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Scale-up Trials Using Weed Suppressive Soil Bacteria in Rangeland Restoration – Design, Methods, and Implementation: An Experts' Workshop

Technical Memorandum

October 2015

Lara M. Aston
Alicia M. Gorton

U.S. DEPARTMENT OF ENERGY
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Scale-up Trials Using Weed Suppressive Soil Bacteria in Rangeland Restoration – Design, Methods, and Implementation: An Experts' Workshop

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Prepared for
the US Fish and Wildlife Service
under Contract F14PG00086

Pacific Northwest National Laboratory
Richland, Washington 99352

Executive Summary

The US Fish and Wildlife Service (USFWS) and Bureau of Land Management (BLM) have assembled an interagency/entity team to accomplish careful evaluation of efficacy, non-target impacts, and feasible techniques needed to deliver *P. fluorescens* as a biopesticide for use in the control of *Bromus tectorum* (cheatgrass), *Aegilops cylindrica* (jointed goatgrass), and *Taeniatherum asperum* (medusahead) at a landscape scale. As a first step toward accomplishing this goal, a workshop was convened by USFWS. Pacific Northwest National Laboratory (PNNL) was contracted to organize, facilitate and prepare a summary report. The goals of the workshop were to 1) review the available body of knowledge on *P. fluorescens* and 2) discuss and evaluate potential issues of scale-up applications of the biopesticide. The invited participants included representatives from federal agencies, university staff, PNNL, and county governments with established expertise in rangeland restoration and soil amendment use.

The group met for two days (August 4th and 5th, 2015) at the USFWS Region 6 office in Lakewood, Colorado. The scope of the workshop was to identify key questions and research needs required to implement the use of a weed suppressive bacteria (WSB) as a land management tool on a regional to national landscape-scale. The workshop was designed to provide a mechanism for a coordinated dialog going forward on regulatory priorities, as well as application appropriate environmental research and monitoring techniques. Significant discussion focused on the following research gaps surrounding the use of *P. fluorescens* as a new land management tool: 1) the limited peer reviewed research, 2) the limited knowledge of the impacts to soil microbial communities, and 3) how to efficiently assess and monitor non-target impacts.

The participants also discussed the need for a more rigorous examination of efficacy and non-target impacts across geographic regions using smaller scale plots. The consensus of the workshop participants was that due to the lack of published data spanning multiple geographic regions where cheatgrass is a land management issue, the next step is to assess efficacy across a broad geographic area.

The two-day Experts' Workshop provided insight on the state of knowledge and understanding of the application of *P. fluorescens* as a weed suppressive biopesticide and new land management tool. The main recommendations are summarized as follows:

- Complete the literature review of application of *P. fluorescens*
- Investigate effects of product application on soil microbial communities
- Develop a user-friendly, centralized, data management system
- Develop a protocol that could be used across all Department of the Interior (DOI) land

Several tasks were identified at the conclusion of the workshop to carry the momentum forward.

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1.0 Introduction

The increase in non-native invasive annual grasses, primarily *Bromus tectorum* (cheatgrass), *Aegilops cylindrica* (jointed goatgrass), and *Taeniatherum asperum* (medusahead) have placed western arid landscapes in peril. Invasive annual grasses have increased fire frequency, which prevents the reestablishment of sagebrush and reduces or eliminates native forbs and grasses (Link et al. 2006). Fire is linked to accelerated loss of sagebrush-steppe habitat and corresponding population declines of greater sage-grouse and other sagebrush obligate species (USFWS 2014; Lockyer et al. 2015). There has been limited success of traditional mechanical and chemical efforts to treat invasive grasses over the past 50 years. Weed suppressive bacteria, *Pseudomonas fluorescens* (*P. fluorescens*), as a host-specific biopesticide is a new technology in the integrated pest management toolbox for invasive annual grass control. *P. fluorescens* is a naturally occurring, ubiquitous, non-pathogenic soil bacterium shown through testing required for registration by the Environmental Protection Agency (EPA), to have specificity for cheatgrass, medusahead, and jointed goat grass (*Aegilops cylindrical*) (Kennedy et al. 1991; Johnson et al. 1993; Tranel et al. 1993; Gurusiddaiah et al. 1994). Unpublished studies have also shown that the biopesticide does not have a negative effect on non-target species or resources such as native grasses and forbs; however, there is a lack of peer-reviewed literature documenting impacts to non-target species and resources at landscape-scales.

P. fluorescens could be a viable option to aid restoration of sagebrush habitat on National Wildlife Refuges and other areas where success remains constrained by limited effectiveness of current approaches, spatial cover, and other knowledge gaps in seedling establishment ecology. *P. fluorescens* could provide a cost-effective, scalable means to modify the seeding environment for desired species and boost success in post-fire restoration projects through reduction in invasive annual grass competition with native seedlings. Field studies of *P. fluorescens* thus far have primarily been conducted on small plots (< 10 acres) demonstrating cheatgrass inhibition (efficacy) and specificity (non-target effects); however, application methods that are scalable to larger landscapes have not been adequately developed due to annual acreage limits imposed by EPA regulations. One strain (D7) of *P. fluorescens* is registered with the EPA as a biopesticide, and the registration package for another (ACK55) was submitted in September 2015 for one other. The US Fish and Wildlife Service (USFWS) and Bureau of Land Management (BLM) have assembled an interagency/entity team to accomplish careful evaluation of efficacy, non-target impacts, and feasible techniques needed to deliver *P. fluorescens* at a landscape scale.

As a first step toward accomplishing this goal, a workshop was convened by USFWS. Pacific Northwest National Laboratory (PNNL) was contracted to organize, facilitate and prepare a summary report. The goals of the workshop were to 1) review the available body of knowledge on *P. fluorescens* and 2) discuss and evaluate potential issues of scale-up applications of the biopesticide. The 30 invited participants included representatives from federal agencies (Department of the Interior (DOI), EPA), university staff (University of Wyoming (UW), Washington State University (WSU)), PNNL, and local county governments with established expertise in rangeland restoration and soil amendment use (Figure 1, Appendix A). The group met for two days (August 4th and 5th, 2015) at the USFWS Region 6 office in Lakewood, Colorado. Many of the participants have had practical experience working with *P. fluorescens* in small scale experiments.

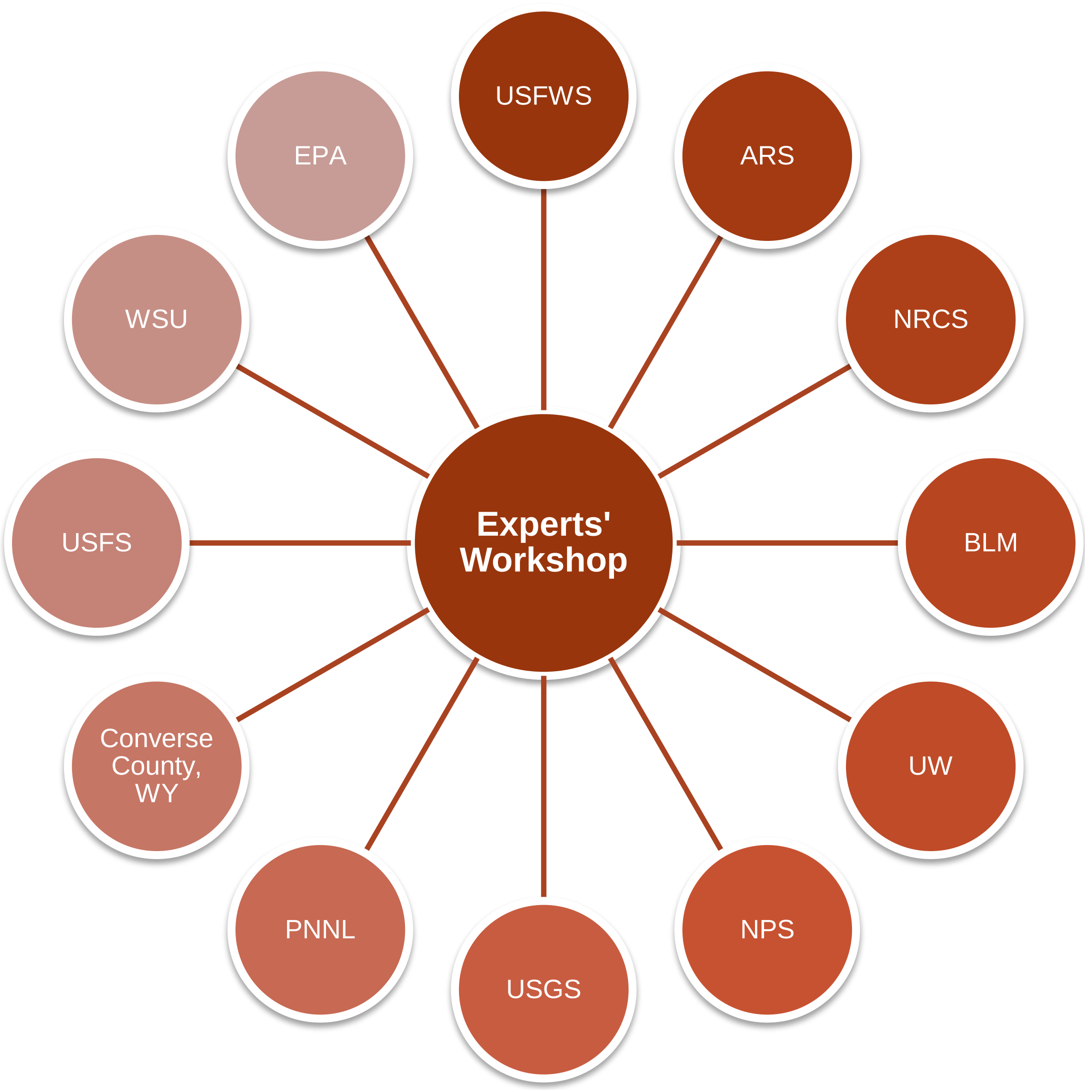

Figure 1. DOI Bureaus/Offices and Other Entities Participating in Experts' Workshop

1.1 Workshop Scope and Objectives

The scope of the workshop was to identify key questions and research needs required to implement the use of a weed suppressive bacteria (WSB) as a land management tool on a regional to national landscape-scale. The workshop was designed to provide a mechanism for a coordinated dialog going forward on regulatory priorities, as well as application appropriate environmental research and monitoring techniques. The workshop facilitated discussion of issues and questions that are regulatory priorities for scale-up and focused participation on developing approaches, methods, and tools that give resource agencies and industry a road map to implementation of this new land management approach in concurrence with stakeholders and the regulatory community.

The workshop had these objectives:

1. Gain a clearer understanding of the process involved in the development and registration of a soil borne organism as a biopesticide.

2. Identify potential sites where scale-up field work across the Great Basin landscape, as appropriate, can be implemented in order to increase understanding of *P. fluorescens* as a tool to control or reduce invasive annual grasses on federally managed lands.
3. Identify WSB projects that have succeeded or failed to date and identify other potential issues related to use of WSB.
4. Develop a draft framework for trials to implement use, including, but not limited to, site selection, application techniques, post-application monitoring, and data collection. Trials can inform future implementation plans.

1.2 Technical Memorandum Contents and Organization

Section 1.0 gives a brief introduction and background on *P. fluorescens* and states the workshop scope and objectives. Section 2.0 provides a brief description of each of the plenary presentations that were presented on the first day of the workshop. Section 3.0 summarizes the main topics discussed during the workshop, while Section 4.0 presents the overarching recommendations. Section 5.0 discusses the "next steps" following the workshop and completion of the Technical Memorandum.

2.0 Plenary Sessions

2.1 Background on biopesticide registration process at EPA

Mike Mendelsohn, Senior Regulatory Specialist in the Microbial Pesticides Branch at the EPA's Office of Pesticide Programs, gave the first plenary talk at the workshop titled "Microbial Pesticide Regulation in the United States" to provide a background on the biopesticide registration process. Topics included a brief discussion of the laws governing pesticide regulation, specific actions within those laws that apply to biopesticides, unique risks associated with microbial pesticides, data requirements for registration, and registration assistance available to registrants.

The five laws that govern pesticide regulation include:

- Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)
- Federal Food Drug and Cosmetic Act (FFDCA)
- Food Quality Protection Act (FQPA)
- Endangered Species Act, Migratory Bird Treaty Act, and Clean Water Act
- Pesticide Registration Improvement Act (PRIA)

Within FIFRA, several sections guide possible registration scenarios and use: Experimental Use Permits, notification for small-scale field testing, registration review, and Special Local Needs and Emergency Exemptions. The unique risk issues associated with microbial pesticides include effects such as pathogenicity and toxicity, and exposure including transmissibility factors and competition.

The types of data required for microbial pesticides includes:

- Project analysis
- Toxicity/pathogenicity
- Non-target organisms/environmental expression
- Residue (i.e. tolerance)
- Efficacy (agency reviews only for public health pests – cockroaches, mosquitoes, ticks, etc.)

Certain data needs can be waived based on the taxonomic identity of the organism (i.e. if it is known to be non-pathogenic) and pre-registration analysis of available literature and/or data on the organism.

Registration assistance is available through pre-submission meetings with EPA that can provide the agency with a background on the product, discuss how best to satisfy data requirements, and provide an estimated timeframe for the review process. Regulatory consultants are also available to help guide registrants through the process and navigate the bureaucracy. Finally, the US Department of Agriculture (USDA) IR-4 Project is available for registrants of minor crop products and can provide regulatory assistance.

2.2 Background on the development of *P. fluorescens* as a tool to target downy brome/cheatgrass, medusahead rye, and jointed goatgrass

Dr. Ann Kennedy, a USDA-Agricultural Research Service soil scientist, gave the second plenary talk titled "*Microbial Control of Cheatgrass, Jointed Goatgrass, and Medusahead*" providing background on the development and use of *P. fluorescens* as a tool for invasive annual grass control. Topics of the presentation included how research on *P. fluorescens* began, the screening process for the bacteria to identify which weeds it would be effective at suppressing, characteristics of the plant-suppressive compounds produced by *P. fluorescens*, culturing and application methods, field studies and results, and a brief discussion on toxicological studies that were conducted as part of the registration process.

The weed-suppressive characteristic of soil bacteria was discovered when poor growth was observed in a winter wheat that had high numbers of bacterium associated with its roots. Further screening identified a specific strain of *P. fluorescens* that had specificity to three invasive annual grasses (cheatgrass, jointed goatgrass, and medusahead) while at the same time did not suppress the growth of native grasses, sedges, forbs, shrubs, and trees.

An important characteristic of *P. fluorescens* that must be considered when planning to use it as a biopesticide is that it requires low temperatures for optimal survival. The species goes dormant during hot, dry conditions so it is not competitive during warmer times of the year. The bacteria does not kill the invasive plants, but rather inhibits root cell elongation, interrupts tiller initiation, and generally reduces vigor of the plant and, thereby, gives the native plants a competitive advantage to regain a foothold and become dominant. Results are not immediate sometimes a 50% reduction is not observed until two years after the initial application.

Six important factors must be considered when planning to use *P. fluorescens* as a biopesticide:

- Understand the system you are working in.
- Choose sites that are not more than 50% infested with cheatgrass, medusahead and or jointed goatgrass.
- It is most effective combined with herbicide treatment at time of application and the following growing season (at a minimum).
- If product is sprayed on to the soil, it is critical that the product be worked into the soil with water; rainfall should occur within two weeks or less; product ability to act is diminished if left on the soil surface.
- Native seeds may be coated with bacteria and then drilled.
- Product is applied when temperatures are 50 degrees or less (but soil not frozen) in fall and winter.

2.3 Results from recent trials in Wyoming

Cheryl Schwartzkopf, District Supervisor of Converse County Weed and Pest in Wyoming, gave the third and final plenary talk at the workshop titled "*Large Scale Cheatgrass Control*" which described the use of *P. fluorescens* as soil amendment that were conducted in Converse County, Wyoming following fire events (5 acres; and 33000 acres) including "check" or untreated plots. There were two main findings with regards to the work completed in Wyoming: 1) herbicides must be used in conjunction with the application of the bacteria and 2) seeding of native vegetation was critical in ensuring other weeds did not colonize land freed up by the treatment and removal of the cheatgrass. The herbicide Plateau was applied to *P. fluorescens* treated acreage. Aircraft were used to apply a large portion of the Plateau and *P. fluorescens*. Since 2007, Wyoming has treated approximately 100,000 acres.

3.0 Discussion Topics

3.1 Research Gaps

Significant discussion focused on the following research gaps surrounding the use of *P. fluorescens* as a new land management tool: 1) the lack of peer reviewed research, 2) the limited knowledge of the impacts to soil microbial communities, and 3) how to efficiently assess and monitor non-target impacts.

3.1.1 Lack of Peer Reviewed Research

The consensus among the workshop participants was that peer reviewed research using *P. fluorescens* as a weed suppressive biopesticide is lacking. While limited peer reviewed research is available for *P. fluorescens*, strain D7, the research is focused on laboratory- and small-scale field tests that do not address efficacy and non-target impacts at landscape-scales. Additionally, most of the studies showing effective use of *P. fluorescens* have been conducted in the Palouse region of Washington State. Published literature for ACK55 is non-existent because this strain is currently in the registration phase. Several workshop participants provided insight on other existing literature citing unsuccessful applications of *P. fluorescens* at the experimental scale that were not represented in the literature list provided in the workshop material. Those studies were conducted outside the Palouse region and therefore the group raised the concern that the bacterium may not be effective in other regions where cheatgrass occurs.

It was agreed that there is limited science to support decision-making regarding the use of *P. fluorescens* on federally managed lands at landscape-scales. Additional study on implementation is needed to establish a foundation upon which agencies can make recommendations and finalize protocols regarding the use of this bacterium as a weed suppressive biopesticide on federally owned and managed lands.

A recommendation of this discussion was to complete a rigorous literature review of all open source and non-open source literature, encompassing both peer reviewed journal articles and unpublished and non-peer reviewed material, examining both successful and unsuccessful applications of *P. fluorescens* as a weed suppressive biopesticide.

3.1.2 Impacts to Soil Microbial Communities

Several workshop participants articulated the need to investigate *P. fluorescens*' impacts to microbial communities. The peer reviewed literature on the use of *P. fluorescens* as a weed suppressive biopesticide in laboratory- and field-scale trials did not explicitly address/discuss the potential impacts to soil microbial communities; potential impacts will need to be investigated and documented during trials.

The importance of soil resources was discussed, including understanding the important effects soil microbial taxa have on communities. A recommendation of this discussion was that scaled-up trials include collection and analysis of soil grab samples at potential scale-up sites during site characterization, product application, and post-application monitoring events. The collection of soil samples will establish a microbial community baseline at each site and provide scientific insight on the heterogeneity within the soil and how the microbial communities are impacted as a result of application. More information on the collection of soil grab samples is provided in the *Consistency Across Sites* section below.

3.1.3 Non-target Resource Impacts

The impacts to non-target resources from the application of *P. fluorescens* across diverse geographic regions are widely unknown. The published literature of laboratory- and field-scale trials of the use of *P. fluorescens* as a weed suppressive biopesticide generally does not address a broad spectrum of non-target impacts. A recommendation of this discussion was to collect non-target resource data and information during site characterization, product application, and post-application monitoring events. More information on the collection of data related to non-target resources is provided in the *Consistency Across Sites* section below.

3.2 Small-scale Trials

The participants also discussed the need for a more rigorous examination of efficacy and non-target impacts across geographic regions using smaller scale plots. The consensus of the workshop participants was that due to the lack of published data spanning multiple geographic regions where cheatgrass is a land management issue, that the next step is to assess efficacy across a broad geographic area. The following sections summarize the discussions related to the development of small-scale trials, including considerations (such as site selection, consistency across sites, and a centralized data management system) and the development of a DOI-wide pilot study protocol.

3.2.1 Site Selection

Various site attributes and characteristics were discussed as considerations during site selection. Table 1 summarizes the main considerations to be weighed and evaluated during the site selection process.

Table 1. Considerations During Site Selection

Site Attribute/Characteristic	Comments
Bureau/office jurisdiction	Selected sites could represent a diverse cross-section of federally managed lands across participating DOI bureaus/office
Plot size	A consensus on “small-scale” plot size was not reached. Significant discussion focused around the ideal plot size (e.g., 2m x 2m versus 10m x 10m versus 50 acres). Plot size must ensure availability for controls and replicates to provide adequate scientific insight and pedigree.
Climate region	Selected sites should represent a diverse cross-section of climate regions (e.g., semi-arid), including diversity in temperature, humidity, and precipitation.
Proximity to local weather stations	Selected sites should be located in close proximity to local weather stations to provide insight into local weather conditions during product application and post-application monitoring events.
Environmental conditions	Selected sites should represent a diverse cross-section of environmental conditions, including local elevation, wind speed, and soil conditions (e.g., type, moisture, nutrients).
Ecological conditions	Selected sites should represent a diverse cross-section of ecological conditions, including the presence of threatened and endangered and non-target species and resources.
Cheatgrass Percent Cover	Selected sites should have 40 – 50 percent

Site Attribute/Characteristic	Comments
	cover of cheatgrass present (50 – 60 percent or greater native species present)

3.2.2 Site Characterization Consistency

One of the largest topics of discussion and agreement among workshop participants was the need for DOI-wide consistency during site selection, product application, and post-application monitoring. Having consistency across all bureaus/offices conducting small-scale trials will ensure that sites are all selected and evaluated using the same criteria and data are collected, evaluated, and stored in a consistent manner. Implementing consistent methods and practices across bureaus/offices will enable effective and efficient communication of the necessary data needed to investigate efficacy and non-target impacts.

Table 2, Table 3, and Table 4 summarize the site information that will need to be collected during site characterization, product application, and post-application monitoring.

Table 2. Data Collection During Site Characterization

Information Collected	Comments
Date/Time	
Photos	Photos of plot area and adjacent areas looking N, S, E, W
Plot size	
Climate region	Note the type of climate region the plot area resides in (e.g., semi-arid)
Precipitation	• Annual • Seasonal • Past 30-years

Soil information	• Soil type
	• Soil profile
	• Surface texture
	• Litter
	• Soil grab sample
	  ○ Soil moisture
	  ○ Total organic carbon
	  ○ Total nitrogen
	  ○ Organic matter
	  ○ Microbial community

Temperature	• Ambient (annual, seasonal) • Soil
Plant diversity/evenness	• Within plot area • Within adjacent area
Plant percent cover	• Target species • Non-target species
Topographic information	• Elevation • Aspect
Land use and disturbance	• Information on how the plot area and adjacent land was used in the past (e.g., agriculture) • Note any disturbance within the plot area and adjacent areas

Information Collected	Comments

Threatened and endangered species	• Reconnaissance level analysis if species are known to occur within the area
	  ○ Within the plot area
	  ○ Within the adjacent area

Table 3. Data Collection During Product Application

Information Collected	Comments
Date/Time	
Photos	• Photos of plot area and adjacent areas looking N, S, E, W • Photos during application
Current precipitation	Note the amount of precipitation (if any) accumulating during the time off application
Time of predicted precipitation	Note the predicted day and anticipated amount of precipitation forecasted for the plot area (must be within two weeks of application)
Soil information	Soil grab sample • Soil moisture • Labile carbon • Litter • pH • Microbial community
Temperature	• Ambient • Soil (at 2” and 6” depths)
Wind information	• Speed • Direction
Relative humidity	
Percent cloud cover	
Leaf surface moisture	
Plant percent cover	• Target species • Non-target species
Type of product applied	• Herbicide • Soil amendment • Other
Application method	• Spray • Seed coating/drilling
Application equipment	
Application rate	
Seed source	If applied via seed coating
Product information	• Lot number • Viability
Certificate of purity	
Chain of custody	

Table 4. Data Collection During Post-Application Monitoring Events

Information Collected	Comments
Date/Time	
Photos	Photos of plot area and adjacent areas looking N, S, E, W
Soil information	Soil grab sample • Soil moisture • Labile carbon • Litter • pH • Microbial community
Biomass	• Target species • Non-target species
Precipitation	• Date of previous precipitation event • Amount of precipitation
Height of the tallest plant by species	• Species • Height • Location
Seed production/viability	
Plant diversity/evenness	• Within plot area • Within adjacent area
Seedling density	• Seeded and non-seeded plots • Non-target species
Non-target species mortality	
Plant abundance/percent cover	• Target species • Non-target species
Canopy gap	
Bio soil crusts	

3.2.3 Centralized Data Management System

Consensus among workshop participants included having a centralized user-friendly data repository that could be accessible by multiple DOI bureaus/offices and other entities.

Several data management systems were briefly discussed, including DIMA[1] (Database for Inventory, Monitoring and Assessment), the USFWS Inventory and Monitoring (I&M) System[2], and CORE[3] (Common Response Operations Environment) developed by PNNL. A recommendation of this discussion was to hold an online webinar in the months following the workshop where representatives of each data management system would present on their respective system. This webinar would provide background information on the available data management systems for DOI bureaus/offices and other entities participating in the pilot study to discuss and evaluate prior to selecting a data management system.

3.2.4 Protocol Development

Agreement and consensus was reached on the development of a DOI-wide protocol for applying *P. fluorescens* as a new land management tool. Participants agreed identifying uniform protocols would

[1] http://jornada.nmsu.edu/monit-assess/dima
[2] http://www.fws.gov/refuges/naturalresourcepc/iandm/
[3] http://readthis.pnl.gov/marketsource/readthis/B3051_not_print_quality.pdf

allow for scientifically sound cross-study result comparisons. Table 5 summarizes the main items discussed that should be included in a DOI-wide protocol.

Table 5. Items to be Included in Protocol

Protocol Item	Reason for Protocol
Site selection criteria	Ensure selection of appropriate pilot sites using consistent evaluation criteria
Site characterization data collection	Ensure consistent data is collected during site characterization
Product application methods	Ensure product is applied in an appropriate and consistent manner across all pilot sites
Product application data collection	Ensure consistent data is collected during product application
Post-application monitoring data collection	Ensure consistent data is collected during post-application monitoring
Data collection practices	Ensure data is collected in a consistent manner across all pilot sites
Quality Assurance/Quality Control	Ensure consistent QA/QC practices across all pilot sites
Data management system	Ensure data is managed and stored in a consistent manner across all sites, ideally in a centralized easy-to-use data management system
Training	Ensure adequate training materials are provided for those conducting site evaluations, applying product, collecting data, and QA/QC-ing data
Data evaluation practices	Ensure that efficacy and impacts to non-target species are evaluated in a consistent manner across all pilot sites

4.0 Summary of Recommendations

The two-day Experts' Workshop provided insight on the state of knowledge and understanding of the application of *P. fluorescens* as a weed suppressive biopesticide and new land management tool. The main recommendations are summarized as follows:

Complete the Literature Review of Application of *P.fluorescens*

- Peer reviewed research using *P. fluorescens* as a weed suppressive is lacking
- Complete the literature review of all open source and non-open source literature, encompassing both peer reviewed journal articles and unpublished and non-peer reviewed material, highlighting both successful and unsuccessful applications of *P. fluorescens* as a weed suppressive biopesticide

Investigate Effects of Product Application on Soil Microbial Communities

- Peer reviewed literature on the use of *P. fluorescens* as a weed suppressive biopesticide in laboratory- and field-scale trials does not address the impacts to soil microbial communities
- Collect soil grab samples at pilot sites during site characterization, product application, and post-application monitoring events to establish a microbial community baseline at each site and provide scientific insight on the heterogeneity within the soil and how the microbial communities are impacted as a result of application

Develop User-friendly Centralized Data Management System

- Consistency in data collection during site characterization, product application, and post-application monitoring is essential to the effective evaluation of the efficacy of *P. fluorescens* as a weed suppressive biopesticide and investigation of non-target effects as a result of application
- Collect data in a consistent manner and develop a centralized user-friendly data repository that could be accessible by multiple DOI bureaus/offices and other entities

Develop Protocol

- Being multiple bureaus/offices and other entities will be participating in the DOI-wide pilot study, consistency in site selection, product application, data collection, and data management is essential to effectively evaluate efficacy and impacts to non-target species
- Develop a protocol and "Best Management Practices" that can be followed by participating entities to ensure consistency across sites and to educate the necessary parties on appropriate methods

5.0 Next Steps

Several tasks were identified at the conclusion of the workshop to carry the momentum forward. These items include:

- Identify representatives from DOI, USDA, and other interested entities to participate on a biopesticide workgroup to evaluate and develop ACK55 as a new land management tool.
- The workgroup will write a study plan to determine efficacy of ACK55 over a broad geographical area.
- This workgroup will also develop protocols for the site selection criteria, and selection of a data management system. Existing, pertinent protocols will be identified as part of this task.
- Conduct an online webinar to demonstrate the potential data management systems to be used for data collection, storage, and sharing during the pilot study.

The execution of these tasks will further progress the goal of USFWS to assemble an interagency/entity team to accomplish careful evaluation of efficacy, non-target impacts, and feasible techniques needed to deliver *P. fluorescens* at a landscape scale.

6.0 References

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Appendix A

Experts' Workshop Participant List

Appendix A
Experts' Workshop Participant List

Full Name	Affiliation	Area of Expertise
Lara Aston	Pacific Northwest National Laboratory	Research Scientist, Restoration Ecology, NEPA
Sidra Blake	US Fish and Wildlife Service	Field biology, study design, data analysis
Chad Boyd	Agricultural Research Service	Research Scientist, Shrub-Steppe
Ian Burke	Washington State University	Associate Professor, Dept. of Crop and Soil Science
Myron Chase	National Park Service	IPM Coordinator
Dawn Davis	US Fish and Wildlife Service	Biologist, Sage Grouse
Carol Dawson	Bureau of Land Management	Botanist
Janelle Downs	Pacific Northwest National Laboratory	Research Scientist
Peter Dratch	US Fish and Wildlife Service	Inventory and Monitoring
Bridgette Flanders-Wanner	US Fish and Wildlife Service	Invasive Species Coordinator
Lindy Garner	US Fish and Wildlife Service	IPM Coordinator
Matt Germino	US Geological Survey	Research Ecologist, Shrub-Steppe
Alicia Gorton	Pacific Northwest National Laboratory	Research Scientist, Environmental and Ocean Engineering
Mike Gregg	US Fish and Wildlife Service	Land Management and Research Demonstration Biologist
Cindy Hall	US Fish and Wildlife Service	National Coordinator Integrated Pest Management
Heather Johnson	US Fish and Wildlife Service	Region 6 PFW Coordinator
Matt Kales	US Fish and Wildlife Service	Senior Advisor, Sage-Grouse Conservation
Ann Kennedy	Agricultural Research Service	Soil Microbiologist
John Klavitter	US Fish and Wildlife Service	Invasive Species Coordinator
Richard Lee	Bureau of Land Management	IPM Specialist
Jeff Mackay	US Fish and Wildlife Service	Refuge Manager
Jeremy Maestas	Natural Resource Conservation Service	Biologist, Sage Grouse
Brian Mealor	University of Wyoming	Extension Weed Specialist

Mike Mendelsohn	US Environmental Protection Agency	Senior Regulatory Specialist, Microbial Pesticides Branch
Susan Meyer	US Forest Service	Research Ecologist, Shrub-Steppe
Heidi Newsome	US Fish and Wildlife Service	Biologist, Habitat Restoration
David Pyke	US Geological Survey	Research Scientist, Shrub-Steppe
Gina Ramos	Bureau of Land Management	Senior Weed Specialist
Dave Repass	Bureau of Land Management	Biologist
Cheryl Schwartzkopf	Converse County	Weed and Restoration Specialist
Nancy Shaw	US Forest Service	Research Botanist
Tony Svjecar	Agricultural Research Service	Research Scientist, Shrub-Steppe

Appendix B

Literature List Provided in Workshop Material

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Literature List Provided in Workshop Materials

The following table is a list of relevant information on the use of *Pseudomonas fluorescens* as a weed suppressive that was included in the workshop materials. The information below includes US patents, journal articles, and meeting proceedings. Hyperlinks are provided for references that are publicly available.

Authors	Title	Publication Information	Date	Document Type
Elliot, Lloyd F. Kennedy, Ann C.	Method for Screening Bacteria and Application Thereof for Field Control of the Weed Downy Brome	NA	07/09/1991	US Patent; Patent No. 5,030,562
Kennedy, A.C. Elliot, L.F. Young, F.L. Douglas, C.L.	Rhizobacteria Suppressive to the Weed Downy Brome	Soil Science of America Journal; Vol. 55, No. 3 p. 722-727	1991	Journal Article
Kennedy, Ann C. Ogg, Jr., Alex G. Young, Frank L.	Biocontrol of Jointed Cheatgrass	NA	11/17/1992	US Patent; Patent No. 5,163,991
Tranel, Patrick J. Gealy, David R. Kennedy, Ann C.	Inhibition of Downy Brome (Bromus tectorum) Root Growth by a Phytotoxin from Pseudomonas fluorescens Strain D7	Weed Science; Vol. 7, No. 1 p. 134-139	1993	Journal Article
Johnson, B.N. Kennedy, A.C. Ogg, A.G	Suppression of Downy Brome Growth by a Rhizobacterium in Controlled Environments	Soil Science of America Journal; Vo. 57, No. 1 p. 73-77	1993	Journal Article
Tranel, Patrick J. Gealy, David R. Irzyk, Gerald P.	Physiological Responses of Downy Brome (Bromus tectorum) Roots to Pseudomonas fluorescens Strain D7 Phytotoxin	Weed Science; Vol. 41, No. 3 p. 483-489	1993	Journal Article
Gurusiddaiah, S. Gealy, David R. Kennedy, Ann C.	Isolation and Characterization of Metabolites from Pseudomonas fluorescens-D7 for Control of Downy Brome (Bromus tectorum)	Weed Science; Vol. 42, No. 3 p. 492-501	1994	Journal Article

Ogg, Jr., Alex G.				
Doty, J.A. Kennedy, A.C. Pan, W.L.	Rapid Bioassay for Inhibitory Rhizobacteria Using Digital Image Analysis	Soil Science of America Journal; Vol. 58, No. 6 p. 1699-1701	1994	Journal Article
Gealy, David R. Gurusiddaiah, S. Ogg, Jr., Alex G.	Isolation and Characterization of Metabolites from Pseudomonas syringae-strain 3366 and Their Phytotoxicity Against Certain Weed and Crop Species	Weed Science; Vol. 44, No. 2 p. 383-392	1996	Journal Article
Gealy, David R. Gurusiddaiah, S. Ogg, Jr., Alex G. Kennedy, Ann C.	Metabolites from Pseudomonas fluorescens Strain D7 Inhibit Downy Brome (Bromus tectorum) Seedling Growth	Weed Technology; Vol. 10, No. 2 p. 282-287	1996	Journal Article
Kremer, Robert J. Kennedy, Ann C.	Rhizobacteria as Biocontrol Agents of Weeds	Weed Technology; Vol. 10, No. 3 p. 601-609	1996	Journal Article
Skipper, Horace D. Ogg, Jr., Alex G. Kennedy, Ann C.	Root Biology of Grasses and Ecology of Rhizobacteria for Biological Control	Weed Technology; Vol. 10, No. 3 p. 610-620	1996	Journal Article
Weaver, J. Donal Hutcheson, Steven H. Lydon, John Kremer, Robert J. Kennedy, Ann C.	The Absence of *hrp* Genes in Deleterious Rhizobacteria Identified as Weed Biological Control Agents	Proceedings of the 53rd Annual Meeting of the Northeastern Weed Science Society; Vol. 53	1999	Proceedings
Kennedy, Ann C. Johnson, Bradley N. Stubbs, Tami L.	Host range of a deleterious rhizobacterium for biological control of downy brome	Weed Science; Vol. 49, No. 6 p. 792-797	2001	Journal Article
Kennedy, Ann C. Stubbs, T.L	Management effects on the incidence of jointed goatgrass inhibitory rhizobacteria	Biological Control; Vol. 40, Issue 2 p. 213-221	2007	Journal Article
Hohnhorst, Amanda Kay	The Effects of Suppressive Bacteria on the Germination and Growth of Cheatgrass (Bromus tectorum L.)	Washington State University	2009	Master's Thesis

Reynecke, Brandy K.	Plant Community Restoration on Mima Mounds at Turnbull National Wildlife Refuge, WA	Eastern Washington University	2012	Master's Thesis
Stubbs, Tami L. Kennedy, Ann C.	Microbial Weed Control and Microbial Herbicides (Chapter 8) in *Herbicides – Environmental Impact Studies and Management Approaches*	ISBN 978-953-307-892-2	2012	Chapter in Book
Stubbs, Tami L. Kennedy, Ann C. Skipper, Horace D.	Survival of a Rifampicin-Resistant *Pseudomonas fluorescens* Strain in Nine Mollisols	Applied and Environmental Soil Science; Vol. 2014 Article ID 306348	2014	Journal Article

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