PEGGY GREB (K9674-2)

Formidable Fungus Goes Toe to Toe With KUDZU

Spreading at the rate of 150,000 acres annually, kudzu can completely envelop the landscape.

Kudzu's lightning-fast growth is the stuff of legend. It was originally introduced into the United States in the 1870s from eastern Asia as a means of controlling soil erosion. Kudzu was also fed to livestock, and some folks planted it as a flowering ornamental. But kudzu followed a master plan of its own evolutionary design and broke free of the plantings of humankind to spread and conquer.

And conquer it did, nudging aside native plants and tipping the ecological balance. Today, kudzu is considered a noxious weed, infesting 8 million acres of land, mainly in the southeastern United States. By one estimate, it spreads at the rate of 150,000 acres annually, easily outpacing the use of herbicide spraying and mowing, as well as increasing the costs of these controls by $6 million annually.

But in Stoneville, Mississippi, ARS plant pathologist C. Douglas Boyette and colleagues are testing a naturally occurring fungus, *Myrothecium verrucaria,* that infects kudzu with NASCAR-like speed.

So fast, in fact, "You can apply it in the morning and see damage in the plants by midafternoon," says Boyette, in the ARS Southern Weed Science Research Unit (SWSRU) at Stoneville. Collaborating with him on this work are chemist Robert E. Hoagland and plant pathologists Mark A. Weaver and Kenneth C. Stetina, all in the SWSRU, and plant pathologist Hamed K. Abbas, who's in the ARS Crop Genetics and Production Research Unit, also at Stoneville.

Boyette first began studying *Myrothecium* (strain IMI 361690) in 1998, after Louisiana Tech University scientist H. Lynn Walker provided him with isolates from diseased sicklepod plants. In greenhouse experiments, spray formulations of the fungus killed 100 percent of kudzu seedlings, and 90–100 percent of older plants in outdoor trials. Disease symptoms—wilted leaves and necrotic stem lesions—appeared on the plants within 24 hours of infection. By 14 days, all but the plants' roots were diseased.

How *Myrothecium* breaches the defenses of the seemingly indestructible kudzu is still being investigated. One telltale clue, though, may be its use of cell wall-degrading enzymes. Besides kudzu and sicklepod, *Myrothecium* attacks hemp sesbania—which is problematic in soybean crops of the southeastern United States—morninggglories, pigweed, redvine, and trumpetcreeper. The last two are native perennial vines that typically infest cultivated and fallow fields, wastelands, fence rows, yards, river banks, swamps, and forests.

The many tests that scientists subjected *Myrothecium* to since 2000 show it can work its antiweed magic under a wide range of environmental conditions, including the absence of dew—a feature that bodes well for bioherbicide uses.

Furthermore, in 2005 host-range studies, the fungus caused little or no injury to 70-plus percent of woody plants known to occur in kudzu-infested habitats. Among others, these included oak, cedar, pine, hickory, pecan, sassafras, and blackberry. The remaining species showed slight to moderate sensitivity but recovered from injury several weeks after the fungus had been applied. Raising *Myrothecium's* bioherbicidal prospects even higher was the researchers' successful formulation of the fungus's chief infective stage, the conidia.

Turning Off Toxins, Ensuring Safety

During their studies, says Boyette, a few companies expressed interest in commercializing *Myrothecium*—but *only* if the researchers could reduce or stop its production of natural toxins called "trichothecenes." In humans, exposure to the toxins can cause skin irritation, such as blistering, and if swallowed, vomiting and diarrhea.

The researchers examined several approaches to tackling the trichothecene problem. These included using natural and synthetic compounds to gum up *Myrothecium's* trichothecene-making machinery, selecting mutant strains incapable of producing the toxins, and using various culturing techniques to remove them from the final bioherbicide formulation.

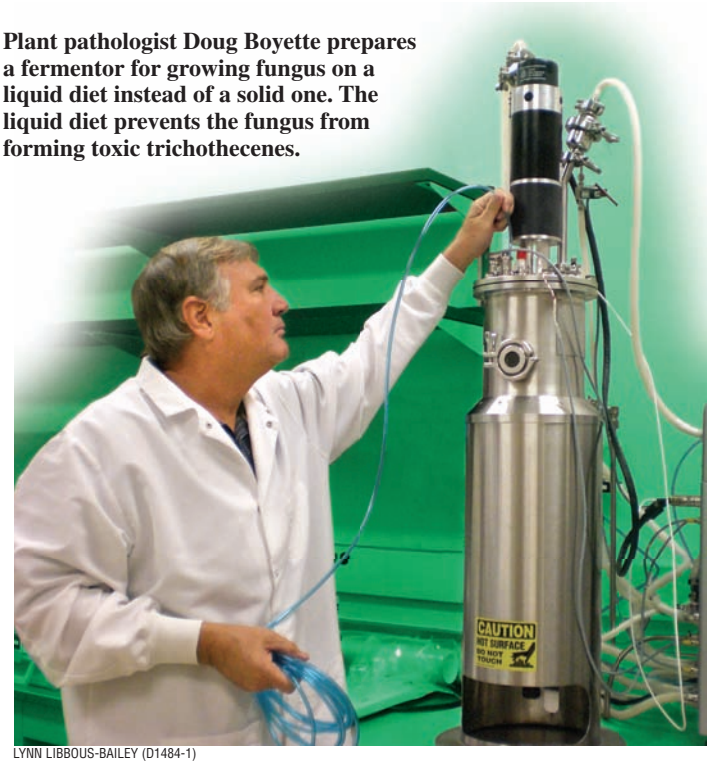
"We found that the toxin could be removed best by cultural methods," says Boyette. One such method is dubbed "spore washing," and uses distilled water. The other method involves growing the fungus inside laboratory fermentors on a liquid diet instead of a solid one.

Using high-performance liquid chromatography (HPLC) and an enzyme-linked immunosorbent assay, the researchers confirmed that the methods either completely silenced *Myrothecium's* trichothecene production or muted it to levels deemed acceptable by the U.S. Environmental Protection Agency for commercially registering the fungus.

"We ran HPLC and other analyses and observed a clear effect," says Boyette. "We now have enough data and evidence to support our claims that the fungus is not producing trichothecene mycotoxins above EPA-approved levels."

Making a Better Bioherbicide

Boyette says their efforts to eliminate trichothecenes led to improvements in how they formulate *Myrothecium*. Initially, they used conidia as their bioherbicide's chief active ingredient. But with liquid fermentation techniques, they were able to use mycelium, a different growth stage of the fungus that's far easier to mass-produce.



Plant pathologist Doug Boyette prepares a fermentor for growing fungus on a liquid diet instead of a solid one. The liquid diet prevents the fungus from forming toxic trichothecenes.

LYNN LIBBOUS-BAILEY (D1484-1)

MARK WEAVER (D1486-1)

The dramatic effect of the *Myrothecium* biocontrol agent on kudzu plants can be seen within 48 hours of application at a test plot in Stoneville, Mississippi.

"Now, we can produce inoculum in 48 hours, and it is toxin free or has a substantially reduced level," says Boyette. "An advantage of a fast production system is that you can produce inoculum as needed." What's more, the mycelium-based formulation lasts longer than the conidia, he adds. Indeed, field tests show that mycelia retain their potency against weeds even after 6 months of storage.

In addition, the fungus has been shown to be effective in killing a wide range of weeds that plague tomato production. Field research has shown that *Myrothecium* has potential as a preemergence bioherbicide, controlling purslane and spurge in transplanted tomatoes. These results have spurred interest from organic growers and offer a potential replacement for some herbicidal uses of methyl bromide.

Though the fungus is ubiquitously distributed in soils, it is not aggressive, and epidemic infestations do not occur. The researchers have seen no recurrence of disease symptoms on plants in areas treated with bioherbicidal amounts of the fungus, indicating no significant problems of persistence or threats to nontarget hosts.

The researchers hope these advances—accompanied by two patents on *Myrothecium*—will rekindle industry's interest in the fungus, both as a kudzu killer and bioherbicide for field use in tomato, soybean, rice, and other crops.—By **Jan Suszkiw,** ARS.

This research is part of Crop Protection and Quarantine (#304) and Methyl Bromide Alternatives (#308), two ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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