food eaten from both tubes by bumblebees was affected by the concentration and the presence of a neonicotinoid pesticide (GLM: $\chi_6^2 = 47.7$, $P < 0.001$, Extended Data Table 2) in one of the food tubes. **d**, Honeybees ate less total food only when it contained 1,000 nM TMX or CLO (GLM: $\chi_2^2 = 10.5$, $P = 0.005$, Extended Data Table 2). White diamonds indicate amount eaten by sucrose control group. *$P < 0.05$ in post hoc comparisons against sucrose. Error bars represent ± s.e.m.

stimulation with nicotine hydrogen tartrate (NHT), KCl and sucrose did (Fig. 2c–f). This effect was the same for all three neonicotinoids in both bee species (Extended Data Table 5). To test whether neonicotinoids are detected via suppression of the neurons' responses to sugars, we applied sucrose solution laced with IMD, TMX and CLO in an ascending series of concentrations from 1 nM to 1 μM (Fig. 2g, h). None of the concentrations we tested altered the spiking activity of sucrose-sensitive gustatory neurons in the bumblebees' or the honeybees' sensilla (Fig. 2g, h, Extended Data Table 5). (Note: we confirmed that the mean spike rates reported in Fig. 2h were not a result of simultaneous excitation of bitter neurons and inhibition of sucrose-sensing neurons by manually spike sorting the records for IMD, Extended Data Fig. 4.) Furthermore, we found that both forager and newly emerged honeybees lack taste neurons that respond to these compounds (Extended Data Fig. 3b). Therefore, the behavioural data and electrophysiological recordings from mouthparts' gustatory neurons lead us to conclude that bumblebees and honeybees cannot taste neonicotinoids in nectar.

The preference of the bees in our assays for solutions containing IMD or TMX probably arises from the pharmacological action of these compounds on nicotinic acetylcholine receptors (nAChRs) in the bees' brains. It does not reflect a generalized enhancement of feeding

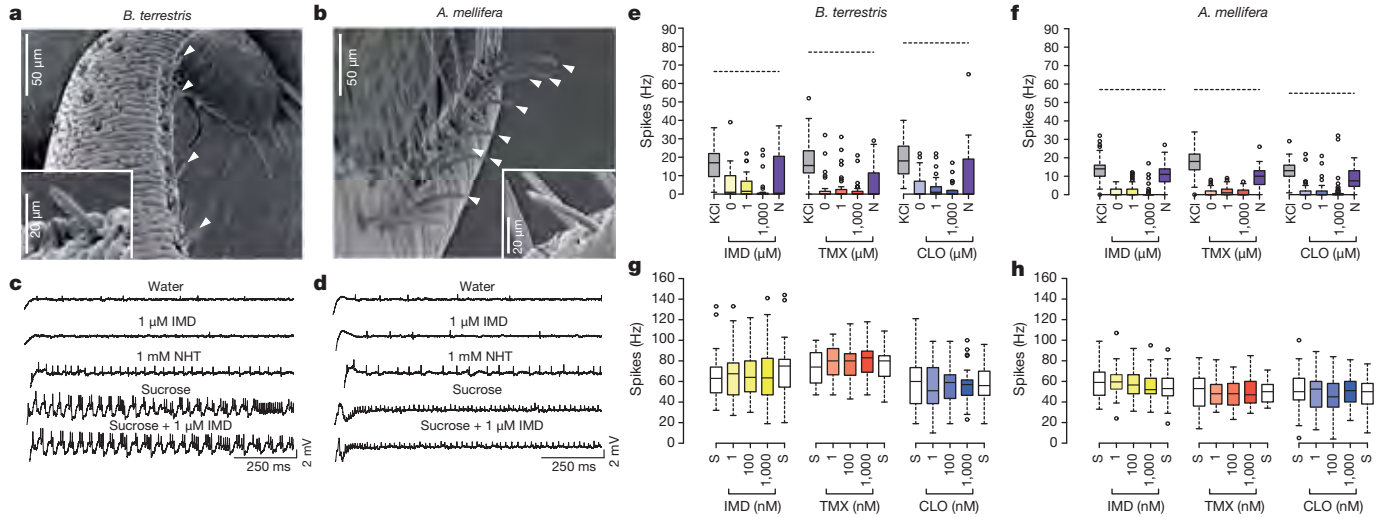

Figure 2 | Electrophysiological recordings of the gustatory receptor neurons from the mouthparts of bumblebees and honeybees during stimulation with neonicotinoids. **a**, **b**, Scanning electron micrographs (SEM) of the galea of bumblebees (**a**) and honeybees (**b**). Recordings were made from the basiconic sensilla of the galea (white arrows); inserts are higher resolution SEM of individual sensilla. **c**, **d**, Spike trains recorded from both species reveal responses to NHT and to sucrose, but not to IMD. **e**, **f**, Boxplots of the spiking responses of gustatory neurons of the mouthparts of bumblebees (**e**) and honeybees (**f**) to KCl, NHT and two concentrations of each of the neonicotinoids. Dashed lines represent the median response to 50 mM sucrose. Solutions of the three neonicotinoids did not elicit activity from gustatory neurons greater than the response to water (indicated as '0' on *x* axis) (Extended Data Table 5, ANOVA: bumblebees: $F_{2,77} = 0.935$, $P = 0.397$; honeybees: $F_{2,144} = 2.38$, $P = 0.096$). (Note: NHT elicited spike frequencies in gustatory neurons greater than those elicited by water in only 11/17 of the bumblebees we tested, whereas NHT elicited spike frequencies greater than water in all of the honeybees tested). Sample sizes: bumblebees: $n_{IMD} = 5$; $n_{TMX} = 7$; $n_{CLO} = 5$. Honeybees: $n_{IMD} = 5$; $n_{TMX} = 5$; $n_{CLO} = 6$. **g**, **h**, The spiking response to sucrose was not reduced by the presence of the neonicotinoids at concentrations in the nectar-relevant range (Extended Data Table 5, ANOVA: bumblebees: $F_{1,86} = 0.579$, $P = 0.449$; honeybees: $F_{1,127} = 2.00$, $P = 0.053$). Bumblebees: $n_{IMD} = 8$; $n_{TMX} = 5$; $n_{CLO} = 6$. Honeybees: $n_{IMD} = 6$; $n_{TMX} = 5$; $n_{CLO} = 6$. Boxplots represent the median (black bars), the 1.5 interquartile range (whiskers) and outliers (circles). Stimuli on *x* axes of **e**–**h** are in order of presentation during the experiment. Bumblebees in both experiments were randomly selected from 8 colonies; honeybees in both experiments were randomly selected from 4 colonies. N, NHT; S, sucrose.

because bees consuming these pesticides ate less food overall. Remarkably, the preference occurred even when bees consuming these solutions were more likely to die. Our data may indicate, therefore, that IMD and TMX affect the neural mechanisms involved in learning about the location of rewarding food. Previous studies have demonstrated that free-flying honeybees prefer to collect sucrose solutions containing low concentrations of nicotine[24]. Nicotine also activates nAChRs[25] expressed throughout the bee brain, including the mushroom bodies required for learning and memory[26,27]. It is notable that several studies have shown that chronic neonicotinoid administration impairs olfactory learning and memory in honeybees[1,8,28,29]. Our finding that bees acquire a preference for food laced with IMD or TMX could be explained by shorter neonicotinoid exposure in our experiments or by differential sensitivity of the nAChRs in the relevant brain regions necessary for each task[26]. It is also plausible that differential sensitivity of nAChRs accounts for our observed avoidance of newly emerged bees towards solutions containing IMD.

Consumption of neonicotinoid-laced nectar by foraging bees could lead to higher attrition in this behavioural caste as well as reducing their foraging efficiency for pollen[2,30]. This would have a greater impact on solitary bee species and on wild bee colonies with relatively few foragers than on honeybee colonies. If foragers prefer to collect nectar containing IMD and TMX, they will also bring more neonicotinoid-laced food back to the colony. For these reasons, whole colonies could be exposed to higher levels of these pesticides in the field than had been predicted previously. Mitigation strategies that rely on planting alternative sources of nectar and pollen, therefore, might not be enough to decrease the risk of poisoning pollinators with pesticides. Instead, long-term changes to policy that include reducing their use may be the only certain means of halting pollinator population decline.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to G.A.W. (jeri.wright@ncl.ac.uk).

METHODS

Behavioural two-choice assays. Experiments were performed at Trinity College, Dublin with *Bombus terrestris dalmatinus* (Unichem Ltd, Co. Dublin, Irish distributor for Koppert). Colonies were maintained at 25–30 °C in 24 h darkness and fed commercial pollen and Biogluc (Agralan Ltd, Swindon) bee food *ad libitum*. Experiments were also performed at Newcastle University, Newcastle upon Tyne with *Bombus terrestris audax* (Biobest, Belgium) and *Bombus terrestris terrestris* (Koppert Biological Systems, NATURPOL, Netherlands). Bees from 3–5 different colonies were used for each neonicotinoid. Individual worker bumblebees were collected as they tried to exit the colony. For the experiments with newly emerged bumblebees, colonies were monitored for newly emerged bees daily; newly emerged adults were identified by their pale colour. These bees were extracted using forceps from within the colony. As previously described in Tiedeken *et al.* (2014)[16], individual bumblebees were cold anaesthetized, weighed and sex-determined, and transferred to individual 650 ml plastic containers (160 × 110 × 45 mm). Containers were fitted with three 3 ml feeding tubes, inserted horizontally. Feeding tubes had four 2 mm holes so bees could alight on the tubes and feed from the openings. The feeding tubes contained one of three solutions: (1) deionized water; (1) 0.5 M sucrose; or (3) 0.5 M sucrose with a specific concentration of a neonicotinoid compound. Whether or not the bee was alive was noted 24 h after the start of the experiment. Bees that did not drink from either tube were excluded from the final analysis; the total number of these subjects was never greater than 3 per treatment (note: these subjects were always dead and likely to have died from stress or other causes).

Experiments with honeybees (*Apis mellifera* var. Buckfast) were performed at Newcastle University during the summer months using 2 free-flying outdoor colonies originally obtained from the UK's National Bee Unit (Sand Hutton, Yorkshire). Foraging adult worker honeybees were collected at the colony entrance as they returned from foraging; newly emerged adult workers were collected from brood comb as they emerged in a purpose-built box kept in an incubator at 34 °C. Bees were cold anaesthetized before placing in rearing boxes. Cohorts of 25 bees were placed in rearing boxes as previously described in Paoli *et al.* (2014)[31]. Five food tubes (as described above) were provided: (1) one with deionized water; (2) two with 1 M sucrose; (3) two with 1 M sucrose containing a specific concentration of a neonicotinoid. The number of bees alive in each cohort was counted at the time of measurement of the food consumption (24 h later).

All of the two-choice experiments were performed experimenter-blind (except IMD with bumblebees). Three neonicotinoid pesticides, imidacloprid (IMD), thiamethoxam (TMX) and clothianidin (CLO), were used in the experiments (Pestanal, Sigma-Aldrich). The neonicotinoid concentrations used were 1 nM, 10 nM, 100 nM, 1 μM (see Extended Data Table 4 for conversions to ppb and ng per bee). Bees were kept in continuous darkness for 24 h at constant temperature and 60% RH (bumblebees: 28 °C; honeybees: 34 °C). Control boxes identical to the experimental boxes (without bees) for each neonicotinoid treatment were placed in the incubator simultaneously with the experiments to measure the rate of evaporation from the food solutions. Feeding tubes were weighed, placed in the experimental boxes with the bees for 24 h, and then removed and weighed a second time. The position of the treatment tubes was randomized across subjects. The amount of solution consumed was determined as the difference in the weight of each tube after 24 h; the average value for the evaporation control for each treatment was subtracted from this final value for each tube. For bumblebees, sample sizes were: IMD: 1 nM = 57, 10 nM = 66, 100 nM = 65, 1 μM = 66; TMX: 1 nM = 38, 10 nM = 39, 100 nM = 36, 1 μM = 40; CLO: 1 nM = 57, 10 nM = 59, 100 nM = 48, 1 μM = 62. For honeybees, $n = 40$ cohorts of 25 bees per treatment. Sample size was chosen as $n \geq 40$ based on previous work[16]; sample size varied because some individuals died from unknown causes at the start of the experiments. No statistical methods were used to predetermine sample size.

Honeybee antennal and mouthparts assays. Honeybees were collected at the entrance of an outdoor colony as they returned from foraging, cold-anaesthetized, and harnessed as described in Bitterman *et al.* (1983)[32]. Each was fed 1 M sucrose to satiety and left overnight in a humidified plastic box and assayed ~ 18 h later. Briefly, two assays were employed: one in which individual honeybees were lightly tapped on the antenna with a stimulating solution (for example, sucrose) to elicit the feeding reflex (that is, proboscis extension reflex, or PER) and a second assay in which a droplet of stimulating solution was placed at the end of the extended proboscis to test whether bees would consume it (further details described in Wright *et al.* 2010[17]). Stimulating solutions were 1 M sucrose containing one of the following concentrations (1 nM, 10 nM, 100 nM, 1 μM, 10 μM) of one of three neonicotinoids (IMD, TMX, CLO).

Electrophysiology. Individual bumblebees (*B. terrestris audax* and *B. terrestris terrestris*) and honeybees were cold-anaesthetized on ice for 3–5 min, and then restrained in a metallic restraining harness as described in Bitterman *et al.* (1983)[32]. To avoid any movements of the mouthparts during recordings, muscles that trigger proboscis retraction were cut by making an incision at the level of the proboscis fossa. Each galea was fixed with a curved metallic wire pinned into dental wax.

Electrophysiological recordings were made from taste neurons located in the first 11 sensilla chaetica[33] located at the tip of the galea on the honeybee's proboscis as in Wright *et al.* (2010)[17] and in the first 6 sensilla in bumblebees. Bees were electrically grounded via a chlorinated silver wire inserted into the head. Sensilla were visualized under a microscope (M205C, Leica, Germany) at a magnification of ×256. To record from gustatory neurons, we used a method first described by Hodgson *et al.* (1955)[34]. Sensilla were stimulated with a recording borosilicate electrode (50 mm long, 20 μm diameter) containing the test compounds diluted in demineralized water. The recording electrode was connected via a chlorinated sliver wire to a high impedance 'non-blocking' pre-amplifier (TastePROBE, Syntech, Germany)[35] mounted on a motorized micromanipulator (MPC-200, Sutter Instrument, USA). The signal was further amplified and filtered with an AC amplifier (model 1800, gain: 100×, band-pass filter: 10–1,000 Hz, A-M Systems, USA). Each stimulus trial was digitized (sampling rate 10 kHz, 16 bits; DT9803 Data Translation), stored on a computer with dbWave software (version 4.2014.3.22) and analysed with Matlab R2012b (version 8.0.0.783) using PeakFinder with fixed thresholds as the peak detection algorithm (PeakFinder.m., Mathworks file ID: 25500). Recordings were made for 2 s, but only data for the first second were included in the analysis. The first 100 ms were removed to avoid the contact artefact. For bumblebees, 2–6 sensilla were sampled per bee; for honeybees, 6–10 sensilla were sampled per bee.

Recording started when the open end of the electrode was placed over the tip of the sensillum. Individuals were repeatedly sampled in one of two protocols: (1) 50 mM sucrose, 100 mM KCl, water, 1 μM neonicotinoid, 1 mM neonicotinoid, 1 mM NHT, 100 mM KCl, 50 mM sucrose; or (2) 50 mM sucrose, 50 mM sucrose + neonicotinoid in one of the following concentrations (1 nM, 10 nM, 1 μM), 50 mM sucrose. The neonicotinoids IMD, TMX, or CLO were used in each protocol. Neonicotinoid (Pestanal, Sigma-Aldrich) solutions were prepared as serial dilutions starting with 1 mM concentration. Sucrose and nicotine tartrate were purchased from Sigma-Aldrich and KCl from Fisher Scientific at purity ≥ 98%. Demineralized water was used to prepare all solutions. Intervals between stimuli were 2–5 min.

Recordings with IMD diluted in sucrose (Extended Data Fig. 4) were further analysed using dbWave (http://perso.numericable.fr/frederic.marion-poll/deterrents/tk/dbwave/index.htm). Predicted spiking neurons or 'units' were sorted from the digitally filtered signals according to their amplitude with the help of interactive software procedures. Electrophysiological recordings were then visually inspected to search for spike doublets, that is, two spikes separated by an interspike interval shorter than the silent period[36,37]. Spike trains were analysed over 1 s following the first 100 ms removed to avoid the contact artefact.

Electron microscopy. Scanning electron microscopy was performed using a Cambridge Stereoscan 240 on samples that had been fixed with glutaraldehyde, washed in phosphate buffer then dehydrated through an ethanol gradient followed by critical point drying. Specimens were then mounted on an aluminium stub with Acheson's silver dag before gold coating with a Polaron SEM coating unit.

Statistics. All analyses were performed using IBM SPSS v 19. The mean total number of spikes in the electrophysiological recordings was analysed using repeated-measures analysis of variance (ANOVA) for each species with neonicotinoid as a main effect, sensillum number and bee as covariates, and stimulus as a repeated measure; a Levene's test was employed to test for equality of variance. Post hoc comparisons were pairwise *t*-tests with a Bonferroni adjustment for experiment-wise error rate. A two-way generalized linear model (GLM) was used to compare the behaviour of bees fed each of the neonicotinoid treatments for each bee species with least squares post hoc comparisons (Note: the sucrose-sucrose choice data were not included because of the requirements of GLM for factorial design). The difference in the amount eaten between the 2 food tubes in the behavioural choice assays was also analysed using a one-sample *t*-test against zero for each treatment; critical values were Bonferroni-adjusted. The proportion of bees alive after 24 h was analysed using logistic regression (lreg). Each individual bee was entered in the analysis for the experiments with bumblebees and with honeybees. For the analysis with honeybees, 'cohort' was entered as a covariate. No statistical methods were used to predetermine sample size.

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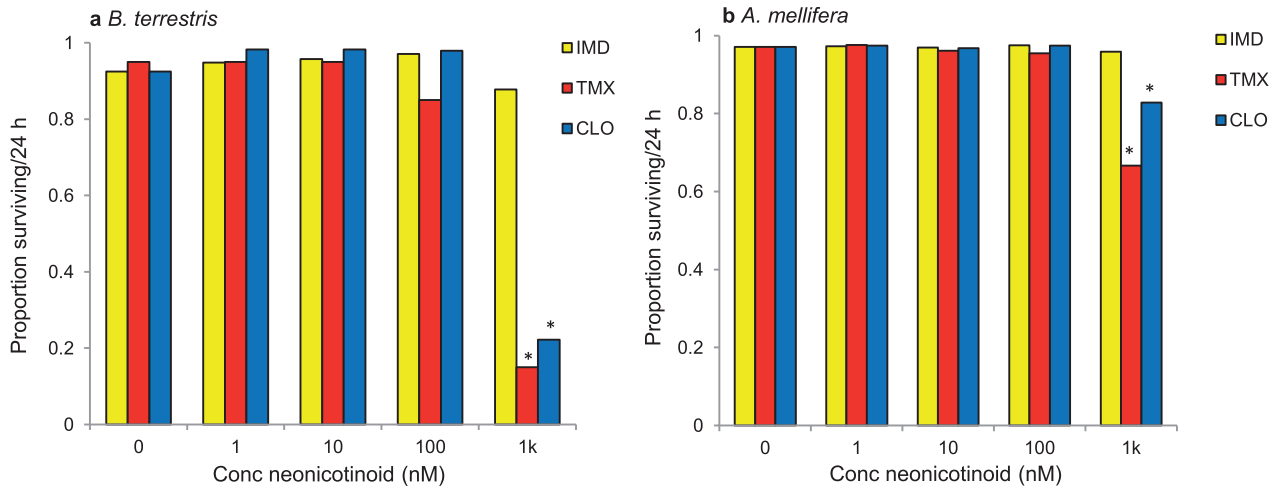
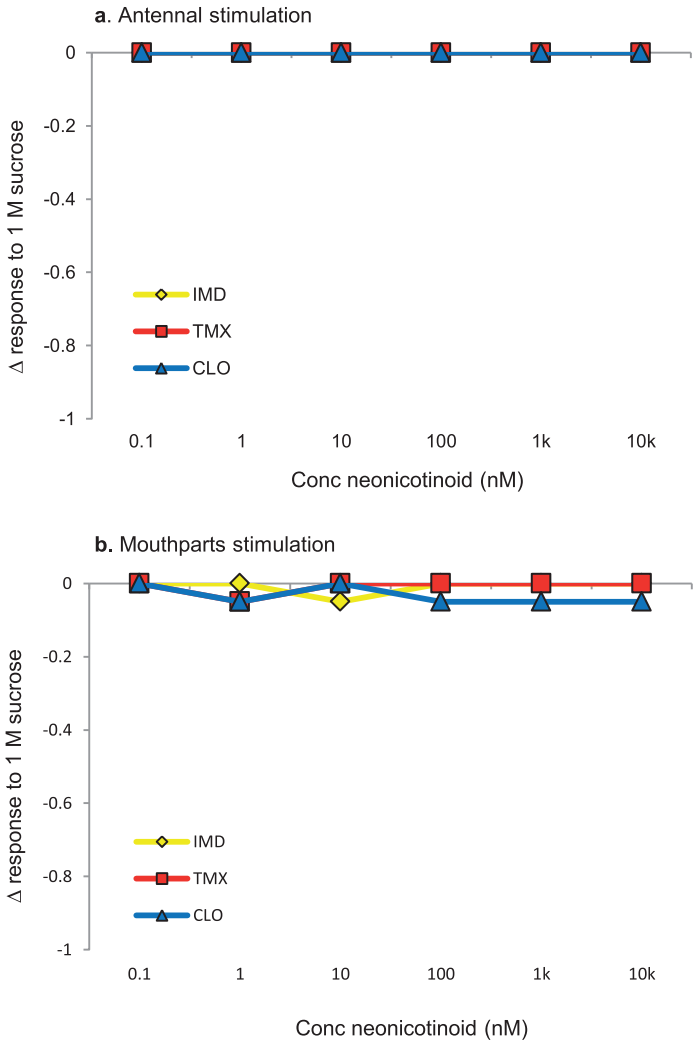
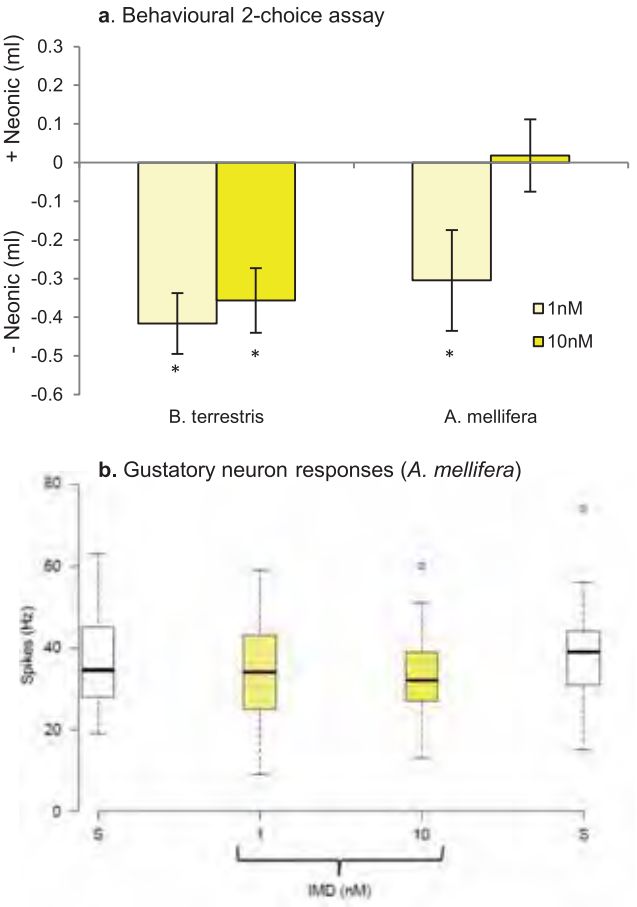


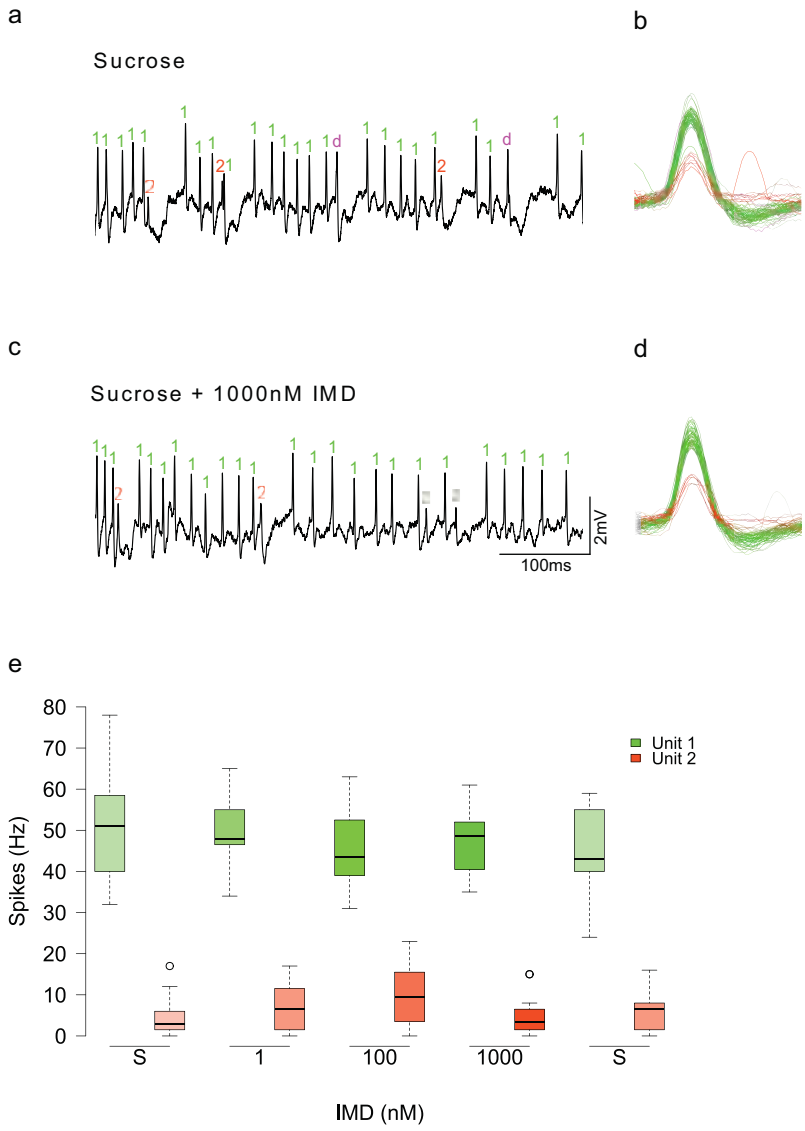
Extended Data Figure 1 | The proportion of bees surviving after 24 h in the two-choice assay. Data from Fig. 1. **a**, Bumblebees given a choice between sucrose and sucrose laced with 1,000 nM TMX or CLO were less likely to survive after 24 h (lreg: IMD: $\chi_4^2 = 4.36$, $P = 0.359$; TMX: $\chi_4^2 = 62.3$, $P < 0.001$; CLO: $\chi_4^2 = 79.7$, $P < 0.001$). **b**, Honeybees given a choice between sucrose and sucrose laced with 1,000 nM TMX or CLO were less likely to survive after 24 h (lreg: IMD: $\chi_4^2 = 5.18$, $P = 0.269$; TMX: $\chi_4^2 = 577$, $P < 0.001$; CLO: $\chi_4^2 = 243$, $P < 0.001$). Cohort (cov) accounted for a significant portion of the variance in survival for all three treatment groups (lreg: IMD: $\chi_1^2 = 22.0$, $P < 0.001$; TMX: $\chi_1^2 = 32.4$, $P < 0.001$; CLO: $\chi_1^2 = 70.2$, $P < 0.001$). Sample sizes are the same as in Fig. 1. *$P < 0.05$ in least squares post hoc comparisons against sucrose in each treatment



Extended Data Figure 2 | Antennal proboscis extension response (PER) and mouthparts assay of honeybees to solutions containing neonicotinoids. **a**, Stimulation of the antennae with 1 M sucrose solutions containing neonicotinoids did not affect the elicitation of PER. **b**, Honeybees did not refuse to consume solutions containing neonicotinoids; only one bee in the CLO treatments failed to drink the solutions. $n = 40$ per neonicotinoid treatment for antennal stimuli and $n = 10$ for each concentration of each neonicotinoid for the mouthparts taste assay. Bees were randomly selected from 2 colonies.



Extended Data Figure 3 | Young bees avoid solutions containing neonicotinoids. **a**, Newly emerged worker bumblebees ($n = 30$ bees per treatment) and honeybees ($n = 20$ boxes per treatment) were tested in the behavioural choice assay with 1 nM and 10 nM IMD in sucrose solution as in Fig. 1. Bumblebees avoided consuming both solutions containing IMD (one-sample t-test against 0, 1 nM: $P < 0.001$, 10 nM: $P = 0.001$), whereas honeybees avoided only the 1 nM concentration (one-sample t-test against 0, 1 nM: $P = 0.003$, 10 nM: $P = 0.773$). Error bars represent ± s.e.m. **b**, The presence of IMD did not alter the spike frequency of gustatory neurons in the galeal sensilla of newly emerged honeybees (repeated-measures ANOVA, stimulus: $F_{1,47} = 0.207$, $P = 0.653$). Recordings were made from the basiconic sensilla on the galea as in Fig. 2. Boxplots represent the frequencies of responses to 50 mM sucrose or to 50 mM sucrose solutions containing 1 nM or 10 nM IMD. $n = 5$ bees, 10 sensilla per bee. Boxplots represent the median (black bars), the 1.5 interquartile range (whiskers) and outliers (circles). Stimuli on x axis are in order of presentation during the experiment.



Extended Data Figure 4 | Spike-sorted recordings. Data from four of the honeybees in Fig. 2h. **a**, To verify that the spike rates we observed in Fig. 2h were not a result in changes in the rates of firing of individual neurons, we spike-sorted recordings from four honeybees stimulated with sucrose and IMD. **b**, Spike sorting revealed two potential spiking neurons (units) characterized by different spike amplitudes; both units spiked in response to sucrose stimulation. (This was also observed previously by Wright *et al.* 2010[17]). One neuron is labelled in green, the other in red. Spike doublets (indicated in pink as 'd') where both neurons spiked nearly simultaneously were also observed. **c**, **d**, These same two spiking neurons continued to respond when stimulated with sucrose containing 1 μM IMD. **e**, Boxplots reveal that the rate of spiking was lower on average for one of the neurons (repeated-measures ANOVA, unit: $F_{1,36} = 596$, $P < 0.001$). The rate of firing of both neurons was not affected by IMD concentration (repeated-measures ANOVA, unit: $F_{1,36} = 0.369$, $P = 0.547$). Spikes from additional neurons (units) were not detected, and so we concluded that no other neurons were recruited during stimulation with IMD. 'S' indicates stimulation with sucrose. Boxplots represent the median (black bars), the 1.5 interquartile range (whiskers) and outliers (circles). Stimuli on *x* axis are in order of presentation during the experiment.

Extended Data Table 1 | Concentrations of neonicotinoids reported in floral nectar

Source	Imidacloprid			Thiamethoxam			Clothianidin		
	ng/g	PPB	nM	ng/g	PPB	nM	ng/g	PPB	nM
Schmuck et al. 2001[7]	1.9	1.9	7.43	-	-	-	-	-	-
Pohorecka et al. 2012[38]	0.6	0.6	2.34	4.2	4.2	14	2.3	2.3	9.2
Dively and Kamel 2012[6]	0.4-11	0.4-11	1.5-43	8.2-9.5	8.2-9.5	28-37	-	-	-
Stoner and Eitzer 2012[39]	10	10	39	11	11	37	-	-	-
Byrne et al. 2013[40]	2.9-39	2.9-39	11-154	-	-	-	-	-	-
Larson et al. 2013[41]	-	-	-	-	-	-	171	171	684
Pilling et al. 2013[42]	-	-	-	0.65-2.4	0.65-2.4	2.2-8.2	-	-	-
Defra 2013[43]	0.13	-	0.5	1-3.9	1-3.9	3.4-13	0.18-4	0.18-4	0.7-16

References 38–43 are cited in this table.

Extended Data Table 2 | Generalized linear models for the neonicotinoid choice experiment and total food consumption

B. terrestris	Choice test			Total food consumption		
Between-subjects contrasts	**df**	**χ^2**	**P-value**	**df**	**χ^2**	**P-value**
Concentration	3	27.9	**<0.001**	3	263	<0.001
Neonicotinoid	2	12.1	**0.002**	2	150	<0.001
Neonic x Conc	6	7.97	0.240	6	47.7	**<0.001**
A. mellifera	Choice test			Total food consumption		
Between-subjects contrasts	**df**	**χ^2**	**P-value**	**df**	**χ^2**	**P-value**
Concentration	3	4.93	0.176	3	37.1	**<0.001**
Neonicotinoid	2	11.1	**0.004**	2	10.5	**0.005**
Neonic x Conc	6	5.89	0.435	6	11.4	0.076

Data from Fig. 1. Values in bold indicate interpreted model parameters. Note: sucrose–sucrose (control) data were not included.

Extended Data Table 3 | One-sample *t*-tests against '0' for each treatment of the 24 h behavioural assay

B. terrestris									
		IMD			TMX			CLO	
	N	t(df)	P-value	N	t(df)	P-value	N	t(df)	P-value
Sucrose	55	-0.24(54)	0.402						
1nM	57	5.13(56)	**<0.001***	38	3.11(38)	**0.002***	57	0.22(56)	0.246
10nM	66	2.39(65)	**0.010**	39	3.11(37)	**0.002***	59	0.26(58)	0.183
100nM	65	2.33(64)	**0.012**	36	1.31(35)	0.099	48	0.09(47)	0.465
1µM	66	-2.6(65)	**0.005**	40	-1.15(39)	0.128	62	-2.36(61)	**0.021**
A. mellifera									
		IMD			TMX			CLO	
	N	t(df)	P-value	N	t(df)	P-value	N	t(df)	P-value
Sucrose	40	-0.85(39)	0.199						
1nM	40	1.93(39)	**0.031**	40	-0.32(39)	0.376	40	-0.288	0.387
10nM	40	1.75(39)	**0.044**	40	3.80(39)	**<0.001***	40	0.882	0.191
100nM	40	2.97(39)	**0.002***	40	3.23(39)	**0.001***	40	-0.221	0.414
1µM	40	2.00(39)	**0.026**	40	3.25(39)	**0.001***	40	0.423	0.337

Data from Fig. 1. *P* values are for 1-tailed tests. *P* values in bold are below $P = 0.05$. *Application of a Bonferroni adjustment criterion alters the *P* value threshold from $P = 0.05$ to $P = 0.002$.

Extended Data Table 4 | Comparison of doses consumed by each bee species for each treatment

B. terrestris												
	1nM			10 nM			100 nM			1μM		
	ml/bee	PPB	ng/bee/24 h	ml/bee	PPB	ng/bee/24 h	ml/bee	PPB	ng/bee/24 h	ml/bee	PPB	ng/bee/24 h
IMD	0.257	0.256	0.064(0.043)	0.167	2.56	0.418(0.337)	0.159	25.6	3.98(3.22)	0.055	256	13.9(18.4)
TMX	0.360	0.292	0.105(0.077)	0.357	2.92	1.05(0.862)	0.354	29.2	10.3(8.74)	0.115	292	33.6(33.9)
CLO	0.279	0.250	0.070(0.065)	0.259	2.50	0.647(0.600)	0.211	25.0	5.28(4.93)	0.041	250	10.3(13.6)
A. mellifera												
	1nM			10 nM			100 nM			1μM		
	ml/bee	PPB	ng/bee/24 h	ml/bee	PPB	ng/bee/24 h	ml/bee	PPB	ng/bee/24 h	ml/bee	PPB	ng/bee/24 h
IMD	0.046	0.256	0.012(0.010)	0.046	2.56	0.118(0.103)	0.045	25.6	1.16(0.974)	0.045	256	11.7(9.95)
TMX	0.040	0.292	0.012(0.011)	0.048	2.92	0.141(0.117)	0.036	29.2	1.07(1.02)	0.035	292	10.3(8.63)
CLO	0.043	0.250	0.011(0.010)	0.044	2.50	0.112(0.101)	0.043	25.0	1.08(0.868)	0.034	250	8.51(7.86)

Data from Fig. 1. Note: ng/bee values were calculated based on the mean values consumed from the neonicotinoid-containing food tubes for each treatment (ml/bee). This calculation is the product of the ng/μl of neonicotinoid in the food solution and the amount of solution eaten (μl) per bee in 24 h. The values in parentheses in the ng/bee/24 h column are the expected values if bees had eaten from both tubes equally. This value was calculated by dividing the total amount eaten for each treatment in Fig. 1c and d by 2 and using this quantity to estimate the dose.

Extended Data Table 5 | Repeated-measures ANOVA

B. terrestris	**Water**			**Sucrose solution**		
Within subjects contrasts	**df**	**F**	**P-value**	**df**	**F**	**P-value**
Stimulus	1	8.60	0.004	1	0.579	0.449
Stimulus x bee (cov)	1	4.45	0.038	1	1.23	0.271
Stimulus x sensillum (cov)	1	0.038	0.846	1	0.558	0.458
Stimulus x neonicotinoid	2	0.935	0.397	2	0.287	0.752
Error(stim)	77			86		
Between subjects contrasts	**df**	**F**	**P-value**	**df**	**F**	**P-value**
Neonicotinoid	2	10.2	0.937	2	0.004	0.996
Bee (cov)	1	0.164	0.686	1	0.871	0.354
Sensillum (cov)	1	5.63	0.020	1	3.35	0.071
Error	77			86		
A. mellifera	**Water**			**Sucrose solution**		
Within subjects contrasts	**df**	**F**	**P-value**	**df**	**F**	**P-value**
Stimulus	1	95.6	<0.001	1	7.47	0.007
Stimulus x bee (cov)	1	4.20	0.042	1	5.31	0.023
Stimulus x sensillum (cov)	1	0.303	0.583	1	0.142	0.707
Stimulus x neonicotinoid	2	2.38	0.096	2	3.00	0.053
Error(stim)	144			127		
Between subjects contrasts	**df**	**F**	**P-value**	**df**	**F**	**P-value**
Neonicotinoid	2	1.23	0.295	2	6.70	0.002
Bee (cov)	1	0.335	0.563	1	1.67	0.198
Sensillum (cov)	1	1.37	0.244	1	12.6	0.001
Error	144			127		

Data from Fig. 2. Note: for 'Water' model, the stimulus variable included: sucrose, KCl, nicotine, water, 1 μM, and 1 mM neonicotinoid. For the 'sucrose solution' model, the stimulus variable included: sucrose, 1 nM, 100 nM, and 1 μM neonicotinoid. The significant 'stimulus × neonicotinoid' term in the sucrose solution experiment for honeybees reflects a slight adaptive effect that occurred in the experiments with IMD, but not with TMX or CLO. Pairwise comparisons of each stimulus applied in the IMD experiment revealed that the 1 μM IMD and the final sucrose control stimulus produced fewer spikes than the first sucrose stimulus ($P = 0.024$ and $P = 0.002$). However, the 1 μM IMD and the final sucrose stimulus were not significantly different ($P = 0.546$) indicating either that the neurons in these experiments exhibited a slight adaptation effect or that the 1 μM IMD concentration had a toxic effect that influenced the integrity of their responses to sucrose.

LETTER

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Seed coating with a neonicotinoid insecticide negatively affects wild bees

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Understanding the effects of neonicotinoid insecticides on bees is vital because of reported declines in bee diversity and distribution[1–3] and the crucial role bees have as pollinators in ecosystems and agriculture[4]. Neonicotinoids are suspected to pose an unacceptable risk to bees, partly because of their systemic uptake in plants[5], and the European Union has therefore introduced a moratorium on three neonicotinoids as seed coatings in flowering crops that attract bees[6]. The moratorium has been criticized for being based on weak evidence[7], particularly because effects have mostly been measured on bees that have been artificially fed neonicotinoids[8–11]. Thus, the key question is how neonicotinoids influence bees, and wild bees in particular, in real-world agricultural landscapes[11–13]. Here we show that a commonly used insecticide seed coating in a flowering crop can have serious consequences for wild bees. In a study with replicated and matched landscapes, we found that seed coating with Elado, an insecticide containing a combination of the neonicotinoid clothianidin and the non-systemic pyrethroid β-cyfluthrin, applied to oilseed rape seeds, reduced wild bee density, solitary bee nesting, and bumblebee colony growth and reproduction under field conditions. Hence, such insecticidal use can pose a substantial risk to wild bees in agricultural landscapes, and the contribution of pesticides to the global decline of wild bees[1–3] may have been underestimated. The lack of a significant response in honeybee colonies suggests that reported pesticide effects on honeybees cannot always be extrapolated to wild bees.

Neuroactive neonicotinoids are commonly used in seed coatings to control herbivorous insect pests in a variety of crops such as corn, cereals and oilseed rape and are taken up systemically by the growing plant and distributed to all tissues[5]. These chemicals account for more than one fifth of the world's insecticide market[14], and this widespread use requires that their effects on non-target organisms are investigated. A particular concern is the effect of neonicotinoids on bees[6,12], because of the bee's role in pollinating crops[4] and declines in bee diversity and distribution[1–3].

These concerns, together with research indicating negative effects of neonicotinoids on bees, have led to a European Union-wide restriction from 1 December 2013 on the use of the three neonicotinoids, clothianidin, imidacloprid and thiamethoxam, as seed coating in crops attractive to bees[6], to allow for studies on their environmental effects. Previous studies have mainly focused on the effects of neonicotinoids on bees artificially exposed to neonicotinoids[8–11], mostly on honeybees[11]. The key question is how wild bees, which may differ from honeybees in response to insecticides[15–17], are affected by neonicotinoids when foraging in real agricultural landscapes[11–13].

Here we investigated how seed coating oilseed rape with Elado (Bayer), including the systemic neonicotinoid clothianidin[5] and the non-systemic pyrethroid β-cyfluthrin[18] as active ingredients, influenced wild and managed bee species in Swedish agricultural landscapes. Because we assessed effects on bees under field conditions, our findings have important implications for policies regulating the use of neonicotinoids as well as for risk assessments of pesticides.

We designed a study with eight pairs of landscapes surrounding 16 geographically separated (>4 km) spring-sown oilseed rape fields (Fig. 1 and Extended Data Table 1). One field in each pair was randomly assigned to be sown with seeds coated with the dose of Elado recommended by the manufacturer and a fungicide, while the other field in each pair, the control field, was sown with seeds coated only with the fungicide. At these 16 fields we estimated: (1) the density of

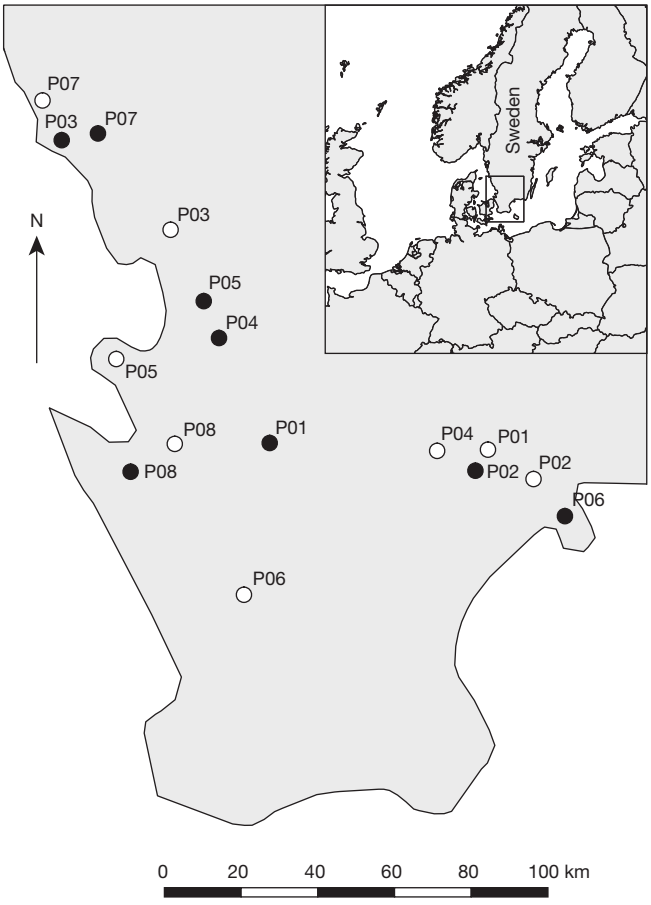

Figure 1 | Paired design with replicated landscapes. Location of the study area in southern Sweden and the eight pairs of landscapes (P01–P08) centred on oilseed rape fields sown with insecticide-coated (open circles) or untreated (control fields, filled circles) seeds. Pairing was based on land use within a 2-km radius surrounding the oilseed rape fields and geographical proximity between fields.

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wild bees; (2) the nesting activity of the solitary bee *Osmia bicornis* L.; (3) the colony development of the bumblebee *Bombus terrestris* L.; and (4) the colony strength of the European honeybee *Apis mellifera* L.

Our first finding was that the insecticide seed coating reduced the density of wild bees, that is, bumblebees and solitary bees, in the flowering oilseed rape fields and adjacent uncultivated field borders (generalized linear mixed model (GLMM), $F_{1,7} = 9.68$, $P = 0.019$; Fig. 2a and Extended Data Table 4). Wild bee density also increased with the size of the focal oilseed rape field, most probably because larger fields attract more bees or support larger colonies[19], but was not significantly related to the proportion of agricultural land in the surrounding landscape (Extended Data Table 4). Flower cover (number and size of flowers) of the oilseed rape had a positive influence on wild bee density ($F_{1,24} = 18.57$, $P < 0.001$) and was higher in fields sown with insecticide-coated seeds (Extended Data Table 5). However, the negative impact of the seed coating on wild bee density persisted irrespective of whether ($F_{1,7} = 9.68$, $P = 0.019$; Extended Data Table 4) or not ($F_{1,6} = 6.36$, $P = 0.044$) flower cover was included as a covariate in the statistical model.

Our second finding was that the insecticide seed coating correlated with reduced nesting of the solitary bee *O. bicornis*. To investigate this we placed three trap nests containing 27 *O. bicornis* cocoons (Extended Data Fig. 1) adjacent to each of the 16 fields before the beginning of oilseed rape flowering and monitored if emerging females started to build brood cells. In six of the eight control fields, but in none of the fields treated with the insecticide seed coating, females started to build brood cells (Wilcoxon test $Z = 2.84$, $P = 0.0045$; Fig. 2b). Although the reasons why the bees failed to build brood cells when exposed to the insecticide treatment remain unclear, a reduced capacity to navigate[8,9,20,21] is a possible explanation.

Our third finding was that the insecticide seed coating was negatively related to colony growth and reproduction of the bumblebee *B. terrestris*. Bumblebees are social and form colonies of one queen and tens or hundreds of workers. At each of the 16 oilseed rape fields we placed six commercially reared *B. terrestris* colonies (Extended Data Fig. 1). During their development, the bumblebee colonies are expected to grow in weight and worker force, and thereafter to switch to producing new queens and males with a resulting decline in colony weight[10]. The seed-coating treatment influenced the weight change of *B. terrestris* colonies (linear mixed model (LMM), day × day × treatment $F_{1,19} = 130.62$, $P < 0.001$, day × treatment $F_{1,21} = 143.00$, $P < 0.001$; Extended Data Table 6 and Fig. 3). As expected, *B. terrestris* colonies at control fields had an initial growth and a following decline (day × day $F_{1,28} = 114.70$, $P < 0.001$, day $F_{1,31} = 129.10$, $P < 0.001$), while those at fields with insecticide seed coating had a considerably smaller weight change ($F_{1,14} = 10.78$, $P = 0.0055$, $F_{1,16} = 0.92$, $P = 0.35$) (Extended Data Table 6 and Fig. 3). While the initial colony weight was the same in the two treatments (Extended Data Table 5), the rate of weight gain of colonies at fields with insecticide-coated seeds was lower than that of colonies at control fields ($F_{1,7} = 115.80$, $P < 0.001$; Extended Data Table 5). Effects of the treatment on colony development may result both from reduced pollen foraging efficiency and insufficient care for the brood[8,20–22]. Bumblebees have an annual life cycle where only the new queens produced at the end of the season hibernate and form new colonies the following spring. At the end of our experiment, fewer queen (GLMM, $F_{1,7} = 7.78$, $P = 0.027$) and worker/male cocoons (LMM, $F_{1,7} = 8.09$, $P = 0.025$) were produced at treated fields compared to control fields (Fig. 2c and Extended Data Table 5). These findings are in line with the reduced colony growth and 85% reduction in queen production reported for bumblebee colonies artificially exposed to imidacloprid under otherwise realistic conditions[8,10].

Our fourth finding was that the insecticide seed treatment had no significant influence on honeybee colony strength. In contrast to the *B. terrestris* colonies, the *A. mellifera* colonies did not differ in strength (number of adult bees) between the treatments after placement at the oilseed rape fields (LMM, $F_{1,7} = 0.01$, $P = 0.94$; Fig. 2d). This finding is

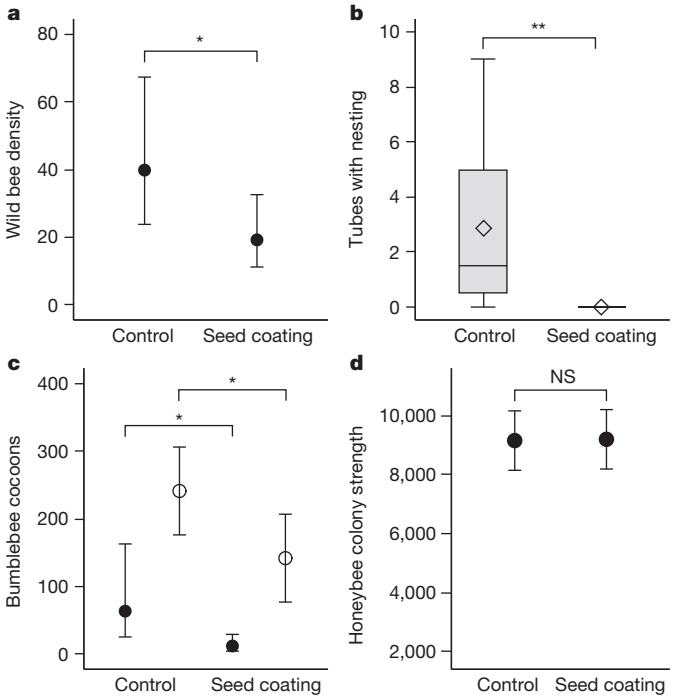

Figure 2 | Bee density and reproduction. **a–d**, Mean (± 95% confidence limits) number of wild bees (solitary bees and bumblebees) per 467 m^2 oilseed rape field and adjacent border (generalized linear mixed model (GLMM)) (**a**), median number of tubes per field with *O. bicornis* brood cells (Wilcoxon test) (**b**), mean (± 95% confidence limits) number of *B. terrestris* queen (filled circles, GLMM) and worker/male (open circles, linear mixed model (LMM)) cocoons per colony (**c**), and mean (± 95% confidence limits) number of adult *A. mellifera* per colony (colony strength) after placement at the fields (LMM) (**d**) in relation to treatment (control or insecticide seed coating) in the oilseed rape fields. $n = 8$ fields per treatment. Means and confidence limits are based on back-transformed, model-estimated least square means. In panel **b**, horizontal line in the box, open diamond symbols, boxes and whiskers indicate median, mean, 25th–75th percentiles and minimum–maximum, respectively. NS, not significant ($P > 0.05$); *$P < 0.05$, **$P < 0.01$.

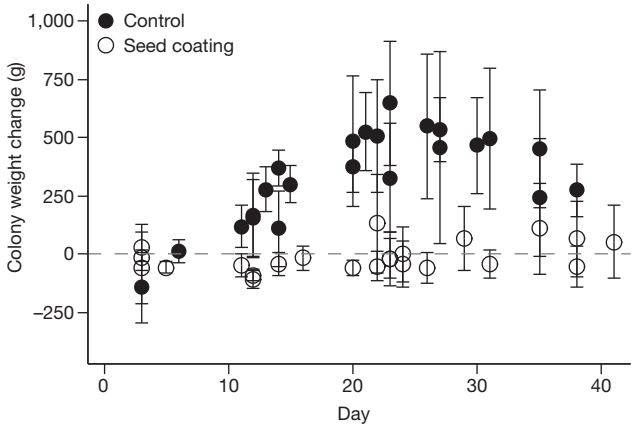

Figure 3 | Bumblebee colony development. Mean (± 95% confidence limits) bumblebee colony weight change (g) per field and survey day since day of placement at the fields (dashed horizontal reference line indicates initial colony weight) in relation to treatment (control (filled circles) or insecticide seed coating (open circles)). $n = 8$ fields per treatment. Dots are means of the six colonies at each field and weighing occasion. Two colonies at different fields (one control field and one treated field) were not weighed at one occasion, resulting in five colonies at those fields and weighing occasions. See Extended Data Table 6 for results from the colony growth model (linear mixed model).

Table 1 | Clothianidin concentrations in bee-collected pollen (ng g^{-1}) and nectar (ng ml^{-1}), and field border plants (ng g^{-1}), and tests of differences between treatments (control or insecticide-coated seeds)

	Control		Insecticide seed coating		Wilcoxon test for difference between treatments (n = 8*)	
	Range	Mean ± s.e.m.	Range	Mean ± s.e.m.	Z	P
Honeybee pollen	0	0	6.6–23	13.9 ± 1.8	−3.16	0.0016
Honeybee nectar	0–0.61	0.1 ± 0.1	6.7–16	10.3 ± 1.3	−3.40	<0.001
Bumblebee nectar	0	0	1.4–14	5.4 ± 1.4	−3.53	<0.001
Field border plants (≤2 days after sowing)	0	0	0–5.9	1.2 ± 0.8	−2.90	0.0037
Field border plants (2 weeks after sowing)	No material collected		0–6.5	1.0 ± 0.8		

*n = 6 for pollen collected by honeybees at control fields, because no such bees with pollen could be found at two fields; and n = 7 for field border plants collected within 2 days of sowing in both treatments, because of lack of communication regarding the sowing date between the farmer and the investigator collecting the samples.

in line with another field study[23] and previous work suggesting that honeybees are better at detoxifying after neonicotinoid exposure compared to bumblebees[17]. However, the lack of short-term effects does not preclude the existence of long-term effects of neonicotinoids[13].

Mass-flowering crops are valuable food resources for wild bees[19,24], but may act as ecological traps if foraging bees are exposed to pesticides such as neonicotinoids. To estimate exposure we assessed the transfer of clothianidin from plant to bee by first estimating the proportion of oilseed rape pollen collected by all three bee species, *O. bicornis*, *B. terrestris* and *A. mellifera* (Extended Data Table 6) and then quantifying the concentrations of clothianidin in bee-collected pollen and nectar (Table 1).

For *O. bicornis*, we found oilseed rape pollen in nine of 17 examined cells, accounting for 35.1 ± 17.0% (mean ± s.e.m.) of the collected pollen (Extended Data Table 5). Because there was no nesting activity at fields with insecticide-treated seeds, we could not assess pollen collection there. For *B. terrestris*, we found that in the 47 pollen samples collected from bees foraging in the oilseed rape fields, 80.1 ± 5.0% of the pollen was from oilseed rape, with similar results for both treated and control fields (Extended Data Table 5). For *A. mellifera* the pollen extracted from pollen traps mounted on the hives contained on average 57.8 ± 5.0% oilseed-rape-type pollen, with similar proportions for both treated and control fields (Extended Data Table 5).

We expected the insecticide seed coating to influence the amount of clothianidin that the bees were exposed to, but not β-cyfluthrin, since β-cyfluthrin, in contrast to clothianidin, is not systemically taken up by plants[5,18]. As expected, no β-cyfluthrin was detected (Extended Data Table 8), but both pollen and nectar collected by *A. mellifera* and nectar collected by *B. terrestris* foraging in the oilseed rape fields contained concentrations of clothianidin that were substantially higher in the treated fields than in control fields (Table 1). Clothianidin levels at treated fields were within the range of neonicotinoid levels quantified in pollen collected by honeybees in other studies (range: <0.1–912 ng g^{-1}; range of mean values per study and compound: <0.1–53.3 ng g^{-1})[25]. We also found higher clothianidin concentrations in plants collected in field borders adjacent to treated fields than adjacent to control fields, a few days and 2 weeks after the oilseed rape had been sown (Table 1), suggesting that plants in uncultivated habitats near treated crops can be an additional source for pesticide exposure[26].

We draw two main conclusions from our study. First, clothianidin seed coating in oilseed rape has negative effects on wild bees, with potential negative effects on populations. This finding is important because of the urgency to understand whether the use of neonicotinoid insecticides pose an unacceptable risk to bees[6]. However, questions remain regarding the mechanisms by which neonicotinoids affect bees, how field exposure varies across crops and seasons, and if effects translate into long-term population consequences, which are the focus of our further research. Second, the impact of clothianidin seed coating in oilseed rape differs between the wild bees studied and the honeybee. This implies that the use of honeybees as model organisms[27] in environmental risk assessments of neonicotinoids may not allow generalizations to other bee species. We question whether prevailing risk assessment standards, where predominantly short-term and lethal effects are assessed on model species under laboratory conditions[27,28], can be used to predict real-world consequences of pesticide use for populations, communities and ecosystems[29,30].

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions R.B., I.F., T.R.P. and H.G.S. conceived the project. M.R. designed the study, coordinated the work, analysed the data, and prepared the manuscript. G.K.S.A., V.H., L.H., B.K.K. and J.Y. collected the data. O.J. quantified the pesticide residues. All authors contributed to the interpretation of results and writing of the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M.R. (maj.rundlof@biol.lu.se).

METHODS

Study design. The design initially included 20 fields matched into pairs based on land use within 2 km (Extended Data Table 1), to cover the foraging distance of most bees[31,32], and geographical proximity. One field in each pair was randomly assigned to be sown with insecticide-coated seeds and the other field was the control field. The matching into field pairs was based on available land-use data for 2011, and the landscapes surrounding the selected oilseed rape fields were inspected for presence of flowering crops (including other spring-sown oilseed rape fields) during 27–28 May 2013. At the same time, establishment and growth stages (using the BBCH scale (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie)[33]) of the oilseed rape plants in the focal fields were inspected. After the field inspections, three fields were excluded from the study, because there were four (total 20.9 ha), five (22.6 ha) or five (46.2 ha) additional spring-sown oilseed rape fields within 2 km from our focal field that, since our study was conducted before the moratorium[6], may have been additional sources of neonicotinoid exposure. One field was excluded because a red clover seed field, known to be very attractive to foraging bumblebees and influence their density in the surrounding landscape[19], occurred adjacent to the focal field. In two cases, we decided to accept a single other oilseed rape field located at distances of 0.9 km (6.5 ha) and 1.0 km (4.4 ha) from the planned location of our bee colonies, to retain as many replications of fields as possible. At this point, the study design included six original field pairs and four fields which had lost their pair field. After reviewing land use in the surrounding landscapes and in geographical proximity of the four unpaired fields, we decided to match these into two new pairs (P07 and P08 in Fig. 1). The final study system included 16 spatially separated (>4 km) spring-sown oilseed rape fields (mean ± s.e.m. field size 8.9 ± 1.4 ha, range 4–27 ha, with all fields but one control field in the range 4–11 ha) located in southern Sweden (Fig. 1). The landscapes surrounding the fields were distributed along a gradient in the proportion of agricultural land, ranging from 6–88%, and the land uses considered often co-varied (Extended Data Table 1).

The field in each pair that was randomly assigned to be sown with insecticide-coated seeds received seeds treated with 25 ml Elado (Bayer; 400 g l^{-1} clothianidin + 80 g l^{-1} β-cyfluthrin) per kg of seed and the fungicide thiram, and the other field in the pair was sown with seeds coated with only thiram (the control). Elado instead of only clothianidin was used, because the pesticide combination was an agronomically realistic scenario for clothianidin use in Sweden and in other parts of Europe[34]. The clothianidin is taken up by the plant, distributed to all parts and protects the whole plant from pest attack[5], while the non-systemic β-cyfluthrin is intended to protect seeds and roots and only a very small amount is found in the aboveground parts of the plant (<0.5% of applied)[18]. We did not detect any β-cyfluthrin in pollen collected by honeybees at fields with insecticide seed coating (Extended Data Table 8). Fungicides alongside neonicotinoids have frequently been used in coating oilseed rape seeds (A. Gunnarson, personal communication)[35,36]. Since our study was conducted before the moratorium[6], no approval for the use of clothianidin-dressed seeds had to be obtained.

All experimental fields were sown with the hybrid oilseed rape cultivar Majong. The amount of seeds sown was 150 plants per square metre, which is the recommended seeding rate for a spring-sown hybrid[37], and corresponds to 7.5 kg ha^{-1} for thiram-treated seeds and 7.7 kg ha^{-1} for Elado + thiram-treated seeds. Sowing time was chosen and carried out by each farmer during the period 6 April to 18 May 2013 (Extended Data Table 2). In two of the pairs, the treated fields were sown considerably earlier (both 21 April) than the control fields (6 and 7–8 May), resulting in a phenological asynchrony between the fields in these pairs.

Farmers were not allowed to use other neonicotinoids in the fields, but they could use the non-neonicotinoid compounds Avaunt (active ingredient: indoxacarb), Mavrik (active ingredient: τ-fluvalinate), Plenum (active ingredient: pymetrozine) and Steward (active ingredient: indoxacarb) to control pollen beetles (Extended Data Table 3). Nevertheless, in one case, at a control field, the farmer applied Biscaya (Extended Data Table 3), where the active ingredient is the neonicotinoid thiacloprid. Thiacloprid has lower acute toxicity for bees than clothianidin, imidacloprid or thiamethoxam[13,22,25] and excluding this field did not qualitatively influence the effect of the insecticide seed treatment on the bees (Extended Data Tables 4–6).

Wild bee monitoring. Wild bees and flower cover were surveyed on three occasions in the flowering oilseed rape fields and adjacent uncultivated field borders, between 17 June and 16 July 2013 (Extended Data Table 2). Four in-field transects of 2 × 25 m located 2–4 m from the edge of the oilseed rape field were surveyed twice (18 June to 12 July and 27 June to 16 July). Transects of 2 × 300 m located at the outer 1-m edge of the oilseed rape field and 1 m of the adjacent, uncultivated field border were surveyed once (17 June to 8 July). Border transects within a field pair were surveyed on the same or subsequent days for the six phenologically synchronous field pairs and at peak flowering at the fields in the two asynchronous field pairs. For in-field transects, at least one of the survey occasions was performed within subsequent days for fields within a pair for all pairs but one (P04), and the other survey at peak flowering within the individual fields (Extended Data Table 2). Surveys were only conducted on warm days with no rain and light winds (<7 m s^{-1}). The observer covered approximately 10 m^2 of transect per minute. All flower visiting and flying solitary bees and bumblebees within the transects were noted and determined to species, genera or taxonomic group (Extended Data Table 7), using the entomological collection at Lund University, and refs 38, 39 and 40. Bumblebees belonging to the *B. terrestris* complex, including *B. terrestris*, *Bombus lucorum*, *Bombus magnus* and *Bombus cryptarum*, could not be separated and were treated as one group (*B. terrestris* ag.). Flower cover was calculated based on measurements of the number and size of flowers within transects.

Solitary bee nesting. Three trap nests (CJ Wildlife), each containing 29 paper tubes with an inner diameter of 6 mm and nine *O. bicornis* (previously *Osmia rufa*) cocoons (four females and five males), in total 27 cocoons (12 females and 15 males) were placed at each field approximately a week before the latest field within a pair was estimated to start flowering (equivalent to stage 55–63 on the BBCH scale, where stage 55 corresponds to individual buds being visible but still closed and stage 63 corresponds to the time when 30% of the flowers on the main raceme has opened[33]), between 10 and 24 June 2013 (Extended Data Fig. 1 and Extended Data Table 2). After emergence from the cocoons, *O. bicornis* individuals mate and the female starts to build cells where she places her eggs on collected pollen[41]. Emergence was the same in both treatments (Extended Data Fig. 1a). Females prefer to return to and build cells in their natal nest, over new equivalent nest cavities[42,43], and there is indication that nest site availability is limiting populations in current agricultural landscapes[44].

The cocoons originated from the study region. We artificially delayed emergence by about a month, by storing cocoons at 2–5 °C, to match the phenology of the spring-sown oilseed rape. In our study region in southern Sweden, observations of the species since 1990 indicate May (255 observations) to be the main activity period of *O. bicornis*, followed by April (94), June (83), July (2) and March (1)[45] (access date 9 February 2014, species: "*Osmia bicornis*", region: "Götaland", period: "1990-2014", "March", "April", "May", "June", "July" and "August"). This indicates that most of the *O. bicornis* at our study fields likely originated from the introduced population. Placement at the fields occurred on the same day at fields within a pair (Extended Data Table 2). Trap nests were mounted on poles in the field borders, approximately 50 m apart, facing southwards and with sheltering vegetation at the northern side.

Nesting tubes were collected 36–43 days after installing the cocoons. Nesting activity was determined in October 2013 by counting the number of tubes with brood cells. Where nesting activity occurred, *O. bicornis* built 4–34 brood cells in total per field (3.5 ± 0.3 (mean ± s.e.m.) cells per nest and field), distributed over 1–9 tubes. Proportion emerging from the cocoons was determined by counting the number of open cocoons. The pupa was considered dead if the cocoon was intact 4 weeks after placement at the fields.

Bumblebee colonies. Six commercially reared *Bombus terrestris* colonies (Natupol N, Koppert Biological Systems) were placed at each field at the onset of oilseed rape flowering, between 14 and 28 June 2013. At this time, the colonies were approximately 10 weeks old and contained one queen, approximately 50 workers and brood in all stages. Placement followed the phenology of the oilseed rape fields and was done on the same day in six of the pairs (or 2 days apart in one case) for fields within a pair (Extended Data Table 2). Placements of colonies at the two field pairs with asynchronous phenology were separated by 8 days between fields within the pairs, to follow the onset of flowering in the individual fields (Extended Data Table 2). Bumblebee colonies were ordered in four batches, with colonies from the same batch in the six synchronous pairs and from batches matching the individual fields for the two asynchronous pairs (Extended Data Table 2). Prevalence of pathogens and parasites in the colonies were not quantified before placement, although commercial colonies can be infested[46], and this could add unexplained noise to our data. Colonies were placed in triplets in two ventilated houses, located in a shaded part of the field borders (Extended Data Fig. 1). The colonies did not receive any supplementary feeding after placement at the fields. The inner plastic boxes and the *B. terrestris* colony content (bees, brood and nesting material) were weighed when placed at the fields and thereafter approximately biweekly. Colonies were closed for exiting bees before weighing and each colony was weighed 3–5 times (including the initial weighing). Two colonies (one at each treatment) were not weighed at one occasion, because they could not be closed for exiting bees. All colonies within a field pair were terminated by freezing (−20 °C) at first sight of emerging new queens in any of the 12 colonies. This happened between 7 July and 5 August 2013, or 23–38 days after the colonies had been placed at the fields. At the asynchronous field pairs, the colonies were collected at different dates from fields within the pair, but were allowed an equal number of days from placement to termination.

The outer two colonies in each triplet box were examined to estimate the number of queen and worker/male cocoons, weight of cocoons, larvae and nest structure and the number of cells used for nectar and pollen storage. Separation between queen and worker/male cocoons were based on the lowest value between the peaks of the bimodal distribution of cocoon width, based on measurement of all cocoons from four of the colonies (Extended Data Fig. 1c).

Honeybee colonies. Six equally sized *Apis mellifera* colonies were placed at each field (in total 96 colonies) at the onset of oilseed rape flowering, on 14–28 June 2013 (Extended Data Table 2), containing an estimated 3,418 ± 123 (mean ± s.e.m.) adult bees per colony (with no statistical difference between treatments (Extended Data Table 5)). Placement at the fields followed their phenology and was done on the same day (or two days apart in one case) for fields in six of the pairs (Extended Data Table 2). At the two field pairs with asynchronous phenology, placements were separated by seven days between fields within the pairs, following the onset of flowering in the individual fields (Extended Data Table 2).

Honeybee colony strength (that is, number of adult bees per colony) was assessed before placement at the experimental fields, on 6–7 June, and again at a common over-wintering location after removal from the experimental fields, on 29 July to 2 August, by a trained observer using the Liebefeld method[47,48]. The colonies were removed from the experimental fields on 2–31 July, at the end of oilseed rape flowering.

The colonies were produced on 27–31 May by a professional beekeeper with 1- or 2-year-old queens of known descent. Colonies were equalized to include two full honeycombs (with bees), two combs with mainly sealed brood (with bees), one queen originating from the same colony as the one from which the split (newly created colony) was taken, bees from two combs shaken into the split, one drawn out empty comb and five combs with wax foundation. The queens in the splits were freely mated and derived from three different mother queens and consisted of four different groups based on queen lineage and age. Queen lineage and age were matched between fields within a pair, but the distribution of colonies was otherwise randomized. The comb size was full Langstroth, with an area of 880 cm^2 per comb side and an estimated 1.25 bees per cm^2 when a comb side was fully covered (a total of 1,100 bees per side)[49]. Parent colonies and the new splits were placed in a 60 ha field of organically grown winter-sown oilseed rape after equalization and before placement at the 16 experimental fields, to minimize the risk of exposure to clothianidin and other pesticides.

Pollen samples. To verify the use of oilseed rape by the bees, pollen samples were taken from pollen traps mounted on the *A. mellifera* colonies, from *B. terrestris* foraging in the fields and from *O. bicornis* brood cells. The pollen traps were mounted on three *A. mellifera* colonies and were activated during the peak flowering of the oilseed rape (stages 65–67 on the BBCH scale[33]). At least 25 ml of pollen was collected from each field. A subsample of 15.0 g of the *A. mellifera*-collected pollen was sorted into separate samples based on colour and the separate samples were weighted. One to five samples from *B. terrestris* were collected per field (2.9 ± 0.3 (mean ± s.e.m.)), giving a total of 47 samples. Pollen was collected, when possible, from *O. bicornis* larval cells, resulting in 17 samples from the six control fields with nesting activity.

50–500 random pollen grains per sample were determined to have originated from either oilseed rape or another plant species using microscopy (10–40× magnification) and the pollen reference collection at Department of Biology, Lund University.

Neonicotinoid residues. Vegetation, pollen and nectar samples were collected to quantify the concentrations of clothianidin, together with β-cyfluthrin and the other four neonicotinoids used in Sweden (Extended Data Table 8), and to confirm the treatments. Samples of herbaceous material (flowers and leaves) were collected, within 2 days of sowing (7 April–20 May), every tenth metre in the transects used for wild bee monitoring in the permanent field borders adjacent to the oilseed rape fields. At the treated fields we also collected similar vegetation samples 13–15 days after sowing (21 April–3 June). In each field, five *A. mellifera* with pollen loads were caught to collect pollen samples and at least five nectar foragers were caught to collect nectar from the honey stomach. At two of the control fields, no *A. mellifera* with pollen loads could be found in the oilseed rape fields. Five *B. terrestris* were caught in the flowering oilseed rape fields, brought to the laboratory and nectar was extracted from the nectar stomachs of 3–5 bees per field, except at one control field where only one bee carried nectar.

Nectar samples were quantitatively handled using the capillary microsampling technique[50–52]. Neonicotinoids were quantified using liquid chromatography coupled with tandem mass spectrometry. β-Cyfluthrin was quantified using gas chromatography coupled with mass spectrometry. See Extended Data Table 8 for limits of detection and quantification.

Observer blind data collection. The people monitoring wild bees in the oilseed rape fields, handling the solitary bee nests, weighing and examining the bumblebee colonies, assessing the honeybee colony strength, and collecting honeybee pollen and nectar samples were blinded with respect to treatment. However, for practical reasons it was not possible to blind the person collecting vegetation samples in field borders during sowing and thereafter monitoring wild bees in the border transects and collecting bumblebees for pollen and nectar samples.

Statistical analyses. All data was analysed in SAS 9.4 for Windows (SAS Institute Inc.).

Wild bee densities were compared between treatments and in relation to flower cover, size of the focal oilseed rape field and proportion of agricultural land in the surrounding landscape using a generalized linear mixed model (GLMM, SAS PROC GLIMMIX) with Poisson error distribution and log link. Pair identity, pair identity × treatment and field part nested within pair identity × treatment were included as random factors, to account for the pairing of sites and the hierarchical study design. To investigate if the difference in phenology between fields influenced the difference in wild bee density between treatments, we also ran a statistical model only including temporally synchronous surveys, that is, surveys not more than 1 day apart for fields within a pair (Extended Data Table 2). In addition, to investigate if the influence of treatment was consistent for strictly wild bees, we ran another two models, but excluded *B. terrestris* ag., which could originate from the commercial colonies, and all bumblebees not determined to species (Extended Data Table 7). Results from all four analyses were qualitatively the same, except for flower cover, which did not relate significantly to the strictly wild bee density (Extended Data Table 4). GLMM with binomial error distribution and logit link were used to test the difference in flower cover between treatments, both for all data and for only temporally synchronous surveys (Extended Data Table 5). Results did not differ qualitatively depending on data set used (Extended Data Table 5).

Differences in emergence of *O. bicornis* from the cocoons between treatments, sexes and their interaction were tested with a GLMM with binomial error distribution and logit link. Pair identity, pair identity × treatment and sex nested within pair identity × treatment were included as random factors. The number of *O. bicornis* nest tubes with nesting activity was compared between treatments using Wilcoxon–Mann–Whitney test (SAS PROC NPAR1WAY).

An individual growth model (Extended Data Table 6) based on a linear mixed model (LMM, SAS PROC MIXED)[53] was used to test the effect of treatments on the weight gain of the *B. terrestris* colonies from placement at the fields (day = 0). The net weight gain was related to day, treatment, day × treatment, day × day and day × day × treatment. Random intercepts and random slopes for day and day × day were included, with the colony identity as the subject and an unstructured covariance matrix. Pair identity and pair identity × treatment were included as random factors to account for the study design. Since the individual growth model was complex and yielded significant two- and three-way interactions between treatment, we decided to: (1) analyse growth over time separate for the two treatments (Extended Data Table 6); and (2) test differences in colony growth rate between treatments only for the positive growth phase, identified as the period until the peak weight at control fields, using a LMM with estimated slope as the dependent variable, treatment as the independent variable and pair identity as a random factor. LMM (with normal error distribution) or GLMM (with Poisson error distribution and log link) were used to compare the number of queen and worker/male cocoons, weight of cocoons, larvae and nest structure and the number of cells used for nectar and pollen storage between treatments (Extended Data Table 5).

Honeybee colony strength (that is, number of adult bees per colony) was compared between treatments using a LMM. Colony strength before placement at the fields was used as a covariate and pair identity and pair identity × treatment were included as random factors. A colony that lost its queen during transport to the field (treated field) and swarmed colonies (eight at control fields and ten at treated field) were excluded from the analysis (which did not qualitatively alter the results).

To investigate if the presence of other spring oilseed rape fields within 1 km influenced the results, *B. terrestris* colony growth (Extended Data Table 5), *B. terrestris* queen and worker/male production (Extended Data Table 5) and *A. mellifera* colony development (Extended Data Table 6) were analysed using the full data set as well as a data set where the two field pairs with other spring-sown oilseed rape within 1 km from one of the fields were excluded, since the other spring-sown oilseed rape fields were within the potential flight range of both bee species[31,32]. The results were qualitatively the same for *B. terrestris* colony growth, weight of produced cocoons and *A. mellifera* colony development independent of including or excluding the two field pairs (Extended Data Tables 5 and 6), but differed for the number of *B. terrestris* cocoons (Extended Data Table 5). The latter could be a result of reduced statistical power to detect differences, since the level of replication is reduced from eight to six when excluding two of the field pairs and queen production in particular is documented to be very variable[10,54–56].

To investigate if the Biscaya used at one control field influenced the results, we analysed wild bee density (Extended Data Table 4), *O. bicornis* nesting activity (results not shown), *B. terrestris* colony growth (Extended Data Table 5), *B. terrestris* queen and worker/male production (Extended Data Table 5) and *A. mellifera* colony development (Extended Data Table 6) in relation to the insecticide seed treatment both including and excluding the field pair where Biscaya was used at the control field. The results were qualitatively the same for all dependent variables independent of including or excluding the field pair (Extended Data Tables 4–6).

The clothianidin concentrations in nectar and pollen collected by honeybees, nectar collected by bumblebees and field border plant material were analysed in relation to treatment using Wilcoxon–Mann–Whitney tests.

Denominator degrees of freedom in the mixed models were estimated with the Kenward–Roger method or, when there was a negative covariance in the random part of the model, the containment method (constraining the variance component to 0), to avoid inflated denominator degrees of freedom[53]. Deviance from the assumption of normal error distribution of the LMM was tested using a Shapiro–Wilks test and visually assessed on residual plots. When deviance was detected ($P < 0.05$ and indicated in plots), data was either square-root transformed or a GLMM, assuming Poisson error distributions, was used. Deviance from the assumption of homogeneous variance between compared groups was tested using Levene's test. When deviance was detected ($P < 0.05$), heterogeneous variance was modelled. Over-dispersion of the data, when the variance is considerably larger than the mean, was assessed by the ratio of the generalized χ^2 statistics and its degrees of freedom[53]. If the ratio was larger than 1.3, an over-dispersion parameter (random _residual_) was added to the model.

Power analysis. We performed a power analysis for honeybee colony strength, to investigate the effect size that we could potentially detect given our design and replication. A power analysis is conditional on the study design and the statistical model used to analyse the data, so we therefore used a power analysis method recommended for mixed models[53]. With the macro (program) MixedTPower[53] we produced a power curve based on the honeybee colony strength model. We assumed $\alpha = 0.05$ and then calculated power for a range of effect sizes. The effect size is initially expressed on the same scale as the dependent variable (that is, number of bees per colony; Extended Data Fig. 2a). By dividing the effect size with the average number of bees per colony at control sites, we obtained effect size expressed as the percentage change in the number of bees per colony (that is, colony strength) between control fields and treated fields (Extended Data Fig. 2b), which made it possible to compare our effect size with the effect sizes stated by the European Food Safety Authority [57] and the power analysis performed by the Centre for Ecology and Hydrology [58]. Our power analysis indicated that, given our design, replication and data analysis method, we would be able to detect an effect size of just below 20% with a power of 0.8 (Extended Data Fig. 2b). This is in line with the estimated effect size for our level of replication given by the Centre for Ecology and Hydrology [58].

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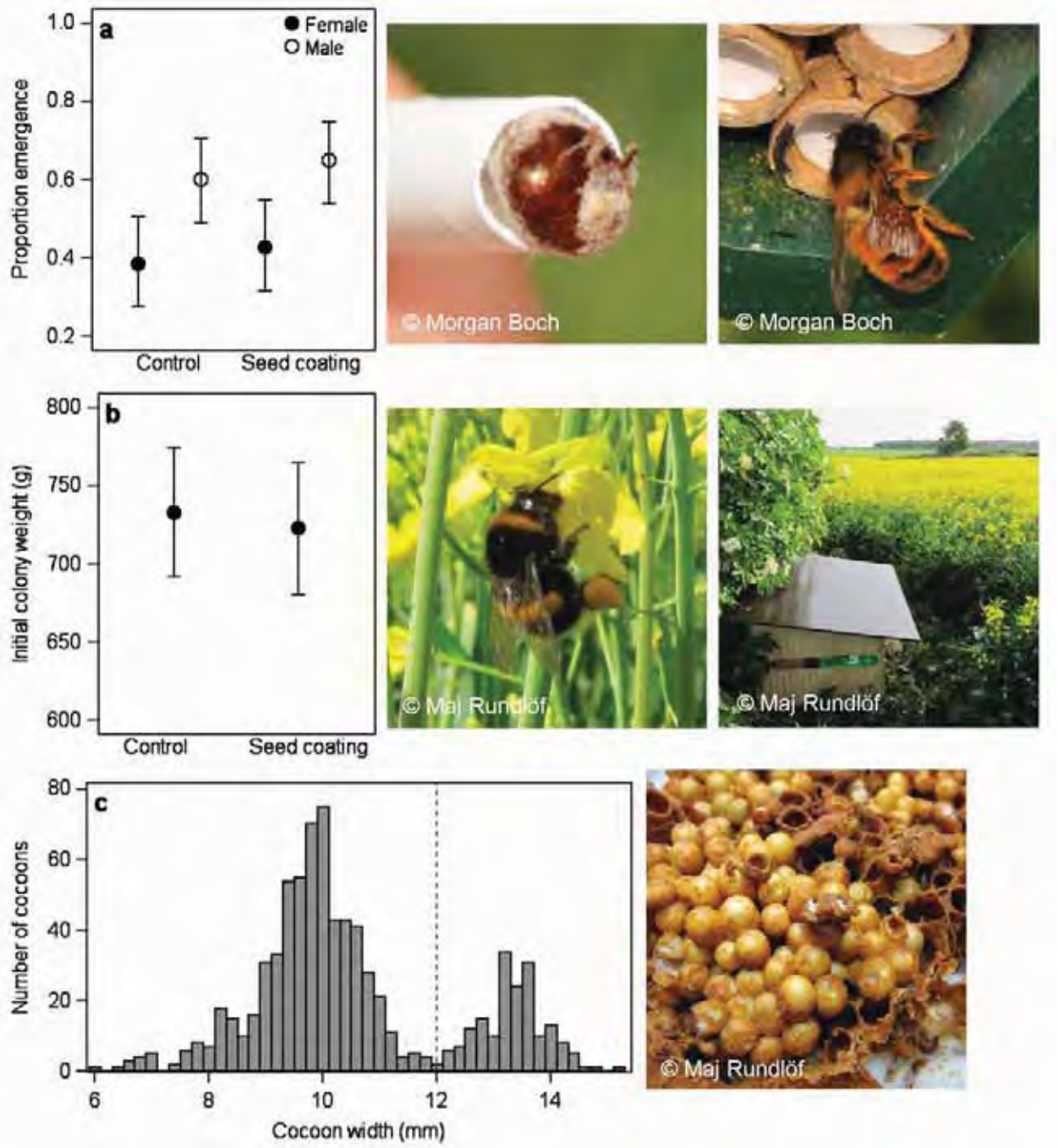
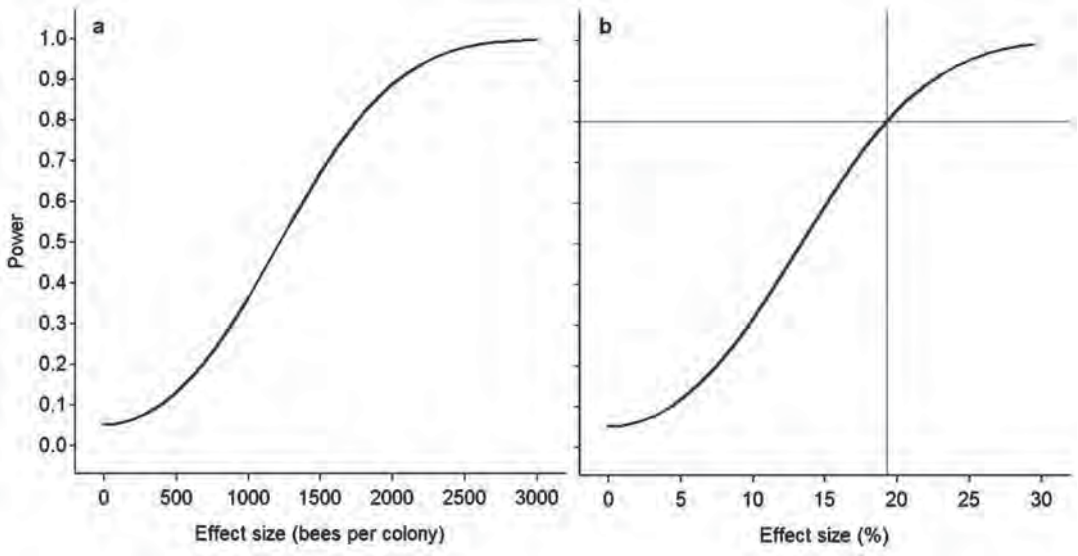


Extended Data Figure 1 | *O. bicornis* emergence and *B. terrestris* colonies. **a**, Mean (± 95% confidence limits) proportion emergence of *O. bicornis* from cocoons in relation to treatment (control or insecticide seed coating), with higher emergence for males than females (generalized linear mixed model, binomial error distribution, logit link; $F_{1, 14} = 14.97$, $P = 0.0017$), no difference between treatments ($F_{1, 7} = 0.71$, $P = 0.43$) and no interaction ($F_{1, 14} = 0.01$, $P = 0.94$). $n = 8$ fields per treatment, with 12 female and 15 male cocoons at each field. Photos (with permission; Morgan Boch): left, emerged *O. bicornis* cocoon; right, *O. bicornis* female at a trap nests filled with cardboard nest tubes. **b**, Mean (± 95% confidence limits) weight of *B. terrestris* colonies at placement at the fields in relation to treatment (linear mixed model, $F_{1, 7} = 0.99$, $P = 0.35$). $n = 8$ fields per treatment, with six colonies at each field. Photos (M.R.): left, *B. terrestris* worker foraging in the oilseed rape; right, house containing three *B. terrestris* colonies. Means and confidence limits in panels **a** and **b** are based on back-transformed, model-estimated least square means. **c**, *B. terrestris* silk cocoon width distribution of all cocoons in four colonies (two from two different control fields and two from two different fields with insecticide seed treatment) initially examined to separate between queen and worker/male cocoons. Dashed vertical line indicates selected cut-off width at 12 mm (the lowest value between the two peaks), with queens larger (or equal) and workers/males smaller. Photo (M.R.): *B. terrestris* colony under examination.



Extended Data Figure 2 | Power curves for honeybee colony strength. **a**, **b**, Relationship between power and effect size estimated for the honeybee model (Extended Data Table 6), with effect size expressed as the difference in honeybee colony strength (number of bees per colony) (**a**) and the percentage change in colony strength (**b**) between colonies at control fields and at fields with insecticide seed coating after placement at the oilseed rape fields. Grey reference lines indicate a power of 0.8 and the corresponding effect size.

Extended Data Table 1 | 2013 field size and 2011 and 2013 land use in the landscapes surrounding (radius = 2 km) the oilseed rape

	Control (n = 8)		Insecticide seed coating (n = 8)		Test of difference between treatments		Correlation matrix									
	mean ± s.e.m.	min-max	mean ± s.e.m.	min-max	F_{df}	P	Agricultural land	Annually tilled arable land	Semi-natural grassland	Length of permanent field borders	Maize cultivation	Spring sown oilseed rape	Winter sown oilseed rape	Mass-flowering crops*	Forest	Urban
Size of focal oilseed rape field (ha)	9.4 ± 2.6	4.0-27.0	8.4 ± 0.9	4.0-11.0	$0.11_{1,7}$	0.75	0.102	0.252	-0.425	0.033	-0.130	-0.174	-0.048	0.543	-0.159	0.300
Agricultural land (%)	58.2 ± 10.6	9.5-88.2	55.8 ± 9.8	5.9-83.3	$0.29_{1,7}$	0.61		0.923	-0.049	0.831	0.401	0.309	0.539	0.744	-0.962	-0.083
Annually tilled arable land (%)	38.8 ± 9.6	3.0-70.9	34.3 ± 8.8	0.3-74.5	$0.64_{1,7}$	0.45			-0.338	0.592	0.173	0.334	0.675	0.870	-0.866	-0.069
Semi-natural grassland (%)	3.1 ± 1.0	0.2-7.4	4.1 ± 1.2	0.2-9.4	$0.16_{1,7}$	0.70				0.381	0.450	-0.259	-0.285	-0.337	0.082	-0.096
Length of permanent field borders (km)	14.2 ± 1.9	3.5-18.5	14.9 ± 2.3	3.2-25.7	$0.11_{1,7}$	0.75					0.688	0.157	0.139	0.462	-0.827	0.009
Maize cultivation 2011 (%)	1.4 ± 0.5	0-3.9	1.7 ± 0.6	0-6.5	$0.02_{1,7}$	0.88						0.272	-0.243	0.107	-0.483	0.203
Maize cultivation 2013 (%)	1.3 ± 0.4	0-3.6	1.3 ± 0.7	0-5.6	$0.42_{1,7}$	0.54										
Spring sown oilseed rape 2011 (%)	0.8 ± 0.7	0-5.7	0.6 ± 0.2	0-1.5	$0.05_{1,7}$	0.83							-0.137	0.785	-0.254	-0.217
Spring sown oilseed rape 2013 (%) – including the focal field	1.8 ± 0.7	0.3-6.2	1.5 ± 0.4	0.3-2.7	$<0.01_{1,7}$	0.98										
Winter sown oilseed rape 2011 (%)	1.4 ± 0.8	0-6.8	1.6 ± 1.0	0-8.2	$<0.01_{1,7}$	0.96								0.566	-0.455	-0.163
Winter sown oilseed rape 2013 (%)	1.5 ± 0.7	0-5.2	2.5 ± 1.2	0-8.6	$0.34_{1,7}$	0.58										
Mass-flowering crops* 2013 (%)	8.2 ± 2.8	0.3-23.6	7.5 ± 2.1	0.8-17.8	$0.01_{1,7}$	0.93										
Forest (%)	25.3 ± 10.6	1.8-74.8	24.0 ± 8.6	0.5-67.2	$0.23_{1,7}$	0.64								-0.693		-0.116
Urban (%)	2.7 ± 1.1	0-8.6	3.3 ± 1.0	0-9.0	$0.53_{1,7}$	0.49								0.026		

*Mass-flowering crops include oilseed rape (46%), potato (28%), pea (18%), bean (4%), fruit and berry cultivation (4%), and herbs and seeds (<1%).

Extended Data Table 2 | Phenology (date, BBCH[33] and flower cover) in the oilseed rape fields and delivery, placement and survey* of bees

Pair	Seed treatment†	Sowing date	Date placement *Osmia bicornis* (oilseed rape growth stage (BBCH))	*Bombus terrestris* delivery date (batch)	Date placement *Bombus terrestris* (oilseed rape growth stage (BBCH))	Date placement *Apis mellifera* (oilseed rape growth stage (BBCH))	Date wild bee survey border (oilseed rape growth stage (BBCH))	Date wild bee survey field 1 (% flower cover)	Date wild bee survey field 2 (% flower cover)
P01	contr	23 April 2013	13 June (59)	18 June (2)	20 June (65)	19 June (65)	25 June (65)	1 July (52)	3 July (43)
P01	treat	28 April 2013	13 June (57)	18 June (2)	20 June (61)	19 June (61)	26 June (63)	1 July (95)	3 July (97)
P02	contr	7-8 May 2013‡	13 June (50)	20 June (3)	26 June (63)	25 June (63)	6 July (65)	28 June (58)	9 July (60)
P02	treat	21 April 2013‡	13 June (61)	18 June (2)	18 June (63)	18 June (63)	20 June (63)	19 June (90)	27 June (49)
P03	contr	18 May 2013	24 June (52)	25 June (4)	28 June (60)	2 July (61)	8 July (63)	12 July (33)	16 July (46)
P03	treat	11 May 2013	24 June (57)	25 June (4)	28 June (61)	2 July (63)	8 July (65)	8 July (53)	12 July (64)
P04	contr	6 May 2013‡	13 June (50)	20 June (3)	26 June (65)	25 June (65)	4 July (65)	7 July (56)	9 July (61)
P04	treat	21 April 2013‡	13 June (61)	18 June (2)	18 June (63)	18 June (63)	20 June (65)	19 June (89)	1 July (37)
P05	contr	29 April 2013	15 June (57)	18 June (2)	20 June (63)	20 June (63)	24 June (65)	24 June (21)	4 July (39)
P05	treat	25 April 2013	15 June (61)	18 June (2)	18 June (63)	18 June (63)	24 June (65)	24 June (57)	4 July (100)
P06	contr	1 May 2013	13 June (57)	18 June (2)	19 June (63)	19 June (63)	28 June (65)	2 July (74)	5 July (94)
P06	treat	25-26 April 2013	13 June (53)	18 June (2)	19 June (63)	19 June (63)	28 June (65)	5 July (89)	9 July (81)
P07	contr	4 May 2013	15 June (55)	20 June (3)	24 June (63)	24 June (63)	1 July (65)	7 July (26)	11 July (33)
P07	treat	2 May 2013	15 June (57)	20 June (3)	24 June (64)	24 June (64)	1 July (65)	7 July (87)	11 July (39)
P08	contr	6 April 2013	10 June (61)	11 June (1)	14 June (65)	14 June (65)	17 June (65)	18 June (43)	28 June (5)
P08	treat	16 April 2013	10 June (61)	11 June (1)	14 June (63)	14 June (63)	18 June (65)	18 June (14)	28 June (72)

*Shaded numbers are surveys selected for analysis of wild bee density data collected at the same time (that is, within subsequent days) within the field pairs.
†contr, control; treat, insecticide seed coating.
‡Highly asynchronous phenology of the fields within the pair.

Extended Data Table 3 | Use of plant protection products in the oilseed rape fields during the 2013 growing season

Pair	Seed treatment*	Date treatment 1	Compound treatment 1	Dose treatment 1	Date treatment 2	Compound treatment 2	Dose treatment 2
P01	contr	04 June 2013	Mavrik	0.25 l/ha			
P01	treat	06 June 2013	Plenum	150 g/ha	15 June 2013	Steward	85 g/ha
P02	contr	31 May 2013	Plenum	160 g/ha	10 June 2013	Mavrik	0.20 l/ha
P02	treat	04 June 2014	Plenum	150 g/ha	10 June 2013	Steward	85 g/ha
P03	contr	no treatment					
P03	treat	12 June 2013	Avaunt	170 g/ha			
P04	contr	16 June 2013	Avaunt	160 g/ha			
P04	treat	07 June 2013	Plenum	150 g/ha			
P05	contr	12 June 2013	Plenum	150 g/ha			
P05	treat	30 May 2013	Plenum	150 g/ha			
P06	contr	12 June 2013	Biscaya	0.30 l/ha	19 June 2013	Mavrik	0.25 l/ha
P06	treat	07 June 2013	Avaunt	170 g/ha			
P07	contr	04 June 2013	Avaunt	170 g/ha	08 June 2013	Plenum	150 g/ha
P07	treat	31 May 2013	Plenum	150 g/ha			
P08	contr	30 May 2013	Avaunt	170 g/ha			
P08	treat	04 June 2014	Plenum	150 g/ha	14 June 2013	Avaunt	120 g/ha

*contr, control; treat, insecticide seed coating.

Extended Data Table 4 | Wild bee density in oilseed rape fields and borders in relation to insecticide seed treatment and covariates

Model	Explanatory variable	Estimate	Degrees of freedom	*F*	*P*
Wild bees (all data)	Intercept	2.55			
	Treatment	0.73	1, 7	9.68	0.019
	Flower cover	1.06	1, 24	18.57	<0.001
	Field size	0.07	1, 7	7.46	0.028
	Proportion agricultural land	-1.20	1, 8	2.35	0.16
Wild bees (synchronized data*)	Intercept	2.03			
	Treatment	0.76	1, 6	6.69	0.043
	Flower cover	1.32	1, 29	26.56	<0.001
	Field size	0.08	1, 7	6.46	0.038
	Proportion agricultural land	-1.00	1, 5	2.76	0.15
Wild bees excluding *Bombus terrestris* ag. (all data)	Intercept	0.79			
	Treatment	1.14	1, 7	12.65	0.0096
	Flower cover	1.06	1, 17	8.52	0.094
	Field size	0.08	1, 6	6.63	0.045
	Proportion agricultural land	-0.33	1, 7	0.20	0.67
Wild bees excluding *Bombus terrestris* ag. (synchronized data*)	Intercept	-16.07			
	Treatment	9.16	1, 4	12.28	0.025
	Flower cover	2.17	1, 7	0.35	0.57
	Field size	1.77	1, 7	54.65	<0.001
	Proportion agricultural land	4.86	1, 7	1.07	0.34
Wild bees (excluding the field pair where Biscaya was used at the control field)	Intercept	0.93			
	Treatment	0.95	1, 3	20.20	0.023
	Flower cover	1.18	1, 15	16.29	0.0011
	Field size	0.20	1, 4	10.04	0.034
	Proportion agricultural land	-0.42	1, 8	0.12	0.74

*See Extended Data Table 2 for identification of synchronized data.

Extended Data Table 5 | Statistical tests and mean values for bee-related variables in relation to the insecticide seed treatment in the oilseed rape fields

Dependent variable	Degrees of freedom	*F*	*P*	Control (mean ± s.e.m.)	Insecticide seed coating (mean ± s.e.m.)
Flower cover (%) - all data	1, 7	9.34	0.018	46.4 ± 7.3	70.2 ± 6.5
Flower cover (%) - synchronized data*	1, 6	8.28	0.028	41.4 ± 9.0	70.9 ± 8.0
Initial *Bombus terrestris* colony weight (g)	1, 7	0.99	0.35	733.2 ± 17.8	722.7 ± 18.6
Slope of *Bombus terrestris* colony growth	1, 7	115.80	<0.001	21.3 ± 1.6	0.4 ± 1.6
Slope of *Bombus terrestris* colony growth - excluding the two field pairs with other spring sown oilseed rape field within 1 km	1, 5	143.02	<0.001	18.9 ± 1.1	-0.5 ± 1.1
Slope of *Bombus terrestris* colony growth - excluding the field pair where Biscaya was used at the control field	1, 6	108.41	<0.001	22.2 ± 1.7	0.5 ± 1.7
Number of *Bombus terrestris* queen cocoons	1, 7	7.78	0.027	70.0 ± 12.3	20.6 ± 8.3
Number of queen cocoons - excluding the two field pairs with other spring sown oilseed rape field within 1 km	1, 5	3.82	0.11	59.7 ± 15.8	22.0 ± 9.6
Number of queen cocoons - excluding the field pair where Biscaya was used at the control field	1, 6	9.46	0.022	69.1 ± 13.7	18.1 ± 7.0
Number of *Bombus terrestris* worker/male cocoons	1, 7	8.09	0.025	241.0 ± 29.8	142.0 ± 29.8
Number of worker/male cocoons - excluding the two field pairs with other spring sown oilseed rape field within 1 km	1, 5	6.57	0.050	206.1 ± 28.3	115.6 ± 20.7
Number of worker/male cocoons - excluding the field pair where Biscaya was used at the control field	1, 6	6.74	0.041	247.6 ± 33.9	144.0 ± 33.9
Weight of *Bombus terrestris* cocoons (g)	1, 7	14.77	0.0061	172.0 ± 32.3	54.0 ± 18.7
Weight of cocoons (g) - excluding the two field pairs with other spring sown oilseed rape field within 1 km	1, 5	12.34	0.017	135.1 ± 25.3	41.6 ± 14.5
Weight of cocoons (g) - excluding the field pair where Biscaya was used at the control field	1, 6	9.62	0.021	201.1 ± 32.3	69.2 ± 32.3
Weight of *Bombus terrestris* larvae (g)	1, 7	0.15	0.71	15.5 ± 6.0	13.6 ± 5.7
Weight of *Bombus terrestris* nest structure (g)	1, 7	12.34	0.0098	261.0 ± 24.7	139.4 ± 24.7
Number of nectar cells	1, 7	2.43	0.16	59.4 ± 23.7	23.5 ± 10.4
Number of pollen cells	1, 7	0.60	0.46	5.5 ± 2.1	3.6 ± 1.4
Initial number of *Apis mellifera* per colony	1, 7	0.12	0.74	3412 ± 192	3325 ± 160
Proportion oilseed rape pollen from *Osmia bicornis* (%)				35.1 ± 17.0	
Proportion oilseed rape pollen from *Bombus terrestris* (%)	1, 8	3.70	0.092	88.1 ± 5.0	74.9 ± 7.7
Proportion oilseed rape pollen from *Apis mellifera* (%)	1, 7	1.09	0.33	52.6 ± 7.2	63.1 ± 5.9

*See Extended Data Table 2 for identification of synchronized data.

Extended Data Table 6 | Bumblebee colony growth (net weight gain) and honeybee colony strength (adult bees per hive) in relation to insecticide seed treatment

Model	Explanatory variable(s)	Estimate	Degrees of freedom	F	P
***B. terrestris* colony growth**					
All fields	Intercept	-51.07			
	Treatment	-434.27	1, 18	51.41	<0.001
	Day	0.23	1, 21	144.31	<0.001
	Day × treatment	72.50	1, 21	143.00	<0.001
	Day × day	0.08	1, 19	102.52	<0.001
	Day × day × treatment	-1.40	1, 19	130.62	<0.001
Only control fields	Intercept	-533.40			
	Day	77.59	1, 31	129.10	<0.001
	Day × day	-1.44	1, 28	114.70	<0.001
Only fields with insecticide seed coating	Intercept	-36.53			
	Day	-1.61	1, 16	0.92	0.35
	Day × day	0.13	1, 14	10.78	0.0055
***A. mellifera* colony strength**					
All fields	Intercept	9834.46			
	Initial colony strength	-0.19	1, 64	1.67	0.20
	Treatment	-41.51	1, 7	0.01	0.94
Excluding the two field pairs with other spring sown oilseed rape field within 1 km	Intercept	9609.95			
	Initial colony strength	-0.18	1, 45	1.33	0.26
	Treatment	199.73	1, 5	0.11	0.76
Excluding the field pair where Biscaya was used at the control field	Intercept	9715.31			
	Initial colony strength	-0.16	1, 57	0.82	0.37
	Treatment	90.68	1, 6	0.02	0.88

Extended Data Table 7 | Number of individuals of wild bee species or groups at control ($n = 8$) and insecticide-treated ($n = 8$) oilseed rape fields

Group	Bee species	Control	Insecticide seed coating
solitary bee	*Andrena* sp.	15	25
solitary bee	*Colletes* sp.	5	2
solitary bee	*Hylaeus* sp.	1	0
solitary bee	*Lasioglossum/Halictus* sp.	10	3
solitary bee	*Macropis europaea*	1	0
solitary bee	*Nomada* sp.	1	3
solitary bee	*Sphecodes* sp.	4	1
solitary bee	unidentified solitary bee (not including *Osmia bicornis*)	10	0
bumble bee	*Bombus hortorum*	3	0
bumble bee	*Bombus hypnorum*	10	5
bumble bee	*Bombus lapidarius*	275	43
bumble bee	*Bombus pascuorum*	18	6
bumble bee	*Bombus pratorum*	3	6
bumble bee	*Bombus ruderarius*	2	2
bumble bee	*Bombus soroeensis*	1	0
bumble bee	*Bombus subterraneus*	1	0
bumble bee	*Bombus sylvarum*	2	0
bumble bee	*Bombus terrestris/lucorum/magnus/cryptarum*	712	403
bumble bee	unidentified bumble bee	190	159

Extended Data Table 8 | Residues of neonicotinoids (n) and a pyrethroid (p) in bee-collected pollen and nectar from control fields and fields sown with insecticide treated seeds

	Control (n = 8 fields*)		Insecticide seed coating (n = 8 fields)			
	Detected in n samples	Highest concentration	Detected in n samples	Highest concentration	LOD†	LOQ†
Honey bee pollen (ng/g)						
Acetamiprid (n)	1	0.34	0		0.080	0.24
Clothianidin (n)	0		8	23	0.50	1.5
Imidacloprid (n)	1	0.23‡	0		0.30	0.90
Thiacloprid (n)	3	1.4§	4	0.29	0.070	0.21
Thiamethoxam (n)	0		0		0.10	0.30
Beta-cyfluthrin (p)			0		1.0	
Honey bee nectar (ng/ml)						
Acetamiprid (n)	0		0		0.033	0.10
Clothianidin (n)	2	0.61	8	16	0.17	0.50
Imidacloprid (n)	3	0.35	0		0.17	0.50
Thiacloprid (n)	2	0.35§	2	0.044	0.033	0.10
Thiamethoxam (n)	1	0.19	0		0.17	0.50

* n = 6 for pollen collected by honeybees at control fields, because no such bees with pollen could be found at two fields.
† LOD, limit of detection; LOQ, limit of quantification.
Pollen LOD and LOQ were estimated from spiking experiments of the average sample weight of 0.056 g.
Nectar LOD and LOQ were estimated for the 0.016 ml sample volume.
‡ Sample weight of 0.091 g explains reported value slightly below the estimated limit of detection, based on a 0.056 g sample weight
§ One oilseed rape field sprayed with Biscaya (12 June 2013), where thiacloprid is the active ingredient (Extended Data Table 3).

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ECOLOGY

Tasteless pesticides affect bees in the field

Two studies provide evidence that bees cannot taste or avoid neonicotinoid pesticides, and that exposure to treated crops affects reproduction in solitary bees as well as bumblebee colony growth and reproduction.

NIGEL E. RAINE & RICHARD J. GILL

Insects such as bees are crucial for the pollination of agricultural crops and wild plants[1,2], helping to ensure food security and maintain biodiversity. Yet a range of environmental stressors are threatening bee populations around the world[3–6]. The impact of pesticide exposure, particularly from neonicotinoid insecticides, has received substantial recent research attention[7,8] and has become a topic of public debate. Studies that have reported adverse effects of neonicotinoids on bees have been criticized for several reasons: that exposure tests are carried out under laboratory or semi-field settings rather than in the field and use pesticide-treated foods containing unrealistically high dosages; and that bees can detect chemical residues on treated crops and avoid foraging on them. Further weight has been added to such criticisms because the few field studies that have investigated potential impacts on honeybees and bumblebees from exposure to neonicotinoid-treated crops have been interpreted to show little or no effect[9–13], although limitations to these studies have been highlighted[7,14]. Two studies published on *Nature*'s website today strike at the heart of these evidence gaps and improve our understanding of pesticide exposure risks to bees.

In their paper, Kessler *et al.*[15] present a carefully controlled laboratory study testing the ability of both honeybees (*Apis mellifera*) and bumblebees (*Bombus terrestris*) to taste the three most commonly used neonicotinoids — clothianidin, imidacloprid and thiamethoxam. When hungry worker bees could choose to collect from feeders containing either a solution of neonicotinoid-treated sugar water or an untreated solution, neither species avoided the treated food, which contained neonicotinoid concentrations comparable to those found in the nectar and pollen of treated crops. Surprisingly, the bees in fact preferred the treated solution in the imidacloprid and thiamethoxam tests, which the authors suggest arises from the pharmacological action of these insecticides on receptors in the bees' brains. The authors corroborated their behavioural results with neurophysiological measurements showing that bees are unable to taste neonicotinoids in sugar water.

Scaling up from the laboratory, Rundlöf *et al.*[16] undertook an ambitious study to assess the impacts of neonicotinoid exposure on bees placed near fields of treated oilseed rape (also known as canola). The experiment — the largest of its kind so far — involved 16 fields across southern Sweden: 8 fields were planted with seeds treated with the systemic insecticide clothianidin, the pyrethroid insecticide β-cyfluthrin and the fungicide thiram, and 8 control fields were treated solely with thiram. Like Kessler *et al.*, these researchers studied both honeybees and bumblebees, but followed entire colonies rather than individuals. Furthermore, they monitored nests of a species of solitary bee (*Osmia bicornis*), as well as surveying wild bees in field margins.

In treated fields, Rundlöf and colleagues found fewer wild bees and observed reduced growth rate and reproduction of bumblebee colonies (which produced fewer males and fewer new queens — consistent with previous semi-field and field studies[14,17,18]) compared to control fields. They also found that none of the solitary bees that emerged from nests placed next to treated fields came back to their natal nest to build new brood cells, whereas

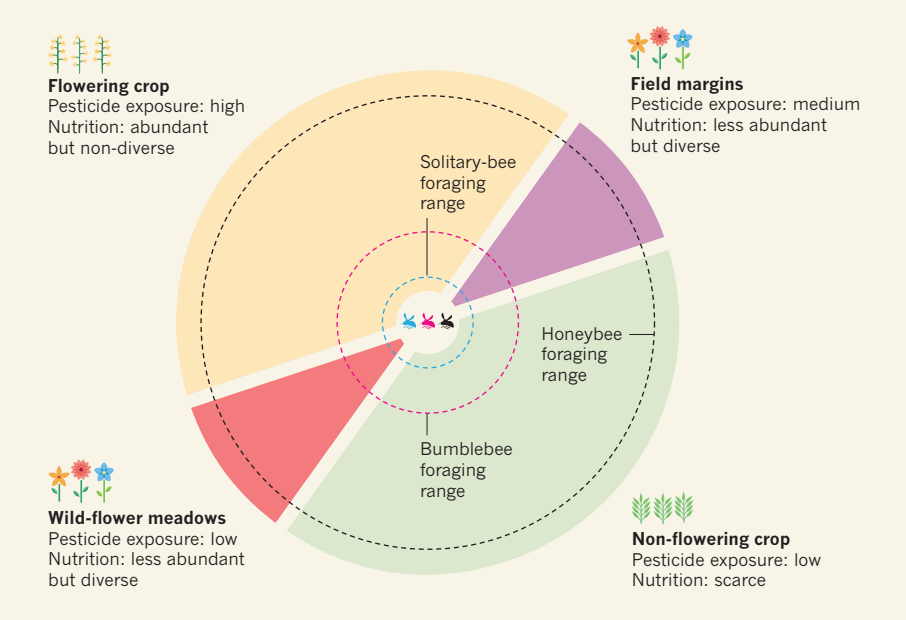

Figure 1 | Bee foraging options and pesticide exposure. Non-flowering crops and pasture cover large areas of rural land, but typically provide limited food resources for bee populations. Flowering crops can provide plentiful (although non-diverse) bee food, but are often treated with pesticides, a direct route for exposure. Flower-rich meadows provide a diverse bee diet, but these are becoming increasingly scarce, and small areas may support only low bee numbers. Furthermore, wild flowers in field margins may contain pesticide residues. Rundlöf *et al.*[16] show that the growth rates and reproduction of bumblebee colonies are lower in neonicotinoid-treated fields than in control fields, and that reproduction of solitary bees can also be affected. However, the authors found no effect on honeybee colonies. These differences may result from different ecologies: honeybees can forage many kilometres from their hive, whereas bumblebees roam over smaller areas, and solitary bees fly less far from their nest. Honeybees also use the waggle dance to communicate the location of rewarding flower patches to nest-mates. Thus, honeybees may have reduced pesticide exposure from visiting a greater mixture of foraging sources or through a greater chance of avoiding treated crops. However, Kessler *et al.*[15] show that neither honeybees nor bumblebees can taste neonicotinoids, suggesting that such avoidance behaviour is unlikely. (Nest sites, foraging ranges and the relative proportion of habitat types vary across landscapes — those depicted are representative only.)

emergent females successfully produced brood cells in six of eight untreated fields. By contrast, there was no significant difference in honeybee colony growth between treated and control fields. However, the authors' power analysis indicated that they would only have been able to detect a minimum effect size of about 19% for honeybees.

These studies provide timely data to address calls for further evidence about the environmental risks of neonicotinoids. The insecticides tested by the authors are currently subject to a European Union moratorium for use as seed treatments on crops attractive to bees, but this usage restriction will be reviewed before December 2015. It is hard to say whether the preferences observed by Kessler and colleagues for nectar containing imidacloprid and thiamethoxam residues would occur in a more complex field setting, where many variables could interfere with foraging decisions. However, their study does imply that foraging bees are unlikely to avoid seed-treated crops in the field, and supports previous reports of honeybees and bumblebees bringing back nectar and pollen from treated fields[9–12,16]. If the preference for treated food does apply in the field, these findings suggest that we could be underestimating the exposure risk to bees from treated crops.

Both studies also highlight the fact that different bee species vary in their responses to exposure. Current pesticide registrations rely on ecotoxicological testing of just one species, the honeybee, when assessing risks for all insect pollinators. Yet Rundlöf and colleagues found negative effects of neonicotinoids on solitary bees and bumblebees in the field, but not on honeybees, suggesting that a single species might not represent the responses of other pollinators. Potential explanations for these apparent differences could include a variable affinity of neuronal receptors for binding neonicotinoids; differences in detoxification capacities; and divergent foraging behaviours, which influence levels of exposure (Fig. 1). Differences could also result from variation in social organization and life-history strategies. Even the smallest perennial honeybee colonies contain a queen and several thousand workers that overwinter as a group, whereas annual bumblebee colonies rarely contain more than a queen and a few hundred workers. Each solitary bee is responsible for its own foraging and reproduction during its few weeks of adult life. The sheer number of workers in the honeybee colony may better enable buffering of stress over long periods, whereas the more severe pinch points that bumblebees and solitary bees experience could render them more susceptible to environmental pressures[19,20].

If field experiments to assess exposure are deemed so important, why have so few been carried out? Limiting factors include the scale of such studies, the levels of replication required to achieve appropriate statistical power, and human and budgetary resources. Even with 16 fields, Rundlöf and colleagues' study had relatively low statistical power and, as with other field studies, many environmental factors probably varied among their sites and could not be standardized. Such studies can provide only correlational evidence of impacts, whereas controlled-exposure studies, such as that of Kessler *et al.*[15], are better suited to determining causative relationships through manipulative experimentation. The complementarity of these two approaches needs to be considered by policy-makers and for future research planning.

Although the two latest studies contribute to our understanding of the risk neonicotinoids pose to bees, knowledge gaps remain. For example, we need further evidence about how neonicotinoid exposure might affect social bee colonies over multiple seasons, how soil residues might affect ground-nesting bees and how neonicotinoid exposure interacts with other environmental stressors. We also need a greater understanding of how neonicotinoids affect other pollinators and natural enemies of crop pests, and of the persistence of these chemicals in soil and their take-up by untreated plants growing in or next to treated fields.

Fundamentally, we must move towards finding the right balance between the risks of neonicotinoid exposure for insect pollinators and the value these pesticides provide to ensure crop yield and quality. Selective use of neonicotinoid seed treatments, on the basis of a demonstrable need for systemic pest protection, might help to reduce non-target exposure and slow the onset of pest resistance. We also need to consider and evaluate alternative options for pest control. It would be unfortunate if the recent focus on the risks from neonicotinoids led unintentionally to broader use of alternative pesticides that prove to be even more harmful to insect pollinators and the essential ecosystem services that they provide. ■

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