PEGGY GREB (D1518-1)

Barcoding Insects

To Control Them

Adults of the native carabid beetle *Lebia grandis* are voracious predators of Colorado potato beetle eggs and larvae.

Mention barcodes and it often brings to mind the sales tags and scanners found in supermarkets and other stores. But Agricultural Research Service scientists are using "DNA barcodes" in their search for ways to control and monitor insects that pose the greatest threats to crops as diverse as wheat, barley, and potatoes.

In DNA barcoding, scientists sequence a designated part of an organism's genome and produce a barcode from it for a systematic comparison with the sequenced DNA of other closely related species. DNA barcodes are being developed on a wide range of plants and animals as part of a global effort to catalog the diversity of life on Earth.

At the Invasive Insect Biocontrol and Behavior Laboratory in Beltsville, Maryland, entomologist Matthew Greenstone is using DNA barcodes in an unconventional way: to identify insect predators best equipped to control the Colorado potato beetle. The Colorado potato beetle is the single most damaging insect pest of potatoes in the eastern United States. It also damages tomatoes and peppers and is known for developing resistance to any pesticides used to control it.

Greenstone is trying to find the insects that are the beetle's worst nightmare. Numerous studies have analyzed the gut contents of predator insects to evaluate their ability to control pests. But predators eat and digest prey at different rates, so simple gut analysis is insufficient for accurately comparing predator effectiveness, Greenstone says. He has fine-tuned that approach and used barcoding to come up with a way to factor in how quickly different predatory insects actually digest the Colorado potato beetle.

"Scientists often use barcoding to distinguish one closely related species from another. We're using it to identify prey in the gut of an insect predator, and in a sense, that's an atypical use," says Greenstone. Greenstone and his colleagues collected four insects that previous studies showed were the most common potato beetle predators. They fed them laboratory-raised potato beetles and looked at the digestion rates of each of the four insects to determine the Colorado potato beetle's DNA "half life"—defined as the point at which at least some DNA of the potato beetle could still be found in half of the fed individuals of each predator species. They used the potato beetle's barcoded DNA to detect it in the predators' guts.

The results, published in the journal *Entomologia Experimentalis et Applicata,* show the importance of taking digestion rates into account when considering different insect predators as biocontrol agents. They may also provide guidance to growers on the most effective control strategies for combating a voracious pest.

"Different pesticides have different effects on different predators, and not all predators are equally susceptible to all insecticides. Based on what you learn, you might delay spraying insecticides, rule out the use of insecticides that harm your most important biocontrol agents, or limit spraying to certain times, depending on the predator's habits," Greenstone says.

ARS researchers are also using barcoding to understand and track the threat of various biotypes of Russian wheat aphid, an insect about the size of a sesame seed, that is a major worldwide pest of wheat, barley, and other cereals. Since it appeared in 1986 in Texas, it has cost U.S. wheat growers alone about $200 million each year.

Gary Puterka, an entomologist in the ARS Wheat, Peanut, and Other Field Crops

Biotech

Russian wheat aphid adult next to its young. This aphid gives live birth to young that are identical clones of itself during the asexual phase of its lifecycle.

GARY PUTERKA (D2491-1)

Research Unit in Stillwater, Oklahoma, periodically surveys Russian wheat aphid populations across eight western states to provide guidance to wheat growers on infestation levels and on the range of biotypes so they can decide whether to implement control measures. He works closely with ARS entomologist Kevin Shufran, who is also in Stillwater, on efforts to control the aphid.

Until recently, the Russian wheat aphid, in North America, was believed to reproduce strictly by asexual cloning, which made it particularly susceptible to resistance mechanisms bred into wheat and barley crops. But Puterka's surveys have turned up evidence showing that it is reproducing sexually.

If the Russian wheat aphid is sexually reproducing, the resulting genetic recombination would produce new "bioytpes" that will be better equipped to counter resistant crops by giving each new generation a varied genetic tool kit, increasing the likelihood that offspring will be able to overcome the plant's resistance mechanisms, feed on it, and survive to reproduce resistant offspring. Sexual reproduction could also broaden the pest's range, enabling it to lay eggs as cooler weather approaches, like other alpine aphids do, and survive harsh winters.

Researchers found a new biotype of Russian wheat aphid in Colorado in 2003 that spread so quickly and caused so much damage—overcoming a new resistance gene that breeders had recently developed for wheat growers—that its success was seen as a new introduction or evidence that the pest had "gone sexual." Subsequent surveys have turned up additional evidence of new biotypes, an evolving threat.

Puterka's survey showed that the Colorado biotype now makes up 90 percent of the population in eight major wheat-producing states. Another study by Puterka found a small, localized population of Russian wheat aphids in a Colorado field that were the offspring of male and female parents. This population of nymphs had a signature trait—missing antenna segments—that was evidence they hatched from eggs and were the result of sexual reproduction. Thirty-nine new biotypes were detected when these nymphs were increased and screened.

"These populations are continually shifting in terms of different biotypes, and as natural selection dictates, the biotypes that are the fittest are the ones that will survive and dominate," says Puterka.

But the extent of the threat posed by the Russian wheat aphid largely remains a mystery, and the field surveys are time consuming. For accurate results, Puterka must make clone colonies from each adult collected and screen them by exposing them to nine different types of resistant germplasm to confirm their biotype.

The most efficient way to determine whether Russian wheat aphids are sexually reproducing would be to find their eggs. When the females reproduce asexually, they give birth to live females, but they lay eggs when they reproduce sexually. The problem is that the eggs of all aphid species look alike, so the scientists cannot distinguish Russian wheat aphid eggs from other aphids' eggs.

Shufran and Puterka have developed a process that uses DNA barcoding to tell the different aphid eggs apart. To establish that it would work, they extracted DNA from the eggs of 10 previously identified species of aphids, including several of the Russian wheat aphid's closest relatives. They sequenced the first 640 base pairs of a gene known as *"CO1."* In a blind test, Shufran compared DNA from eggs provided by Puterka, who masked the identities of the different species. With help from various aphid genetic databases, Shufran was able to correctly distinguish the different aphid species by comparing their *CO1* sequences. Results were published in *Annals of the Entomological Society of America.*

With the new tool, Puterka and Shufran will be able to identify Russian wheat aphid eggs for the first time and can better track the biotypic diversity of an aphid that poses a major threat to wheat and other crops.—By **Dennis O'Brien,** ARS.

This research is part of Crop Protection and Quarantine, an ARS national program (#304) described at www.nps.ars.usda.gov.

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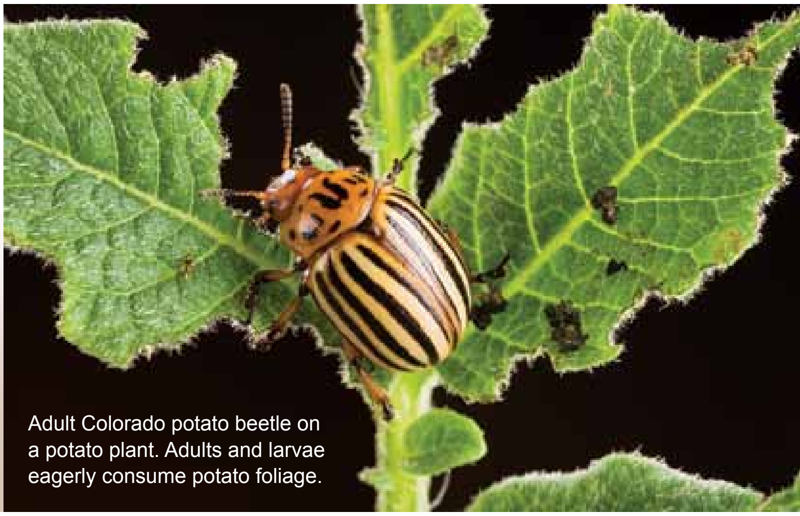
PEGGY GREB (D2486-1)

Entomologist Matt Greenstone examines DNA analysis results of Colorado potato beetle removed from the gut of an insect predator collected from the field. He is looking for evidence that the predator mainly consumes Colorado potato beetle.



Adult Colorado potato beetle on a potato plant. Adults and larvae eagerly consume potato foliage.

PEGGY GREB (D1515-1)