Fungal Protein Slows Broadleaf Weeds

You have to give credit to stubborn weeds like dandelion or knapweed. No matter how many times you pull, spray, or plow them up, they seem to come back for more.

Now researchers are trying a different approach. Using a natural protein from a soil fungus, they've developed a potential herbicide that causes the weeds' leaf cells to kill themselves. In greenhouse experiments, this mass cellular suicide killed the plants' leaves within 24 hours.

"It's the first case I know of where a protein like this has been used as a foliar herbicide to induce a hypersensitive response," says Bryan Bailey, a plant pathologist with USDA's Agricultural Research Service in Beltsville, Maryland.

"The idea here," he adds, "is to get the plant to kill itself rather than using chemical herbicides that may be less environmentally friendly."

Bailey and ARS colleagues Nichole O'Neill, Jim Anderson, and Patricia Birkhold, now with DowElanco, will report their findings in the January/February issue of *Weed Science.*

They first discovered the protein, called Nep1, about 5 years ago in secretions of the fungus *Fusarium oxysporum*. Some forms of this fungus cause wilt diseases that diminish yields of corn, cotton, tomatoes, and other crops.

However, by removing the protein-making gene of *F. oxysporum* and then infecting plants with the altered fungus, the researchers showed that Nep1 plays no part in causing disease. Harmless *Fusarium* strains also produce the protein with no ill effect to plants, notes Bailey.

Yet, when purified from a broth culture of *Fusarium* and sprayed on or injected into dandelions, yellow starthistles, and several other broadleaf plants, Nep1 becomes a powerful natural herbicide. The team first observed the phenomenon in studies late last year. However, not all broadleaf plants respond the same.

One way weeds fight off disease-causing organisms is with a built-in defense called a hypersensitive response. It triggers the sudden collapse of cells immediately around an invading pathogen. This helps to cordon off further infection. With Nep1, however, this defense mechanism goes into maximum overdrive, sacrificing so many cells that the leaves die 3 to 24 hours later.

PEGGY GREB (K8703-3)

The Nep1 protein is purified from culture filtrates (shown in the petri dishes) of the fungus *Fusarium oxysporum*. Above, plant pathologist Bryan Bailey holds a damaged spotted knapweed that was sprayed with Nep1 a day earlier. The healthy spotted knapweed was not sprayed.

"I look at this as a different mechanism than that triggered by an herbicide," says Anderson, a plant physiologist at ARS' Weed Science Laboratory in Beltsville. The weed "turns on its defensive response to such a high degree that it kills itself."

Potential Uses of Nep1

Because it isn't harmful to monocot crops—such as corn, wheat, barley, rye, or turfgrass—Nep1 may prove to be a natural alternative to some conventional synthetic herbicides. It could be used to control or kill off cover crops, for example, hairy vetch, which is very sensitive to the protein.

"If you could use this in an organic farming situation, I think that would be great because organic farmers have limited weed control choices right now," says Bailey.

According to Mark Lipson, policy program director for the Organic Farming Research Foundation (OFRF), "Biopesticides in general are very important transitional tools to organic systems. But they are not, and should not be, essential in the long run."

An organic farmer, he explains, might apply a biopesticide as a last resort or to complement other organic weed controls, such as using cover crops, mulches, burning, or cultivation.

The ARS researchers envision using Nep1 to kill or weaken dicot (broadleaf) weeds like yellow starthistle, northern joint vetch, and spotted knapweed. In rangeland or pasture areas, for example, this could give grasses and other forages a chance to reclaim lost ground or resources.

"If you could knock the weed back and give it a 1- or 2-week delay," says Bailey, "then competing plants could shade it out." Nep1 could also be sprayed on weeds between rows of monocots like corn and have the same effect, he adds.

Another potential use is combining Nep1 with the herbicides RoundUp (glyphosate) and 2,4-D, or 2,4-dichlorophenoxy acetic acid.

Used as an additive, Nep1 appears to give these herbicides a boost that damages weeds faster than in instances when the chemicals are used alone. This may also reduce the amount of chemical needed, a possibility the scientists are now exploring in lab and greenhouse experiments.

In trials last summer, RoundUp applied alone took about 2 weeks to kill the weeds. But with small doses of Nep1 added, damage was visible overnight and the plant didn't grow back, says Bailey.

Between 5 and 20 parts per million of Nep1 are used with water, along with Stilwet, a synthetic surfactant. It helps Nep1 stay glued to the leaf surface where it can penetrate natural openings like stomata.

For organic farming purposes, though, a naturally derived surfactant would be needed, notes Anderson. While common biopesticide ingredients, surfactants of a synthetic nature "can make a formulation unacceptable" for organic farming, says Jane Sooby, OFRF's technical director.

Biodegradable Weed Wacker

Outdoors, Nep1 is stable on plant leaves for about 24 hours. Though environmental factors like sunlight or microbes can break apart its amino acid structure, the protein is generally absorbed by plants within 30 minutes of contact. Researchers believe the havoc begins when Nep1 physically contacts receptors on cells, setting off a biochemical chain reaction that soon kills the leaf.

Researchers also suspect broadleaf plant cells are more likely than monocot cells to harbor receptors for detecting Nep1. Monocots may escape harm because their defenses either don't recognize Nep1 or don't react with it in the same way as dicots.

The initial damage caused in dicots by Nep1 is restricted to the leaves. That's because other areas, like the stem, don't have stomatal openings. That same feature, Bailey notes, may allow Nep1 to be used as a natural leaf dessicant in certain agricultural situations, like harvesting lint from cotton—a dicot crop.

A nice feature of Nep1 is that it won't hang around long in the environment. Though it can last a few months stored in a refrigerator, it readily breaks down in a field. It's also unlikely to be toxic to humans or animals.

However, because Nep1 is a protein, researchers will still have to check for potential allergenicity. And the cost of mass-producing the fungus is uncertain, says Anderson.

On the flip side, Nep1 "makes up 90 percent of all protein the fungus produces," Bailey notes. "It's readily available and doesn't require a lot of processing, and you can take its partially purified form and use it."

For dandelions, spotted knapweeds, and other pesky weeds, this can only mean more troubled times ahead.—By **Jan Suszkiw**, ARS.

This research is part of Crop Protection and Quarantine, an ARS National Program (#304) described on the World Wide Web at http://www.nps.ars.usda.gov/programs/cppvs.htm.

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PEGGY GREB (K8704-14)

Technician Dave Clark (left) and research associate Sarah Keates of the Weed Science Laboratory in Beltsville, Maryland, apply Nep1 to a planting of the ground cover hairy vetch. Nep1 killed the vetch foliage within 24 hours of treatment (insert shows treated and untreated vetch).