RNA-based pesticides aim to get around resistance problems

Leah Shaffer, *Science Writer*

The half-inch-long corn rootworm larva packs a surprising punch. It feasts on the root system of corn before pupating into a black and yellow beetle that does further damage to the plant's leaves. Before the advent of genetically modified (GM) crops that produce insecticidal proteins to fight rootworm, these insects cost US farmers an estimated $1 billion annually in damage and control measures.

But as insects such as the corn rootworm evolve resistance to the suite of traits baked into commodity crops, scientists are queuing up a new application for a biotech tool that targets the protein-making machinery of insects. First identified in 1990, RNA interference (RNAi) entails using double-stranded RNA (dsRNA) to block messenger RNA from its usual function (i.e., sending out instructions to make proteins). With impressive specificity, RNAi can potentially block nucleotide sequences that are only found in a target pest and not in friendly insects or humans. As a result, some scientists are keen on making RNAi the next big tool in agricultural science.

The EPA first approved an RNAi pesticide in 2017. That product, called SmartStax Pro, is a GM corn seed that will deploy both transgenic insecticidal proteins and RNAi to fight western and northern corn rootworm. It's expected to be released in the United States in the next few years (1), according to its maker, Bayer AG, which is headquartered in Leverkusen, Germany.

But GM crops are just one of many agriculture-related applications for RNAi. As the cost of producing dsRNA has dropped precipitously, biotech companies are developing dsRNA formulations that could also serve as spray pesticides, making the technology more affordable to smaller farms.

"RNAi is really different from everything that's come before because you can pick your target," says Bruce Tabashnik, professor of entomology at University of Arizona. But he cautions that this new tool should not be considered a silver bullet against invertebrate pests. "Insects are the champions of adaptation," he adds. "They will adapt to any challenge we can throw at them but if we do a combination of challenges simultaneously, we have a much greater expected durability of our strategy."

As destructive agricultural pests such as the corn rootworm evolve resistance to conventional pesticides, researchers and farmers are looking to RNAi-based treatments as a promising possible alternative. Image credit: Science Source/USDA/Nature Source.

The Old Guards

That combination of challenges is what's commonly known as "integrated pest management." The idea is that by combining many different mechanisms to kill pests, farmers can counter the evolution of resistance to any particular method while also making less use of conventional pesticides that can potentially harm ecosystems.

This mix of strategies can include crop rotation, traditional small-molecule synthetic pesticides, and a variety of biopesticides, or what the EPA classifies as "plant incorporated protectants," meaning plants genetically modified to produce their own pesticide. It can also entail setting up "refuges," sections of untreated crops in a given field or adjacent plot that allow a normal population of insects to breed with any resistant survivors. Depending on the type of crop, the EPA can require that farmers establish a minimum of 5 percent of the crop as a refuge, either planted adjacent to or mixed in with the majority treated crop.

Over the past decade, one of the focal points of integrated pest management in commodity crops has been a soil bacterium called *Bacillus thuringiensis* (Bt).

First published December 9, 2020.

The root system of a product called SmartStax Pro is equipped to kill beetle larva that normally decimate the roots. Genetically modified corn seed releases insecticidal proteins and messenger RNA to kill the larva. Image credit: Brian Leake.

Bt pesticides have been around for a century since they were first deployed against flour moths in Germany in the 1920s. Harmless to people and most nonlepidopteran insects such as honeybees, the bacteria are a staple of organic farming as well. Researchers isolated a cadre of insecticidal proteins called Cry toxins from Bt in the late 1990s and incorporated them into GM crops starting in 2003, making for a potent defense against pests such as the corn rootworm or the pink bollworm in cotton crops (2). But less than two decades later, there are increasing instances of "practical resistance," meaning more than 50 percent of a targeted species is resistant to a particular Cry toxin. Whereas there were only four cases worldwide of practical resistance to Bt crops found in 2006, that number rose to 19 in 2016, according to research by Tabashnik, published in the *Journal of Economic Entomology* (3).

Tabashnik says GM crops should be built with both RNAi and Bt toxins to drastically reduce the number of resistant survivors in a targeted pest population. "The hypothetical ideal would be you're killing such a high proportion of pests that the frequency of resistance is extremely low and it makes it unlikely for resistance to evolve," he says.

Because no single insect control approach can kill every pest, scientists pyramid or stack traits into a GM crop, in hopes that at least one mechanism will work. According to Tabashnik, pyramiding traits is even more effective the larger the refuge of nontreated crop, as he found when he tested Bt cotton crops against the cotton bollworm in China. In that study, Tabashnik and colleagues used computer models to determine the effect of different sizes of refuges for bollworm resistance to GM cotton crops with Bt toxins and RNAi pesticides built in. The greater the refuge (up to 50 percent of the crop), the slower the evolution of resistance (4). "The advantage of a pyramid is magnified the bigger the refuge is," says Tabashnik.

Although avoiding resistance is a big part of developing RNAi pesticides, the technology could also potentially provide a more environmentally friendly way to treat crops. Small molecule compounds such as organophosphate, carbamate, or neonicotinoid pesticides act as a neurotoxin. But these types of pesticides can affect nontarget creatures, including pollinators and the natural predators of pests. A 2016 meta-analysis published in *Peer J-Life and Environment* found that use of neonicotinoid pesticides applied to seeds was associated with 16 percent reduced abundance in a treatment area of pest predators such as spiders and wasps (5).

With RNAi pesticides, biochemists can manufacture dsRNA that will silence the nucleotide sequence responsible for making proteins crucial to the development of one particular insect. Tabashnik's research has explored using RNAi to silence the production of proteins used to synthesize or transport juvenile hormones, something unique to a particular species of insect. Using RNAi as opposed to conventional pesticides is the difference between taking a hammer to a whole row of bugs versus using tweezers to cripple part of one particular insect. And the juvenile hormones are not the only target.

Scientists can use the tools of genomics to examine nucleotide sequence variants between different insects, notes Brad Fabbri, chief science officer at TechAccell, a Kansas City-based private equity biotech firm investing in RNAi technology. "You should be able to design something that has a high degree of specificity," says Fabbri.

The Unknowns

Big questions still remain as to how insects may evolve resistance to RNAi pesticides and how those pesticides will affect the environment. Tabashnik is worried about the worst-case scenario: Insects will evolve resistance to the manufactured dsRNA in general.

If the insect can adapt to RNAi machinery, "it doesn't matter how many targets there are," he adds, noting that scientists have confirmed the possibility of this potential scenario in lab experiments. In a 2018 paper from Monsanto (now acquired by Bayer), crop scientists did find cross resistance to other dsRNA traits tested with a corn rootworm engineered to be resistant to one trait. The resistance came about because insect's gut walls did not absorb the dsRNA; essentially, the larva did not digest the poison pill in its various forms. It was a lab test to probe the weaknesses of dsRNA, not necessarily replicate real world-circumstances in which resistance would evolve. And the same test also found that an insect resistant to the dsRNA insecticide was not resistant to the Bt insecticides, backing the notion that stacking insecticide traits is a good way of killing potentially resistant survivors (6).

Bayer global head of resistance management Graham Head says they aim to anticipate the different forms resistance could take as they release RNAi pesticides. Tests like those reported in the 2018 work purposely breed resistant insects in the lab. But the real-world use of RNAi will include all the tools to keep that worst case from happening, combining as many different insecticides as they can to ensure few resistant survivors, in addition to leaving refuges for nontreated insects to breed with any few survivors. The expectation is that farmers will manage resistance at the field level with integrated pest management, and agriculture companies can "optimize the available technology to create the strongest pyramid possible with RNAi being the core piece of that," he says.

After Effects

Others are investigating how dsRNA will filter into the environment, how it will degrade, and whether those degraded molecules still pose a risk to target or nontarget organisms.

Kimberly Parker, assistant professor of environment and chemical engineering at Washington University, has had to come up with an entirely new technique to detect particles of dsRNA in the environment because they are much larger than the molecules in traditional pesticides (7). "Our big concerns are actually understanding what happens to them in the environment and how they undergo different degradation processes," she says.

DsRNA is a heavy molecule, meaning it's unlikely to spread through the air. But it could potentially move via groundwater, she speculates. The molecule remains in the environment at much lower concentrations than something like Bt toxins. Preliminary tests of dsRNA in soils have found that it degrades faster than conventional pesticides. Parker and colleagues are looking to quantify that loss into three categories: absorption, chemical degradation, and biological degradation. "As these molecules continue to be more commercially available and used more widely, we do have a role to play in the continued evaluation of their safety," she adds.

Such studies may not convince GM skeptics, however. "The regulation and acceptance landscape of [GM crops] makes the widespread adoption [of RNAi] very difficult," says Neena Mitter, director of the Centre for Horticultural Science, at the University of Queensland, Australia, who has been studying RNAi for 20 years. Whether it's used to protect against viruses, pests, or fungi, the technology provides a powerful tool, she says. But in her native Australia, for instance, no food-grade crops are genetically modified—only commodity crops such as cotton or canola.

Because of the slow regulation process for approving a GM crop, Mitter expects the first dsRNA products in Australia to be a topical application. She and her colleagues have been working on a type of spray that combines the dsRNA with layered double hydroxide (LDH) nanosheets to allow for the slower and more stable of uptake of the dsRNA product on a plant's surface (8). Called BioClay, the product is meant to help the dsRNA glom onto the plant's surface longer so that a pest is more likely to ingest it. And it's safe for human consumption because it's been used for biomedical applications over the past decade.

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—Kimberly Parker

Because not all crops lend themselves easily to GM tinkering, Mitter is interested in providing the sprayable option for farmers. A topical spray would not be considered a GM product, at least among Australian regulatory authorities, she notes.

The GM distinction isn't so clear, however. One of the main ways to make dsRNA is to genetically modify a bacterium to produce it. Companies such as TechAccel use genetically modified microorganism to make dsRNA. And it's becoming increasingly affordable to do so. Just four years ago, production of dsRNA could cost as much as $1,000 per gram, estimates Fabbri. That's an economic nonstarter. But their system of using vats of microorganisms to spew out dsRNA has allowed them to lower that price to just under a dollar per gram. "Once you get that active ingredient cheap enough, it really opens up the door," he says. It's still early days, as their products are at least a few years away from starting the regulatory process. But he's hopeful for the environmental and food production benefits that could emerge.

Mitter agrees, saying RNAi is "one of the very powerful tools we can use, especially in the space of crop protection, whether it is protection [from] viruses, insect pest, or fungi or nematode."

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