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Cheating Cheatgrass: New Research to Combat a Wily Invasive Weed

Cheatgrass and its cousin, red brome, are exotic annual grasses that have invaded and altered ecosystem dynamics in more than 41 million acres of desert shrublands between the Rockies and the Cascade-Sierra chain. A fungus naturally associated with these *Bromus* species has been found lethal to the plants' soil-banked dormant seeds. Supported by the Joint Fire Science Program (JFSP), researchers Susan Meyer, Phil Allen, and Julie Beckstead cultured this fungus, *Pyrenophora semeniperda*, in the laboratory and developed an experimental field application that, in some trials, killed all the dormant soil-banked *Bromus* seeds, leaving none to germinate the following year. The team's work opens the way to a commercial biocontrol product that may be capable of safely eliminating the seed bank of persistent invasive grasses. Biocontrol could be used in conjunction with other weed control measures and conservation strategies to make sagebrush-steppe lands less susceptible to reinvasion. A biocontrol tool effective against *Bromus* seeds would be a boon to managers working to restore the native bunchgrasses, forbs, and shrubs that characterize an intact shrub-steppe ecosystem.



Introduction

Weeds are a costly nuisance everywhere. They are the target of much of the pesticide applied throughout the world; herbicides constitute nearly half of the world's agrochemical sales, and more than half of the preharvest labor in the developing world goes for weeding, mostly by hand.

A notoriously troublesome weed in the arid and semiarid rangelands of the U.S. Intermountain West is cheatgrass, or downy brome (*Bromus tectorum*). Cheatgrass is a fast-spreading annual grass that migrated from Eurasia in the 19th century, about the same time cattle were beginning to graze large expanses of western range. Cheatgrass' aggressive growth cycle and habit of copious seed production gave it an advantage over native perennial bunchgrasses. Cheatgrass grabbed a toehold in areas where heavy cattle grazing had depleted the native vegetation community, and it is now present on about 100 million acres—about three-quarters of the Great Basin. On nearly 41 million acres, it has largely replaced the patchy sage- and bunchgrass-dominated landscape with its own continuous tawny pelt.

Cheatgrass germinates with fall or late winter moisture and grows rapidly into the spring. Early in the season, its blades and stems are palatable forage for livestock. Cheatgrass sets seeds in late spring and sows them on the dry summer soil. The seeds sprout when winter's rain and snow return. Some do not germinate the next season, but cycle in and out of dormancy for up to 2 years, or maybe longer—they are the plant's survival insurance in a high-risk environment.

Cheatgrass foliage dries with early summer's heat—cattle won't eat it then—and becomes extremely flammable. The invasive grass has spread widely enough to dramatically alter the fire cycle; some sagebrush-steppe lands that once experienced fires every 60-100 years are now burning every 3-5 years. More frequent fires, along with cheatgrass' adeptness at colonizing burned soil, make reestablishing difficult for native grasses and shrubs.

Abandoning the range to annual grasses has unfortunate environmental and economic consequences. Many wildlife species rely on the sagebrush-bunchgrass-desert forb community; among them are the pygmy rabbit, sage-grouse, sage sparrow, sage thrasher, Brewer's sparrow, and gray flycatcher.

In addition, most ranchers would prefer to graze their cattle on native perennial grasses, which are both more nutritious than cheatgrass and more consistent in their production from season to season.

More frequent fires bring more soil erosion. "We had a huge fire in 2007, the Milford Flat Fire," says Susan Meyer, U.S. Forest Service research ecologist with the Rocky Mountain Research Station, "and we're still getting dust storms all the way to the Wasatch Mountains." The fires also release pulses of CO₂ into the atmosphere, carbon that might otherwise stay locked up in the leaves and roots of sagebrush and other plants.

Curbing cheatgrass would lessen the frequency of fires, reduce CO₂ emissions, and allow the sagebrush-steppe ecosystem to recover. That, says Meyer, might help the Great Basin become a carbon sink, absorbing more carbon than it gives off. "It will never sequester as much as a forest, of course," she says, "but you'd be surprised how much carbon can be stored in root masses down there in the soil."

Killer Fungus

None of these benefits will happen until range managers get a handle on cheatgrass. Cheatgrass is tough, fast spreading, and exceedingly difficult to dislodge. The main tools available for battling cheatgrass are fire, tillage, and herbicides. Each method has a role in combating cheatgrass, but each also has disadvantages. Burning early in the spring, before seeds form, may eliminate the current season's foliage, but it doesn't kill most of the seeds banked in the soil. Also, fires produce smoke that may bother nearby communities, and there's always a risk that prescribed fire will escape its boundaries. Tillage disturbs the soil and can harm desirable plants and microorganisms, and it's expensive to undertake across vast acreages. Herbicides are effective against

annual weeds, but they can be expensive when used on a large scale and may harm native species. Even more notable, none of these remedies can eliminate the banked seeds. With its cache of seeds intact, cheatgrass will always have the upper hand.

That's where *Pyrenophora semeniperda* shows promise, says Meyer. It's a naturally occurring pathogen, found wherever cheatgrass is found, and it likes to kill and eat grass seeds. "If we can use this fungus to develop a biocontrol agent that's really

The invasive grass has spread widely enough to dramatically alter the fire cycle . . .

effective on seeds,” says Meyer, “we could create a window for restoring the native shrub-steppe plant community, even on sites that have burned repeatedly and are in persistent, annual brome monocultures.”

Black Fingers

Meyer has been studying the ecology of cheatgrass for 20 years. Ten years ago she formed a team that included her former graduate student Julie Beckstead and Brigham Young University colleague Phil Allen, and they set out to probe the mysteries of the cheatgrass seed bank. How many seeds did the plant store in the soil every year? How long were they viable? How many germinated the following year?

They started by collecting and sprouting cheatgrass seeds in the laboratory, and there they made a startling discovery: after a couple of weeks of exposure to moisture, some of the seeds started to grow stubby black protuberances from their slender sides. The infested seeds did not germinate—they were dead. “We knew it was a fungus, but we didn’t know what kind,” says Meyer. “Since we didn’t have a name for it, we dubbed it ‘Black Fingers of Death.’”



Bitsy Schultz

Susan Meyer, U.S. Forest Service research ecologist, and Julie Beckstead, a member of Meyer’s research team, install a longevity study of the *Pyrenophora semeniperda* fungus in the field.

The researchers were also collecting samples of cheatgrass seed banks and counting the seeds in a given volume of soil. Many of the seeds in these samples, they discovered, were infested with BFOD, as they’d taken to calling it. “A large proportion of dormant seeds had this fungus growing out of them, and it was the same fungus we’d seen in the germination experiment,” says Meyer. “That was our ‘Aha’ moment. We saw that this fungus was killing lots of seeds, thousands and thousands per square meter.” The fungus appeared to affect only seeds—the plants themselves showed no symptoms.

They still didn’t know what to call the pathogen, so Meyer and Beckstead got in touch with a retired Canadian plant pathologist, Robert Shoemaker. Beckstead emailed Shoemaker a photo of dead seeds with the protruding black fingers. Shoemaker immediately identified the fungus as *Pyrenophora semeniperda*, whose species name means “lost seed.” (The fungus is also known in another form as *Drechslera campanulata*; fungi are tricky to classify because they can take many different forms in their life cycle.)

“Then when we had a name for it, we discovered literature on it,” says Meyer. Scientists in Australia had identified *P. semeniperda* on seeds of weedy *Bromus* grasses. They had been trying to develop it as a biocontrol for ripgut brome (*B. diandrus*), a weed that’s troublesome in cereal croplands there.

Australian scientist M.A. Campbell had developed a method to produce a fungal inoculum in the lab. Campbell and colleague R.W. Medd applied the inoculum to mature *Bromus* seeds but could not get it to kill them. One of the Meyer team’s early experiments, led by Julie Beckstead, revealed why: the germinating seeds were outracing the pathogen. Fast-germinating seeds usually escaped the pathogen’s clutches, while slow-germinating ones were often killed.

Natural Presence

P. semeniperda is a dry-sporulating fungus and a necrotroph, meaning it colonizes by killing tissues of its host. It invades dormant seeds by secreting toxins that eat into the seed’s endosperm tissue, which the fungus then consumes and metabolizes. In the process, it kills the seed’s embryo.

The Meyer team’s 3-year, JFSP-supported study (JFSP Project No. 07-1-3-10) became a multidimensional field and laboratory examination of *P. semeniperda* and its *Bromus* hosts. The researchers

wanted to know which other plants might be susceptible to the fungus, how virulent it could be, how many seeds it killed in its natural environment, and how long it persisted in a seed bank. They wanted to perfect techniques for culturing the fungus in the laboratory and for creating an inoculum that could be applied and tested in the field.

The team sampled the composition of seed banks at five study sites where cheatgrass or red brome was present, and they counted and incubated the seeds they found, including those of the native grass species. They found *Bromus* seed in densities ranging from 6,000 to 25,000 per square meter in August. Between 40 and 70 percent of these seeds germinated the following season; 3 to 35 percent carried over as viable dormant seeds; and 10 to 53 percent were killed by the fungus.

It seemed clear that *P. semeniperda* was an important natural presence in these seed banks. But obviously, many dormant seeds were escaping infection and remaining viable into the next season. By contrast, seed banks of native bunchgrasses had very few seeds killed by *P. semeniperda*, which suggested

that *Bromus* species were probably the fungus' main hosts.

To explore this question further, the researchers inoculated seeds of more than 50 plant species that live in semiarid western environments with high loads of the *P. semeniperda* fungus. They found that most of the grasses—including many of the natives that managers are working to restore—had some susceptibility; although a few, including Indian ricegrass (*Achnatherum hymenoides*) and needle and thread (*Hesperostipa* spp.), seemed to be quite resistant. But even highly susceptible species were usually able to escape through rapid germination, especially at the lower inoculum loads achievable in biocontrol treatments. “This means that the inoculum levels we’re likely to use [in a biocontrol product] will have low impact on native grasses,” Meyer says.

The researchers also wanted to identify the likeliest strains of *P. semeniperda* for development into a commercial seed-killing agent. After some experimentation, Suzette Clement, U.S. Forest Service microbiology technician, developed an efficient method for growing the fungus in culture



Susan Meyer

Thomas Stewart, Brigham Young University graduate student on Susan Meyer's research team, inoculates field plots with *P. semeniperda* inoculum.

Susan Meyer



Suzette Clement, U.S. Forest Service microbiology technician, harvests conidia spores from *P. semeniperda*.

and harvesting its spores, called conidia. Then they tested 92 fungal strains gathered from different sites to determine their virulence levels. They measured virulence in terms of how capable a strain was at killing not only dormant but nondormant seeds—a good way to flush out the most efficient killers. They found a wide variation; some strains of the fungus couldn't kill any nondormant seeds, whereas one was able to kill more than 40 percent of seeds inoculated.

Slow and Mean

Clement and Brigham Young University graduate student Thomas Stewart tested the growth rate of the various strains by measuring how long it took for a single spore to grow into a colony of mycelia (a fungus' vegetative part). To their surprise, they found that the most virulent strains were the slowest-growing ones. "We thought, this is a race between the fungus and the seed, and whichever races fastest, wins," says Meyer. "But we found that the slowest-growing strains are the meanest, and the fastest-growing ones are the least mean."

In fact, most of the fungal strains were at the faster-growing, lower-virulence end of the spectrum, which suggests that these qualities are evolutionarily better fitted to life in a *Bromus* seed bank. That may be because, as a necrotroph, the fungus has to produce toxins that kill its food. More-virulent fungal strains can produce more toxins, says Meyer, but they pay a price in growth. "The meaner poisons kill and disable more quickly," she says, "but poisons are metabolically expensive to produce. So if you're a *P. semeniperda* fungus, you can either grow fast or you can make lots of poison, but you can't do both. That's our hypothesis."

The team is still working out the evolutionary implications of the variation in virulence. But Meyer says this slower/meaner–faster/milder correlation bodes well for the prospects of a commercial biocontrol product. "It creates the interesting possibility," she says, "that if we can select or breed a highly virulent strain, that strain would grow so slowly that, once it does its job in eliminating the cheatgrass seed bank, it would fail to persist in competition with the less virulent but faster-growing wild strains."

This would be an invaluable trait for a biocontrol agent: throw a heavyweight punch that knocks out the cheatgrass seed bank, and then die out, leaving the field open for desirable grasses and shrubs—whose fast-germinating seeds would be able to outgrow any less-virulent wild strains of the fungus that might remain.

Potato Soup

Clement led the team in producing the test biocontrol product. She made a broth of potato dextrose and seeded it with selected fungus strains. She set each batch to ferment for 2 or 3 days at room temperature, letting it develop a mycelial culture. The mixtures were spun in a centrifuge to concentrate the mycelial mass, moistened with fresh potato dextrose broth, mixed with sterile granulated clay, and set to dry slowly for 1 or 2 days, encouraging spores to form. Then the crumbly spore-laden clay was forced through a sieve.

The resulting granulated inoculum, in a range of virulence levels, was sprinkled by hand in varying quantities on field plots. All the treatments reduced the proportion of viable cheatgrass and red brome seeds in the seed bank beyond the approximately 54 percent that the endemic fungus killed naturally. Heavy applications of the most virulent inoculum killed an average of 89 percent of the seed bank, and in some treatments, the kill rate reached 100 percent.

"Complete eradication of the seed bank may not be absolutely necessary," says Meyer, "but you need to get close to that, because cheatgrass is very plastic in its growth responses. That's what makes it such a good weed." A seed-carpeted square meter of ground will produce many small cheatgrass plants; a pinch of seeds in a square meter of ground will produce a few big cheatgrass plants. "And in either case, they make a gazillion seeds, and you're right back where you started."

This is why other tools, like burning and herbicides, will likely be used in conjunction with

The Evolution of Virulence

The team's finding that the most virulent strains of *P. semeniperda* are also the slowest growing presents an intriguing puzzle. Why shouldn't the strongest also be the fastest? In a race where the prize is long-term availability of food, one might expect the winner to be just fast enough to get the resources to keep reproducing itself, just mean enough to disable some host seeds without keeping the host from reproducing—and just fast and mean enough to beat out its competitors.

Meyer's team knew that *P. semeniperda* was most effective in killing dormant seeds—the ones already germinating were the ones that got away. So they hypothesized that the most virulent strains would have a competitive advantage on the moister sites, where there were more germinating seeds and fewer dormant ones. This did not prove to be the case. Instead, they found a range of virulence across all sites, and the most virulent strains so far have actually come from the drier sites, where more seeds were dormant.

Why should there be a range of virulence on a single site? The researchers got a glimpse at the answer when they discovered, using molecular genetics tools, that single seeds were often infected by multiple strains of the pathogen. This suggested that the strain that kills a seed is not necessarily the one that eats it. The slower-growing, more virulent strain may kill the seed only to

have the faster-growing, less virulent strain jump in and scavenge the resources.

For *P. semeniperda*, the researchers speculate, high virulence is an advantage only when the prey is a fast-germinating seed. A dormant seed is not going anywhere, so a mean pathogen has little advantage over a mild one in killing it. And slow growth, even if it's coupled with virulence, is no advantage on fast-germinating seeds if there are faster (albeit weaker) strains in the neighborhood that can gobble the seed's resources once it's dead.

This intraspecies competition may help explain why high virulence is rare. But why should it exist at all? The fact that virulence varies randomly across populations and habitats, the researchers say, suggests that highly virulent strains may result from mutations or recombination events that persist in a limited environment for a limited time, before their fitness advantage vanishes with changing conditions.

Cheatgrass seeds vary considerably in the timing of dormancy and germination, and this variability probably encourages a reciprocal variability in the *P. semeniperda* pathogen. Meyer and her team are continuing to delve into the environmental and genetic factors that govern virulence in *P. semeniperda*.

biocontrol. Burning before cheatgrass sets seed doesn't eliminate the carryover seed bank, but it can reduce it considerably without killing off the natural fungus population. In addition, the team found that combining the test inoculum with herbicide applications gave essentially complete control of cheatgrass and red brome for a season—a generous window for reestablishing native vegetation.

Persistence

Could a highly virulent, laboratory-created fungus somehow mutate into a “Godzilla” strain that would escape and infect desirable plants? Based on findings thus far, says Meyer, that's highly unlikely. “If we could somehow breed a strain that will kill germinating seeds very well, it should have a slow growth rate, which means that it's maladapted to the real world.”

Once the carryover dormant seed bank is eliminated, the researchers say the pathogen is unlikely to persist on all but the driest sites. In the field trials, enough fungal inoculum was applied to significantly

knock back the cheatgrass seed banks, and native grasses that were sown a year later showed minimal harmful effects.

Just in case, however, the team tested three common agricultural fungicides and found they were able to kill *P. semeniperda* in the field. Fungicides, applied either as a soil drench or a seed treatment, could be an effective line of defense in case a lab-created biocontrol product needed to be curtailed after it had done its work.

“In any event, most of the native grasses of the Great Basin are either resistant or fast germinating,” says Meyer, “and in the loads of inoculum that you see [naturally] in the field, they outrace the pathogen. If we find ourselves developing strains strong enough to take out the cheatgrass seed bank completely, then we'd recommend waiting a year to plant desirable grasses, because we have good evidence that the fungus doesn't persist on most sites.”

The team also tested the herbicides glyphosate (Roundup®) and imazapic (Plateau®) and found that neither impaired the seed-killing ability of the pathogen. Thus, herbicide treatments could be

combined fruitfully with fungal inoculum to eliminate both vegetative cover and seed bank.

Breeding a Mean Strain

The next step, says Meyer, is to develop a method for breeding a super-mean strain in the laboratory. “We know this thing sometimes reproduces sexually, but its sexual stage is hard to produce in culture,” she says. “If we could get it to crossbreed in the lab, we’d be in a position to breed for a hypervirulent strain.” The team’s ongoing genetic studies of *P. semeniperda* and its *Bromus* hosts are helping them understand the selection processes that produce the pathogen’s natural range of virulence.

In the meantime, Meyer, Beckstead, and Clement have applied for a patent to develop a commercial product using naturally occurring virulent strains. They’re continuing to refine their production technique, experimenting with more effective ways to concentrate the pathogen and put it in a form that’s easy to apply.

“We have a couple of nibbles from industry, companies that might be interested in helping us develop the product and bring up production to the operational scale,” Meyer says. She hopes to file the patent application soon and expects to see a fully

operational biocontrol product within a couple of years. The “Black Fingers of Death” nickname won’t be part of the package, she adds, since it might not inspire the greatest public confidence in the product.

Improving Odds of Success

When it’s ready, a new biocontrol tool, after thorough field testing, might be used as part of an integrated strategy to restore native rangeland vegetation and maintain the community’s resiliency. A possible process for a cheatgrass-infested parcel might go something like this: burn off the foliage in the spring, before it has a chance to make seed; then apply the new biocontrol product to kill the dormant seeds; spray herbicide as needed to kill any remaining plants; let the site lie fallow for a year, ensuring that the fungus has died out and the cheatgrass is gone; then in the fall, as the rains are beginning, seed or plant the area with the best suited natives—wheatgrass and ricegrass, needlegrass and bluegrass, fescue and squirreltail, globemallow, lomatium, lupine, penstemon, buckwheat, balsamroot, hawksbeard, sweetbush, and brittlebush; and use fungicide-treated seeds if necessary to dispel any lingering fungal effects.

Biocontrol in History

Biological control of weeds has a long and mostly successful history, according to the Australian biologist Rachel E. Cruttwell McFadyen. The predominant biocontrol method—what McFadyen calls classical biological control—has been the importation of exotic insects, mites, or pathogens to attack a problem weed. A widespread example of classical biocontrol in the western valleys of the Pacific Northwest is the release of cinnabar moths in pastures infested with tansy ragwort, an exotic weed of the Asteraceae family. The moths lay their eggs on the plant, and the larvae feed on the flowers and young foliage.

Meyer’s team is exploring an alternative biocontrol method, called the augmentative or inundative approach, in which the goal is to increase the abundance of a naturally occurring pathogen or pest to a level that achieves adequate control of the target weed. The use of fungi as bioherbicides has been much explored in theory. However, few fungal products have been brought to market, and none have been introduced that target weed seeds.

Developing a commercial biocontrol agent is expensive and time consuming. A new product must not only be

effective, but it must satisfy a host of safety concerns. In particular, scientists have to be certain that the agent—especially if it’s imported from somewhere else—will not spread to unintended hosts and become an invasive problem of its own.

While the risks of biological control are real, they have often been overstated, McFadyen writes. Most agents are host specific or nearly so. Any damage that has been caused by currently approved biocontrol agents, she asserts, has been minor, and is far outweighed by their benefits in controlling problem weeds and reducing the need for chemical herbicides.

P. semeniperda is not an exotic species, but part of *Bromus*’ natural ecology, and high virulence seems to be an evanescent mutation that sooner or later fades, imposing a natural threshold. “This type of biocontrol doesn’t involve introducing an exotic enemy to attack cheatgrass,” says Meyer. “Rather, we’re giving a leg up to a pathogen that’s already there. And our studies suggest pretty clearly that the virulent strains we’d be using are the ones that would tend to naturally self-destruct after they complete their mission.”

Additional Key Findings

Here are other findings from Meyer and her team:

- ▶ In experimental prescribed burns, *P. semeniperda* survived higher temperatures than cheatgrass seeds. However, fires rarely became hot enough to completely kill off either one; although, burning reduced the number of viable seeds.
- ▶ *Pseudomonas fluorescens* D7, a root-colonizing bacterium that inhibits growth in cheatgrass, did not enhance the effects of *P. semeniperda* when the two were applied simultaneously. In fact, some combinations showed a weaker effect than that of *P. semeniperda* alone.
- ▶ Many more pathogen-killed seeds were found on sites with a heavy cover of cheatgrass litter, suggesting that dead cheatgrass leaves and stems can harbor viable *P. semeniperda* inoculum.
- ▶ *P. semeniperda* spores are present not only in the seed bank but are also dispersed from the soil onto the covering structures of cheatgrass seeds still on the stalk. The highest inoculum levels on undispersed seeds were found on the driest sites and sites with high levels of inoculum in the soil. The seed covering is probably a vector transmitting the fungus, along with the seeds, into new territory.
- ▶ Sometimes large cheatgrass monocultures in the Great Basin experience “die-off,” in which the grass cover fails to establish. The cause remains a mystery. The density of seeds killed by the black fingers pathogen was the same or lower in these areas than in adjacent healthy cheatgrass stands, and the fungus was no more virulent. The researchers are using their seed bank data to investigate the die-off phenomenon further.

“At that point,” says Meyer, “if you’ve done it right—and especially if you’re blessed with a couple of good moisture years—you should have near-complete control.”

Any new commercial product, of course, will add expense to an already costly enterprise. “But if it works,” Meyer says, “it will be worth it. Seeding is expensive, and you can’t know in advance if it’s going to be successful. If this little fungus could improve our odds of success by, let’s say, a factor of 10, it could prove to be a very, very valuable tool.” The JFSP has funded additional research by Meyer and her team to further explore the use of *P. semeniperda* as a biocontrol for cheatgrass (JFSP Project No. 11-S-2-6).

Suggested Reading

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Web Resources

Cheatgrass Biocontrol (the Meyer team’s website).

www.cheatgrassbiocontrol.org/index.html

GU Cheatgrass Research a ‘Hot’ Topic in News.”

Gonzaga University. www.gonzaga.edu/News-and-Events/newsdetail-print.asp?PrinterFriendly=true&EventID=3113

SageSTEP: Sagebrush Steppe Treatment Evaluation Project. www.sagestep.org