Scientists learn how horseweed shrugs off herbicide

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Everyone has seen *Conyza canadensis*, also known as mare's tail, or horseweed (top), growing through cracked concrete, in a vacant lot or on a freeway shoulder. In the fall it produces tiny dandelion-like puffs of seeds, setting roughly 200,000 seeds per plant Credit: Luke Samuel, Phillips Co., via Monsanto collaborator

As everyone knows, the pharmaceutical industry is struggling to deal with bacteria that have become resistant to common antibiotics. Less well known is the similar struggle in agribusiness to deal with weeds that have become resistant to a herbicide that is widely used in farming practice.

The herbicide, first introduced in 1974, is glyphosate, the active ingredient in Monsanto's Roundup products and also in herbicides produced by other manufacturers. The first case of glyphosate resistance was documented in 1997, and today more than 20 weed species globally are reported to be resistant. (For more on the history of the herbicide read "The Back Story" here.)

Recently a team of scientists from Washington University in St. Louis and Monsanto, the St. Louis-based company that makes Roundup herbicides, were able to follow molecules of glyphosate as they entered a resistant variant of horseweed to discover exactly how the plant disarms the herbicide. Their work was published in *Pest Management Science* last year.

In a second paper published in April 2011, also in *Pest Management Science,* they describe herbicide application conditions that can be used to overcome the resistance mechanism they had discovered.

This is not the end of the story, the scientists say, because some weed species are resistant to the herbicide in ways different from horseweed. Still the scientists are glad to have won this round even if they know the contest will be prolonged.

Caught in the act

"The story begins when I received a phone call from Doug Sammons, who directs a research group at Monsanto tasked with uncovering the mechanisms leading to glyphosate resistance in weed species," says D. André d'Avignon, PhD, director of Washington University's High Resolution Nuclear Magnetic Resonance Facility.

"He was calling because, under favorable conditions, it is possible to track the chemical fate of phosphorus in a living system with nuclear magnetic resonance (NMR)," says d'Avignon. (As its name implies, glyphosate consists of a phosphonate group (PO3) attached to the amino acid glycine.)

"I was initially very skeptical," says d'Avignon. "My feeling was that, because a living plant would present a very heterogeneous environment, we would not observe well-resolved phosphorus NMR signals from glyphosate, let alone pinpoint glyphosate's cellular handling.

"I was proved wrong," he says.

To attack the problem, d'Avignon assembled a team of chemists skilled in the field of NMR. Along with Sammons from Monsanto, he enlisted Xia Ge, PhD, a postdoctoral research associate, and Joseph Ackerman, PhD, the William Greenleaf Eliot Professor of Chemistry in Arts & Sciences.

D'Avignon's team focused its initial efforts on Conyza canadensis, also called mare's tail or horseweed, a fibrous biennial plant that can grow to be six feet tall with sparsely hairy stems and pale-green irregularly nicked leaves. (When it grows in a soybean field, it overtops the crop and can reduce yields by more than 80 percent.)

It is the most persistent of the glyphosate-resistant weeds and is already found in 19 states in the United States and separately on five continents.

Spraying resistant horseweed under cold conditions circumvented the resistance mechanism. All of the cold-acclimated horseweed treated with herbicide at normal field-rate levels or higher succumbed to its lethal effects (top). Resistant horseweed plants maintained under warm conditions survived treatment (bottom), with the exception of plants sprayed at 10 times the usual field-use rate (dead plants to right in bottom block). Credit: Andre d'Avignon/Pest Management Science

The scientists sprayed horseweed plants with glyphosate and then examined living leaf tissue in the NMR instrument. They immediately saw that they could distinguish the glyphosate signal from those of other phosphorus-bearing plant metabolites, including the ubiquitous energy-storing molecule adenosine triphosphate (ATP).

Then they had a stroke of luck. During the time course of the data collection, a second glyphosate signal appeared at a slightly different resonance frequency. The first signal was coming from glyphosate in the cell cytoplasm and the second from glyphosate in the plant vacuole, a large water-filled compartment found in all plant cells that can serve as a garbage disposal for chemicals foreign to the plant.

There were two phosphorus NMR signals because the resonant frequency of a chemical species depends upon the local chemical environment and the plant vacuole is significantly more acidic than the cytoplasm.

Within 24 hours, resistant horseweed had managed to shuttle 85 percent of the glyphosate into the vacuole. Sensitive horseweed, on the other hand, had disposed of only 15 percent of the glyphosate in this way.

Scientists believe resistant horseweed has a pump in the tonoplast (the membrane surrounding the vacuole) that actively shuttles glyphosate into this storage compartment where it can no longer interfere with the critical biological reactions taking place in the chloroplast (the small green organelle to the upper left).

Meanwhile, the glyphosate remaining in the cytoplasm was being transported together with sugars to rapidly growing parts of the plant, such as young leaves and root tips.

Once glyphosate reaches such "metabolic sinks," it interrupts the critical shikimate pathway and kills the plant. Within 24 hours, sensitive plants had translocated 35 percent of the glyphosate from the source leaves to these sink tissues, whereas resistant plants had allowed only 15 percent to move to the sinks and much of this was shuttled into the vacuole of the sink tissue, thus further reducing chloroplast exposure.

"It's really a race," says d'Avignon. "Once glyphosate gets to the vacuole it is trapped," he says. "Because resistant horseweed rapidly shuttles glyphosate into the vacuole, there's less of it available for translocation to rapidly growing parts of the plants."

The scientists believe resistant horseweed has a pump, or transporter, that actively moves glyphosate across the tonoplast (the vacuole membrane).

"The existence of a glyphosate transporter is a surprise," says d'Avignon. "People had thought glyphosate moved into plant cells and organelles passively, along a diffusion gradient."

D'Avignon warns that not all weeds use this particular resistance mechanism. "We have since screened a number of weeds," he says. "Some use a similar mechanism, but we also find that others use other mechanisms."

According to a recent article in the Journal of Agricultural and Food Chemistry describing work by scientists at Colorado State University, the U.S. Department of Agriculture and the University of Adelaide in Australia, certain variants of Palmer amaranth, or pigweed, for example, have become resistant by overproducing the EPSPS enzyme — to the point that it cannot all be bound by glyphosate.

Gaming the system

The scientists used a simple trick to make resistant horseweed sensitive again. "If a plant that had been sprayed with glyphosate at room temperature was put into a warm, high-light greenhouse, we noticed much more rapid vacuole sequestration than if the plant was treated at room temperature and maintained at room temperature," d'Avignon says.

"As chemists we knew that many reactions are temperature dependent. What would happen, we wondered, if we put the plant in a cold environment rather than a warm one? Could we inhibit the flow of glyphosate into the vacuole?"

To find out, they cold-acclimated resistant horseweed at 10 degrees Celsius (about 50 degrees Fahrenheit) and then sprayed the plants with glyphosate. Sure enough, the plants succumbed to the herbicide.

The scientists also found the records of a field trial in Monsanto's archives where a field of resistant horseweed was sprayed with glyphosate in early spring. Although the trial had an unrelated purpose, the data from the trial showed that kill rates correlated with temperature under field conditions, just as they had under laboratory conditions.

These experiments suggest farmers might be able to stave off resistant horseweed by spraying in early spring, when the weather is cooler.

"I'm a chemist by training and never paid much attention to plants," says d'Avignon. "I never thought plants were that sophisticated or interesting or complicated. But since I have been working on this project, I have certainly had an attitude adjustment. Plants are extraordinarily complex, and they're masters at survival."

Provided by Washington University in St. Louis