

**UNITED STATES DEPARTMENT OF AGRICULTURE  
NATURAL RESOURCES CONSERVATION SERVICE  
BRIDGER, MONTANA**

**and**

**MONTANA AGRICULTURAL EXPERIMENT STATIONS  
MONTANA STATE UNIVERSITY  
BOZEMAN, MONTANA**

**and**

**WYOMING AGRICULTURAL EXPERIMENT STATIONS  
UNIVERSITY OF WYOMING  
LARAMIE, WYOMING**

**NOTICE OF RELEASE OF COPPERHEAD SELECTED CLASS GERmplasm  
SLENDER WHEATGRASS**

The U.S. Department of Agriculture Natural Resources Conservation Service-Bridger Plant Materials Center, Montana Agricultural Experiment Stations-Montana State University, and Wyoming Agricultural Experiment Stations-University of Wyoming announce the selected class pre-varietal germplasm release of slender wheatgrass *Elymus trachycaulus* (Link) Gould ex Shinnery ssp. *trachycaulus* for the intermountain foothills and valleys, with particular emphasis on areas with soils with low pH and high concentrations of heavy metals.

**Collection Site Information:** The original seed collection was made by Leslie Marty (8/16/98) near the Wisdom Junction along Highway 1, 5 km east of Anaconda, Montana. Seed was collected from at least 20 plants. The site was severely impacted by smelter fallout and surface wind and water-transported contaminants, as well as historic overflow from the canal transporting waste material to the Opportunity Sediment Ponds. The surface pH of the soil was 4.3. This slender wheatgrass (original accession number 9081620) was found growing in association with big bluegrass *Poa secunda*, redtop *Agrostis giganteus*, scarlet globemallow *Sphaeralcea coccinea*, and western wheatgrass *Pascopyrum smithii*. This original collection site has since been reshaped and replanted.

**Description:** Slender wheatgrass is a short-lived perennial bunchgrass that often acts as a pioneer species on disturbed sites. The individual plants of this slender wheatgrass are 60 to 100 cm tall, reaching mature heights by early July. Slender wheatgrass has mostly cauline leaves and does not develop dense basal forage. The leaves are flat with prominent nerves and clasping auricles. At maturity, the stems may turn reddish purple at the base of the plant and just below the inflorescence. This species produces a narrow spicate inflorescence, with the 5-7 flowered spikelets that are strongly imbricate (overlapping like shingles). Slender wheatgrass has short anthers making it strongly self-pollinated. Seeds of this slender wheatgrass are awnless or awn-tipped (2-3 mm).

**Method of Selection:** Copperhead Germplasm slender wheatgrass is being released as a 'Natural-track' germplasm and increased with no purposeful manipulation. This accession was compared to two other collections from acid/heavy-metal-impacted sites (9081621—Stucky Ridge north of Anaconda and 9078455—Lulu Pass near Cooke City, MT) and four released cultivars ('Pryor' [Montana], 'San Luis' [Colorado], 'Revenue' [Canada], and 'Highlander' [Canada]). This material has been field tested at three impacted sites (two deep plowed only, one deep plowed and amended) in the Anaconda area and in a controlled greenhouse environment utilizing contaminated soil. This collection of slender wheatgrass has exhibited superior emergence, survival, and biomass production on amended acid/heavy-metal-impacted soil under the ambient climatic condition of the Upper Clark Fork Watershed (Deer Lodge County, MT).

**Testing:** Testing has been done in the field (acid/heavy-metal-impacted sites) and under controlled environmental conditions (greenhouse).

**Greenhouse Replicated Trials--2001:** The growth media utilized in the greenhouse study was collected (surface 15 cm) from a site approximately 6.5 kilometers southeast of Anaconda, MT. This site was typical of sites impacted by past smelter emission fallout and the ongoing deposition of fugitive dust. The sandy clay loam soil had a low pH and high levels of heavy metals (table 1). Organic mater content ranged from 1% to 3%.

Table 1. Soil analysis of growth media used in the greenhouse study at the Bridger PMC.

	pH	Arsenic As <i>mg/kg</i>	Cadmium Cd <i>mg/kg</i>	Copper Cu <i>mg/kg</i>	Lead Pb <i>mg/kg</i>	Zinc Zn <i>mg/kg</i>
	4.5	427	10	614	208	404
Phytotoxic Levels	<5	136-315	5.1-20	236-750	94-250	196-240

The study was established in a 20-replication, Randomized Complete Block Design, utilizing a single replication of a control media (Sunshine Mix #1). In this study, the cultivars outperformed the native collections from impacted sites (table 2). This may be attributed, in part, to the fact that the seed of the native indigenous accessions were wildland collected seed, while the cultivars were from commercially produced seed that may have had better seedling vigor because of the higher quality seed. Highlander is a high elevation collection from north of Jasper Park in Alberta, Canada. Although Highlander had poor germination, the surviving plants exhibited the best performance on these contaminated soils. Under controlled conditions, Copperhead Germplasm did not outperform the four released cultivars of slender wheatgrass.

Table 2. Survival, height, vigor, and biomass production of slender wheatgrasses after 90 days in acid/heavy-metal-impacted soils grown in Cone-tainers™ in the Bridger PMC greenhouse.

Accessions/ Cultivars	Trmt. Germ. %	Trmt. Survival %	Trmt. Ht. <i>cm</i>	Ctrl. Ht. <i>cm</i>	Trmt. Vigor <sup>‡</sup>	Ctrl. Vigor <sup>‡</sup>	Trmt. Dry Wt. <i>grams</i>	Ctrl. Dry Wt. <i>grams</i>
Highlander	20	75	10.5	21.5	1	3	0.0762	0.2316
San Luis	100	100	6.8	28.5	3.9	3	0.0088	0.1819
Revenue	100	95	6.5	26.5	4	3	0.0078	0.2651
Pryor	100	90	6.8	28.5	3.9	3	0.0151	0.2819
<b>Copperhead</b>	<b>100</b>	<b>85</b>	<b>5.0</b>	<b>24.0</b>	<b>4</b>	<b>3</b>	<b>0.0065</b>	<b>0.2485</b>
<b>9081621</b>	<b>100</b>	<b>70</b>	<b>4.6</b>	<b>27.5</b>	<b>3.9</b>	<b>3</b>	<b>0.0061</b>	<b>0.2958</b>

‡ Rated 1-5 with 1 best.

**Upland/Lowland Seed Mixture Trials--2001:** In October 2001, two replicated plantings were established near Anaconda, MT--one on a lowland site (Mill Creek) and one on an upland site (Stucky Ridge). Eight seed mixtures were utilized; four mixes made up exclusively of native indigenous grasses and forbs (Mixes A, B, C, D) and four mixes of the same or similar species of native cultivars (Mixes E, F, G, H). Copperhead Germplasm slender wheatgrass was used in Mixes A, B, and C, while Pryor slender wheatgrass was used in Mixes E, F, and G. The plots were established on contaminated sites that were deep plowed (depth of 25 cm) and not amended (table 3).

Table 3. Soil analysis (0-15 cm samples) of Mill Creek and Stucky Ridge Seed Mixture Trial plots following deep plowing.

Site/lab	pH	As <i>mg/kg</i>	Cd <i>mg/kg</i>	Cu <i>mg/kg</i>	Pb <i>mg/kg</i>	Zn <i>mg/kg</i>
<b>Mill Creek</b>						
Energy Lab	6.2	386	7.8	676	173	464
HKM Lab	5.8	493	10.3	858	212	650
<b>Stucky Ridge</b>						
Energy Lab	5.5	131	2.0	502	44	133
HKM Lab	4.7	178	1.8	779	66	161
Phytotoxic Levels	<5	136-315	5.1-20	236-750	94-250	196-240

The combined effect of the unamended soil conditions, below average precipitation, and some surface soil erosion on the bare soil resulted in poor establishment of all mixtures at both sites. Although grass seedlings were not identified by species, it was noted that most of what emerged and survived in Mixes A, B, and C was Copperhead slender wheatgrass; and most of what emerged and survived in Mixes E, F, and G was Pryor slender wheatgrass. Therefore, any comparisons of these groups of mixes can be utilized as a comparison of the performance of Copperhead (indigenous) and Pryor (cultivar) (table 4).

Table 4. First-year plant density of eight mixtures established on two acid/heavy-metal-impacted sites near Anaconda, MT.

Mixture	Lowland Mill Creek		Upland Stucky Ridge	
	June 4 <i>seedlings/m<sup>2</sup></i>	July 23 <i>seedlings/m<sup>2</sup></i>	June 4 <i>seedlings/m<sup>2</sup></i>	July 23 <i>seedlings/m<sup>2</sup></i>
<b>Indigenous</b>				
<b>A</b>	<b>1.5</b>	<b>1.0</b>	<b>18.3</b>	<b>4.7</b>
<b>B</b>	<b>16.8</b>	<b>9.5</b>	<b>20.3</b>	<b>1.7</b>
<b>C</b>	<b>2.5</b>	<b>3.3</b>	<b>20.0</b>	<b>2.7</b>
D	0.5	0.3	1.8	0.6
<b>Cultivar</b>				
<b>E</b>	<b>4.5</b>	<b>0.3</b>	<b>14.0</b>	<b>1.1</b>
<b>F</b>	<b>12.5</b>	<b>1.7</b>	<b>15.6</b>	<b>0.3</b>
<b>G</b>	<b>4.3</b>	<b>1.0</b>	<b>18.6</b>	<b>0.6</b>
H	1.8	0.6	0.2	0.3

Although the mixtures that contained Pryor slender wheatgrass (E, F, G) had plant density comparable to the plots that contained Copperhead Germplasm (A, B, C) early in the growing season, there was significantly better survival later in the season with the mixtures containing Copperhead Germplasm.

**Stucky Ridge Comparative Evaluation Planting--2003:** On May 13, 2003, a replicated trial was established to evaluate 36 accessions of 11 species of grasses, 16 accessions of 11 species of forbs, and 4 different grass/forb mixtures. Within this trial were included two accessions of indigenous accessions and three released cultivars of slender wheatgrass. Plots were established on a site that was deep plowed (September 2002), followed by amending with ~20 metric tons per hectare of lime, which was disked (four passes) into the surface 30 cm (table 5). In April 2003, fertilizer was applied and incorporated at a rate of 67 kg/ha nitrogen, 90 kg/ha P<sub>2</sub>O<sub>5</sub>, and 280 kg/ha K<sub>2</sub>O. The planting was done with a four-row cone-seeder equipped with double-disk opens and adjustable depth bands.

Table 5. Soil analysis of the amended Stucky Ridge Comparative Evaluation Trial site north of Anaconda, MT.

	pH	As <i>mg/kg</i>	Cd <i>mg/kg</i>	Cu <i>mg/kg</i>	Pb <i>mg/kg</i>	Zn <i>mg/kg</i>
Pre-Treatment	4.7	495		1530		380
Post-Treatment	7.8	145	1.3	832	43	194
Phytotoxic Levels	<5	136-315	5.1-20	236-750	94-250	196-240

The plots were sampled for seedling density the first year on June 24 and August 25, 2003 (table 6 next page). During the 2004 growing season, sampling for percentage stand, vigor, and plant height was done on July 1 and September 23; biomass production was sampled only on September 23. Grab-samples from the biomass were used for tissue analyses. In 2005, sampling was done on August 30 for plant cover, vigor, height, and biomass. Copperhead Germplasm (9081620) has been the top performer at the Stucky Ridge site all 3 years. Highlander had comparable plant density the seedling year, but has since deteriorated. The percentage stand, vigor, height, and biomass production of the Copperhead Germplasm was significantly better than all other accessions of slender wheatgrass, as well as any of the other ten grass species tested at this site.

In 2004 and 2005, grab-samples (25 grams each) were sent to Energy Laboratories, Inc. in Billings, MT for analysis of heavy-metal levels. The tissue samples were unwashed so the heavy-metal concentrations are a combination of intercellular and plant surface accumulations.

Concentrations of cadmium (Cd), lead (Pb), and arsenic (As) were very low or nondetectable in all of the accessions (table 7). Copperhead Germplasm and San Luis (origin southern Colorado) slender wheatgrass had the lowest levels of zinc (Zn), copper (Cu), and aluminum (Al). Copper was the only element that was high enough to be of concern for wildlife use or plant toxicity.

Table 7. Tissue analysis of slender wheatgrasses in the Stucky Ridge Comparative Evaluation Trial. Samples are unwashed so heavy-metal values are both intercellular and plant surface.

	Al		As		Cd		Cu		Pb		Zn	
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
	<i>mg/kg</i>		<i>mg/kg</i>		<i>mg/kg</i>		<i>mg/kg</i>		<i>mg/kg</i>		<i>mg/kg</i>	
<b>Copperhead</b>	<b>242</b>	<b>285</b>	<b>ND</b>	<b>8</b>	<b>ND</b>	<b>ND</b>	<b>26</b>	<b>47</b>	<b>ND</b>	<b>ND</b>	<b>14</b>	<b>16</b>
9081621	197	283	ND	ND	ND	ND	38	41	ND	ND	21	22
Pryor	301	413	ND	8	ND	ND	25	62	ND	ND	37	35
Revenue	280	445	ND	7	ND	ND	48	65	ND	ND	50	47
San Luis	441	193	ND	6	ND	ND	45	27	ND	ND	40	33
<u>Max. tolerance</u>												
Livestock			50		0.5		100		30		500	
Wildlife			50		2.0		55		40		300	
<u>Metal levels in plants</u>												
Deficient							2 to 5					
Sufficient to Normal			1 to 1.7		.05 to 0.2		5 to 30		5 to 10		27 to 150	
Excessive or Toxic			5 to 20		5 to 30		20 to 100		30 to 300		100 to 400	

ND=Not Detectable

Table 6. Stand, vigor, plant height, and biomass production of slender wheatgrass accessions in the Stucky Ridge Comparative Evaluation Planting north of Anaconda, Montana.

Genus & Species	Seedling Density		Stand			Vigor <sup>‡</sup>			Height		Biomass	
	6/24/03	8/26/03	6/30/04	9/22/04	8/30/05	6/30/04	9/22/04	8/30/05	6/30/04	8/30/05	9/22/04	8/30/05
	#/sq.ft.	#/sq.ft.	%	%	%				cm	cm	kg/ha	kg/ha
<b><i>Elymus trachycaulus</i></b> <b>Copperhead</b>	<b>22.6</b>	<b>23.2</b>	<b>61.3</b>	<b>61.3</b>	<b>75</b>	<b>3.4</b>	<b>1.8</b>	<b>2.1</b>	<b>54.4</b>	<b>87.5</b>	<b>2,083</b>	<b>8,211</b>
<i>Elymus trachycaulus</i> 9081621	15	9.8	28.4	30	34.1	4.8	2.7	2.7	34.2	76.3	544	4,100
<i>Elymus trachycaulus</i> Pryor	24	19.4	26.9	27.5	23.1	4.8	4.6	5.3	18.5	47	386	1,578
<i>Elymus trachycaulus</i> Revenue	14	13.4	19.7	23.8	11.9	4.8	4.3	5.4	14.3	36.9	172	578
<i>Elymus trachycaulus</i> San Luis	21.8	21.5	18.6	19.1	20.9	4.8	4.4	4.6	14.5	46.3	100	622

<sup>‡</sup> rating 1-9, with 1=best

**Germination in Contaminated & Diluted Soils (MSU)-2005:** Seed of Copperhead germplasm (9081620) slender wheatgrass was provided to Tara Martin, a graduate student in the Land Resources and Environmental Sciences Department at Montana State University-Bozeman, to evaluate the germination potential of seed of this accession of slender wheatgrass in amended heavy metal contaminated soils that are diluted to varying degrees with non-impacted native soils. The native soils originated from German Gulch and Little Blackfoot drainages and the contaminated soils were labeled Arts and Lamberts. The native and contaminated soils were mixed at rates of 0, 6.25, 12.5, 25, 50, 75, and 100 % by volume of contaminated soil (table 8). Concentration of all measured heavy metals increased with increasing percentage mixtures. Because of amending with lime, pH was uniform in all soil mixtures.

Table 8. Organic matter, heavy metal levels, and pH of native soils and the varying dilutions with amended contaminated soils used for germination trials (MSU) 2005.

	pH	Pb	Cu	Zn	Cd	As	Total	OM
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%
Soil Samples								
German Gulch	7.48	42	71	92	2.6	36	244	4.16
ARTS 6.25%	7.3	60	237	184	2.9	75	559	3.88
ARTS 12.5%	7.45	81	458	304	3.5	127	974	3.93
ARTS 25%	7.91	106	791	482	4.5	201	1585	3.84
ARTS 50%	7.71	168	1463	833	6.8	358	2828	3.39
ARTS 75%	7.74	245	2284	1263	9.5	558	4360	3.45
ARTS 100%	7.53	311	3043	1678	12.2	739	5783	2.79
LittleBlackfoot	7.9	57	25	151	1.2	16	251	2.91
Lamberts 6.25%	7.71	123	247	200	1.6	78	650	3
Lamberts 12.50%	7.72	224	627	299	2.2	182	1334	3.86
Lamberts 25%	7.3	250	947	370	2.7	330	1900	3.94
Lamberts 50%	7.44	420	1912	553	4	636	3525	5
Lamberts 75%	7.29	660	3185	968	5.8	1066	5885	5.53
Lamberts 100%	7.22	823	4314	1058	7	1319	7521	7.71

Germination of slender wheatgrass seed decreased significantly with increased percentage of contaminated soil in the Little Blackfoot soil mixes, but exhibited no significant germination decrease on the German Gulch soil (table 9). The germinated seedlings had similar growth responses to increases in the percent contaminated soil in both soil mixtures i.e., decreasing root/shoot elongation and biomass production with increases in the percent of contaminated soil. Copperhead germplasm was able to establish and survive, albeit at reduced rates, at the higher percentages of contaminated soils.

Table 9. Germination, shoot height, root length, shoot biomass, and root biomass of seedlings grown in native soils and amended impacted soils with varying dilution. MSU Germ. Study-2005. (Preliminary data).

Treatment	Total		Shoot		Root		Shoot		Root	
	Germ.		height		length		biomass		biomass	
	(%)		(mm)		(mm)		(mg)		(mg)	
	G.Gulch	Blackfoot	G.Gulch	Blackfoot	G. Gulch	Blackfoot	G.Gulch	Blackfoot	G.Gulch	Blackfoot
0	36	92	25.5	26.2	21.1	22.8	29.6	36.5	12.8	27.3
6.25	76	96	25.4	25.9	20.3	21.9	48.2	44.8	23.3	25.5
12.5	76	88	22.4	25.6	20.4	21.3	26.8	42.6	14.4	20.2
25	76	72	18.4	22.7	18.7	18	10.8	22	7.1	13.7
50	76	64	15.9	22	17.9	20	8.2	20.8	5.2	15.2
75	76	24	14.7	12.7	13.2	9	7.2	5.4	6.2	5.3
100	88	32	15.2	10.4	15.7	12.4	9.7	4.3	7.8	7.1

**Seed Increase:** Only one seed increase field has been established and harvested, yielding 450 kg/ha at its peak performance during the second growing season (first crop). Average seeds per pound of commercially produced seed is 145,000, while wildland collected seed has been found to be somewhat lighter with 175,000 seeds per pound. Maximum production can be expected by maintaining in 50- to 60-cm spaced rows, with supplemental irrigation and fertilization. Average date of harvest at the Bridger PMC has been July 14. The standard method of harvest is to swath when the seeds are in the firm dough stage (approximately 22% seed moisture) and combine from a cured windrow. Copperhead Germplasm, like all other releases of slender wheatgrass, is susceptible to seed shatter, making this species moderately difficult to harvest or to get high yields.

**Ecological Considerations and Evaluation:** Copperhead Germplasm slender wheatgrass is a low seral, short-lived perennial bunchgrass. It is a prolific seed producer, but will not become weedy. This species is strongly self-pollinated so does not readily cross-pollinate with other slender wheatgrasses nor hybridize with closely related wheatgrasses. However, slender wheatgrass will hybridize with foxtail barley (*Hordeum jubatum*), resulting in Macoun's barley (*Elyhordeum macounii*). It is very important that native reclamation species adapted to the Upper Clark Fork watershed be made commercially available for use on areas impacted by mining and smelting in the northern Rocky Mountain region.

**Anticipated Conservation Use:** It is intended for use on severely impacted sites with low pH and high concentration of heavy metals, as well as on disturbed sites. At high elevations, Highlander may be better adapted, and in the short-grass prairie region, Pryor would be the preferred accession. Copperhead Germplasm, as with any slender wheatgrass, is best used in native reclamation mixtures for its quick establishment and site stabilization.

**Anticipated Area of Adaptation:** This accession of slender wheatgrass originated in the upper Clark Fork River basin of western Montana where it was growing on soil impacted by acid/heavy-metal contamination resulting from historic copper smelter emissions, and currently by wind and surface water erosion. The testing of this accession has been limited to the immediate area of its origin and at the Bridger Plant Materials Center in south-central Montana. This accession is adapted for use on drastically disturbed acidic/heavy-metal-impacted areas in mountain valleys and low to mid-mountain elevations in the northern Rocky Mountain region.

**Increase and Distribution:** One generation ( $G_2$  equivalent to Certified) beyond  $G_1$  (equivalent to Foundation) are recognized.  $G_1$  seed of Copperhead germplasm slender wheatgrass is available from the USDA-Natural Resources Conservation Service (NRCS) Plant Materials Center (PMC) in Bridger, Montana, through the Foundation Seed Program at Montana State University-Bozeman or the University of Wyoming.  $G_1$  seed stock will be available in the spring of 2006.

**Prepared by:** Mark E. Majerus, USDA-NRCS Plant Materials Center, 98 South River Road, Bridger, MT 59014.

**Signatures for release of:**

**Copperhead Germplasm Slender wheatgrass *Elymus trachycaulus***

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