Washington Grain Commission
Wheat and Barley Research Annual Progress Report / Final Report

Project # 3019-5599

Progress Report Year: Third Year (2014-2015) of Three Years (2012-2015) **FINAL**

Title: Suppression of Downy Brome (Cheatgrass) in Wheat Using a Soil Bacterium

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Deliverables:

1. Added data collected over the past three years on the bacteria application in winter wheat to the EPA registration packet that will be submitted to EPA by ARS in January 2015. The bacterial bioherbicide should be available to growers by the fall of 2016.
2. Filed patent for the weed-suppressive bacterium (*Pseudomonas fluorescens* strain ACK55 in May 2014.
3. Scheduled to file a patent on another weed-suppressive bacterium in spring 2015.
4. Published the manuscript: Stubbs, T., Kennedy, A.C., Skipper, H. 2014. Survival of a rifampicin-resistant *Pseudomonas fluorescens* strain in nine Mollisols Applied and Environmental Soil Science ID306348, 7 pages http://dx.doi.org/10.1155/2014/306348
5. Obtained EPA Registration for our weed-suppressive bacterium (*Pseudomonas fluorescens* strain D7 in August 2014. The company who obtained the registration is Verdesian from Cary, North Carolina. We are not sure of the timeline or future of this bacterial bioherbicide.
6. Developed draft application and restoration documents for the use of bacterial bioherbicides in wheat and rangeland.

Executive Summary: Downy brome (cheatgrass) is a serious weed in wheat causing reductions in wheat yield even when only a few weed plants are present. Bacteria suppressive to downy brome have the potential to reduce downy brome populations for several years and reduce viable seed in the seed bank. If a competitive crop is maintained, downy brome will be limited and annual applications of grass-weed herbicides should not be necessary. Our objectives are to: 1.) Evaluate the impact of downy brome suppressive bacteria with or without herbicide to reduce downy brome and improve winter wheat yields; and 2.) Optimize the delivery of the bacteria for maximum downy brome reduction and increased winter wheat growth and yield. We established studies in wheat fields each fall from 2011 through 2014 to determine the effect of these weed-suppressive bacteria on downy brome populations at more than nine Washington sites with wheat in rotation. At each site, for each year, the bacteria were applied in plots designated as multiple year application plots at same sites as well as on new adjacent land. We monitor the plants in those plots in the spring of the year following application (6 months) and the subsequent springs as well. Over all sites after 3 years, the bacteria reduced downy brome an average of 89% in winter wheat studies. Application of the bacteria resulted almost complete

suppression of downy brome by 5 years, when winter wheat was in rotation or cereals continuously grown (Figure 1). Multiple year application did not necessarily increase the speed of downy brome reduction. The bacteria inhibited downy brome at most of the sites within 6 months of application, but multiple applications did not always result in greater weed reduction in the second spring. Vegetation, soil, climate, bacteria survival, and wheat growth are factors controlling the bacteria's ability to reduce downy brome and we are still analyzing the data on that part of the study.

Bacterial Information

These weed-suppressive bacteria:

- are applied in the in the fall and establish in the soil microbial community as weather cools;
- inhibit radicle formation, root growth and tiller initiation of these weeds;
- do not hurt native plants or crops;
- grow well in fall and spring coinciding with the early root growth of the fall annual weeds; and
- grow down roots and deliver the weed-inhibitory compound.

The bacterium, *Pseudomonas fluorescens* strain ACK55 (*P.f.* ACK55), inhibits only:

- cheatgrass (downy brome, *Bromus tectorum*),
- medusahead (*Taeniatherum caput-medusae)* and
- jointed goatgrass *(Aegilops cylindrica).*

Pseudomonas fluorescens strain ACK55 does not inhibit any economically important plants nor does it injure any native plant species found in the United States. Replicated field plots ($3m^2$ to 10A size) across many different years, and locations have been established by several collaborators. These field studies consistently show a minimum of 50% reduction in downy brome within three years of one bacterial application. In long-term field trials of quarter acre size in Washington, application of the bacteria resulted almost complete suppression of downy brome in 5 years, when winter wheat was in rotation or cereals continuously grown (Figure 1). Additional applications of the bacteria may be needed in 3 to 6 years if downy brome or weed-laden soil is transported into the site. With the reduction of downy brome, cereals are even more competitive and yields increased 5 to 30%. The bacteria suppress downy brome roots at a time when the weed is increasing its competitive root growth. Application of a herbicide in year 1 along with the bacteria increased the rate of downy brome reduction. In addition, no downy brome could be found in the seed bank three to seven years after a single application. Herbicides are able to kill the standing plant, but not able to work on the seed bank. The herbicide/bacterial interaction can rid a field of downy brome. These bacteria provide a novel means to reduce downy brome while limiting the need for tillage and chemical use for weed control.

The Toxicology/Pathology study by an independent lab showed that the weed-suppressive bacterium presents no mammalian toxicity or pathology. The EPA registration document for the one bacterium that inhibits cheatgrass, medusahead and jointed goatgrass will be submitted to EPA this month. The bacteria are being integrated into weed-management plans for croplands using both spray and seed coat technologies. We are developing rangeland restoration plans that include the bacteria. We have several research studies on the ground to test these plans. Other weeds that we have been studying are wild oats, Ventanta grass, bulbous bluegrass, rattail fescue, annual bluegrass, and several other emerging annual grass weeds. Because of its selectivity, this bacterium can be used in management of downy brome in rangeland, cropland, pasture, turf, sod production, golf courses, road sides and road cuts, construction sites, and right-of-ways (road, rail, pipeline, electrical).

Excerpts from a Potential Label
Active Ingredient:
***Pseudomonas fluorescens* strain ACK55**
Crop/Site: rangeland, cereal, pasture, turf, sod production, golf courses, road sides and road cuts, construction sites, and right-of-ways (road, rail, pipeline, electrical).

For selective suppression of the fall annual grass weeds cheatgrass/downy brome (*Bromus tectorum* L.), medusahead (*Taeniatherum caput-medusae* [L.] Nevski)**, and jointed goatgrass (*Aegilops cylindrica* L.) in rangeland, cereal, pasture, turf, sod production, golf courses, road sides, and road cuts, construction sites, and right-of-ways (road, rail, pipeline, electrical).**

ACTIVE INGREDIENT
0.23% *Pseudomonas fluorescens* strain ACK55; 2.6% spent medium; 97.2% water. *Pseudomonas fluorescens* strain ACK55 contains a minimum of 30 million cells mL^{-1}. This product is a naturally occurring *Pseudomonas* bacterium from soil, which selectively reduces or suppresses the growth of the weeds cheatgrass/downy brome (*Bromus tectorum* L.), medusahead (*Taeniatherum caput-medusae* [L.] Nevski), and jointed goatgrass (*Aegilops cylindrica* L.) and does not hurt crops or rangeland plants. It is a preemergent bioherbicide.

MODE OF ACTION
This bacterium specifically inhibits downy brome/cheatgrass and DOES NOT inhibit other grass and broadleaf crops and plants. The bacterium suppresses weed growth by the production of a labile secondary metabolite that inhibits root-cell elongation and tillering; reduces seedling vigor and overwintering; lowers seed production; and reduces viability of the seed bank.

Once applied to the soil surface the bacterium can be carried into the soil by rain or irrigation. The bacteria grow well on residue, seeds, and roots. The bacteria move to the roots of the target weeds, seeds, or young seedlings and inhibit root-cell elongation. The suppressive compound inhibits lipopolysaccharide production in the cell wall and membrane and reduces root-cell wall elongation. If this inhibition of cell elongation occurs early in the seedling life, tiller initiation can be reduced. Visual effects of the bacteria working are a red color of the plant leaves due to stress and anthocyanin production, stunted plants with few tillers, and few seeds produced. With application of the weed-suppressive bacterium, the soil seed bank is reduced. The bacteria need to establish in the soil and on roots for suppression. The suppression of cheatgrass, medusahead, and jointed goatgrass by this bacterium may take two to five years. Dry conditions do not allow the bacteria to grow in the soil and colonize soil, residue, seed, and roots, and result in only minor suppression of the weed.

DIRECTIONS FOR USE

This product is a preemergent bioherbicide and should be applied in late fall. For best results, make initial application in the late fall when daily high temperatures are less than 55^{o}F and more than 0.2 inches of rain is imminent or will occur within 2 weeks. Activity may be low when applied to dry soil or heavy residue. Lack of rainfall within 2 weeks of application may reduce weed suppression. Application in late spring or summer will not allow the bacterium to establish in the soil and will result in low weed suppression.

When sprayed on the soil surface or coated on seed, this product will suppress the growth of downy brome/cheatgrass, medusahead or jointed goatgrass over time and is not likely to harm other plant species. Apply 1 pint of material or the equivalent of 40 billion cells acre^{-1}) in 100 or 400 gallons of water (5 to 20 gallons acre^{-1}) with mixing and spray the solution on the soil surface. Optimum conditions for application are cool air temperatures ($<50^{o}$F) and wet conditions (0.25 inches of rain). Hot and dry conditions and dry soil surface limit the effectiveness of the application. For best results, apply the bacterium in the fall or very early spring before annual grass weed seed germination and when day-time temperatures are below 50^{o}F.

The coverage characteristics of the spray equipment will determine the volume of water needed. Use 5 to 30 gallons of solution acre^{-1} for conventional tillage or direct seed application. If there is dense vegetation or residue use 20 to 50 gallons acre^{-1} of spray solution. Once mixed with water use immediately.

APPLICATION METHODS
Select one of the application methods below.
Method 1: Seed
For cheatgrass, medusahead, or jointed goatgrass suppression, use 0.25 to 1 gallons of liquid acre^{-1} or a minimum of 40 billion cells acre^{-1}. Apply bacteria to seed in 16 oz. of cells for 60 lb. seed rotating in a drum. Direct drill or broadcast seed at recommended seeding rates, which should be 60 lb. acre^{-1} wheat seed or 30 lb. acre^{-1} native seed. Species of seed can be crop or native plant or other species and drilled or broadcast.

Method 2 Liquid Spray
For cheatgrass, medusahead, or jointed goatgrass suppression, use 0.25 to 1 gallons of liquid acre^{-1}. For 20 acres, apply 5 to 20 gallons of liquid acre^{-1}. Final minimum concentration would be 10 million bacterial cells square foot soil surface^{-1}. The liquid spray can be applied by back pack sprayer, ground sprayer or aerially applied.

APPLICATION INSTRUCTIONS
GENERAL: Avoiding spray drift at the application site is the responsibility of the applicator. The interaction of many equipment- and weather-related factors determines the potential for spray drift. The applicator and the grower/treatment coordinator are responsible for considering all of these factors when making decisions. Where states have more stringent regulations, they should be observed. Note: This section is advisory in nature and does not supersede the mandatory label requirements.

GROUND: Be sure to maintain agitation during mixing and application to assure uniform product suspension. Thorough coverage of the soil surface is essential for effective disease control. *Pseudomonas fluorescens* strain ACK55 can be applied with commonly used ground equipment, including hose-end, pressurized, greenhouse and hand-held sprayers. To achieve good coverage, use proper spray pressure, gallons acre^{-1}, nozzles, nozzle spacing and ground speed. Consult spray nozzle and accessory catalogues for specific information on proper equipment calibration.

AERIAL: This product can be applied by aerial application. Refer to the Aerial Drift Reduction Advisory Information section of this label for general directions and precautions. Use the application rate indicated for the appropriate crop in sufficient water to achieve thorough coverage, typically between 3 to 20 gallons of water acre^{-1} depending upon the crop. Three gallons of water acre^{-1} is the minimum.

CHEMIGATION: This product can be applied through sprinkler (center pivot, lateral move, end tow, side (wheel) roll, traveler, solid set, and hand move) or drip type irrigation systems. Refer to the Chemigation Directions for Use section of this label for general directions and precautions.

COMPATIBILITY

Do not tank mix with toluene or surfactants that are used to reduce microbial growth. This product is compatible with most herbicides and bioherbicides. Adjuvants and surfactants may reduce the viability of the bacteria.

STORAGE AND DISPOSAL

Product must be stored in the original container at temperatures less than 60^{o}F. Store the container unopened until ready to apply. Do not contaminate water, food, or feed by improper storage and disposal.

CONTAINER DISPOSAL

Do not reuse or refill the container. Dispose of empty container. Material can be sanitized with a 1:10 solution of bleach or caustic material to water or steamed or autoclaved to kill any remaining active bacterial cells.

Management Guidelines

Application guidelines and management systems are being constructed for wheat and rangeland systems.

1. **Apply the bacteria in that fall when the air temps are below 50^{o}F with rain or rain in forecast (mid October to mid November).**

2a. Treatment - An early fall application of a herbicide to get rid of any cheatgrass that germinated with late summer or early fall rains.

2b. Why-The bacteria will not inhibit the fall germinated plants. This bacterium is well suited for application after burns because the soil is exposed and the bacteria can fall onto the soil instead of the residue.

3a.Treatment - A light harrow before or after the herbicide might be beneficial.
3b. Why- to expose more seed to germination (before) and disturb the residue to expose more routes for the bacteria to enter the soil (after). Disturbing the residue or a burn can also increase the amount of weed seed germination, which is both good and bad, the residue is reduced, but the volume of seeds that germinate might be large, too large for the bacteria contact and inhibit.
4a. Treatment- Drill seed desirable plant species.
4b. Why - Desirable plant species are needed to compete further with the weed plants stressed by the bacteria.
5a. Treatment- In the spring of each year, check for broadleaf weeds and use herbicide as needed. May need to spot spray, if reasonable, to reduce the broadleaf weeds but not kill desirable broadleaf plants.
5b. Why – As cheatgrass is eliminated, voids are created, other weeds will grow in those voids.
6. Treatment - If fall germinated grass weeds are still a problem the next fall, then another application of a grass weed herbicide may be needed.
7. Treatment - Walk the land each month to see what weeds are appearing and spray when appropriate.

Delivery Systems
Delivery systems showed mixed results in cheatgrass reduction. Winter wheat coated with the bacteria and then seeded did not reduce the down brome in the first spring, but downy brome populations were reduced to near zero in subsequent years. Bacteria applied in pellets did not reduce weed numbers sufficiently in these studies and the bacteria did not spread through the soil. From these field studies, surface characteristics, laboratory analyses and weather data, we will determine the effects of interactions among bacteria, surface cover, soil disturbance, soil type, weed seed bank, seed coating, and winter wheat growth and yield on inhibition of downy brome. This research illustrates the use of these bacteria in weed management systems and hastens product development and lead the way for other weed-suppressive bacteria.

I**mpact:** Downy brome is a winter annual grass weed that thrives in all soils, is very competitive, and has a negative effect on crop growth. Its mat-like rooting system and ability to grow further into the winter than wheat allows the weed to extract much of the soil water and nutrients before wheat produces its roots in the fall. Downy brome is conservatively estimated to cost growers over 400 million dollars annually. Herbicide costs that reduce the growing downy brome, but not the seed bank can be well over \$60 $A^{-1}yr^{-1}$. Bacteria native to Washington soils reduce downy brome populations over a few years and reduce viable seed in the seed bank. If a competitive crop is maintained, downy brome will be limited and annual application of grass-weed herbicides should not be necessary. Additional field information is needed on the best methods to apply these bacteria to inhibit downy brome in wheat. Delivery methods such as seed coating, pelleting or encapsulation and raking are potential means to increase bacterial survival and are being studied.

These bacteria have the potential to reduce invasive weeds, improve winter wheat yields and restore western landscapes while reducing tillage and chemical use in crop production (5yr goal). The research provided information on these bacteria in cropland situations and hastened registration by the federal government and EPA approval of this bioherbicide. This research assisted the commercialization process for cropland use with solid scientific data on application methods and risk assessments. A long-range goal (10yr+) for this research is to pave the way for additional bioherbicides against emerging weeds such as Ventenata grass, rattail fescue, Italian ryegrass and feral rye.

Outputs and Outcomes: WGC project number: 3019-5599

WGC project title: Suppression of Downy Brome (Cheatgrass) in Wheat Using a Soil Bacterium

Project PI(s): Ann C. Kennedy

Project initiation date: July 1, 2012 Project year: 2014-2015

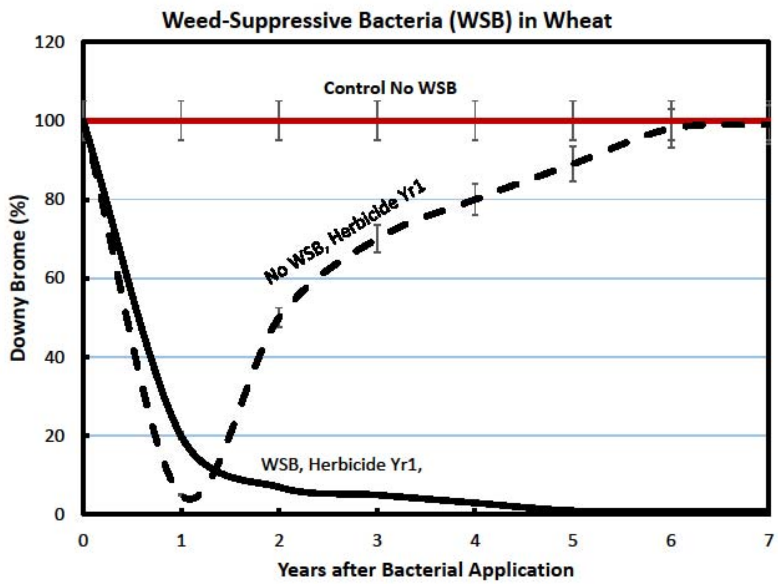
Objective 1: Evaluate the efficacy of downy brome suppressive bacteria with or without herbicide to reduce downy brome and improve winter wheat yields;

Objective 2: Optimize the delivery of bacteria for maximum downy brome reduction and increased winter wheat growth and yield.

Objective	Deliverable	Progress	Timeline	Communication
Obj.1: Downy brome suppression by the bacteria				
Field application	Information on the efficacy of downy brome-suppressive bacteria on downy brome populations.	Weed-suppressive bacteria were applied to 9 locations in the fall of 2011,2012,2013,2014 and again at same sites and new adjacent land in the fall of 2012, 2013, and 2014.	We found significant reductions in downy brome at each site over time. Data will be collected from each plot in spring and harvest 2015 and continue with monitoring in subsequent years to obtain a full data set over 3 more years.	Manuscript published: *Stubbs, T.*, Kennedy, A.C., Skipper, H. 2014. Survival of a rifampicin-resistant *Pseudomonas fluorescens* strain in nine Mollisols Applied and Environmental Soil Science 7 pages http://dx.doi.org/10.1155/2014/306348 Field data were added to the documents needed for EPA registration that will be submitted by the IR4 group who will submit the packet to EPA in January of 2015.
Monitoring of survival of applied bacteria	Data indicating the survival of the bacteria in different soils and various climatic conditions.	We monitored the bacterial populations over time. The bacteria are in soil at sufficient numbers to cause downy brome inhibition (> 10^8 cells per g soil).	We will continue to monitor the weed-suppressive bacteria in soil over time.	BLM pesticide certification classes Albuquerque, NM (2/26/14) and Boise ID (4/2/14); NRCS CIG, Pullman, WA (5/2/14).
Soil analyses	Characterization of each soil at each site and climatic data.	We are characterizing the soil at each location and have completed the analyses. The soils are silt loams and other characteristics will be analyzed using multivariate analyses not yet completed.	We collected soil and climatic data in 2011, 2012, 2013, and 2014 to characterize soil quality. We will continue to sample and analyze soil in the plots to monitor any changes in the soil for 3 more years.	Presented seminars on Weed-Suppressive Bacteria at Lind Field Day (6/12/14); Worley, ID (7/14/14); Ag Tech, Pullman, WA (7/16/14); Baker City, OR (8/22/14); Monument OR (9/29/14); Baker City, OR (9/30/14);

Cheatgrass reduction	Reduction in cheatgrass in the field.	The bacteria reduced cheatgrass an average of 89% in winter wheat studies. At all sites we found significant reductions in cheatgrass	We will continue to monitor the cheatgrass populations in these plots for 3 more years to follow the effect of the bacteria on cheatgrass populations.	Presented seminars on Weed-Suppressive Bacteria at Idaho Department of Transportation Idaho Falls, ID (11/28/14); SGI Risk Assessment webinar (2/5/14). In 2014, we presented information to over 500 growers, producers or land managers.
Obj. 2: Delivery of bacteria to soil and downy brome roots				
Spray	Survival and efficacy of downy brome-suppressive bacteria when sprayed on the soil surface.	We applied the bacteria as spray at 9 locations in 2011, 2012, 2013, and 2014.	Data will be collected in each plot each spring (downy brome) or at harvest (wheat growth and yield) for another 3 years past this funding.	Met with producers and land managers throughout the year to discuss the use of weed-suppressive bacteria as a spray.
Seed treatment	Survival and efficacy of downy brome-suppressive bacteria when coated on seed and drilled.	Bacteria were applied as a seed coat and drilled at three locations. Reduction in cheatgrass was not evident the first year.	Next fall we will coat seed with the bacteria and drill in plots. Data from plots in which seed was coated with the bacteria will be collected in each plot each spring and at harvest for the next three years past this funding.	Met with producers, grain elevator personnel, ag industry, and land managers throughout the year to discuss the use of weed-suppressive bacteria as a wheat seed treatment.
Pellets	Survival and efficacy of downy brome-suppressive bacteria when incorporated into a protective pellet and broadcast.	We impregnated pellets with the bacteria. The pellets released the bacteria into the soil directly under the pellet but no movement occurred.	We terminated this area of research as the bacteria were not distributed into the soil when pelleted.	Discontinued

Figure 1. The reduction of downy brome by weed-suppressive bacteria in winter wheat fields in Washington. Data are means of 5 replicates at each location and 9 locations.



Budget

Amount allocated by the Commission in fiscal year 2015 (July 2014 to June 2015): $ 34,191.

Amount requested for FY 2016 (July 1, 2015-June 30, 2016) $0.

Category	2012-2013	2013-2014	2014-2015	Total
Salaries[1] – 00	9,918	10,315	**10,315**	30,548
Wages[2] - 01	5,760	5,990	**5,990**	17,740
Goods/Services[3] - 03	5,730	5,959	**8,498**	19,187
Travel[4] - 04	5,950	5,950	**5,950**	17,850
Benefits[1,2] - 07	4,700	4,438	**4,438**	14,027
Total Direct Costs	32,058	33,103	**34,191**	99,352

[1] Part-time skilled personnel for field data collection $35/hr; benefits at 40%.

[2] Undergraduate wages for lab and field work $10/hr,; benefits at 2.0%

[3] Field and lab supplies, field monitoring, soil analyses.

[4] Travel to field sites for applications and monitoring, field ($0.50 per mile), lodging and MIE ($146/day).

Budget approved by Jason Croyle 12.18.2013.