FEATURES

THE PERFECT PESTICIDE?

Insecticides made of RNA could offer a safer and more targeted weapon against crop pests

By **Erik Stokstad**

The Colorado potato beetle is so destructive that East German propaganda once accused the United States of dropping the ravenous insects onto the country's potato fields. "*Halt amikäfer*"—meaning "Stop the American beetle"—read a pamphlet in 1950. There's no evidence the colorful pest was used as a form of biological warfare. But German potato farmers—and those in many other countries—continue to battle the beetle to this day.

A native of the Rocky Mountains, the beetle now occurs across the Northern Hemisphere, causing more than half a billion dollars of crop losses each year. It's a master of resistance, making it hard to control. The pest was an early driver of research into chemical pesticides starting in the 1930s. Ever since, it has evolved immunity to one compound after another—now more than 50 pesticides, representing all major types of active ingredients.

"They were chewing through treated plants like it was nothing," Andrei Alyokhin, an entomologist at the University of Maine, says of the moment, in 2001, when farmers in Maine noticed that a still-new class of pesticides, neonicotinoids, were no longer controlling the beetle. Finding additional tools has been "increasingly difficult," he adds.

This year, however, U.S. farmers will have a new weapon against the pest, one that works in an entirely different way from traditional pesticides and that proponents say should be safer for people and the environment. Based on a mechanism called RNA interference (RNAi), the spray targets a vital gene in the Colorado potato beetle. The gene target is unique to the pest and its close relatives, which should prevent damage to pollinators and other species. "You can … hit the insect you want to kill with precision," says Subba Reddy Palli, an entomologist at the University of Kentucky who published a review last year in *Frontiers in Insect Science* describing the development of RNA-based pesticides. "You cannot get anything better than this."

Marketed as Calantha by the company GreenLight Biosciences, the pesticide won approval from the U.S. Environmental Protection Agency (EPA) in January after a 4-year review. More RNA-based products are in the pipeline. GreenLight has applied for regulatory approval for a pesticide targeting the varroa mite, the main plague of honey bees, which can resist almost all available pesticides. Other companies have products in field trials. Meanwhile, researchers at various universities are exploring RNA as a tool to combat Asian citrus psyllids, bark beetles, mosquitoes, and other species.

The technology has limitations: It falters with lepidopterans, a group of insects that includes the diamondback moth and many other pests, which have powerful enzymes in their gut that break down RNA. And it has critics. During the regulatory review of Calantha, environmental groups raised concerns about potential harm to nontarget species. The endangered Hungerford's crawling water beetle, for example, can live close to potato fields. The groups called for broader risk assessments. (EPA only requires tests on a few indicator species, such as honey bees and ladybugs.) They also pointed out that it's not clear whether the formulation in the spray that keeps the RNA stable is safe because the ingredients are confidential.

But many are hopeful the technology can usher in a new era of pest control. "I think RNAi is going to explode," says William Moar, an entomologist at Bayer who has long worked on RNA-based controls. Compared with most of the chemicals currently used, adds Ana Maria Vélez, an insect toxicologist at the University of Nebraska–Lincoln, "it's way safer."

PHOTO: EDWIN REMSBERG/VWPICS VIA AP IMAGES

The Colorado potato beetle (adult and larva at right) has evolved resistance to most agricultural pesticides.

PHOTO: THOMAS MARENT/NPL/MINDEN PICTURES

THE SCIENCE BEHIND RNA pesticides began decades ago with some puzzling lab results. In the 1980s, researchers studying DNA were surprised to find they could effectively silence the expression of a gene if they added more copies of that gene. In one experiment, petunias were genetically engineered to have several copies of the gene responsible for their purple hue. The biologists had assumed the alteration would deepen the color. Just the opposite happened: Some of the flowers no longer had any pigment at all. How the additional genes silenced the original one was a mystery, but other researchers began to suspect it had something to do with RNA.

Messenger RNA (mRNA)—the genetic material that carries the information encoded by a gene to the cell's proteinmaking machinery—typically exists as a single strand. Sometimes, though, a strand combines with a complementary partner. In the 1990s, one hypothesis to explain gene silencing was that adding an extra gene somehow led to the production of a strand of complementary RNA. If this strand paired up with mRNA from the original gene, it might prevent cellular machinery from attaching to it and building the protein.

To test this idea, a graduate student at Cornell University, Su Guo, injected single-stranded RNA into *Caenorhabditis elegans*—a millimeter-long worm that's a stalwart of laboratory investigations in developmental and molecular biology. Part of the experiment went as expected: When the injected RNA was complementary to mRNA from a gene important for embryo development, the gene was silenced. Puzzlingly, though, Guo found that RNA identical to the worm's mRNA also silenced the gene.

A breakthrough came in 1998, when Andrew Fire of the Carnegie Institution for Science and Craig Mello of the University of Massachusetts Chan Medical School led a group that injected *C. elegans* with RNA coding for a protein that helps muscle cells contract and relax properly. When they added a step to ensure the RNA wasn't contaminated with unwanted genetic material, they discovered that the single-stranded RNAs didn't silence the gene; silencing only happened when both kinds of strands were injected together as double-stranded RNA (dsRNA). As it turned out, the previous, confusing results were due to minute contamination with dsRNA. Fire and Mello were awarded a Nobel Prize for this discovery in 2006.

Later work revealed that the phenomenon, now called RNAi, occurs because of cellular machinery that, among other things, defends cells from infection by viruses that require dsRNA for their life cycles. A large enzyme known as DICER locates long pieces of dsRNA inside the cell, then cuts them into pieces called short interfering RNA (siRNA). These short pieces are picked up by a protein complex called RISC—the RNA-induced silencing complex—which searches the cell for single-stranded RNA that matches the sequence of its siRNA. (dsRNA viruses also need single-stranded RNAs for part of their replication.) If RISC finds any such RNA, it triggers its destruction.

The discoveries raised hopes that dsRNA could be used as medicine. During a lecture at the Nobel Prize ceremony, Fire—who by then was at Stanford University—mused that dsRNA might be helpful for shutting off disease-related genes, such as those that are essential for tumor growth in cancer patients. A few RNAi-based drugs have been approved; inclisiran, for example, treats high cholesterol and atherosclerosis. But progress has been slow, in part because enzymes in human blood break down dsRNA.

Meanwhile, yet another experiment with *C. elegans* gave scientists hope that RNAi might be helpful for dealing with an entirely separate problem: pest control. Lisa Timmons, then a postdoc working with Fire, genetically altered *Escherichia coli* bacteria to produce dsRNA that would interfere with the worm's muscle contraction gene. When the worms ate the bacteria, they began to twitch—the telltale sign the gene had been silenced. Until that point, no one had expected that dsRNA could be taken up through the digestive tract and make it into cells to silence genes. "This is something very astonishing," says Karl-Heinz Kogel, a plant pathologist at Justus Liebig University Giessen. It suggested the right dsRNA, when eaten, could kill a pest.

RNA-BASED PEST CONTROL first hit the market last year as a genetically modified crop. U.S. farmers started to plant SmartStax Pro, a corn variety Bayer had genetically engineered to resist the western corn rootworm. The plant produces dsRNA that disrupts the expression of *DvSnf7*, a rootworm gene that's crucial for the movement of proteins across cell membranes. In field trials with severe infestations, the plants had 95% less root damage by corn rootworm larvae compared with conventional corn, according to a 2017 study. The variety was approved by EPA that year, but it didn't reach the U.S. market until 2023 because Bayer also sought approvals in countries that import corn from the U.S.

Many hope the new variety will tamp down the impact of corn rootworm, which has evolved resistance to other forms of control. "We definitely need new modes of action," Moar says. The benefits of the method are numerous: By genetically engineering a plant to produce dsRNA, the farmer doesn't need to spray, the pesticide is always ready, and only insects that eat the crop are exposed.

Creating a genetically modified crop and getting it approved, however, can take more than a decade and cost upward of $200 million. Europe presents a particular challenge because the regulatory hurdles are higher and consumer acceptance is lower. So some companies are developing dsRNA as sprays, a faster and cheaper process. A spray might also be more versatile, as it could be authorized for use on any crop that a pest frequents.

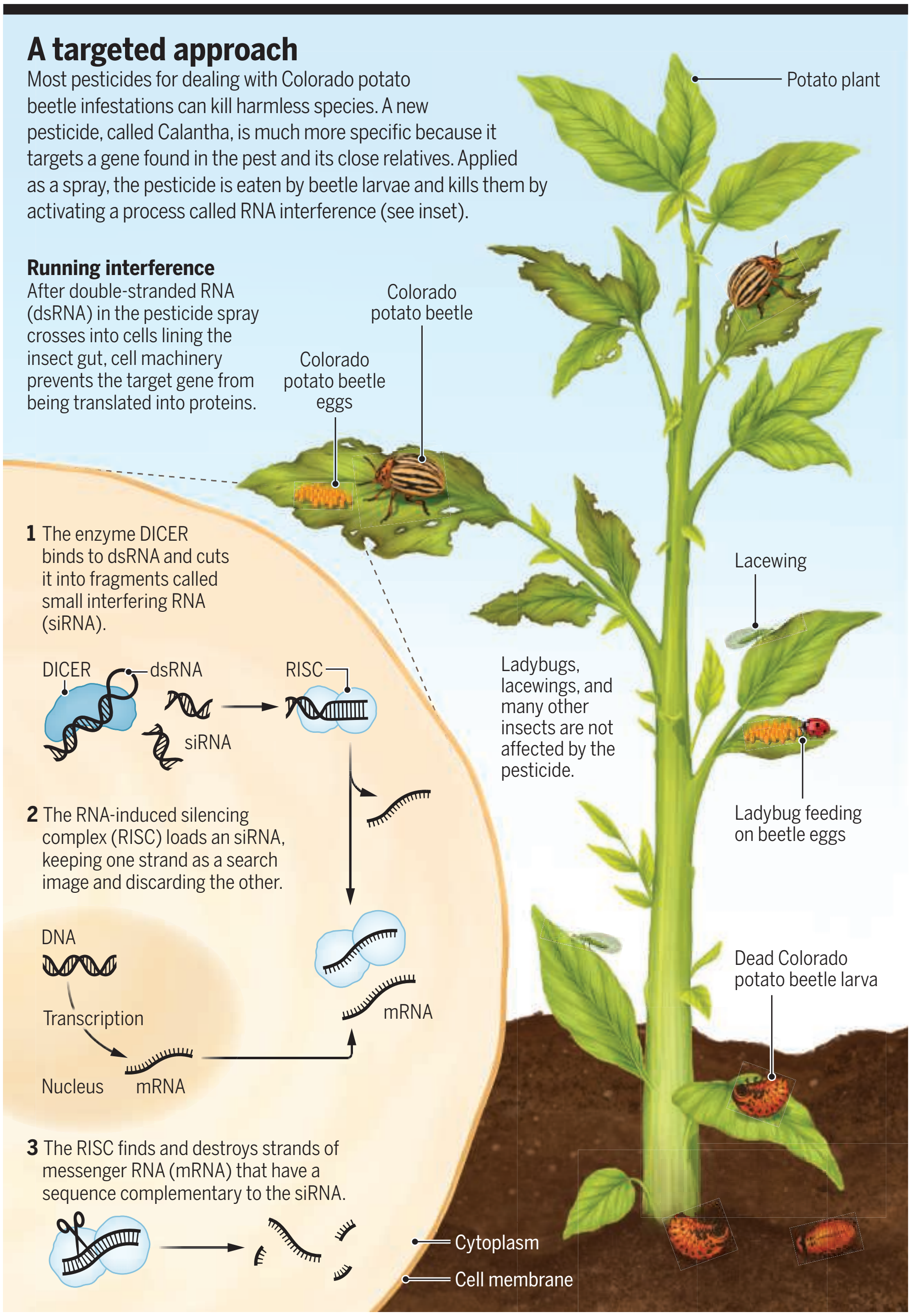
The Colorado potato beetle was a good target because the voracious critters damage not just potato, but also tomato, eggplant, and bell peppers. Research had also shown that feeding the pests dsRNA can effectively silence targeted genes.

After exploring various genes in the beetle, GreenLight's Ken Narva and his team settled on *PSMB5*, which codes for part of the cellular machinery that removes damaged proteins. When it's silenced, cells accumulate nonfunctional proteins and die. The dsRNA for *PSMB5* was effective in lab and greenhouse tests, killing 90% of larvae within 6 days, according to a 2021 study.

To test whether the pesticide could harm other insects, Ron Flannagan and colleagues at GreenLight checked bioinformatics databases to see how much *PSMB5* in the potato beetle differed from the versions in other insects. Four closely related beetles had some sequence matches. But toxicity tests on those species showed that only two are affected by the pesticide, and both are agricultural pests. Tests on more distantly related insects—honey bees, green lacewings, ladybugs, and others—showed no ill effects.

Those results are encouraging, says Alyokhin, who worked with GreenLight in the development of Calantha. But, "We should not assume that just because it's RNAi it will never have nontarget effects," he adds.

As the technology was developing, some researchers questioned whether dsRNA could be produced cheaply enough, and in sufficient quantities, to be practical. The solution, GreenLight says, lies inside a former Kodak factory in Rochester, New York. The company opened a plant there in 2021 to scale up its production of dsRNA. Inside, workers tend large bioreactors, where a broth



A targeted approach

Most pesticides for dealing with Colorado potato beetle infestations can kill harmless species. A new pesticide, called Calantha, is much more specific because it targets a gene found in the pest and its close relatives. Applied as a spray, the pesticide is eaten by beetle larvae and kills them by activating a process called RNA interference (see inset).

Running interference
After double-stranded RNA (dsRNA) in the pesticide spray crosses into cells lining the insect gut, cell machinery prevents the target gene from being translated into proteins.

GRAPHIC: A. MASTIN/*SCIENCE*

A GreenLight Biosciences factory in Rochester, New York, can produce RNA pesticides in bulk.

PHOTO: GREENLIGHT BIOSCIENCES

of *E. coli* churns out the valuable reagents: rings of DNA called plasmids that contain the instructions for the dsRNA in Calantha, as well as enzymes that will synthesize it. Once purified, the plasmids and enzymes are piped into other tanks, where a biochemical reaction produces the dsRNA. The RNA is then mixed with chemicals in a proprietary solution, which, among other things, helps it adhere to leaves.

The plant can produce 2 tons of dsRNA per year, a number that CEO Andrey Zarur expects to rise to 20 tons by the end of 2025. And it can churn out that dsRNA for less than $1 per gram, allowing GreenLight to sell its new pesticide at a price that compares with top-of-the-line commercial pesticides.

Juan Luis Jurat-Fuentes, an entomologist at the University of Tennessee, Knoxville (UTK) who has studied RNAi for nearly a decade, says the "most exciting moment" was when he heard GreenLight could make cheap dsRNA in bulk. "That's when I felt this is doable."

NOW THAT THE BEETLE-KILLING dsRNA spray is for sale, a key question is how long before pests develop ways to render the weapon ineffective. "These bugs are so crazy," Vélez says. "Sometimes, they just surprise us."

Researchers already know some pests can, at least in the lab, evolve ways to elude dsRNA. In 2018, Moar and colleagues published a paper showing that the western corn rootworm evolved to stop taking up the dsRNA from its gut. In doing so, the insects effectively became resistant to any dsRNA approach, a result Moar calls "sobering" because there is no easy workaround.

Swati Mishra, a Ph.D. student at UTK, is finding a similar phenomenon with the Colorado potato beetle. In a lab environment that constantly exposed larvae to dsRNA, the insects dramatically reduced their uptake of the genetic material within 11 generations. How long the beetles might take to evolve resistance in the field, where they aren't exposed to as much dsRNA, is hard to predict.

To reduce the risk that resistance will emerge, EPA requires farmers who grow SmartStax Pro—the genetically modified corn—to plant refuges for pests. These patches of unprotected corn raise the odds that rootworm populations will maintain the genes that render them susceptible to dsRNA. Potato farmers don't face the same requirement with Calantha because it's not a genetically modified crop. Flannagan says GreenLight is aware of the risk and will encourage farmers to alternate spraying dsRNA with other pesticides. "We're trying to make sure RNA is managed as part of the toolkit."

The prospect of the beetles evolving resistance to all available pesticides, as happened in the 1990s, still haunts the industry, says Karl Ritchie, agronomist for Walther Farms, which grows potatoes on more than 3000 hectares and was involved in trials of Calantha. "Everybody's nervous." For now, though, Ritchie is grateful to have another pesticide at his disposal. To help prevent resistance, he is coordinating pesticide applications with neighboring farmers; they will all spray the same pesticide one year and something else the next.

Meanwhile, researchers want to expand the use of RNA pesticides to lepidopterans, which include major moth pests such as corn borers and the fall armyworm. Many have already evolved resistance to chemical insecticides—yet so far they do not appear vulnerable to dsRNA. "Controlling Lepidoptera with RNA is sort of the Holy Grail," Moar says. "But no one has been able to make it work commercially."

Companies are trying, with some of the research aimed at packaging dsRNA to survive the lepidopteran digestive and immune systems. "It's one of the hottest areas in RNAi right now," Jurat-Fuentes says.

AgroSpheres has genetically engineered bacteria to make both dsRNA and tiny protective shells, derived from their cell wall. Results of a field trial, reported in 2022, suggested "commercially acceptable" control of the diamondback moth on cabbage. Another company, Trillium Ag, has developed a different package. Each small strand of RNA is surrounded by even shorter molecules called aptamers that serve as anchors for a covering made of proteins or lipids. The company is currently testing its effectiveness on the fall armyworm and two other pests.

In general, it's hard to know how well the protective technologies work, experts say, because most companies have published few results. But Palli is cautiously optimistic about the future of these unconventional pesticides. "As we learn more and more, we'll find a way to overcome some of these hurdles. It will happen." ■