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An Experimental Test of Weed-Suppressive Bacteria Effectiveness in Rangelands in Southwestern Idaho, 2016–18

Open-File Report 2019–1050

U.S. Department of the Interior
U.S. Geological Survey

Cover: Photograph showing rangeland at Wildcat study site, southwestern Idaho.
Photograph by Brynne Lazarus, U.S. Geological Survey, May 30, 2017.

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DAVID BERNHARDT, Secretary

U.S. Geological Survey
James F. Reilly II, Director

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Conversion Factors

U.S. customary units to International System of Units

Multiply	By	To obtain
	Length	
foot (ft)	0.3048	meter (m)
	Area	
acre	4,047	square meter (m^2)
acre	0.4047	hectare (ha)
	Volume	
ounce, fluid (fl. oz.)	0.02957	liter (L)
gallon (gal)	3.785	liter (L)

International System of Units to U.S. customary units

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
meter (m)	3.281	foot (ft)
	Area	
square kilometer (km2)	247.1	acre
square kilometer (km^2)	0.3861	square mile (mi^2)
	Mass	
g (gram)	0.03527	ounce(oz)

Abbreviations

ANOVA	analysis of variance
DIMA	Database for Inventory Monitoring and Assessment
pf	*Pseudomonas fluorescens*
WSB	weed-suppressive bacteria

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Abstract

Approaches and techniques for control of exotic annual grasses are a high priority in sagebrush-steppe and other rangelands. Strains of the soil bacterium *Pseudomonas fluorescens* (Pf) have been proposed to be selectively pathogenic to multiple species of exotic annual grasses with effects evident by the second year, and with no effect on native or desirable species including native bunchgrasses. However, scientifically defensible tests of the target and non-target/risk effects of these hypothetically weed-suppressive bacteria (WSB) strains in the field have been lacking in rangelands and other environments. We evaluated the effects of two strains of Pf WSB (D7 and MB906) sprayed on the surface in autumn 2016 at three sites in sagebrush steppe across southwestern Idaho that had cheatgrass (*Bromus tectorum*), medusahead (*Taeniatherum caput-medusae*), and other exotic annual grasses. Treatments also were replicated within each site (n=3, 8.3×8.3 meter plots) and included evaluation of the WSB strains with and without herbicides (imazapic and rimsulfuron) and with or without discing to mix surface-spray of the WSB into deeper soils. By the second year following application (spring 2018), neither strain of WSB affected exotic annual grasses, perennial bunchgrasses, or total community cover, either with WSB alone or in combination with herbicides or discing. We conclude that neither the D7 nor MB906 strains of Pf WSB have a negative effect on exotic annuals at the sites we evaluated.

Introduction

Exotic annual grasses are affecting numerous ecosystem types, particularly semiarid rangelands such as sagebrush steppe, where they are causing increased wildfire activity, loss of biotic diversity, and diminished ecosystem services on about one-half of the approximately 1 million km^2 of the original range of the habitat type (Miller and others, 2011). Once exotic annual grasses dominate a site, it is very difficult to restore the native species assemblage and abundance using current approaches (Monaco and others, 2017). This difficulty is due partly to strong feedbacks that exotic annuals have on the environment (for example, disturbance patterns, soils, nutrient cycling) that directly enhance their own establishment but also diminish the competitive ability of displaced species (Germino and others, 2016). Management strategies and techniques that can be used to prevent plant communities from becoming dominated by exotic annual grasses are a priority for land managers. Management actions that increase the resistance of sites to invasion by exotic annual grasses, and their resilience to disturbances (ability to recover quickly) are increasingly used. Preserving or restoring resprouting bunchgrasses, which generally are better able to compete with exotic annuals (Chambers and others, 2016), is considered central to sustaining or increasing the resistance and resilience of a native plant community.

Where sites are co-dominated by bunchgrasses and exotic annuals, there is a particular need for treatments to reduce competition to bunchgrasses from exotic annual grasses and thereby increase bunchgrass populations. Although pre-emergent herbicides can be used to diminish exotic annuals without effects on bunchgrasses (for example, Applestein and others, 2018), their effectiveness is short-lived, generally less than 2 years. Population growth of bunchgrasses (maturity of existing individuals plus new recruits) typically requires longer time frames, such as multiple years to a decade in the warmest and driest sites within sagebrush steppe where exotic annuals are most problematic. Thus, a tool of high value to land managers would be one that can provide sustained control of exotic annual grasses after the initial action of herbicides (or perhaps in place of herbicides) for 2–5 years while bunchgrass populations become better established.

Most or all plant species are subject to pathogens, although the rapid expansion of exotic plant species as they invade new areas often results in escape from their natural "enemies" (Blumenthal, 2005). Biocontrol agents, including invertebrates and microbes, have been identified and used successfully on many exotic species including exotic invasive forbs in rangelands. Exotic annual grass communities, like most low-diversity grass stands (for example, wheat fields), are known to be periodically prone to pathogenically driven stand failure (Hulbert, 1955). A complex of five soil/plant fungal species are known to interact in ways that cause "cheatgrass dieoff" at large scales, and although the biology and ecology of the phenomenon are well known, dispersion of the fungi does not seem feasible in such as manner as to induce predictable dieoffs (Meyer and others, 2016). A less-well-studied but more strongly endorsed biocontrol prospect has emerged with strains of the widespread bacterium *Pseudomonas fluorescens* (Pf), which has reportedly reduced root growth in laboratory, greenhouse, and wheat field trials of exotic annual grasses including *Bromus tectorum* (cheatgrass), *Taeniatherum caput-medusae* (medusahead), and *Aegilops cylindrica* (jointed goat grass), but has had no effect on a long list of native or agriculturally important species (Kennedy and others, 1991, 2001; Johnson and others, 1993; Kennedy and Stubbs, 2007; Kennedy, 2018). Only one report of the effectiveness of the bacteria in rangelands is available (Kennedy, 2018; strain ACK55), and although the report showed very high levels of control from the first growing season following application to more than 5 years after application, report data are insufficient to determine whether the results are reproducible. Numerous other trials have been implemented but few have all the components of replication, proper controls, and quantitative measurement of vegetation responses.

The appeal of Pf weed-suppressive bacteria (WSB) is that it is considered native—although the species is known to be very diverse and thus local adaptation seems very likely—and can be dried and rehydrated for relatively inexpensive spraying across large landscapes at low densities. The bacterium supposedly requires cool, wet conditions for significant population growth, and the concept is that late autumn application of small quantities of Pf WSB is followed by growth increase in soils to levels pathogenic to exotic annual grasses. Soil surfaces in sagebrush steppe can have extreme growth conditions for bacteria owing to large temperature variation, low moisture, and low nutrient availability. Whether incorporating surface soils in deeper horizons that have more favorable growth conditions would enhance any potential effects of Pf WSB is an important question for its application in sagebrush steppe. Co-application of WSB with herbicides also is an attractive management option, and whether WSB effects interact positively or negatively with herbicides is another key question (especially because early trials only tested the combined effects of herbicide and WSB rather than taking a factorial approach).

In this study, we tested the efficacy for annual weed control of two *P. fluorescens* strains, two application methods (surface spraying and incorporation by discing), and application in combination with two herbicides (imazapic and rimsulfuron) for three southwestern Idaho sites differing in climate, soils, and plant community. Three dominant strains of Pf WSB were available at the inception of this study (and still are in various ways):

1. ACK55, which is not permitted for use on public lands and was not available at the inception of the experiment;
2. D7, which was shown to be effective against cheatgrass or jointed goatgrass in the laboratory, greenhouse, and wheat fields (Kennedy and others, 1991, 2001; Johnson and others, 1993; Kennedy and Stubbs, 2007) and is a registered biopesticide in the United States and at the time was commercially available through Verdesian Life Sciences; and
3. MB906, which was commercially available as a soil amendment through Bio-West Inc. but was not permissible for use on Federal land.

Bio-West Inc. has since removed the MB906 product from the market and is planning release of a similar, registered product.

Methods

Study Sites and Design

The study was replicated at three southwestern Idaho sites differing in climate, soils, and vegetation community (fig. 1, table 1). The Wildcat site, the lowest and driest of the three sites, is located on state grazing land near Marsing, Idaho, and was shrub steppe occupied primarily by shadscale saltbush (*Atriplex confertifolia*) before it burned during the 2015 Soda Fire. A subsequent drill seeding with crested wheatgrass (*Agropyron cristatum*) was initially unsuccessful, and the site was occupied primarily by cheatgrass and Sandberg bluegrass (*Poa secunda*) at the time of treatment initiation in autumn 2016. The Orchard site, intermediate in elevation and precipitation, is located east of Bureau of Land Management Birds of Prey National Conservation Area in an area without cattle grazing for many years but that is heavily used by smaller herbivores. At the time of treatment initiation, the Orchard plant community consisted primarily of crested wheatgrass, cheatgrass, and sixweeks fescue (*Vulpia octoflora*), a native annual grass. The highest and wettest of the three sites, Avimor, is on private easement land in the Boise foothills. At the time of treatment initiation, the Avimor plant community consisted primarily of the warm-season perennial bunchgrass three-awn (*Aristida purpurea*) and several invasive annual grasses—primarily medusahead and *Ventenata dubia* but also field brome (*Bromus arvensis*), cheatgrass, and rattail fescue (*Vulpia myuros*), and the invasive biennial bulbous bluegrass (*Poa bulbosa*). MB906 treatments could not be applied at the Orchard site because MB906 has not been approved for use on Federal lands.

Site Preparation and Treatment Application

Treatment application occurred in autumn 2016. Sites were fenced to exclude grazing and pre-burned to simulate post-fire conditions and to allow the bacteria to make contact with the soil. Herbicides were applied in October. Each of the 10 treatments (table 2) was randomly applied in three replicate 25×25-ft plots at each site (fig. 2). Herbicides and bacteria were sprayed with a pressured hose and dye to ensure even application. Imazapic was applied at a rate of 6 oz/acre with 24 oz/acre of the adjuvant methylated seed oil (MSO) in 100 gal/acre of water. Rimsulfuron was applied at 4 oz/acre with 24 oz/acre MSO in 100 gal/acre of water. Bacteria were applied in November, when temperatures were cool and conditions were moist. D7 (freeze dried) was resuspended in tap water and applied at a rate of 2g/acre in 100 gal/acre of water. MB906 was received as a liquid suspension of live cells and was applied at a rate of 1 gal/acre in 100 gal/acre of water. Bacteria and herbicide could not be co-applied simultaneously because the weather conditions and phenological response of exotic annuals occurred earlier in autumn (thus requiring spraying earlier) than the time at which conditions were optimal for the spraying of bacteria. Discing treatments occurred following bacterial application with a mini rangeland drill pulled by an all-terrain vehicle.

Data Collection

Data collection occurred (1) prior to treatment (September 2016), (2) in year 1 spring (May–June 2017), and (3) in year 2 spring (May–June 2018). We measured foliar cover using Line Point Intercept (Herrick and others, 2009) in 1 × 0.5-m frames (36 points per frame × 5 frames per plot = 180 points per plot at each collection interval). A pin was dropped at each point on the frame, and all species touching the pin from the canopy down to the soil surface were recorded. The presence of litter and the condition of the surface (for example, soil, rock, gravel, lichen crust) also were recorded. Data were entered directly into a DIMA database (Database for Inventory Monitoring and Assessment, Jornada Experimental Range, New Mexico) in the field using a Mesa^2 field tablet (Juniper Systems, Logan, Utah). The cover values we report include the subcanopy (that is, “any hit” rather than “first hit” in DIMA).

Statistical Analyses

We used analysis of variance (ANOVA) to determine treatment effects on 2018 (year 2 spring, which provided the most amount of time for treatments to become evident) total foliar cover, cover of invasive annual grasses targeted by WSB (cheatgrass and medusahead), and perennial grass cover. For treatments that were applied at all sites and did not show site x treatment interactions in preliminary analyses (D7, discing), we analyzed all sites together using ANOVA with a full factorial with main effects site, D7, and discing as independent variables (“all-garden analyses” results in table 3). We used Tukey-Kramer analyses to understand differences among sites. For treatments that were not applied at all sites (MB906), or that showed significant site x treatment interactions in preliminary analyses (imazapic, rimsulfuron), we calculated ANOVAs for individual sites with “treatment” as the independent variable and then used planned linear contrasts to investigate treatment differences (“individual garden analyses” results in table 4A-4C). Invasive annual grass cover was arcsine square root transformed to homogenize variance. All analyses were completed in JMP 12.1.0 (SAS Institute, Cary, North Carolina).

Results

All-Garden Analyses (D7, Discing)

The application of D7, either as a surface spray or incorporated with discing, did not decrease total foliar cover or cover of invasive annual grasses targeted by WSB, nor did it increase perennial grass cover for the three sites in year 2 spring (table 3, fig. 3). Discing (with or without D7) resulted in lower total foliar cover at the Orchard site only (significant site x discing interaction; see table 3 and fig. 4). Sites varied significantly in cover—cover of annual grasses targeted by WSB was highest at Wildcat, intermediate at Orchard, and lowest at Avimor (fig. 5); perennial grass cover was higher at Avimor than at the other two sites (fig. 6). Cover averages for the three gardens combined for all treatments and all years are plotted in figure 3. Cover averages for individual gardens for all treatments and all years are plotted in figures 4–6.

Individual Garden Analyses (MB906, Imazapic, Rimsulfuron)

The application of MB906, either as a surface spray or incorporated with discing, did not decrease total foliar cover or cover of invasive annual grasses targeted by WSB, nor did it increase perennial grass cover at any of the three sites in year 2 spring (table 4A–4C; figs. 4–6). Imazapic decreased total foliar cover and cover of invasive annual grasses targeted by WSB but did not increase perennial grass cover for all three sites in year 2 spring (table 4A–4C; figs. 4–6). The effect of imazapic was not modified in any way by additional application of D7 (table 4A–4C; figs. 4–6).

Rimsulfuron decreased total foliar cover substantially at Orchard, slightly at Avimor, and did not decrease total foliar cover at Wildcat in year 2 spring (table 4A; fig. 4). Rimsulfuron decreased cover of invasive annual grasses targeted by WSB at Avimor and Orchard but not at Wildcat in year 2 spring (table 4B; fig. 5), although it did reduce annual grass cover strongly at all three sites in year 1 spring (fig. 5; statistics not shown). Rimsulfuron significantly decreased perennial grass cover (primarily *Poa secunda*) at Wildcat but did not affect perennial grass cover at the other two sites (table 4C; fig. 6). The effect of rimsulfuron was not modified in any way by additional application of D7 (table 4A–4C; figs. 4–6).

Discussion

According to Kennedy (2018), WSB effects can be pronounced by years 1 and 2 after spraying. However, neither strain of WSB led to decreases in targeted invasive annual grasses or increases in perennial grass cover when sprayed on the surface, incorporated into the soil by discing, or combined with herbicide (although herbicide was effective; see fig. 7) by year 2, when at least some effect ought to be visible according to other reports (Kennedy and others, 1991; Kennedy 2018). Our application methods carefully followed guidelines regarding the freshness and viability of WSB and highly suitable cool/wet conditions prevailed during and after our applications of the treatments; thus, faulty application likely did not cause the observed results. WSB populations possibly did not increase in soils after our application to levels that can be pathogenic, and efforts are underway to determine whether detectable changes to the soil microbial community have occurred, and whether molecular genetic markers can be developed to enable us to track the WSB strains in soil after application.

There is a slight chance that WSB effects may become evident in upcoming years, and we intend to continue measurements though the fifth post-treatment year. Of the approximately 1 million km^2 of sagebrush steppe that once were present, about one-half are currently invaded by exotic annual grasses (Germino and others, 2016). These areas, both invaded and not invaded, span a wide range of soil and climate conditions (for example, mean annual precipitation ranging from 170 to 400 cm/yr, with none to nearly one-half of annual precipitation occurring in summer). The sites we studied spanned a wide range of climate (and, specifically, precipitation) conditions, although even wetter conditions than we evaluated may be necessary for WSB to have effects. In comparison to WSB, imazapic continued to be effective in controlling invasive annual grasses at all sites in year 2, whereas the decreases in annual grasses through application of rimsulfuron appeared to be lessening considerably by year 2, particularly at the driest site we studied. These interim findings suggest that imazapic can more reliably lead to short-term control of annual grasses than WSB or rimsulfuron.

Acknowledgments

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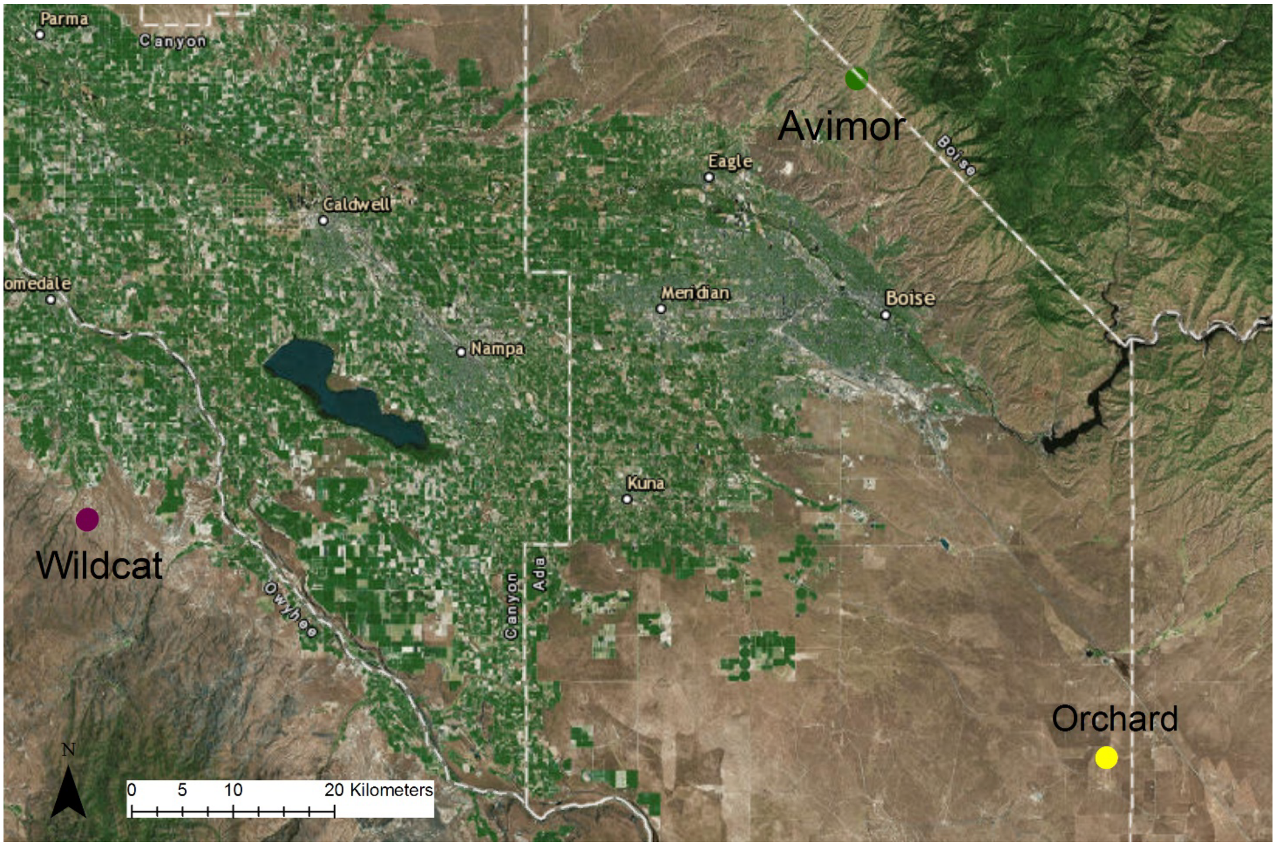

Figure 1. Image showing Wildcat, Avimor, and Orchard study site locations, southwestern Idaho, 2016. Image sources: Esri, Digital Globe, GeoEye, Earthstar Geographics, CNES/Airbus, U.S. Department of Agriculture, U.S. Geological Survey, AEx, Getmapping, Aerogrid, IGN, IGP, swisstopo, HERE, DeLorme, ©OpenStreetMap contributors, and the geographic information system user community.

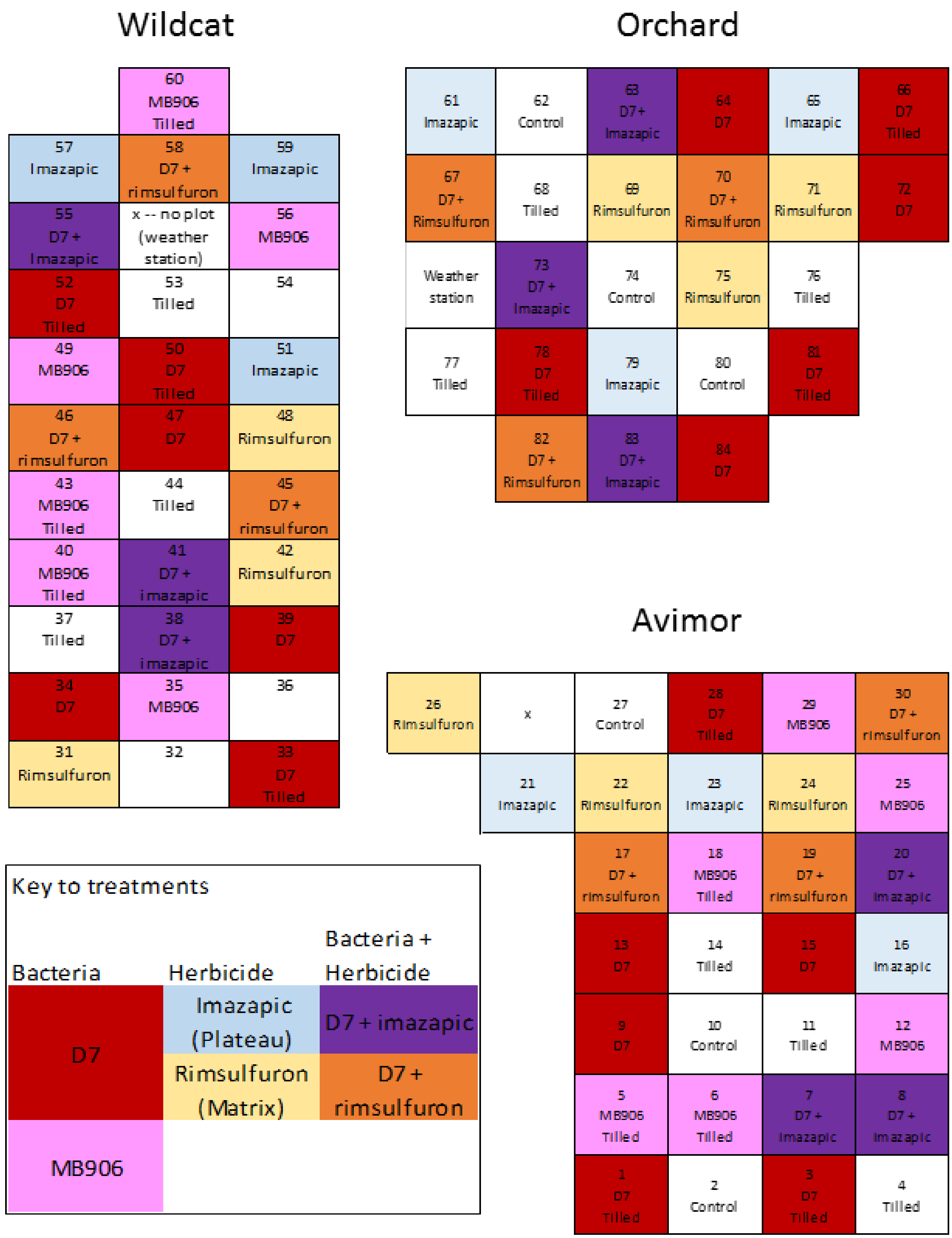

Figure 2. Experimental design of treatments applied at Wildcat, Orchard, and Avimor study sites, southwestern Idaho, autumn 2016. Plot numbers are indicated at the top of each square.

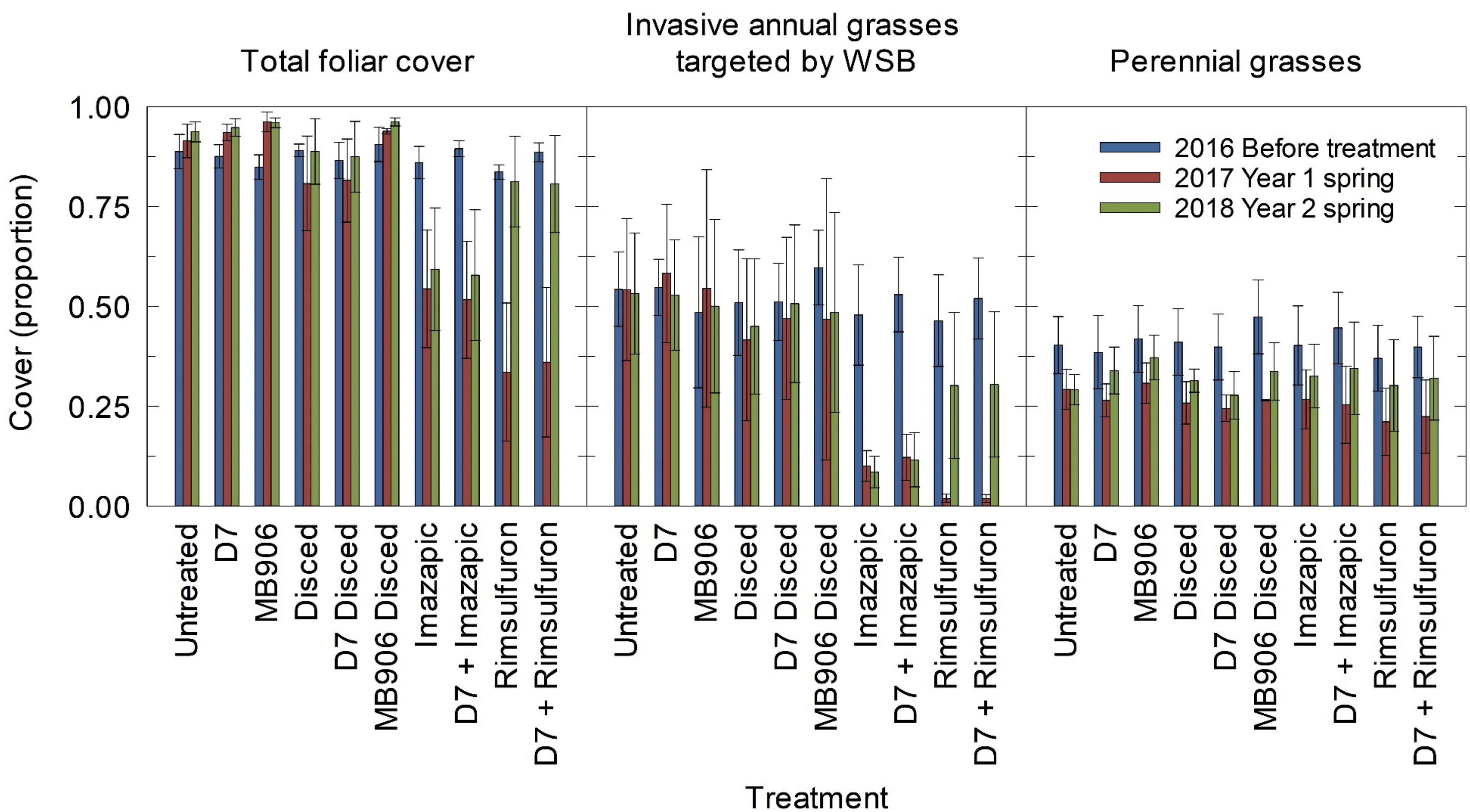

Figure 3. Graphs showing cover of the whole plant community (left graph), or of the annual grasses that were previously reported to be affected by weed-suppressive bacteria (WSB; middle graph), and perennial grasses (right graph) for all three study sites combined, southwestern Idaho, 2016–18.

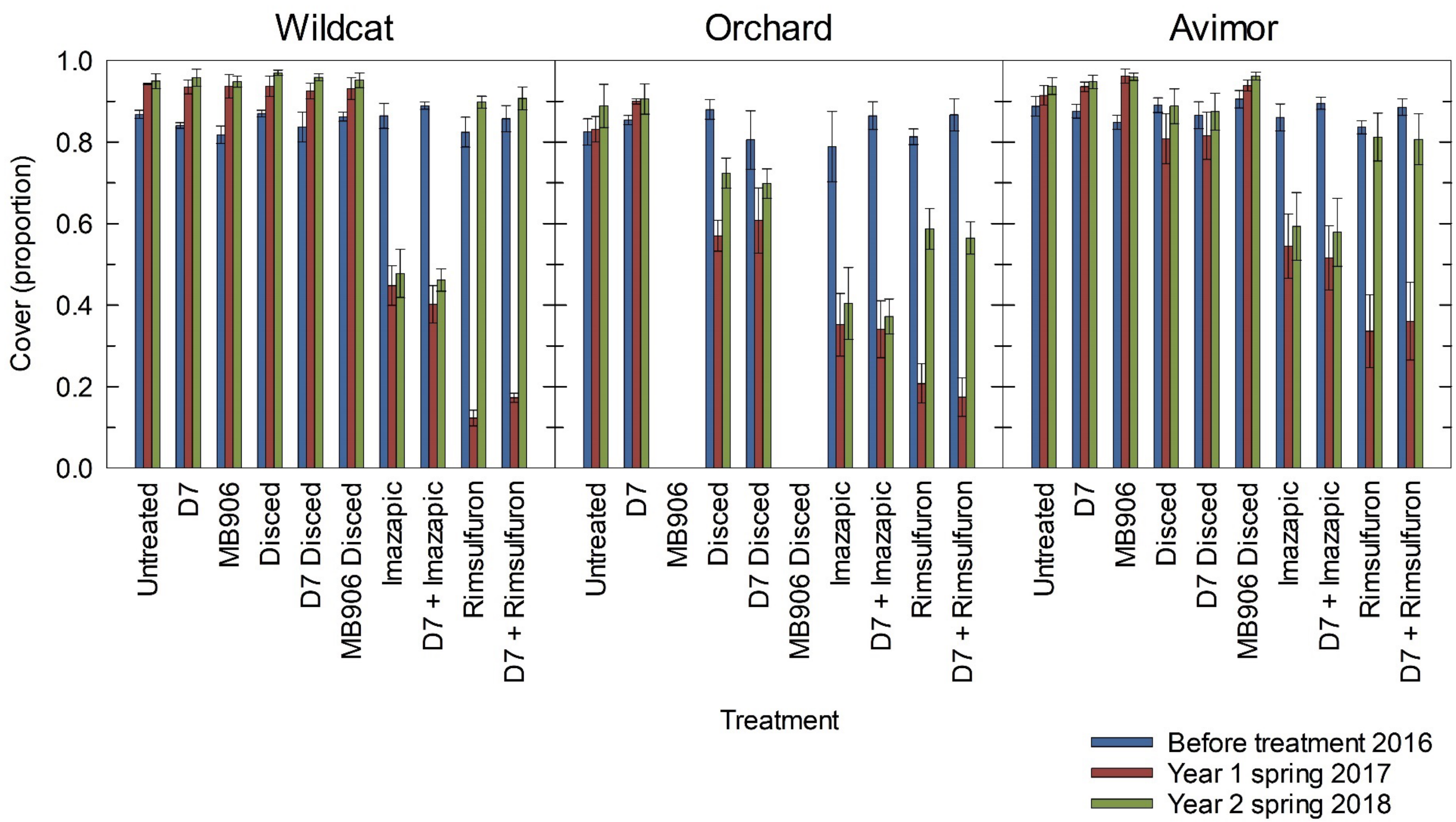

Figure 4. Graphs showing total community foliar cover by Wildcat (left graph), Orchard (middle graph), and Avimor (right graph) study sites, southwestern Idaho, 2016–18.

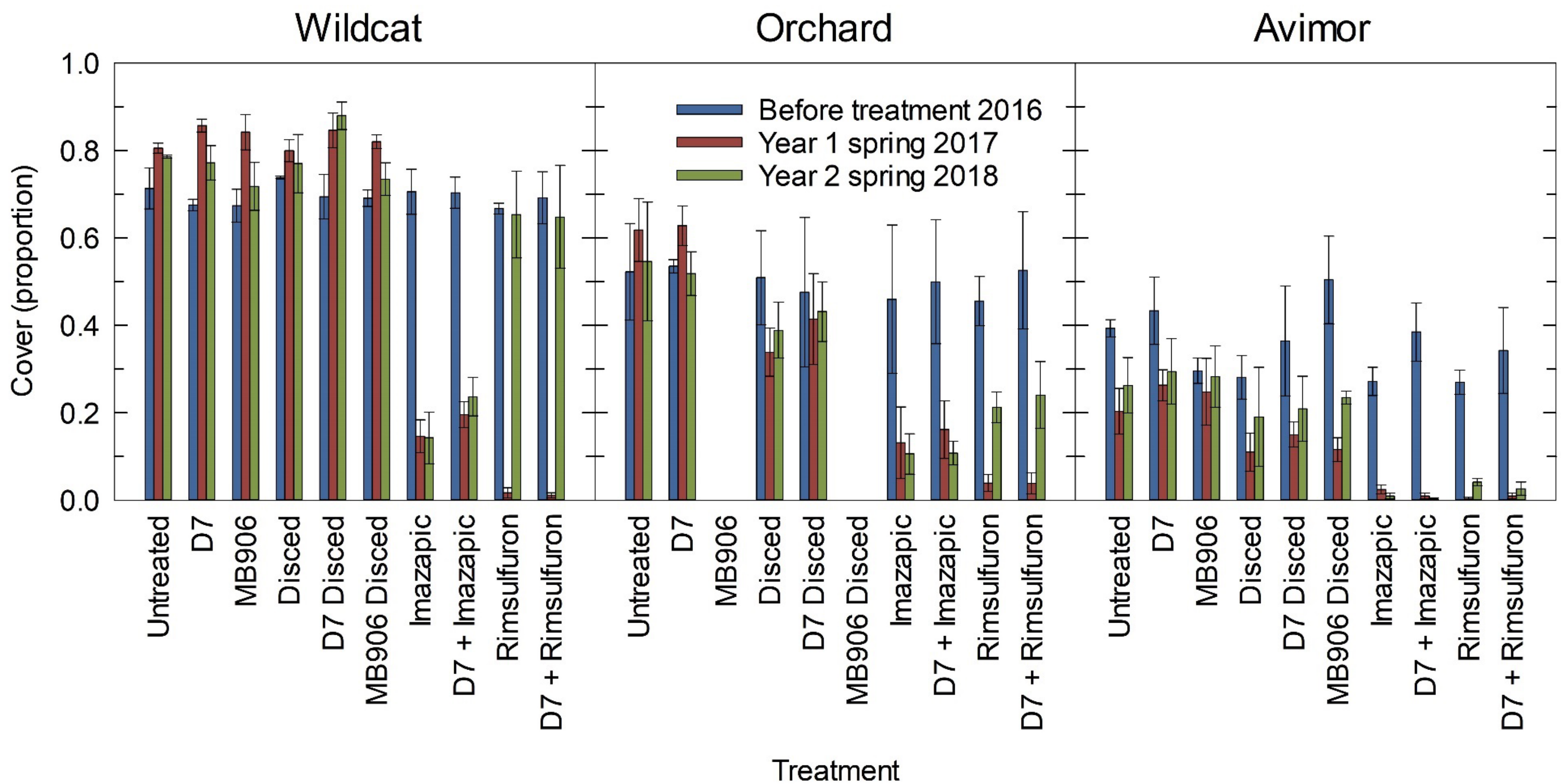

Figure 5. Graphs showing cover of invasive annual grasses targeted by weed-suppressive bacteria by Wildcat (left graph), Orchard (middle graph), and Avimor (right graph) study sites, southwestern Idaho, 2016–18.

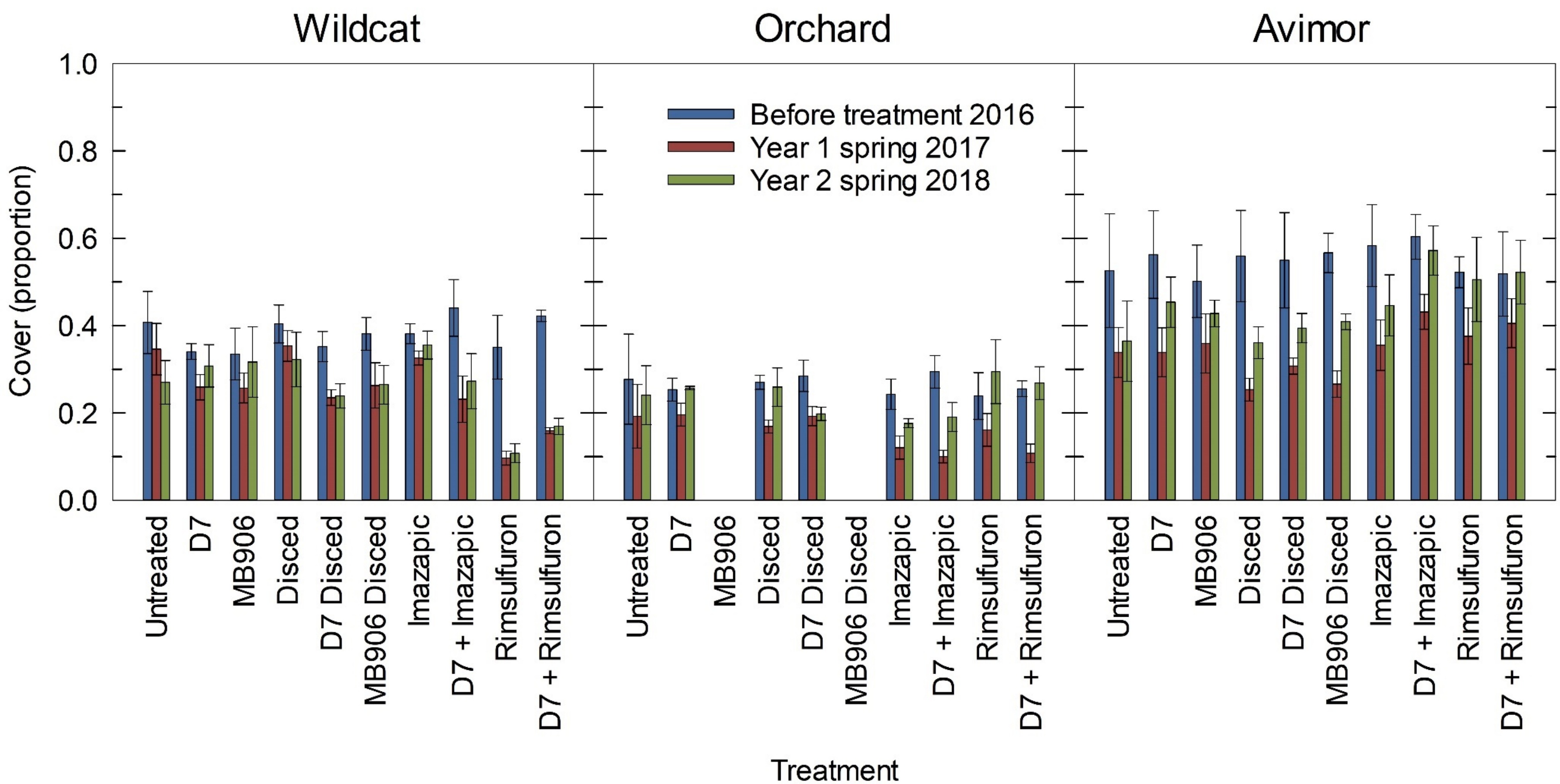

Figure 6. Graphs showing perennial grass cover by Wildcat (left graph), Orchard (middle graph), and Avimor (right graph) study sites, southwestern Idaho, 2016–18.

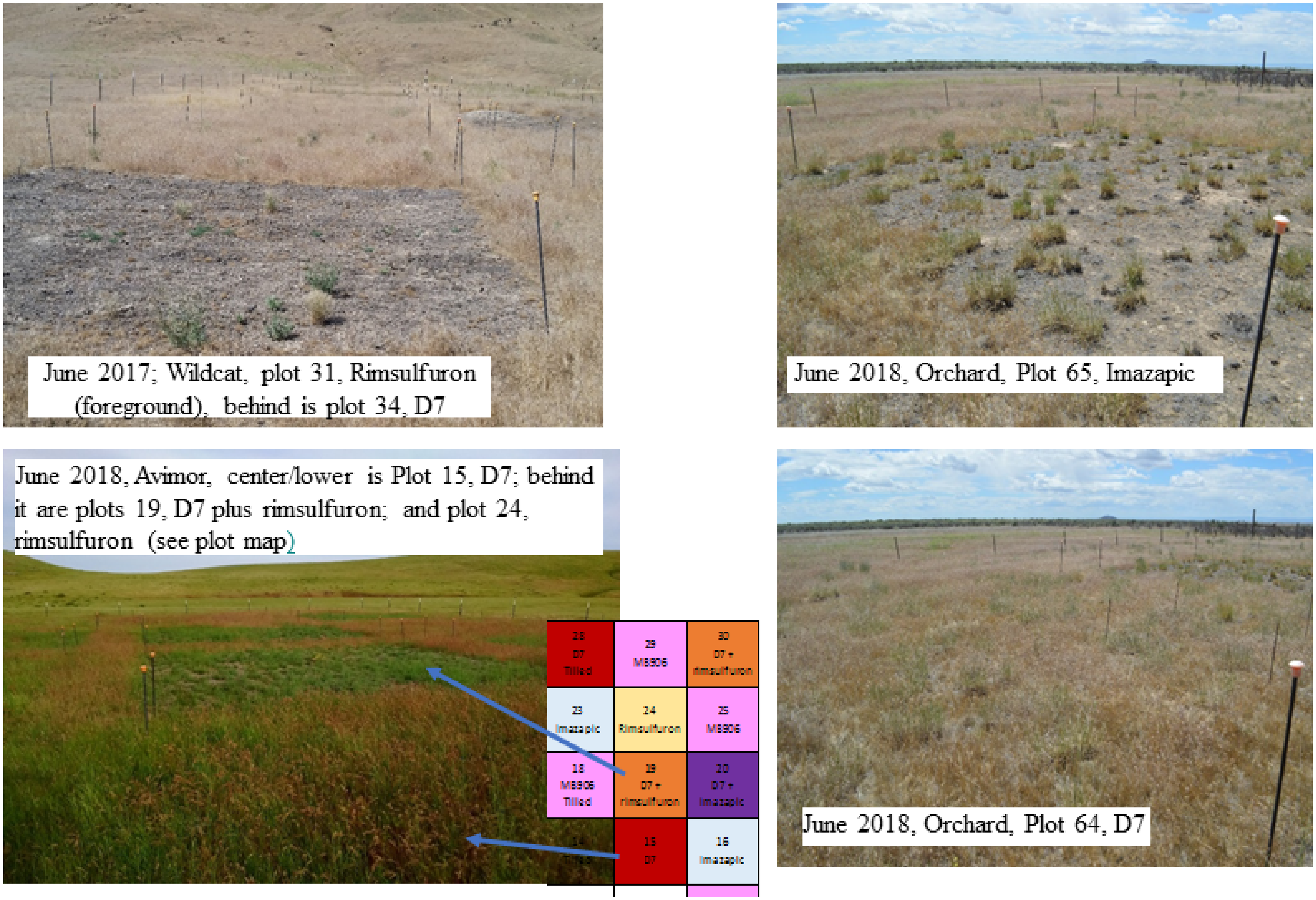

Figure 7. Photographs showing representative plots at the Wildcat, Orchard, and Avimor study sites, southwestern Idaho. Photographs by Merry Davidson, U.S. Geological Survey, June 2017 (upper left photograph) and June 2018 (all other photographs).

Table 1. Elevation, climate, soil texture and type, ownership, and plant community data at Wildcat, Avimor, and Orchard study sites, southwestern Idaho.

[Precipitation estimates are from Natural Resources Conservation Service web soil survey page (https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm). Soil textures are percent means (plus or minus standard error, in parentheses) of six samples per site measured using the hydrometer method (Bouyoucos, 1962). **Abbreviations:** m, meters above North American Vertical Datum of 1988; mm/yr, millimeter per year; %, percentage]

Site	Elevation (m)	Precipitation (mm/yr)	Soil texture			Soil type	Ownership	Plant community
			Sand (%)	Silt (%)	Clay (%)			
Wildcat	808	152–254	36 (1)	53 (1)	11 (0.4)	Silt loam	State (Idaho)	Sandberg bluegrass and cheatgrass
Orchard	973	203–279	46 (2)	46 (2)	8 (0.6)	Loam	Federal (Bureau of Land Management)	Crested wheatgrass and cheatgrass
Avimor	1,177	355–432	56 (2)	32 (1)	12 (0.9)	Sandy loam	Private	Three-awn, medusahead, and *Ventenata dubia*

Table 2. Weed-suppressive treatments applied at the three study sites, southwestern Idaho, autumn 2016.

[Treatments 2 and 3 were not applied at the Orchard site because MB906 is not approved for use on Federal lands]

Treatments
1. Untreated (control to treatments 2, 4, 6).
2. *P. fluorescens* MB906 sprayed on surface.
3. *P. fluorescens* MB906 incorporated in soil (by disc cut with rangeland drill seeder).
4. *P. fluorescens* D7 sprayed on surface.
5. *P. fluorescens* D7 incorporated in soil (same technique as treatment 3).
6. Disc only (control to treatments 3, 5).
7. *P. fluorescens* D7 spray/applied after imazapic.
8. Imazapic sprayed only (control to treatment #7).
9. *P. fluorescens* D7 spray/applied after rimsulfuron.
10.) Rimsulfuron only (control to treatment 9).

Table 3. Results of analysis of variance testing effects of site and treatment (D7, discing) on total foliar cover, invasive annual grass cover, and perennial bunchgrass cover at Wildcat, Avimor, and Orchard study sites, southwestern Idaho.

[*P* values less than (<) 0.05 are in bold text and indicate statistical significance. *P* value, probability value; r^2, coefficient of determination]

	Total foliar cover	Cover of invasive annual grasses targeted by D7 (including subcanopy)	Cover of perennial bunchgrasses (including subcanopy)
Whole model r^2	0.86	0.84	0.51
Source	**Individual effect *P* values**		
Site	**<.0001**	**<.0001**	**0.0007**
D7	0.92	0.53	0.85
Site*D7	0.99	0.93	0.41
Discing	**0.0005**	0.23	0.50
Site*Discing	**<.0001**	0.26	0.95
D7*Discing	0.44	0.48	0.16
Site*D7*Discing	0.90	0.80	0.90

Table 4A. Planned linear contrasts in total foliar cover by study site, southwestern Idaho, spring 2018 (year 2).

[**Abbreviations:** D7 and MB906, two strains of soil bacterium *Pseudomonas fluorescens*; DenDF, denominator degrees of freedom; DxM, Discing x MB906; F Ratio, ratio of two mean square values; Imaz, Imazapic; ImxD7, Imazapic x D7; NumDF, numerator degrees of freedom; Prob >F, probability is greater than F ratio; Rim, Rimsulfuron; RxD7, Rimsulfuron x D7; SS, sum of squares, Std Error, Standard Error]

	Discing	MB906	DxM
Control	0.5	0.5	0.5
MB906	0.5	-0.5	-0.5
MB906 + Disced	-0.5	-0.5	0.5
Disced	-0.5	0.5	-0.5

	Imaz	D7	ImxD7
Control	0.5	0.5	0.5
D7	0.5	-0.5	-0.5
D7 + Imazapic	-0.5	-0.5	0.5
Imazapic	-0.5	0.5	-0.5

	Rim	D7	RxD7
Control	0.5	0.5	0.5
D7	0.5	-0.5	-0.5
D7 + Rim	-0.5	-0.5	0.5
Rim	-0.5	0.5	-0.5

Avimor

	Discing	MB906	DxM
Estimate	0.0017	-3.00E-04	0.0017
Std Error	0.011	0.011	0.011
t Ratio	0.1515	-0.03	0.1515
Prob>|t|	0.8811	0.9761	0.8811
SS	8.30E-06	3.30E-07	8.30E-06

SS	NumDF	DenDF	F Ratio	Prob > F
2.00E-05	3	20	0.0156	0.9973

	Imaz	D7	ImxD7
Estimate	0.0767	-0.005	-0.001
Std Error	0.011	0.011	0.011
t Ratio	6.9681	-0.424	-0.091
Prob>|t|	**9.20E-07**	0.676	0.9285
SS	0.0176	0.0001	3.00E-06

SS	NumDF	DenDF	F Ratio	Prob > F
0.018	3	20	16.2475	**<.0001**

	Rim	D7	RxD7
Estimate	0.0267	-0.001	-0.005
Std Error	0.011	0.011	0.011
t Ratio	2.4237	-0.091	-0.424
Prob>|t|	**0.025**	0.9285	0.676
SS	0.0021	3.00E-06	0.0001

SS	NumDF	DenDF	F Ratio	Prob > F
0.002	3	20	2.0208	0.1434

Orchard

No MB906 was applied at Orchard

	Imaz	D7	ImxD7
Estimate	0.509	0.0073	-0.024
Std Error	0.0507	0.0507	0.0507
t Ratio	10.038	0.1446	-0.48
Prob>|t|	**2.60E-08**	0.8868	0.6378
SS	0.7772	0.0002	0.0018

SS	NumDF	DenDF	F Ratio	Prob > F
0.779	3	16	33.6699	**<.0001**

	Rim	D7	RxD7
Estimate	0.3213	0.0027	-0.02
Std Error	0.0507	0.0507	0.0507
t Ratio	6.3369	0.0526	-0.388
Prob>|t|	**9.90E-06**	0.9587	0.7032
SS	0.3098	2.10E-05	0.0012

SS	NumDF	DenDF	F Ratio	Prob > F
0.311	3	16	13.4366	**0.0001**

Wildcat

	Discing	MB906	DxM
Estimate	-0.012	0.0098	-0.008
Std Error	0.0257	0.0257	0.0257
t Ratio	-0.473	0.3825	-0.318
Prob>|t|	0.6412	0.7062	0.7541
SS	0.0004	0.0003	0.0002

SS	NumDF	DenDF	F Ratio	Prob > F
0.001	3	20	0.157	0.9239

	Imaz	D7	ImxD7
Estimate	0.4843	0.004	-0.012
Std Error	0.0257	0.0257	0.0257
t Ratio	18.838	0.1556	-0.48
Prob>|t|	**3.00E-14**	0.8779	0.6366
SS	0.7037	4.80E-05	0.0005

SS	NumDF	DenDF	F Ratio	Prob > F
0.704	3	20	118.372	**<.0001**

	Rim	D7	RxD7
Estimate	0.0512	-0.009	0.0005
Std Error	0.0257	0.0257	0.0257
t Ratio	1.9901	-0.344	0.0194
Prob>|t|	0.0604	0.7348	0.9847
SS	0.0079	0.0002	7.50E-07

SS	NumDF	DenDF	F Ratio	Prob > F
0.008	3	20	1.3596	0.2837

Table 4B. Planned linear contrasts in cover of invasive annual grasses targeted by weed-suppressive bacteria by study site, southwestern Idaho, 2018 (year 2).

[**Abbreviations:** D7 and MB906, two strains of soil bacterium *Pseudomonas fluorescens*; DenDF, denominator degrees of freedom; DxM, Discing x MB906; F Ratio, ratio of two mean square values; Imaz, Imazapic; ImxD7, Imazapic x D7; NumDF, numerator degrees of freedom; Prob >F, probability is greater than F ratio; Rim, Rimsulfuron; RxD7, Rimsulfuron x D7; SS, sum of squares, Std Error, Standard Error]

Discing / MB906 contrasts

	Discing	MB906	DxM
Control	0.5	0.5	0.5
MB906	0.5	-0.5	-0.5
MB906 + Disced	-0.5	-0.5	0.5
Disced	-0.5	0.5	-0.5

Avimor

	Discing	MB906	DxM
Estimate	0.0797	-0.054	0.0293
Std Error	0.073	0.073	0.073
t Ratio	1.092	-0.738	0.4022
Prob>|t|	0.2878	0.4693	0.6918
SS	0.019	0.0087	0.0026

SS	NumDF	DenDF	F Ratio	Prob > F
0.03	3	20	0.6328	0.6025

Orchard

No MB906 was applied at Orchard

Wildcat

	Discing	MB906	DxM
Estimate	-0.002	0.0608	0.0142
Std Error	0.0735	0.0735	0.0735
t Ratio	-0.026	0.8269	0.1931
Prob>|t|	0.9799	0.418	0.8488
SS	1.10E-05	0.0111	0.0006

SS	NumDF	DenDF	F Ratio	Prob > F
0.012	3	20	0.2406	0.867

Imazapic / D7 contrasts

	Imaz	D7	ImxD7
Control	0.5	0.5	0.5
D7	0.5	-0.5	-0.5
D7 + Imazapic	-0.5	-0.5	0.5
Imazapic	-0.5	0.5	-0.5

Avimor

	Imaz	D7	ImxD7
Estimate	0.4851	-0.005	-0.029
Std Error	0.073	0.073	0.073
t Ratio	6.6492	-0.072	-0.397
Prob>|t|	**1.80E-06**	0.9437	0.6953
SS	0.706	0.0001	0.0025

SS	NumDF	DenDF	F Ratio	Prob > F
0.709	3	20	14.792	**<.0001**

Orchard

	Imaz	D7	ImxD7
Estimate	0.5002	0.0041	0.0253
Std Error	0.0801	0.0801	0.0801
t Ratio	6.2481	0.0506	0.3167
Prob>|t|	**1.20E-05**	0.9602	0.7556
SS	0.7505	4.90E-05	0.0019

SS	NumDF	DenDF	F Ratio	Prob > F
0.752	3	16	13.047	**<.0001**

Wildcat

	Imaz	D7	ImxD7
Estimate	0.6439	-0.058	0.0727
Std Error	0.0735	0.0735	0.0735
t Ratio	8.7546	-0.788	0.9882
Prob>|t|	2.80E-08	0.4399	0.3349
SS	1.2437	0.0101	0.0158

SS	NumDF	DenDF	F Ratio	Prob > F
1.27	3	20	26.08	**<.0001**

Rimsulfuron / D7 contrasts

	Rim	D7	RxD7
Control	0.5	0.5	0.5
D7	0.5	-0.5	-0.5
D7 + Rim	-0.5	-0.5	0.5
Rim	-0.5	0.5	-0.5

Avimor

	Rim	D7	RxD7
Estimate	0.3732	0.0093	-0.044
Std Error	0.073	0.073	0.073
t Ratio	5.1151	0.1279	-0.597
Prob>|t|	**0.0001**	0.8995	0.5573
SS	0.4178	0.0003	0.0057

SS	NumDF	DenDF	F Ratio	Prob > F
0.424	3	20	8.8456	**0.0006**

Orchard

	Rim	D7	RxD7
Estimate	0.3286	0.0009	0.0285
Std Error	0.0801	0.0801	0.0801
t Ratio	4.1043	0.0114	0.3559
Prob>|t|	**0.0008**	0.991	0.7266
SS	0.3238	2.50E-06	0.0024

SS	NumDF	DenDF	F Ratio	Prob > F
0.326	3	16	5.6572	**0.0077**

Wildcat

	Rim	D7	RxD7
Estimate	0.1344	0.0056	0.0091
Std Error	0.0735	0.0735	0.0735
t Ratio	1.8274	0.0763	0.1237
Prob>|t|	0.0826	0.9399	0.9028
SS	0.0542	0.0001	0.0002

SS	NumDF	DenDF	F Ratio	Prob > F
0.055	3	20	1.1202	0.3645

Table 4C. Planned linear contrasts in cover of perennial grasses by study site, southwestern Idaho, spring 2018 (year 2).

[**Abbreviations:** D7 and MB906, two strains of soil bacterium *Pseudomonas fluorescens*; DenDF, denominator degrees of freedom; DxM, Discing x MB906; F Ratio, ratio of two mean square values; Imaz, Imazapic; ImxD7, Imazapic x D7; NumDF, numerator degrees of freedom; Prob >F, probability is greater than F ratio; Rim, Rimsulfuron; RxD7, Rimsulfuron x D7; SS, sum of squares, Std Error, Standard Error]

	Discing	MB906	DxM
Control	0.5	0.5	0.5
MB906	0.5	-0.5	-0.5
MB906 + Disced	-0.5	-0.5	0.5
Disced	-0.5	0.5	-0.5

	Imaz	D7	ImxD7
Control	0.5	0.5	0.5
D7	0.5	-0.5	-0.5
D7 + Imazapic	-0.5	-0.5	0.5
Imazapic	-0.5	0.5	-0.5

	Rim	D7	RxD7
Control	0.5	0.5	0.5
D7	0.5	-0.5	-0.5
D7 + Rim	-0.5	-0.5	0.5
Rim	-0.5	0.5	-0.5

Avimor

	Discing	MB906	DxM
Estimate	0.0115	-0.056	-0.007
Std Error	0.0617	0.0617	0.0617
t Ratio	0.1864	-0.9	-0.122
Prob>|t|	0.854	0.3789	0.9044
SS	0.0004	0.0092	0.0002

SS	NumDF	DenDF	F Ratio	Prob > F
0.01	3	20	0.2864	0.8346

	Imaz	D7	ImxD7
Estimate	-0.1	-0.107	0.0183
Std Error	0.0617	0.0617	0.0617
t Ratio	-1.616	-1.74	0.2972
Prob>|t|	0.1218	0.0972	0.7694
SS	0.0298	0.0346	0.001

SS	NumDF	DenDF	F Ratio	Prob > F
0.065	3	20	1.9092	0.1606

	Rim	D7	RxD7
Estimate	0.0115	-0.056	-0.007
Std Error	0.0617	0.0617	0.0617
t Ratio	0.1864	-0.9	-0.122
Prob>|t|	0.854	0.3789	0.9044
SS	0.0004	0.0092	0.0002

SS	NumDF	DenDF	F Ratio	Prob > F
0.01	3	20	0.2864	0.8346

Orchard

No MB906 was applied at Orchard

	Imaz	D7	ImxD7
Estimate	0.0657	-0.016	-0.001
Std Error	0.0429	0.0429	0.0429
t Ratio	1.5311	-0.365	-0.023
Prob>|t|	0.1453	0.7197	0.9817
SS	0.0129	0.0007	3.00E-06

SS	NumDF	DenDF	F Ratio	Prob > F
0.014	3	16	0.8261	0.4986

	Rim	D7	RxD7
Estimate	0.0657	-0.016	-0.001
Std Error	0.0429	0.0429	0.0429
t Ratio	1.5311	-0.365	-0.023
Prob>|t|	0.1453	0.7197	0.9817
SS	0.0129	0.0007	3.00E-06

SS	NumDF	DenDF	F Ratio	Prob > F
0.014	3	16	0.8261	0.4986

Wildcat

	Discing	MB906	DxM
Estimate	-2.00E-04	0.0055	-0.052
Std Error	0.0488	0.0488	0.0488
t Ratio	-0.003	0.1128	-1.063
Prob>|t|	0.9973	0.9113	0.3005
SS	8.30E-08	0.0001	0.0081

SS	NumDF	DenDF	F Ratio	Prob > F
0.008	3	20	0.3808	0.7679

	Imaz	D7	ImxD7
Estimate	-0.025	0.0225	-0.06
Std Error	0.0488	0.0488	0.0488
t Ratio	-0.509	0.4614	-1.234
Prob>|t|	0.6162	0.6495	0.2316
SS	0.0019	0.0015	0.0109

SS	NumDF	DenDF	F Ratio	Prob > F
0.014	3	20	0.6647	0.5835

	Rim	D7	RxD7
Estimate	0.1505	-0.05	0.0122
Std Error	0.0488	0.0488	0.0488
t Ratio	3.0859	-1.022	0.2495
Prob>|t|	**0.0058**	0.3191	0.8055
SS	0.068	0.0075	0.0004

SS	NumDF	DenDF	F Ratio	Prob > F
0.076	3	20	3.5431	**0.0332**

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