Pseudomonas spp. as Bio-herbicides Is this a fit for Wyoming?

Kyle N. Race, University of Wyoming Masters Student
William Stump, University of Wyoming Assistant Professor, Plant Sciences, Extension Specialist of Plant Pathology
Daniel R. Tekiela, University of Wyoming Assistant Professor, Plant Sciences, Extension Specialist of Invasive Plant Ecology

There has been considerable interest and scientific research looking into the use of certain *Pseudomonas* species and their respective strains as bio-herbicides for almost three decades now.

Pseudomonas is a large genus of bacteria that contains at least 191 different species and is characterized as having an outer membrane that provides protection against toxic compounds, including antibiotics like penicillin.

Various studies have been conducted; from a systematic approach in selecting soil bacteria to manage winter annual grasses, to using *P. syringae pv. phaseolicola* a well-known plant pathogen of the common bean (*Phaseolus vulgaris L.*) to control kudzu (*Pueraria lobate*) in the southeastern United States, to controlling green foxtail in Canada with a different strain of *P. fluorescens*. Recent work found additional strains that suppressed cheatgrass (*Bromus tectorum*) over a long-term study. Bio-herbicides like *Pseudomonas* spp. have been proposed to be an additional tool for Wyoming land owners when it comes to controlling invasive species. This fact sheet is to better understand *Pseudomonas* spp. in the context of weed control and determine if they may be utilized in Wyoming.

The way *Pseudomonas* spp. works on plant species, which scientists call the 'mode of action', depends on the specific species. In the kudzu example, *P. syringae* has a mode of action that is the actual parasitic infection

Identifying times that meet the correct conditions to apply *P. fluorescens* can be difficult in Wyoming. During this research the application of *P. fluorescens* was applied successfully.

UW College of Agriculture and Natural Resources Extension

of the invasive plant. It is hypothesized that secondary compounds produced after the colonization of the root tips of cheatgrass (*Bromus tectorum*) inhibit their growth when the *P. fluorescens* D-7 strain was used in the cheatgrass suppression study. The exact compounds and mode of action that cause this bio-herbicidal activity are still not known according to a Canadian study that used the *P. fluorescens* BRG 100 strain to control the invasive green foxtail.

Current uses

The most relevant use of *Pseudomonas* spp. for current invasive weed issues in Wyoming is the use of *P. fluorescens* to control invasive annual grasses. Recent studies from Washington and Idaho showed that particular strains of *P. fluorescens* significantly reduced cover of cheatgrass, medusahead (*Taeniatherum caput-medusae*) and jointed goatgrass within a single year and reduced cover to nearly zero by five years while not impacting over 100 other plant species.

Psudomonas syringae pv. *phaseolicola* was used in Alabama to control kudzu, a problematic invader of the eastern U.S. Although short-term injury was observed in the form of stunted growth, within the growing season treated plants were indistinguishable from the non-treated plants. No control in terms of reduction of the target invasive plant species was realized.

In a controlled environment study, *P. fluorescens* BRG100 applied to green foxtail (*Setaria viridis*) seedlings resulted in reduced root lengths of 73 to 79 percent compared to the non-inoculated control. This study investigated the potential of formulating the *P. fluorescens* organism in what the authors referred to as a pesta formulation (a wheat gluten-based product). Pesta formulation is frequently used with fungal bio-pesticides and found to be effective in the delivery and subsequent colonization of *P. fluorescens* BRG100 on green foxtail roots. These findings concluded that *P. fluorescens* BRG100 has considerable potential as a bio-herbicide.

Pseudomonas spp. has also been used to suppress aquatic organisms and pathogenic fungi. Zebra and Quagga mussels (*Dreissena polymorpha*, *Dreissena bugensis*) are two invasive species that rapidly reproduce and have caused monetary damages to aquatic infrastructure and greatly increased management costs to agencies across 23 eastern states in the U.S. Current control methods for these species of mussels, such as chlorinating water, pose an environmental concern. This has led to an effort to develop an environmentally friendly bio-pesticide using the Pf-CL145A strain of *Pseudomonas fluorescens*, a common soil inhabitant all over the world. Out of ten strains of *P. fluorescens* tested, the Pf-CL145A strain showed the most toxicity to the target mussel species without showing effects on other aquatic species. The dead bacterial cells act as a capsule for the toxin which the mussel's intake through filter feeding. Once the toxins enter the mussel's gut, it destroys the digestive system. This bio-pesticide can only be used in enclosed structures and is not designated to be used to control the invasive bivalves in entire lakes or river systems.

Specific strains of *Pseudomonas* spp. can also be utilized for post-harvest disease management. There are many opportunities for fruits and vegetables to become damaged and infected by decaying organisms before it reaches consumers after harvest. These post-harvest diseases can result in economic losses in transport, storage and unappealing rotting produce for the consumer. Fungicides such as imazalil (Freshguard ™) and thiabendazole (Mertec®) are used to manage fungal decay organisms, either as direct applications to produce or incorporated in produce wax coatings. As an alternative, bio-pesticides that use *Pseudomonas syringae* (Bio-Save 10 LP® and Bio-Save 11 LP®) and *Pseudomonas fluorescens* (BlightBan® A506) are applied post-harvest to prevent decay causing organisms from damaging produce.

Bio-Save 10 LP® can be effectively used on cherries to suppress decay from blue mold (*Penicillium expansum*) and gray mold (*Botrytis cinerea*). It's also used on apples and pears to suppress blue mold, gray mold and mucor rot (*Mucor piriformis*). This bio-pesticide is used on potatoes to control tuber decay caused by dry rot (*Fusarium sambucinum*). Bio-Save 11 LP® is used to suppress Rhizopus soft rot decay (*Rhizopus stolonifer*) in sweet potatoes. BlightBan® A506 is used to reduce frost and frost damage on cherry, apple, pear, almond, peach, tomato, potato and strawberry. It is also used to prevent fire blight and fruit russeting in apples and pears.

The MB906 strain of *P. fluorescens* is sold as a liquid soil inoculant by Biowest Ag Solutions that when applied to soil, may increase biodiversity. The label makes no claims for weed suppression and clearly states that it may enhance biodiversity. There is no evidence to support these claims or to disprove them in this region. Theoretically, increasing the biodiversity of the soil community may make it resistant to more pathogens and increase overall soil health, but there is no solid evidence of this happening in our region.

Possible benefits

Bio-herbicides could allow growers to rely less on traditional chemical herbicides, which could reduce or eliminate the development of herbicide resistance. Additional benefits include decreasing off-target effects and soil residues. Because some chemical herbicides have residual effects, which limit crop rotation options, using a bio-herbicide can result in increased crop rotation diversity in production systems. Chemical herbicides also have the potential for non-target plant damage, especially in natural areas and rural landscapes. Increased selectivity would decrease potential off-target impacts although Kennedy showed harm to several desirable plant seedlings in the lab.

Current challenges

Little information exists about the efficacy of Pseudomonas spp. in Wyoming for challenges such as annual grass control, therefore, it is difficult to know if any current products could be effective management solutions. Although recent studies have reported promising long-term control of cheatgrass in the Pacific Northwest, it is not known if the same results will be seen in Wyoming's climate and more generally the intermountain West.

Cheatgrass control is only effective when *Pseudomonas* spp., *P. fluorescens* remains alive in the soil. Application conditions, weather and climate may play an important role in its success. D7 strain applications require maximum daily air temperatures below 50 F and imminent precipitation to safely incorporate into the soil. These conditions are not always common in the fall, when most cheatgrass management occurs, and may force applicators into a narrow window between being too dry or having equipment freeze if too late. Some challenges include avoiding non-target effects, the overall survival and efficacy of microorganisms out in the field, delivery and storage methods, interactions with chemical herbicides, EPA regulations and mass production.

Even in ideal conditions, bio-herbicides have been reported to take longer to be effective when compared to other synthetic chemical herbicides. In a long-term field study visible suppression did not occur until two years after application but up to 90 percent weed decline was observed after six years when combined with seeding of competitive grasses. In these cases, if bio-herbicide applications failed due to application conditions, the results would not be known until two years later, meaning follow-up management would be delayed.

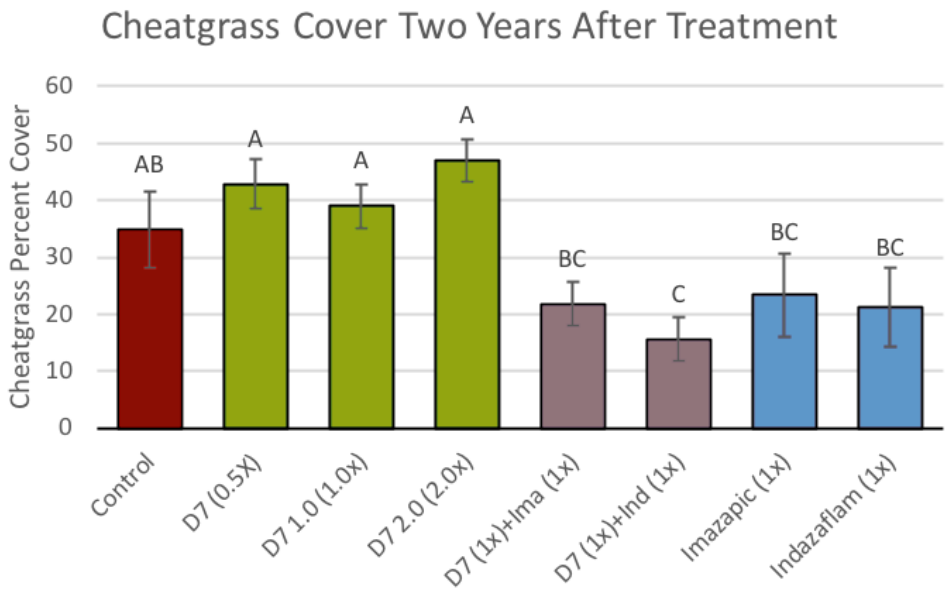

Figure 1. Cheatgrass percent cover two years after treatment. Ima is Imazapic, Ind is Indaziflam. Bars that share the same letter are not significantly different.

Some *Pseudomonas* spp. can act as human pathogens and must be extensively vetted if developed as bio-control products. The most common species of the genus that causes human infection is *Pseudomonas aeruginosa*. In healthy people, infection is usually only a skin rash or ear infection. In patients that have pre-existing illness or weakened immune systems, these infections can cause serious injury or even death.

Wyoming field test of *P. fluorescens* cheatgrass control

To test the effect of *P. fluorescens* on cheatgrass, the EPA labeled product D7 was applied in three separate locations in Wyoming in the fall of 2016. D7 was applied following the recommended rate (1 oz/acre), half rate and double rate; and labeled suggestions of below 50 F air temperature daily maximum and preceding a precipitation event. Additionally, two common cheatgrass control synthetic products, imazapic (Plateau®) and indazalflam (Esplanade 200SC ®) were applied at labeled rates either alone or in combination with D7 to identify possible synergies. In the summer of 2018, approximately two years after treatment, plots were monitored for the percent cover of cheatgrass. Plots treated with D7 alone had consistently greater cheatgrass than those treated with either imazapic or indaziflam. Additionally, the combination of synthetic and bio-herbicides did not significantly increase the level of cheatgrass reduction (Figure 1). These results suggest D7 alone or in combination with other products is ineffective at least for the first two years after treatment.

References

Caldwell, C. J., Hynes, R. K., Boyetchko, S. M., & Korber, D. R. (2012). Colonization and bioherbicidal activity on green foxtail by *Pseudomonas fluorescens* BRG100 in a pesta formulation. *Canadian Journal of Microbiology*, 58(1), 1–9. https://doi.org/10.1139/w11-109

Gurusiddaiah, S., Gealy, D. R., Kennedy, A. C., & Ogg, A. G. J. (1994). Isolation and characterization of secondary metabolites from Pseudomonas flourescens-D7 for control of Downy Brome (Bromus tectorum), 42(March), 492–501. https://doi.org/10.1002/hlca.201500032

Kennedy, A. C. (2018). Selective soil bacteria to manage downy brome, jointed goatgrass, and medusahead and do no harm to other biota. *Biological Control*, *123*(May), 18–27. https://doi.org/10.1016/j.biocontrol.2018.05.002

Orloff, N. (2017). Weed Suppressive Bacteria for Cheatgrass Word Scramble : Test your knowledge of weed suppressive bacteria for cheatgrass, (March), 1–2.

Stubbs, T. L., & Kennedy, A. C. (2012). Microbial Weed Control and Microbial Herbicides. *IntechOpen*. http://dx.doi.org/10.5772/32705

Zidack, N. K., & Backman, P. A. (1996). Biological Control of Kudzu (Pueraria lobata) with the Plant Pathogen Pseudomonas syringae pv. phaseolicola. Weed Science, 44(3), 645–649.

UW College of Agriculture and Natural Resources Extension

B-1337 • April 2019

Editor: Katie Shockley. Graphic design: Tanya Engel.

Issued in furtherance of extension work, acts of May 8 and June 30, 1914, in cooperation with the U.S. Department of Agriculture. University of Wyoming Extension, University of Wyoming, Laramie, Wyoming 82071.