Novel Alfalfa Cleans Fertilizer Spill

This alfalfa takes nitrogen from soil, not air.

Alfalfa, the queen of forages, may soon hold court in a new realm of environmental cleanup.

Scientists in the ARS Plant Science Research Unit at St. Paul, Minnesota, envision a new use for an unusual type of alfalfa that extracts its nitrogen from soil rather than from the air. It could be useful in removing nitrogen from soil contaminated by fertilizer spills, overfertilization, or excess application of livestock manure, municipal sludge, or food-processing wastes.

Plant physiologist Carroll P. Vance says that research on the unusual alfalfa has been under way for about 10 years and has progressed to a field trial under a cooperative research and development agreement with Canadian Pacific Rail Systems of Minneapolis, Minnesota.

"We call the alfalfa ineffective, because it blocks bacteria from effectively fixing atmospheric nitrogen," Vance says. "This alfalfa depends primarily on nitrogen from fertilizer, soil, or water in order to grow."

This makes ineffective alfalfa a prime candidate for bioremediation or cleanup of excess nitrogen in soil or irrigation water. JoAnn Lamb, a plant geneticist, began working in 1991 to develop alfalfa plants to be used for bioremediation.

A nitrogen-fixing legume like beans and peas, alfalfa normally forms a symbiotic relationship with *Rhizobium meliloti*, a bacterium that lives in the soil. *Rhizobium* converts nitrogen gas, which makes up about 80 percent of the atmosphere, into nitrogen fertilizer that the alfalfa then converts into plant proteins. Because normal alfalfa can satisfy most of its nitrogen requirement from the atmosphere, it needs very little from other sources.

The ineffective germplasm is a naturally occurring mutant and was identified in the 1980's as a research tool for use in the greenhouse and laboratory. Not until recently was it determined to be useful in bioremediation. Then scientists saw an opportunity to use the mutant in a new way.

"To be able to address remediation, we felt we needed a plant that was not available on the market," says Lamb. "We wanted a plant that would deal with buried or surface contaminations. Alfalfa is typically a deep-rooted crop. In a well-drained soil, roots grow down about 6 feet each year."

Armed with this knowledge, Lamb began searching for germplasm that exhibited the traits needed for bioremediation: specialized root growth patterns, good forage yield, winter hardiness, and disease resistance. "Our goal was to build a good alfalfa plant for remediation, so we had to go back to some very old genetic material," she says.

By conducting field trials over a 5-year period, ARS researchers were able to identify alfalfa plants with either a deep taproot or a highly branched root system.

Lamb and soil scientist Michael Russelle are currently identifying rapidly elongating taproots in field trials using the herbicide Fluridone. Fluridone is usually applied to control weeds in aquatic systems, but it is ideal for root growth studies because it does not move readily in

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At a train derailment site near Bordulac, North Dakota, plant geneticist JoAnn Lamb and soil scientist Michael Russelle monitor the biological cleanup of nitrogen fertilizer with an unusual alfalfa. Yellowing leaves in the foreground indicate an area of cleaner soil because this alfalfa cannot use bacteria to extract nitrogen from the air. (K7531-2)

soil and produces distinctive, visible symptoms in alfalfa.

Scientists dug a 60-by-200-foot plot to a depth of 10 feet, laid down an organic layer, and sprayed it with Fluridone. Then they refilled the plot with soil, replaced the topsoil, and planted over it.

Alfalfa plants that contacted the herbicide showed a bleached-white appearance indicating the roots reached the chemical layers. The faster roots grew, the faster the symptoms appeared. Scientists think plants selected for fast root growth may be able to catch mobile contaminants, like nitrate, before they move into groundwater.

In a field experiment, the researchers discovered that ineffective alfalfa takes up to 30 percent more nitrogen from the soil than normal alfalfa. "This is particularly surprising because the ineffective alfalfa yielded less herbage than the effective germplasm," Russelle says.

"Crop nitrogen uptake usually corresponds quite closely with crop yield. This research showed that much of the nitrogen in normal alfalfa still comes from the atmosphere, even when nitrate is available in the soil."

Ineffective alfalfa can also be used as an indicator of nitrogen depletion. Since it can't fix nitrogen gas, it will turn yellow when the nitrogen supply in the soil has been depleted.

Put to the Test

Scientists got an unexpected opportunity to test their new alfalfa last year, when they learned of a 1989 train derailment near a small town in North Dakota. It had spilled thousands of pounds of anhydrous ammonia and granular urea onto land adjacent to the tracks.

"The railroad used the normal procedures for remediation: excavation and land-spreading of the contaminated soil," says Russelle.

However, tests conducted at the spill site still showed very high levels of nitrate in both the soil and groundwater. Even though the site itself was less than an acre, the potential existed for surface water and further groundwater contamination.

So groundwater from the spill site is being pumped out and irrigated

onto a plot planted with three different types of alfalfa: a standard commercial variety called Agate, an ineffective germplasm called Ineffective Agate, and an experimental population. The scientists will compare how well the three types remove nitrate from the water.

"There are two major forms of nitrogen: ammonium and nitrate," says Russelle. "Ammonium doesn't move in the soil. It has a positive electrical charge and is attracted to the clay, which has a negative charge.

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Braun Intertec Corporation's supervisor of Remedial Services Earl Windahl (kneeling), ARS plant geneticist JoAnn Lamb (center), and LeeAnn Thomas, Canadian Pacific Railway remediation specialist, examine first-year root development of alfalfa irrigated with high-nitrate groundwater. (K7531-1)

"But nitrate has a negative charge, so it doesn't form a bond with the negatively charged soil particles. Nitrate that is not absorbed by plants moves freely through the soil and can easily go beyond the rooting depth of typical farm crops like corn and wheat."

High concentrations of nitrate could threaten groundwater if allowed to leach down through the soil and into the water table.

"Most groundwater is not static," says Russelle. "It is moved along by gravity and subsoil pressure differences. It often bubbles to the surface in springs, streams, and lakes, while in other places it receives water from lakes and streams."

Although alfalfa does have its limits in terms of how much nitrogen it can absorb, development of the ineffective germplasm means remediation can be done in an environmentally safe and more expedient manner.

"Our overall goal is to produce a plant that performs well as a remediation agent, yields well enough to be cultivated as an economically valuable crop for farmers, and reduces the cost of remediation to industry and the public," says Lamb.

Looking to the future, researchers want to design plants that remediate other chemical compounds and can be adapted and grown in different geographic regions.—By **Dawn Lyons-Johnson,** ARS.

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A Speedier Transformation

Necessity is said to be the mother of invention pushing many scientific advances. For Deborah Samac, a plant pathologist in the ARS Plant Science Research Unit at St. Paul, Minnesota, the need was to make an already effective process even better.

She and other plant scientists had long known alfalfa could be improved using the soil bacterium *Agrobacterium tumefaciens* to transfer engineered genes into plant cells. The transformed cells would then be grown in culture and regenerated into whole plants carrying the new genes.

Although effective in achieving research objectives, the method was time-consuming. Scientists might wait 6 months for the transformed plants they needed to continue with their research.

While still using *A. tumefaciens,* Samac developed a quick and efficient method of transforming alfalfa cells for regeneration of whole plants with new genetic material in just half the time.

"We need to know the effect of the introduced genes as soon as possible," Samac explains. "But the published protocols for transforming alfalfa were inefficient and slow.

"By building off a rapid method developed by Sandra Austin-Phillips at the University of Wisconsin and optimizing it for a particular project, we developed a method as rapid and efficient as those for model systems like tobacco."

To produce regenerated alfalfa plants with new genetic material, the researchers cut sterilized alfalfa leaves into squares less than one-half inch in diameter and dip them in a suspension of *Agrobacterium* cells.

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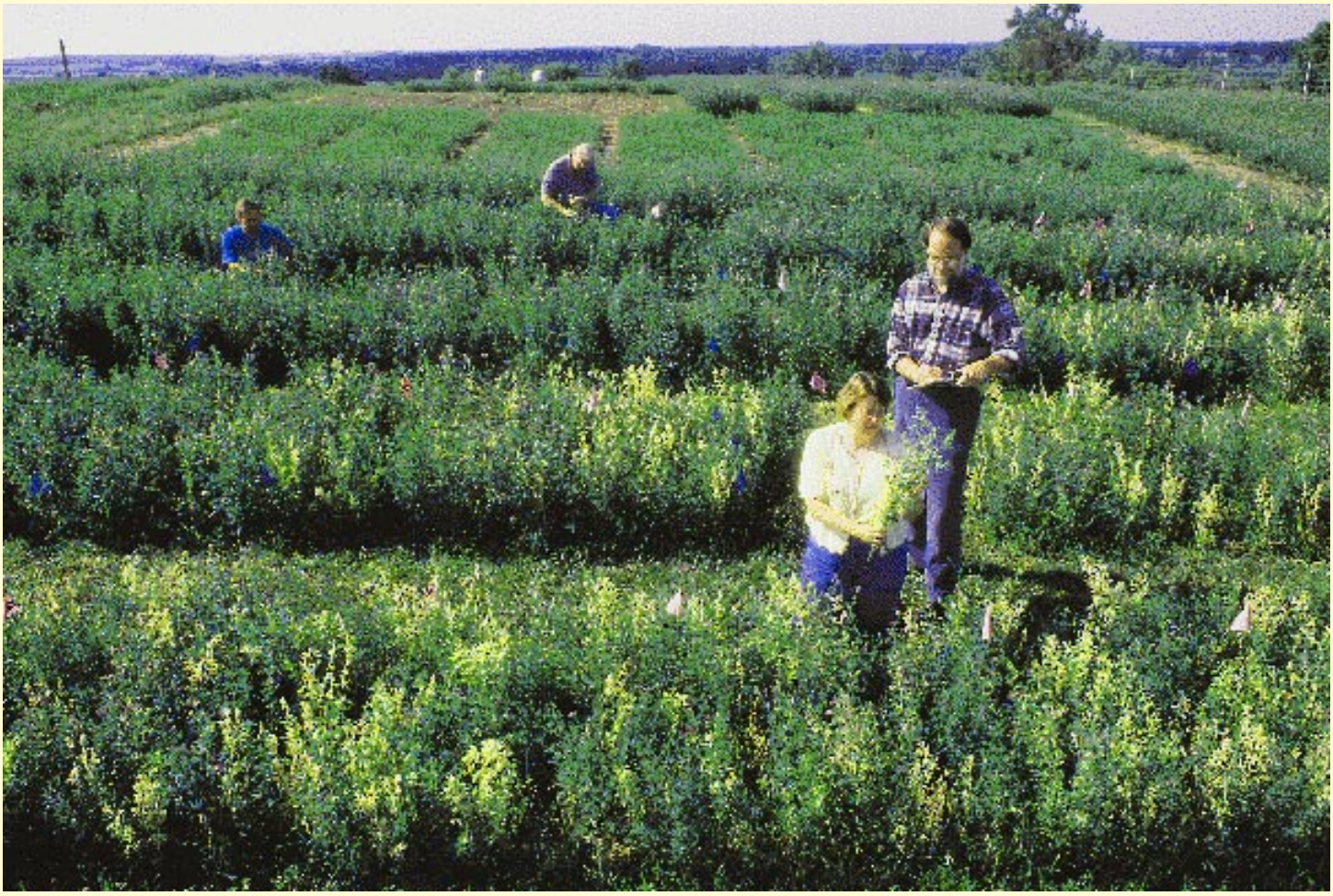

At a St. Paul, Minnesota, test site, plant geneticist JoAnn Lamb (kneeling) and soil scientist Michael Russelle evaluate alfalfa for root growth. Plants with the quickest growing roots turn yellow first when their roots reach a specially treated layer of subsoil. (K7532-1)

The leaf pieces and bacteria are cultured together, to allow DNA transfer to take place.

Next, they wash the leaf pieces and place them on a culture medium containing antibiotics that kill the bacteria and any leaf cells that did not receive the new DNA. Leaf cells with the new DNA proliferate and are then moved to a new culture medium that induces the formation of plant embryos. Researchers remove the embryos and germinate them to form alfalfa plants that are grown to a level of maturity needed to test for gene expression.

This method is being used to develop alfalfa with increased leaf retention and disease resistance and for still another project aimed at developing alfalfa suitable for detoxifying soil contaminated with the chemical herbicide atrazine.—By **Dawn Lyons-Johnson,** ARS.

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