plant pathologist William Bruckart examines a yellow starthistle weed for rust symptoms. The purple-flowered cornflower plants are a close relative of the weed and are used to evaluate whether potential biological control pathogens attack nontarget plants.

KEITH WELLER (K8882-1)

A casual observer might think the five greenhouses that Frederick, Maryland, plant pathologist William L. Bruckart visits each day are a bit unusual. Rather than housing something expected, like rare, tropical flowers, the greenhouses are home to an unruly tangle of noxious weeds.

Actually, "unruly" is a bit misleading. The weeds, in fact, are all potted and neatly lined up along tables inside the greenhouses. Each greenhouse is part of a microbial containment (quarantine) facility operated by the Agricultural Research Service's Foreign Disease-Weed Science Research Unit in Frederick.

There, inside the facility, Bruckart and colleagues like plant pathologist Norman W. Schaad infect some of America's most noxious alien weeds with foreign bacteria, fungi, and other pathogens that sicken these pesky plants with disease.

KEITH WELLER (K8878-1)

Biologist Craig Cavin examines safflower leaves inoculated with *Puccinia carthami* (left), an indigenous safflower pathogen, and *Puccinia jaceae* (right), a candidate for biological control of yellow starthistle.

KEITH WELLER (K8882-14)

Inside the containment greenhouse facility, technician Mary Winter sprays test plants with spores before placing them in a dew chamber.

Led by Douglas G. Luster, the Frederick lab is the first stop in a national campaign to reunite exotic weeds like yellow starthistle and Russian knapweed with their homeland's natural enemies.

Like their hosts, the plant pathogens are also of foreign origin and are thus kept in quarantine. There, Frederick researchers can determine the microbes' safety and potential as biological control agents.

"When exotic weeds come to the United States, they're usually healthy and don't have their normal complement of natural enemies—namely, diseases and insects," explains Bruckart, a plant pathologist. "We're trying to reintroduce these pathogens to their natural hosts and then allow them to become part of the ecosystem."

War of the Weeds

This approach—classic biological control—is only one front in America's multibillion-dollar war on invasive weeds.

Invaders like yellow starthistle have already displaced native flora and tipped the ecological balance. On rangelands, exotic weeds have displaced forage eaten by cattle and extended harm to other aspects of American agriculture, including those who earn their living from it.

In California alone, yellow starthistle infests over 10 million acres of rangeland. Yet it's just one of 4,000 to 5,000 exotic plant species that have become established in the United States.

Even more startling is the speed with which they've spread. From 1958 to 1985, California's infestation of yellow starthistle increased from 1.2 million acres to 7.9 million. By 1996, infestations had crept their way into 42 percent of all California townships.

Since the mid-1970s, scientists at Frederick and elsewhere have sought to slow the advance with biological, instead of chemical, means. Cost, environment concerns, and the extent of infestations are three reasons for taking this approach.

KEITH WELLER (K8883-1)

A 2-day-old safflower seedling being inoculated with rust spores of *Puccinia jaceae* to clarify whether the plant is susceptible.

All three come into play when trying to chemically treat an infested area that may cover hundreds of thousands of acres.

Also, weeds like yellow starthistle are notoriously resilient. Several years of repeated spraying are often needed to bring an infestation under control. This, in turn, can raise the likelihood that chemical residues will accumulate in the environment, causing more harm than good over the long haul.

"You need to think about ecological approaches," says Margaret Mellon, director of The Union of Concerned Scientists' Agriculture and Biotechnology Project, in Washington, D.C. "It's too big a problem for chemicals alone," she adds.

Tipping the Scale of Nature's Checks and Balances

One way around the problem is to encourage nature's choke-hold on weeds by finding diseases that can weaken a population's ecological grip over native competitors.

As Bruckart says, "It provides the opportunity to apply constant pressure" that chemical or cultural controls like tillage sometimes can't.

KEITH WELLER (K8881-1)

Yellow starthistle flower.

Classic biocontrol of weeds involves importing a natural enemy from abroad and then releasing it in regions overrun by its host.

But there are some risks. One concern is that an introduced insect or weed pathogen might attack a native plant or nearby crop.

"You have to consider the possible downside of introducing one exotic organism to control another," comments Mellon. "In general, biocontrol has worked. It's a reasonable thing to do, but you have to do it with your eyes wide open."

That's why special care is taken in the selection, importation, study, and release of foreign weed fighters.

"When we evaluate a pathogen, we're looking for two things: One is virulence (infectivity)," says Bruckart. "We also want to make sure it's safe to release in the United States. That centers on a host-specificity study."

The results of such studies are packaged into a proposal for the microbes' use, along with risk assessment data. Both are then submitted to various state agriculture departments and the Technical Advisory Group.

Called TAG for short, its members represent such organizations as the Weed Science Society of America and federal agencies like the U.S. Environmental Protection Agency. TAG makes a recommendation on the proposed release of an organism to USDA's Animal and Plant Health Inspection Service (APHIS).

After considering input from agriculture officials in affected states, APHIS makes a final decision whether to allow the proposed activity.

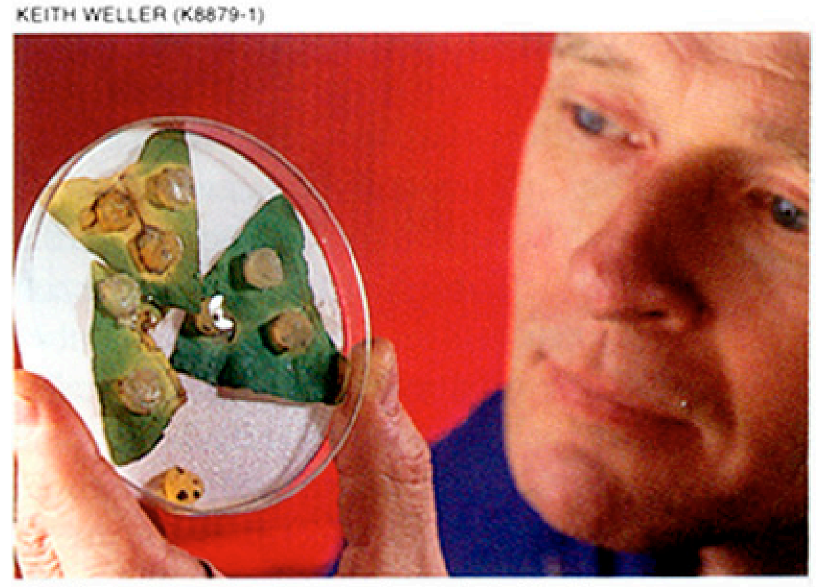
According to APHIS records from 1945 to the present, nearly 68 species of insects and plant pathogens have been released to biologically control 31 species of exotic weeds in the continental United States.

One of these is the rust fungus *Puccinia cardorum* Jacky. It was released in 1987 as part of a 3-year field study by Frederick researchers and colleagues at Virginia Polytechnic Institute and State University in Blacksburg. Modeled after a field study in Switzerland, the Virginia trial helped determine the fungus' outdoor safety and effectiveness against exotic musk thistle in the field.

The weed typically encroaches on pasture and rangeland. Unchecked until now, it has crowded out forage and native grasses eaten by cattle and other livestock.

Since its Virginia release, the fungus has since turned up in thistle populations

KEITH WELLER (K8879-1)



William Bruckart examines detached leaves of the weed mile-a-minute, *Polygonum perfoliatum*, used to screen a test fungus in disks of agar.

as far away as Wyoming and California. Luster confirmed the fungus' identity on samples by using genetic fingerprinting techniques that he developed.

"We've been watching this organism all across the United States," Bruckart says. "It has really spread through the musk thistle population substantially."

Interestingly, the largest reductions, of more than 90 percent, have coincided with attacks by a tiny, imported seedhead weevil, *Rhynocyllus conicus*. "We've found that the rust actually enhances the weevil's feeding damage," says Bruckart.

Much of the credit for the microbial collection in Frederick should go to collaborating scientists elsewhere in the United States and other countries, such as Hungary, Switzerland, Croatia, the United Kingdom, and China. Last, but certainly not least, are colleagues at ARS' European Biological Control Laboratory in Montpellier, France. As Bruckart puts it, "They're the ones beating the bushes for useful disease agents."

Through this network, the Frederick lab recently acquired four good candidate pathogens—two on Russian thistle, one on Russian knapweed, and another on common groundsel. Chinese researchers have also sent them several fungi for screening on "mile-a-minute," a fast-growing vine with sharp spines.

Fort Detrick: Biocontrol Bootcamp

On receiving a sample from abroad, Frederick researchers begin work immediately. It's imperative a new arrival be in peak condition for the battery of tests it will be subjected to. But first, each specimen gets an identification number for tracking purposes. Part of it is also put in long-term storage.

The scientists' next step, for most pathogens, is to collect the spores. Using an atomizer, they then spritz the spores onto the leaves of a host weed grown inside the greenhouses. A sample of the weed's closest native U.S. relatives is also treated. The containment facility, Luster notes, "is the largest research facility where we can work with whole plants in a microbial quarantine greenhouse."

Generally, 2 to 3 weeks pass before disease symptoms appear in plants. A 300-ton air-conditioning unit helps the process along by maintaining desired air temperatures. A high-tech filtration system also keeps tiny, floating particles and airborne spores from leaving the greenhouse.

KEITH WELLER (K8880-16)

Early symptoms of safflower rust, a disease native to the United States, appear on the stem of a safflower seedling.

To further ensure that nothing escapes the facility, a waste disposal system sterilizes any effluent generated. This includes water from plant beds, toilets, and showers where scientists and technicians wash off any seeds, pollen, or spores.

Putting Pathogens Through the Paces

One method of checking a weed pathogen's virulence is counting the number of pustules or lesions that pepper a plant's leaves. Scientists also measure plant height, weight, growth rate, mortality, and seed production.

They also determine the environmental conditions under which pathogens are most likely to grow best and cause disease. Once a candidate meets all the desired safety criteria, scientists apply for an APHIS permit to examine the pathogen's weed-fighting prowess outside the greenhouse.

"When you are dealing with the ecosystem," says Bruckart, "it's a different story" than what actually occurs in the laboratory.

For example, a weed pathogen or insect can vanish after it's been released, only to reappear in force months or even years later. Classic biocontrol is generally slower acting than chemical pesticides, so noticeable results may take several years.

But once those results generally kick in and are observed, they're permanent. One exception is if a weed marshals a natural defense against the pathogen recruited to fight it, though evidence of potential resistance is usually noted in greenhouse evaluations.

In Idaho, for example, scientists knew that some skeleton weed populations were resistant to the rust fungus *Puccinia chondrillina,* which was released in 1976. Additional biocontrol agents have been sought to combat these hardy new strains.

In the end, no single weapon is likely to vanquish or eradicate such pesky, invasive plants. Rather, management is the more likely scenario.

"We're learning that it's going to take multiple agents—diseases and insects—in an arsenal of strategies that includes chemicals and management, like mowing, crop rotation, and plowing," says Bruckart. "It's part of an integrated system, really."—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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