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Judy Wu-Smart, University of Nebraska-Lincoln assistant professor and extension specialist, works with bees this spring as part of a massive research study to monitor the effects of a toxic ethanol plant over the next decade.

SEEKING CLUES

Birds, bees, water helping researchers trace environmental damage near Mead

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Lincoln Journal Star

MEAD — Honey bees will fly miles seeking clumps of Dutch clover in a pasture or the yellow flowers of wild mustard in a roadside ditch, sources of nectar and pollen that grow plentiful in eastern Nebraska.

When a bee discovers a good source of food on the landscape — milkweed flowering on the edge of a field, to give another example — it will return to the hive to recruit other foragers to the spot.

Using a series of waggles, turns and head butts to communicate the quality of the resources, what direction and how far to find it with its nest mates, the honey bee will return to the discovery with a foraging party.

They will then go to work packing pollen into baskets woven from the hair on their

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■ **Online:** *Watch "Bad Seed: Mead's fight against toxic ethanol plant," a documentary on the environmental disaster facing Mead @JournalStar.com.*

■ **Coming Monday:** *Environmental regulators foundered for years without an effective way to bring AltEn to heel, government records reveal.*

A multi-year study will use honey bees, redwing blackbirds and surface water monitoring to track how pesticides from the former AltEn ethanol plant are moving through the environment near Mead.

Research

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back legs and slurping up nectar from the base of a flower using their straw-like tongue, loading themselves like a fleet of miniature cargo planes.

The resources gathered are distributed across the colony: The queen and her attendants get to eat, as do the nurse bees caring for larvae and the undertakers who throw dead and dying bees from the hive.

So, too, do the housekeepers charged with maintaining a tidy hive, and the guards who protect the colony from wasps and other predators.

What isn't eaten is stored carefully in sealed honeycomb cells for the long winter months when nothing blooms on the horizon, and the whole process starts over, repeating itself again and again.

Honey bees' foraging efficiency can also work against them.

If the bees bring something harmful from the environment into the colony, like pollen or nectar laced with pesticides, the work ethic critical to a successful hive can be interrupted, sometimes with disastrous effects.

Queen bees who come into contact with neonicotinoids, a pesticide class similar to nicotine that overstimulates the insect's nervous system to the point of paralysis, will stop laying eggs, potentially creating a future labor shortage in the colony.

Nurse bees exposed to pesticides will become agitated to the point where they will furiously groom themselves instead of caring for the newly hatched babies. The same thing will happen to housekeeper bees, which can allow diseases to spread.

Foraging bees that fly into pesticide-contaminated plants may die away from the hive or become severely impaired, forcing younger bees into those roles before they are physiologically ready, causing a shortage of pollen and nectar.

Jennifer Weisbrod, a pesticide safety education coordinator at the University of Nebraska-Lincoln, said the death of a thousand bees out of tens of thousands living in a colony might not seem significant until you consider it from a different perspective.

"If a thousand people die in a city of 60,000, that's a lot of people," Weisbrod said. "That's a lot of jobs that are then unfilled."

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UNL integrated sciences major Patrick Niyitugize tests water in a drainage ditch east of AltEn in late April. Researchers will test the surface water for pesticides to look for changes in the concentrations.

The resourcefulness and susceptibility bees demonstrate interacting with their surroundings has led to their use as a bioindicator species, one that can help monitor the spread of pollution in the environment.

Since 2017, the loss of dozens of honey bee colonies has indicated something was wrong in the environment at the Eastern Nebraska Research and Extension Center, the UNL's agricultural research station south of Mead.

Ultimately, AltEn, a biofuel plant just north of the research farm, was identified as the reason bees were dying en masse.

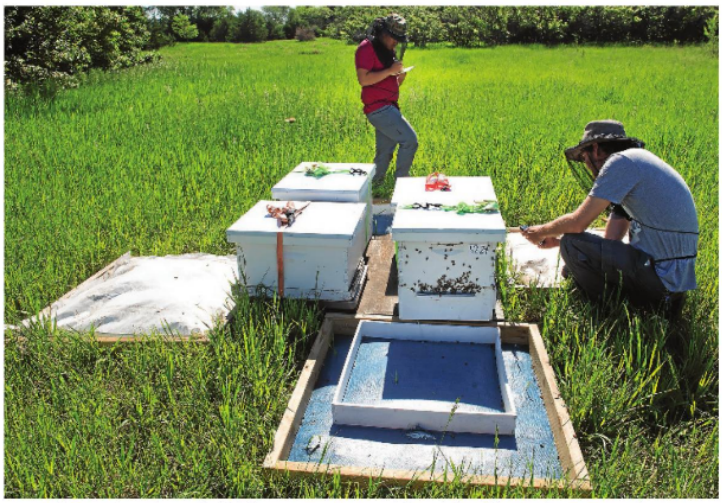
While it was in operation, AltEn was processing between 500,000 and 900,000 pounds of pesticide treated seed into ethanol every day, creating hazardous solid and liquid byproducts that were spread on farm fields for miles.

The Nebraska Department of Environment and Energy ordered the plant to shut down in February following years of violations of the state's environmental regulations. Now, attention has turned to determining how far the pesticide contamination spread — and at what level.

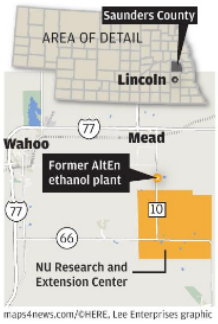
The University of Nebraska and Creighton University have launched a massive study to monitor the plant's effects on the environment, wildlife and humans over the next decade, a project that could cost $10 million.

Judy Wu-Smart, an assistant professor of entomology at UNL, said harnessing honey bees' natural instincts to find food sources will be the starting point for a massive environmental study that is taking place in and around Mead.

"We always talk about bees being canaries in the coal mine, but nobody has ever tried to use them as a method of identifying hot spots," she said. "We know (the pesticides) are all over. We're trying to find out where and at what level."



Insect science major Luke Norris (right) uses an app to record the weight of hives at the Eastern Nebraska Research and Extension Center's Bee Lab as fellow student Earl Agpawa takes notes before the pair collect dead bees in late May.



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Rite of spring

The honey bees destined for Mead arrive in Lincoln on the back of a flatbed truck in late April, survivors of a brutal February polar vortex that stretched its icy fingers all the way to their winter home in Texas.

Among the blooming flowers and trees of East Campus, the bees — darker, tiger-striped Italians and the more golden Carniolan — will be given time and space to recover from their long trip and settle into their new hives, white boxes stamped with the red letters "UNL."

Two weeks later, in mid-May, the hives will be moved once again, loaded onto a pickup christened "Bertha" for a 40-mile journey north to UNL's agricultural research station.

It's a rite of spring that has taken place almost annually since 1995, when Marion Ellis launched the Bee Lab at UNL.

"There was a steel storage building on the property they gave to us to convert to a lab," Ellis recalled, referring to a faded yellow shed that rests about a mile and a half south of AltEn, "so we had the carpenters, electricians and plumbers employed at the station build us one."

Until his retirement in 2015, Ellis managed the lab and studied parasitic mites — which remain the most pressing management issue for most beekeepers — from the heart of the 16,000-acre farm, which kept his bees isolated from other colonies.

"It was a really good place to keep bees and do research," Ellis said.

But changes to the farm economy also meant changes to the landscape. Once uncultivated land would be plowed and sown with corn and soybeans, shrinking and shifting the areas available for bees to gather food.

"It wasn't a dramatic thing," Ellis said, "just something that happened over time."

Bees will visit corn tassels and soybean flowers — researchers have found evidence to suggest pollinators can increase yield in soybean acres — but the window for doing so is narrow, typically just a few short weeks, leaving them to look for food elsewhere.

As row crop acres expanded, honey bees deployed in the eastern reaches of Nebraska and other Corn Belt states find themselves in vast "agricultural deserts" for much of the year, said Autumn Smart, a research assistant professor at UNL who studies how changes in land use affect pollinators.

Many of those deserts are also "glowing" with neonicotinoids through common agricultural practices, Smart added.

That has forced bees to forage creeks, waterways and ditches, Smart said, the same channels where contaminated runoff from AltEn traveled away from the plant toward the Platte River.

Previous research done by Wu-Smart's team at the Eastern Nebraska Research and Extension Center discovered high levels of clothianidin, a widely-used neonicotinoid, in milkweed samples clipped in a waterway near one of the hives.

The goal of the current research is to determine if those concentrations remain high enough to impact bee colonies or other wildlife, if they have diminished, or if they have moved elsewhere.

Detecting hot spots

Luke Norris and Earl Agpawa unload the final hive boxes from Bertha under a light drizzle, carefully carrying the colonies one-by-one to a long forgotten concrete slab in a clearing of trees nearly 4 miles southeast of AltEn.

It's the last of four locations spread out over miles south and east of AltEn where Wu-Smart has opted to put a forward operating base for honey bees to start seeking out pesticide hot spots in the environment.

At each location, Norris and Agpawa, both insect science majors who work in the UNL Bee Lab, position four hives on top of a wood pallet, one facing each of the cardinal directions.

Underneath two of the hives are bathroom scales to monitor the weight of the hives, a data point that can help determine whether or not the colony is thriving — individual bees

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Ian Hoppe (right), a University of Nebraska-Lincoln graduate student studying applied ecology, hands a red-winged blackbird egg to Callie McCright, a UNL undergraduate studying environmental science in fisheries and wildlife, as the pair collect information on the newly discovered nest on June 17 near Mead.

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gaining weight, stockpiling food, and hatching new bees to replace older bees, for example.

Fixed below the other two hive boxes are wooden drawers designed to catch any pollen the bees inadvertently kick off their back legs when they return from foraging.

Every two weeks for the next several months, the Bee Lab will gather the contents of the pollen trap over a 48-hour period and send them to a U.S. Geological Survey lab in West Virginia for DNA metabarcoding, which identifies different plants within the same sample.

The pollen will then go to a U.S. Geological Survey in Colorado to match the DNA sequences to a database, giving researchers a readout of what plants the bees foraged during the time in question.

Neonicotinoids were developed to dissolve into water easily so they could be absorbed into the corn or soybeans, offering specific protection from the pests that feed on those plants.

But neonicotinoids can also be absorbed into the pollen and nectar on plants commonly visited by insects, both beneficial ones like honey bees or wild bees, as well as pests.

Smart said the pollen trap data won't give a precise location of where the bees deployed by UNL may have come into contact with pesticide, but it allows researchers to narrow down what kinds of plants the bees were visiting and when.

"It's a piece of the puzzle," Smart said, "but once enough are assembled, it gives us a clearer picture."

Wu-Smart said the Bee Lab will then do some good, old-fashioned fieldwork. Researchers will walk out into the field in the same direction as the bees on a hunt for the flowers that produced the pollen found in the trap and map the blooms.

"If we find the vegetation collected in the field is highly contaminated, and we see the same thing in the hive, there is evidence the bees showed us a hotspot."

Tracking changes in water

Sitting on the banks of Johnson Creek east of AltEn, Patrick Niyitugize kicks off his Nike Air Force Ones and slips into a pair of rubber hip waders.

Niyitugize, an integrated sciences major from Rwanda who works in UNL's Water Sciences Lab preparing samples for analysis, saw the research happening around AltEn as an opportunity to get into the field himself.

On a late April morning with overcast skies and the wind whipping out of the north, that means wading into a cold, murky creek to wrestle with a metal post driven deep into the muddy bottom several weeks earlier.

Chained to the post is a small cage that holds a passive water sampler, a white disc several inches in diameter that mimics the gills of a fish, absorbing organic chemicals carried in water.

Student Callie McCright holds a red-winged blackbird egg. The birds are plentiful in the countryside around Mead.

UNL professor Shannon Bartelt-Hunt (left) and student Patrick Niyitugize prepare to test the pH, temperature and conductivity of water in a drainage ditch east of AltEn near Mead on April 23.

Niyitugize rocks the post back and forth and side to side until it's loose, dragging it to the bank where Shannon Bartelt-Hunt, chair of UNL's Department of Civil and Environmental Engineering, is waiting with a replacement sampler.

The membrane will give researchers insight into the chemicals traveling through surface water over a period of weeks, detecting long-term trends to go along with the direct sample Niyitugize will grab to look at the changes in the water month-to-month.

After sinking the post into the creek bottom once again, Niyitugize and Bartelt-Hunt repeat the process at other sites, some downstream from AltEn and more likely to show the presence of high levels of pesticides, as well as upstream from the plant, which can help provide a baseline for water quality in the area.

"Surface water is the most transient and will change quickly," Bartelt-Hunt explains. "It's difficult to get the whole picture with one sample, so we want to build a record over time and understand what's happening with trends in concentrations as well as spatially."

An analysis of the early samples found decreasing amounts of neonicotinoids in the surface water after the plant shut down in February, Bartelt-Hunt said in an interview in August.

But, the same analysis found the presence of several metabolites, chemical compounds created when neonicotinoids break down in water, soil or sunlight, sometimes becoming more toxic and persistent in the process.

Samples collected in August still show neonicotinoids like clothianidin and imidacloprid present in surface water, Bartelt-Hunt said, as well as higher levels of a slew of degradation products like imidacloprid desnitro, which studies have found is more toxic to mammals than its parent compound.

Hunting blackbirds

In addition to breaking down when in water or exposed to sunlight, the routine digestive processes of wildlife can also degrade neonicotinoids into metabolites.

And in some cases, animals who swallow a pesticide-treated seed, eat an insect or plant that absorbed the chemical or drink contaminated water can pass that metabolite along to their offspring.

A small selection of red-winged blackbird eggs collected from nests in giant plum thickets and cattails that grow out of ditches and creeks on Eastern Nebraska Research and Extension Center property this year have shown the presence of neonicotinoid metabolites.

Finding those eggs to better understand how pesticides can move through the ecosystem is an exercise in patience and persistence, however.

Dead bees from hives are collected and labeled. Bees are used as a bioindicator species meaning they can help monitor the spread of pollution in the environment.

Before sunrise on a hot morning in late July, Larkin Powell, a professor of conservation biology and associate dean of UNL's College of Agricultural Sciences and Natural Resources, is on the hunt for blue ribbons tied to low-hanging branches.

Each of the markers notes the location of a documented nest and detail where in the egg-laying stage the mother was when last observed by students or faculty researchers.

Red-winged blackbirds — males are black with red wings bordered in white, while females are brown and resemble sparrows — are everywhere in the countryside around Mead.

During their late summer nesting, females will lay three to four eggs in their nest built low to the ground and near water, which makes them good candidates to study how contaminants move through the environment, as well as a meal for hungry predators.

After noting the status of several nests — only about 30%-40% of eggs will ultimately hatch — and even plucking a secondary feather from each wing of a fledgling red-winged blackbird that flew out of a thicket during the search, Powell ends his hunt in a ditch just outside of AltEn.

In the shadow of twin hoop buildings, less than a football field away from the massive pile of wet distiller's grains, the solid byproduct of AltEn's ethanol production found to be highly contaminated with pesticides, Powell uses a tracking device to locate a nest tucked low among the sturdy grasses.

Notes from a previous week's observations state the nest should contain one egg, with more possibly being laid by the mother. Instead, the nest is empty, laying on its side on the bottom of the ditch.

Powell keeps searching, gently pushing aside the tall grass as his eyes sweep side to side. A female red-winged blackbird flies out of the ditch about 20 yards to his north, a flare signaling the location of another nest.

In it are three eggs: "We got a sample," Powell says, adding later that the confirmation that the "We now seem to have a sampling method that will work, and a fairly simple one — assuming we are patient in watching bird behavior to find nests," he said.

The big picture

Tracking the movement of pesticides over and through the landscape is a slow, painstaking business, involving countless hours of work — some of it mundane.

Wu-Smart's team, for example, must carefully empty the honeycomb of its stores of pollen and nectar, separating each to be sampled individually for the presence of pesticides.

Identifying the flowers foraged by the bees will take months, while searching for the chemical signature of neonicotinoids in those plants, bird eggs and water can also require weeks, if not months, to complete.

But with unprecedented contamination coming from an ethanol plant, researchers say it's important to begin understanding the effects, layer by layer, as well as how those layers are interconnected.

"To understand a complex health challenge, you need a diverse array of skills and perspectives to begin appreciating the connections and impacts," said Liz Van Wormer, an assistant professor in UNL's School of Natural Resources.

Van Wormer, who is also coordinator for Nebraska One Health, said the understanding brought by ongoing research will eventually help inform the potential impacts on humans living near the plant, as well as what the future might hold for the local ecosystem.

Insects, both helpful and harmful, who survived exposure to high levels of neonicotinoids coming from AltEn over a long period may develop resistance to the pesticide, which could present future challenges both to agriculture and to human health.

Earlier this year, the University of Nebraska Medical Center conducted a voluntary survey to start looking for any common health problems that have emerged among the more than 500 residents of the village.

Obtaining blood or urine samples to look for the presence of neonicotinoids is the next phase, pending approval from the university, according to Eleanor Rogan, chair of the Department of Environmental, Agricultural and Occupational Health at the University of Nebraska Medical Center.

Rogan said it could take all of the planned 10-year study to fully understand where the pesticide pollution originating from AltEn has moved, as well as what impacts it has had and will continue to have.

"Some toxic exposures take some time to manifest themselves," she said.

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