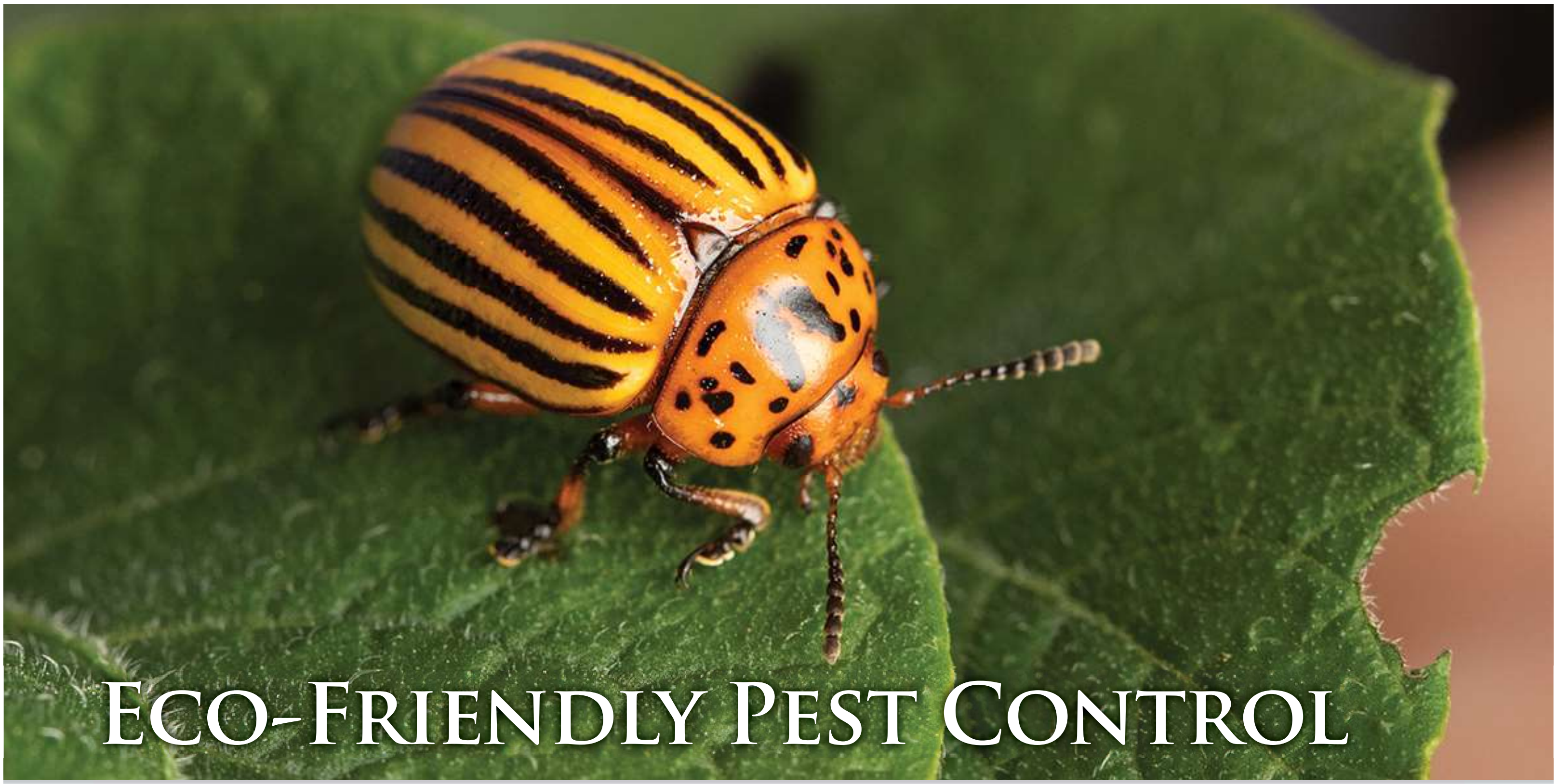


Eco-Friendly Pest Control

Russ Groves develops environmentally sustainable options for growers

By Nicole Miller, GROW, Wisconsin's Magazine for the Life Sciences, https://grow.cals.wisc.edu

Wisconsin has a long history of vegetable production. It's a leading producer of the nation's processing vegetables, such as snap beans, sweet corn, carrots, and potatoes.

As a professor and extension specialist in the UW-Madison Department of Entomology, it's part of Russ Groves' job to protect these vegetable crops from insect pests, and that includes safeguarding the state's potato crop from the voracious Colorado potato beetle (CPB).

The beetle is one of the most harmful pests of potato, capable of munching so many leaves that it reduces tuber yields, so growers turn to insecticides to keep the bugs at bay.

Groves notes that some of the state's surface water and groundwater reflect an unfortunate legacy of long-term pesticide use in select areas with high agricultural intensity.

That's a big motivator behind his efforts to develop more environmentally sustainable pest control options for growers.

In recent years, Groves has been working with biotech industry partners to develop and fine-tune

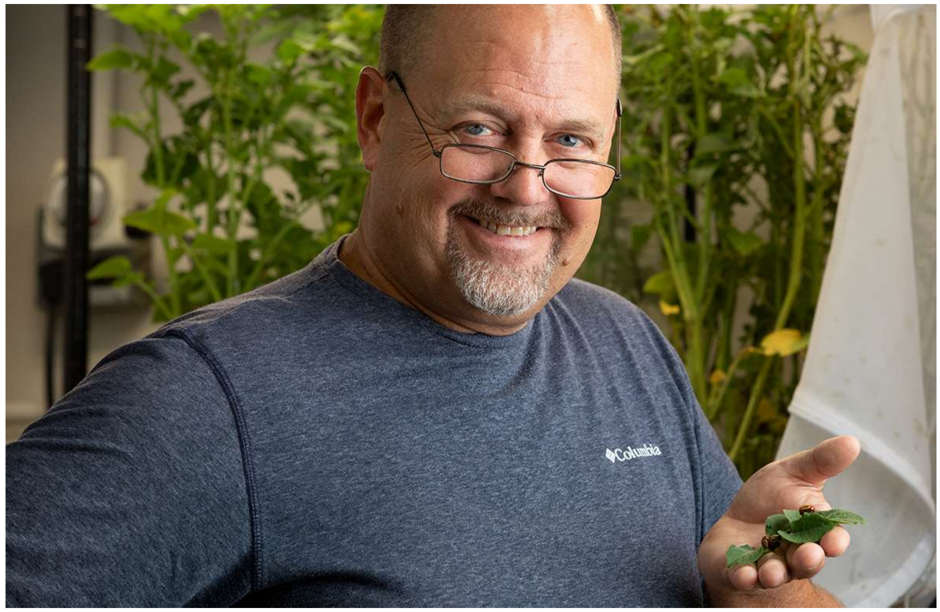
Above: **A Colorado potato beetle clings to plant leaves in the lab of Russ Groves.** ***Photo courtesy of Michael P. King***

a new, more eco-friendly insecticide option involving RNA interference (RNAi) technology.

The approach, which utilizes double-stranded RNA (dsRNA) to silence critical genes in the beetle, is much more targeted than traditional insecticides.

Under Groves' supervision, it's being tested on Wisconsin fields this coming summer. He anticipates seeing the first RNAi-based insecticide on the market later this year.

Groves answers some questions regarding his work.



Russ Groves, University of Wisconsin (UW)-Madison professor of entomology, poses for a portrait with Colorado potato beetles in his lab. *Photo courtesy of Michael P. King*

What makes the Colorado potato beetle such a formidable foe? The Colorado potato beetle evolved to live on a poisonous plant. It eats the foliage of members of the nightshade family of plants, which are full of alkaloids, so it was important for populations of this insect to evolve detoxification mechanisms to cope with all the toxins.

Because of these innate coping mechanisms, the insect has a high potential to develop insecticide resistance.

When a Colorado potato beetle is subjected to a new insecticide, it is already equipped to cope with these compounds.

continued on pg. 18

continued from pg. 17

As with the plant-based toxins that the beetles have evolved to deal with, they sequester insecticides, detoxify them, and excrete them.

Consequently, over the years, the potato industry has lost more and more insecticidal options. So, growers are always looking for novel approaches, especially more environmentally conscious, reduced-risk approaches.

What does your work studying insecticide resistance involve? For the past 8 to 10 years, we've been studying the response of CPB to new and existing insecticides, and, more recently, have attempted to discover the principle set of genes involved in insecticide resistance.

We've investigated different populations of Colorado potato beetle collected in several states, knowing that the insect has various patterns of resistance in separate places.

In collaboration with Sean Schoville, a professor of entomology, we continue to learn about the suite of genes that encode for this resistance.

In recent research, we have observed that a discrete set of genes appears to be involved in this resistance. And to further confirm the function of these genes, we use loss-of-function analyses based on RNAi to better determine gene function.

Very simply, this means we synthesize and introduce a piece of double-stranded RNA that is analogous to a critical gene inside that insect.

Once inside the cells of the target insect, in this case CPB, its cellular machinery is "tricked" into knocking out, or knocking down, the function (in other words, the translation) of its own genes.

Several of the genes identified turned out to be multifunctional but were mostly associated with metabolic detoxification.

Much of this work has been the focus of recent publications, which triggered new contacts and relationships with biotechnology companies interested in developing RNAi-based approaches to pest management.

HOW RNAi WORKS

Protein synthesis is the process through which genetic material in every living organism is turned into proteins, the building blocks of life.

At its most basic, there are two steps involved: transcription, which is the transfer of genetic instructions from DNA to messenger RNA (mRNA); and then translation, where cellular components use the genetic code in mRNA to make proteins.

RNAi, also known as Post-Transcriptional Gene Silencing (PTGS), stops this process.

Through RNAi and PTGS, scientists

exploit our understanding of how to regulate or turn off the production of a given protein.

Over the past decade or more, researchers regularly capitalize on this natural process, which occurs in most eukaryotic organisms, utilizing it for numerous kinds of experiments and technological applications.

In the lab, these are the main steps:

1. Design a piece of double-stranded RNA that contains genetic sequences analogous to the target gene.
2. Introduce this dsRNA into the cytoplasm of the organism's cells.
3. Inside the cell, an enzyme called Dicer chops up the dsRNA into small pieces.
4. These small pieces get loaded into a multiprotein complex called the RNAi silencing complex, or RISC.
5. RISC, loaded with an RNA guide strand, explores the cytoplasm, hunting for strands of mRNA that complement the small piece of dsRNA it's carrying.
6. When RISC finds a matching mRNA, it cleaves the nucleic acid, thereby preventing the translation of a target protein.

What has happened since these initial contacts? Our publications led to three biotech companies reaching out to our group.

These biotech groups explore recently published genome sequence databases, such as the Center for Biotechnology Information, in efforts to identify genes unique to select groups of organisms like the Colorado potato beetle.

While our goals in utilizing RNAi approaches have largely been to ascertain resistance gene function, these companies are exploring gene targets that result in a lethal phenotype.

In other words, they wish to develop RNAi-based approaches that can be used in pest control to kill the insect.

continued on pg. 20

Eco-Friendly Pest Control. . .
continued from pg. 19

In our lab, we use very small needles to inject the dsRNA into individual beetles, but you can't do that on a field scale.

LEAF-EATING BEETLES

Fortunately, Colorado potato beetles are leaf-eating beetles, and they have the capacity to process and transport RNA across their midgut and move these molecules into their bloodstream, which allows the RNAi effect to become systemic throughout the insect.

> *"We will always be in an 'arms race' with Mother Nature. There is no silver bullet."*
>
> **– Russell L. Groves,**
> *professor and extension specialist, UW-Madison Department of Entomology*

Biotechnology companies have formulated dsRNAs that can be sprayed directly onto the plants. And once on the leaves of potato plants, then the Colorado potato beetle will consume these small molecules, where they make their way into the bodies of insects and mortality results.

It's quite amazing that it functions in this way.

Importantly, these dsRNA on the leaves have a very short functional life of about three to four days. After this time, the compounds denature relatively quickly.

Equally important is that this technology is target specific. It only kills Colorado potato beetle. In addition to being very targeted, we view these kinds of pest management approaches as reduced-risk and quite environmentally friendly in terms of pest management outcomes.

That sounds amazing. Does this spell the end for CPB and other pests? Of course, you can't rely on a single technology. We all knew, and we can anticipate in the future, that CPB will continually develop resistance to selections like pesticides, even RNAi-based approaches.

We will always be in an "arms race" with Mother Nature. There's no silver bullet.

In fact, research has already demonstrated the Colorado potato beetle's ability to generate resistance to select RNAi approaches.

When resistance to these new technologies develops in the future, we will need to consider other gene targets and develop new dsRNA molecules that will target other important genes (to account for mutations in the beetle population).

Unfortunately, (simply spraying dsRNA on potato leaves) doesn't work to control all pests of the crop, such as aphids and leafhoppers, potato's other top insect pests.

These insects, which possess piercing-sucking mouthparts like mosquitoes, simply avoid the dsRNA on the leaf surface, as their mouthparts go right past it.

What's coming up next for this project? We continue to work with pest management and agribusiness companies to develop and test these new technologies.

We have several projects at the Arlington and Hancock agricultural research stations in 2022, as we have for the past four to five years, looking at timing of delivery, adjuvants that can help extend the life of the compounds on the leaves, and how the dsRNA works in tank mixes with other active ingredients like fungicides and fertilizers.

Simple, but very practical investigations are still warranted.

The very first sprayable RNAi targeting the Colorado potato beetle is anticipated to be registered later this year. It will be called Calantha, and Wisconsin's potato growers are eager for this. BC'T