KEITH WELLER (K9923-1)

The Alfalfa Factory

A remarkable perennial legume finds many uses.

Alfalfa, the preferred feed for thoroughbred horses, dairy cows, and other livestock, has always been a pioneer plant. Armed with the ability to produce its own nitrogen fertilizer, alfalfa can establish a front line on poor soils. It enriches the soil so much that it paves the way for grasses and shrubs to thrive and even dominate the landscape.

So it should be no surprise that alfalfa is still pioneering today. Scientists say it has the potential to be the first

In a growth chamber, plant pathologist Deborah Samac examines alfalfa plants that provide the starting material for inserting new genes into alfalfa. Scientists are working to improve the crop's digestibility and ethanol yield, among other traits.

dual-purpose biofuel plant, that is, the stems would be harvested for fuel and the leaves for feed and other products.

Alfalfa may also be grown as a renewable replacement resource for other petroleum-based products, such as plastics and nitrogen fertilizer. And it can reduce the need for other nonrenewable resources, such as phosphorus fertilizer, which is mined. What's more, this forage plant can clean land and water of contaminants and even prevent such contamination.

JoAnn F.S. Lamb, a plant breeder who serves on a team of five scientists at the ARS Plant Science Research Unit, in St. Paul, Minnesota, is devoted to "putting more alfalfa on the landscape," she says. She encourages farmers to plant alfalfa for its environmental benefits, but she knows its dollar value must rise before more farmers will consider it as a crop.

ARS plant physiologist Carroll P. Vance says, "We are developing new alfalfa varieties for this changing world in which fertilizers and gasoline may one day be priced out of range or unavailable."

Says Lamb, "We think using alfalfa to produce ethanol on the side is part of the answer to making alfalfa a more profitable plant." The Plant Science Research Unit has new bioenergy funding from U.S. Department of Agriculture.

Breeding Alfalfas for Diverse Uses

Ethanol production is just one of many ways to make alfalfa a more profitable crop. The team hopes to accomplish this by breeding many new varieties of alfalfa—one for bioenergy and forage; one with a higher nutritive value in its leaves for cattle forage; another for growing on marginal soils; another for fixing more nitrogen in the soil for successive crops; others for catching excess fertilizer and pesticides; and one for producing industrial products in its leaves, whether medicine, industrial enzymes, or plastics.

These features could be combined into varieties for niche or mainstream markets. For example, bioenergy alfalfa would ensure its profitability by producing a better forage or an industrial product in its leaves.

Alfalfa for Fuel

New alfalfas will likely trace their heritage to the third generation of a new bioenergy alfalfa being bred by the team. It has unusually thick stems so the plant doesn't lodge (fall over) and so there is more material to make ethanol from.

DOUG WILSON (K7198-13)

Alfalfa field in the Shasta Valley near Yreka, California.

To get the parent material, Lamb and colleagues crossed European varieties bred for lodging resistance with modern alfalfa varieties developed for dairy cattle feed. ARS plant pathologist Deborah A. Samac works with Lamb's group to make sure that the European hybrid is given resistance to U.S. plant diseases and insect pests.

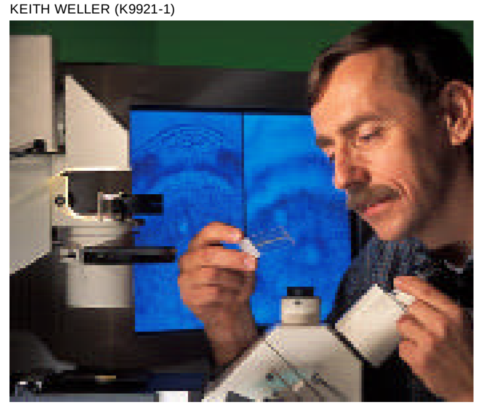
With the new bioenergy funding, ARS dairy scientist Hans Jung will develop tests to screen alfalfa types for variations in amounts of different carbohydrates and their ease of conversion to ethanol. Jung, Lamb, and Samac hope to enhance this conversion by putting more sugars and starches in the stems, which would make it more digestible both for livestock and for the microbes that convert it to ethanol.

Jung points out similarities in what would at first seem to be disparate areas: ethanol production and animal digestion. Indeed, cows' rumens are really fermentation vats, natural versions of the industrial vats used to produce ethanol.

KEITH WELLER (K9912-1)

Flowering alfalfa.

KEITH WELLER (K9921-1)



Dairy scientist Hans Jung examines alfalfa stem sections before and after digestion by rumen bacteria. Genetic modification of nondigestible xylem tissue would make stems better cattle feed and enhance their conversion to ethanol.

And microbes do the digesting in both. Jung also studies limitations to the breakdown of cell wall carbohydrates to identify ways to improve alfalfa.

Using selection and breeding methods, Lamb is developing alfalfa lines that incorporate beneficial traits such as more sugars, larger stems, and improved fermentation. The first cycle of this selection process has already been completed for several traits that will ultimately result in more digestible alfalfa and efficient ethanol production.

With part of the new bioenergy funds, ARS will hire a biochemist/geneticist to investigate what traits are needed for conversion of the stem to ethanol through fermentation and find the genes for these traits.

"This is new territory. We don't know what traits are most desirable for conversion to ethanol," says Samac. "The conversion process is complex, involving sugars, starches, cellulose, and yeasts."

Vance wondered if part of alfalfa's roots and crowns could also be used for ethanol production. His colleague, ARS soil scientist Michael P. Russelle, is working with Lamb, Jung, and University of Minnesota colleagues to test the idea. They will analyze these plant parts to see what percentage can be harvested without harming the plant's ability to store nitrogen and carbon in soil.

A Fungus-Resistant Alfalfa

Like the rest of the team, Samac has years of experience with alfalfa. Her role is finding and inserting genes into alfalfa that will make it a better traditional crop and a more profitable industrial crop for fuel, plastics, or other value-added products.

One area of interest is resistance to disease, particularly *Phoma medicaginis,* a fungal disease of leaves. Team members are identifying alfalfa types that are highly resistant to the fungus so they can "tease out the mechanisms that inhibit the fungus," Samac says. She has a University of Minnesota graduate student investigating natural variations in the fungus, information important for selecting more resistant alfalfa plants.

Samac is also evaluating the use of biocontrol bacteria that produce an antifungal antibiotic. Applied as a dust to soil, the bacteria coat the leaves of emerging seedlings and fight the fungus. Results in growth chamber studies have been good; the next step is to see whether the concept works as well in the field.

KEITH WELLER (K9918-1)

Plant physiologist Carroll Vance evaluates roots of alfalfa, *Medicago truncatula,* as part of his efforts to help the crop fix more nitrogen and take in more phosphorus.

Alfalfas for Plastics, Bioremediation

To create an industrial crop, Samac has added genes that have made alfalfa into a plastics factory, manufacturing beads of a raw, biodegradable plastic in its leaves. She thinks there is a good chance that the alfalfa makes enough plastic to be commercially viable; it just may not be moving outside the cell walls during the extraction process, where it can be harvested from the leaf. Samac is looking for research partners with skills in overcoming the cell wall barrier.

In helping to give alfalfa an even better edge in marginal soils, Samac and Vance have added a gene that produces an enzyme called malate dehydrogenase, or MDH. Samac has found it helps alfalfa tolerate aluminum, which becomes toxic in marginal, acidic soils.

She has also added a gene to alfalfa that enables it to detoxify the herbicide atrazine. Samac worked with University of Minnesota scientists to create plants that detoxify atrazine. The university patented the technique. Soon, another graduate student there will work with Samac on expressing a new gene so that plants can degrade enough atrazine to be useful in cleaning up contaminated soil and water.

Russelle sees alfalfa as a safety valve in the nitrogen cycle: "Everyone thinks that legumes like alfalfa add nitrogen to soil only by fixing it from the air. But actually they're flexible. They absorb nitrate from the soil and fix the remainder of what they need from the air."

Russelle has worked for several years on using alfalfa and other perennials to bioremediate (clean up) nitrate and other potential pollutants while also finding ways to use legumes to prevent contamination in the first place. He led a team that used alfalfa to clean up a spill of ammonia fertilizer from a train wreck in North Dakota after all other cleanup techniques had failed.

He argues that tremendous water quality benefits can be achieved by the strategic placement of alfalfa on America's landscape. “It’s particularly suited for soils that leak nitrates easily.” He is using computer modeling and mapping to identify the locations of these sandy or shallow soils.

“Perennials, like alfalfa, can nearly eliminate the losses because they start growing in early April—when the soil thaws in our region—taking in water and nitrates. Annual crops don’t start growing until June, giving nitrates two extra months in which to leach toward groundwater,” Russelle says. “Perennials also continue to take in water and nitrates later in the fall.”

To achieve the same reductions in nitrate leaching, farmers would have to reduce their nitrogen fertilizer use so much that they would have unacceptably low yields, Russelle says. “So it makes sense to plant a perennial like alfalfa on soils at high risk of nitrate leaching.”

He is working with the Lincoln-Pipestone Rural Water Board, of Lake Benton, Minnesota, on using alfalfa to lower nitrate levels in well water in rural southwest Minnesota. The community blends water from various wells to lower nitrate levels, but it still can’t get the level below the U.S. Environmental Protection Agency’s 10 parts per million standard for drinking water. Russelle and David W. Kelley, a former ARS postdoctoral scientist now at the University of St. Thomas, St. Paul, Minnesota, are producing maps of the wellhead protection areas that show where alfalfa should be planted to protect groundwater.

In some places, strategic planting of perennials like alfalfa may not be enough to protect groundwater quality, so Russelle is developing an approach to remove nitrate from shallow aquifers. Called phytofiltration, it involves running contaminated groundwater through alfalfa’s root zone to remove nitrate and allow clean water to flow back into the aquifer. In east central Minnesota, Russelle and colleagues used the technique to clean irrigation water from 50 parts per million (ppm) of nitrate-nitrogen to well below 5 ppm.

KEITH WELLER (K9915-1)

Geneticist JoAnn Lamb evaluates different genetic sources of alfalfa to identify plant traits that would increase growth and enhance the conversion of plant tissues into biofuel.

“These are very promising results,” says Russelle. “We produce a high-value forage or energy crop and cleaner water. The water is safe to drink in terms of nitrate levels, but we’ll have to check for other problems, such as off-flavors caused by contact with plant roots.”

Russelle is also beginning to experiment with an idea he had several years ago to control nitrate leaching—perennial biocurtains. These are narrow strips of alfalfa or other perennials planted above buried soil-drainage pipes. In the United States, such pipes lie under more than 75 million acres of poorly drained soils. A lot of the nitrate contributing to the Gulf of Mexico’s Dead Zone apparently comes from Midwest farmland through the pipes, which pour into ditches that eventually flow into streams and rivers.

Russelle and several University of Minnesota colleagues measured losses of 30 to 80 pounds of nitrogen per acre from drained soils where corn and soybean were grown. Less than 5 pounds per acre were lost under alfalfa or perennial grasses.

“Perennial biocurtains are another example of a strategic use of alfalfa, where major environmental benefits could be gained through reduction in nitrate losses,” Russelle says. He is working with the University of Minnesota and the ARS National Soil Tilth Laboratory in Ames, Iowa, to test this idea.

Russelle, Vance, and Samac are also analyzing data from alfalfa grown on soils mixed with sludge from Chicago’s municipal sewage treatment plant. Sludge typically contains zinc, nickel, and other heavy metals that can be toxic. Samac’s MDH-producing variety may be a good candidate for metal uptake.

Vance’s focus is on improving biological nitrogen fixation for alfalfa and other legumes as well as improving how plants acquire more phosphorus from the soil. He has isolated many genes, including the one that produces MDH. He found that this gene also helped alfalfa fix more nitrogen and take in more soil phosphorus.

“That’s how genetic engineering research goes,” Samac says. “You turn one wheel and see what other wheels might turn. It’s nice when you find a gene with multiple benefits.”

Discovering the relationships between genes is one way scientists can give alfalfa an even greater pioneering role in the future.—By **Don Comis,** ARS.

This research is part of Rangeland, Pasture, and Forages, an ARS National Program (#205) described on the World Wide Web at http://www.nps.ars.usda.gov.

To reach scientists mentioned in this article, contact Don Comis, ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; phone (301) 504-1625, fax (301) 504-1641, e-mail comis@ars.usda.gov. ◆