pioneer species for later recruitment of sagebrush, (5) determine the effect of AM on moisture stress tolerance of big sagebrush seedlings, and (6) evaluate pre-1985 reclamation methods on shrub establishment.

D. Methods

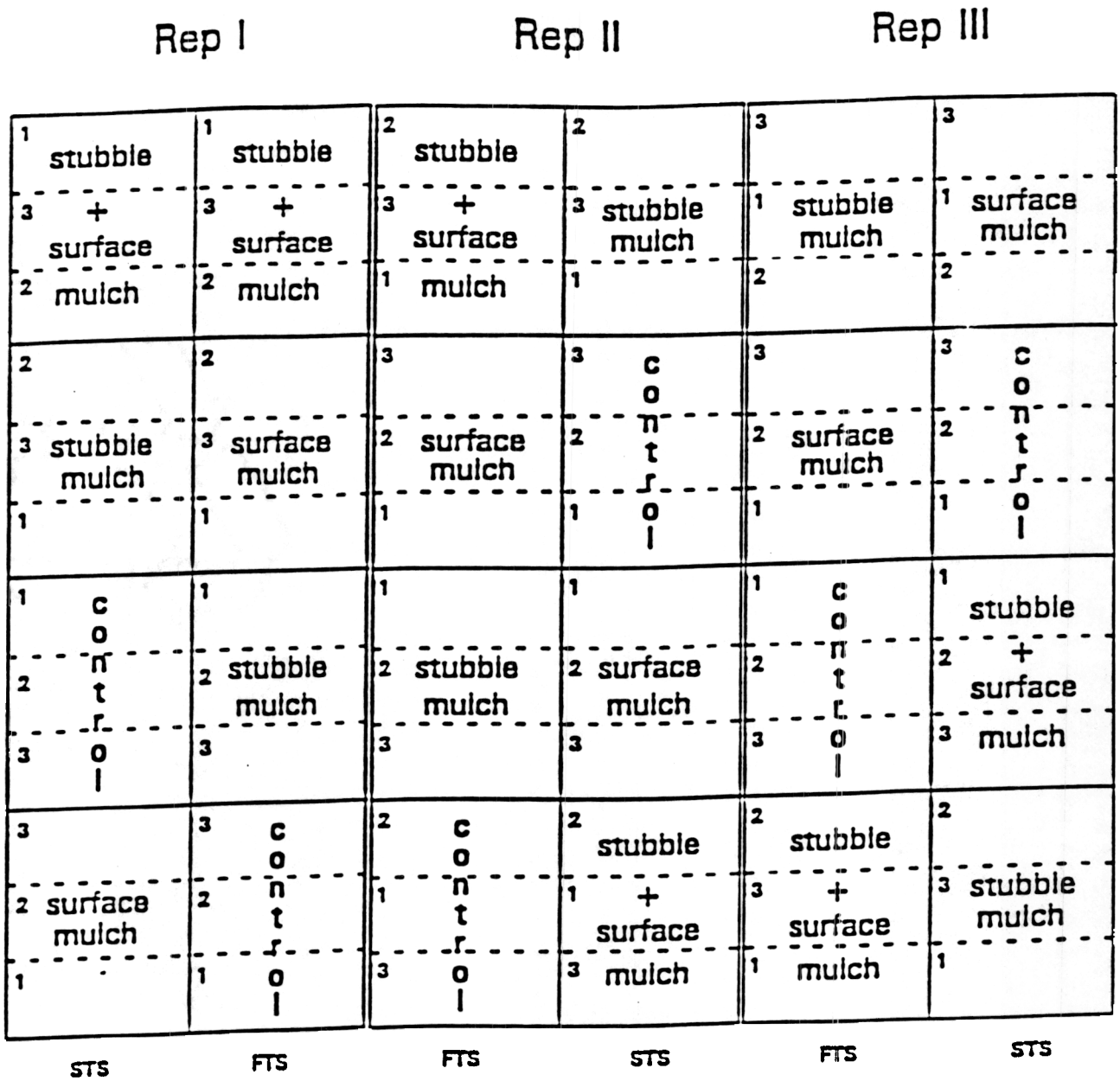
This project was composed of three field studies and one laboratory/greenhouse study. The field studies are identified as the "Establishment Study," the "Pioneer Plant Study," and the "Survey Study." The Establishment and Pioneer studies were implemented simultaneously at the North Antelope Coal Company mine about 100 km north of Douglas, Wyoming. The field plots were established in August 1990 on approximately 1.2 ha of leveled coal mine spoil. The greenhouse study was conducted at the Plant, Soil, and Insect Sciences Department facilities and the Survey study was conducted at 8 mines throughout Wyoming.

Establishment Study

This study was designed to evaluate three sets of main treatment variables: (1) topsoil management procedures (direct-applied or stockpiled), mulch methods (stubble, straw, stubble + straw, and no-mulch), and level of herbaceous species competition (two levels of concurrently seeded native grass mixture). Three replicate main plots (15 x 60 m) of each topsoil management treatment were established by replacing 30 cm of either direct-applied or stockpiled topsoil on regraded good quality spoil material. Mulch subplots (15 x 15m) were established on each topsoil management main plot. All treatments were assigned at random in a randomized block, split-split plot design (Figure 1). All plots were ripped and disked to relieve compaction during spoil regrading and topsoil replacement. In late April 1991, 'Steptoe" barley (*Hordium vulgare*) was seeded at the rate of 60 kg/ha on the mulch subplots that had been assigned the stubble or stubble + straw mulch treatments. In mid-July the barley was clipped to reduce seed maturation and subsequent year competition from volunteer barley plants. Annual weeds on the other mulch treatment plots were controlled with 0.55 kg/ha glyphosate [N-(phosphonomethyl) glycine] and tillage.

In late November 1991, the competition treatments were imposed by drill seeding a mixture of 'Rosana' western wheatgrass (*Pascopyrum smithii*), 'San Luis' slender wheatgrass (*Elymus trachycaulus*), and 'Critana' thickspike wheatgrass (*Elymus lanceolatus*) composed of equal seed numbers of each of the grasses. Grass seeding was made as a dormant seeding after soil temperature had dropped below that conducive to germination. The competition sub-subplots were seeded at 0, 16, and 32 kg PLS(pure live seed)/ha. After seeding the grass mixture, wheat (*Triticum aestivum*) straw was added at 1000 kg/ha to the stubble + straw treatment and 4500 kg/ha of straw was applied to the straw mulch treatment sub-subplots and crimped into the soil surface using a vertical disk crimper.

In early March 1992, all plots were broadcast seeded with sagebrush (2.63 kg PLS/ha) without any seed covering. The sagebrush was seeded at this time of year to reduce the potential for spurious germination during the winter if temperatures became adequate and also to reduce the length of time the seed might be displaced by the wind. Rice hulls were mixed with the sagebrush seed to improve seed flow in the seeder. The sagebrush seed was collected in the



Grass Seeding Rate:

1 = 0 lbs PLS/hectare

2 = 16 kg PLS/hectare

3 = 32 kg PLS/hectare

FTS = fresh topsoil

STS = stored topsoil

Figure 1. Field plot diagram for Establishment Study.

Powder River Basin area of Wyoming in early December 1991 and threshed with a debearder (Booth et al. 1995).

In the spring of 1992, nine 1-m^2 quadrats were established in each mulch by grass competition (15 x 5m) subplot, for a total of 216 quadrats per replication. These quadrats were used to assess grass and sagebrush seedling numbers in 1992 and sagebrush seedling numbers in 1993 and 1994. Grass seedling numbers were not assessed after 1992 because of the difficulty of distinguishing individual plants from new culms. Sagebrush seedling numbers were assessed in June and October 1992 and 1993 and in late September 1994.

Topsoil and spoil samples were obtained in April 1991 to assess electrical conductivity, soluble cations (calcium, magnesium, potassium and sodium), total Kjeldahl nitrogen, sodium bicarbonate-extractable phosphorus, cation exchange capacity, and AM spore levels. Topsoil samples (0 to 7.5 cm) were taken every 2-3 weeks, in each subplot of one replication, throughout the growing season in 1992 to evaluate the effects of the treatments on soil moisture. Two samples were collected from each subplot and averaged to represent the subplot.

In June of 1993, sagebrush seedlings located outside of the quadrats were excavated, their roots fixed and stained to evaluate percentage AM infection (Kormanick and McGraw 1982, Giovenetti and Mosse 1980).

Because many of the quadrats had no sagebrush seedlings present, particularly in the spring and fall of 1992 evaluations, analysis of variance was accomplished on the sagebrush seedling means of the 9 quadrats collected on each replicate sub-subplot. This is a conservative evaluation of the data; therefore, it strengthens interpretations resulting from any statistical differences observed. The data were analyzed as a split-split plot design. Fisher's Protected LSD was used to separate treatment means. All statistical comparisons were evaluated at the $P \leq 0.10$.

Arbuscular Mycorrhizae Study

A greenhouse study was accomplished to examine the influence of AM and seedling age on soil moisture stress tolerance of Wyoming big sagebrush. The soil used in this study was collected at the North Antelope Coal Company mine and was classified as a Ustic Torriorthent. The soil was passed through a 1 cm screen before being used in the study. The study included a soil with no mycorrhizae present and a natural soil. Arbuscular mycorrhizae were eliminated from the non-mycorrhizal treatment by pasteurizing at 115°C for 4 hr. The pasteurized soil was subsequently treated with a finely sieved (24um) water extract of non-autoclaved soil to restore indigenous soil microorganisms other than the AM fungi. A moisture retention curve was determined for the soil using a pressure plate apparatus (Klute 1986). Each soil treatment (-AM and +AM) was used to fill 108, 15-cm pots with about 1.4 L of soil. Approximately 10 sagebrush seeds were placed on the surface of each pot. After germination and emergence, sagebrush seedlings were thinned to 2 per pot. To evaluate the effects of AM and seedling age on moisture stress tolerance, seedlings of varying age were subjected to moisture stress by discontinuing water application to the pots. Moisture stress evaluations were conducted on 30, 45, 60, 90, 120, and 150 day old sagebrush seedlings. At each sampling interval, 18 pots of each soil treatment were subjected to moisture stress. Pots were allowed to dry until the seedlings died and they were then harvested immediately to examine root system mycorrhizal infection

rate. Soil moisture stress of the 36 pots that were not receiving water was monitored daily. Soil water potential was estimated gravimetrically using the soil moisture retention curve established for the soil used in the study (Figure 2). Soil moisture potential was estimated by relating the percent soil moisture in the pot to the moisture retention using the following equation:

$$\text{Soil Water Potential} = 24.2 + (-[illegible]7)(\text{soil moisture content,\%})$$

After careful harvest of the sagebrush seedlings, roots were prepared for examination of mycorrhizal status by washing with distilled water and clearing in warm (ca. 60°C) 10% KOH for 30 minutes. Root samples were then stained with 0.166% trypan blue in lactoglycerol for 1 hr and then destained in clear lactoglycerol. Levels of AM infection were quantified using the method of Allen and Allen (1980). Paired t-tests were used to compare the response of mycorrhizal and non-mycorrhizal sagebrush seedlings in each age group to soil moisture stress (i.e. level of soil moisture resulting in death). Analysis of variance was used to test the influence of AM and seedling age on sagebrush seedling drought stress tolerance and the interaction of these two factors.

Pioneer Study

This phase of the research was initiated to evaluate the role of fourwing saltbush as a pioneer plant to enhance the later establishment of big sagebrush and to assess the role of this plant in excluding big sagebrush when planted at seeding rates in excess of 2.2 kg/ha. The research was conducted as a companion study to the Establishment Study at the North Antelope Coal Company mine in Campbell County Wyoming. The project was begun in August 1990 on 1.2 ha of leveled coal mine spoil. Direct-applied or stockpiled topsoil was replaced on 15 x 60 m plots and the seeding treatment subplot (15 x 15 m) randomly assigned within each topsoil-management treatment (Figure 3). This study is north and has a slight north facing aspect as compared to the adjacent Establishment Study. The stubble mulch treatment was the sole mulch treatment evaluated in this study and was seeded at the same time as on the Establishment Study plots. No grass was seeded in this study. Sagebrush seeding treatments were as follows: (1) sagebrush seeded in 1992 and in 1993, (2) fallow in 1992 and sagebrush seeded in 1993, (3) fourwing saltbush seeded in 1991 and sagebrush overseeded in 1993, and (4) control (nothing seeded). The fourwing saltbush seed was "Wytana" (*Atriplex canescens* ssp. *aptera)*. The seed was harvested in 1986 and had 92.4% purity and 14% germination. The seeding rate of 26 kg/ha resulted in 281 PLS/m^2 and was drill seeded in November 1991. The sagebrush seed was from the same source as used in the Establishment Study. Nine permanent 1-m^2 quadrats were systematically located in each treatment plot to make seedling assessments in 1992 (Mueller-Dombois and Ellenberg 1974). Fifteen 1-m^2 quadrats were used in 1993 and thereafter. Shrub seedling-density evaluations were made each spring. Colored markers were placed by each seedling as it was identified, the markers counted, and the plot photographed using color print film.

The color prints were used to evaluate seedling population changes during the study period, and to provide a quantitative record of plant development. The prints were scanned with a Hewlet-Packard ScanJet 4C color scanner. "MapInfo Professional" software was used to monitor each seedling in the 360 quadrats evaluated during the 4 years and to calculate seedling density by age class.

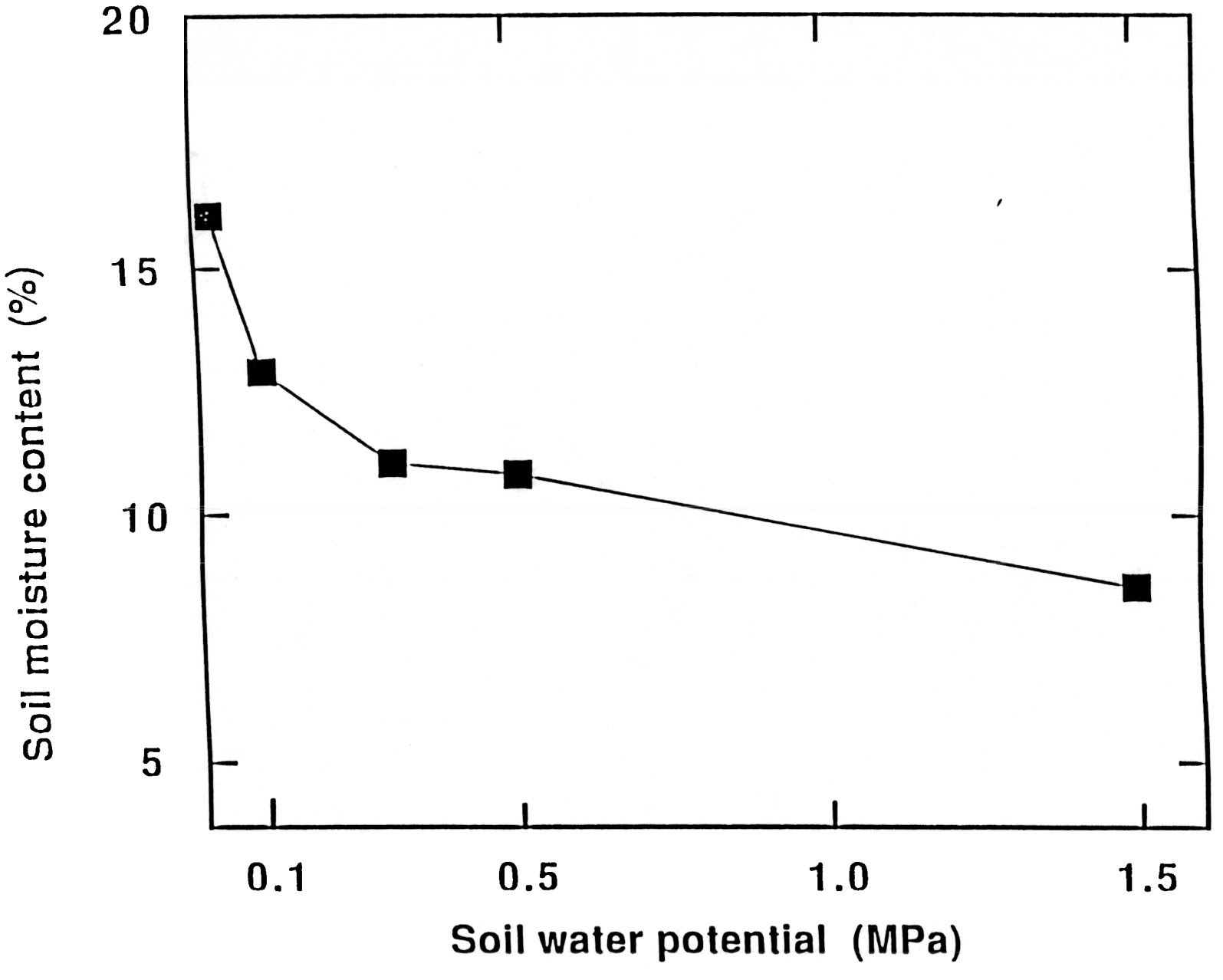


Figure 2. Soil moisture retention curve for arbuscular mycorrhizae study.

Rep		Rep II		Rep III	
Atriplex canescens	delayed Artemesia tridentata	Artemesia tridentata	control	Atriplex canescens	Atriplex canescens
Artemesia tridentata	Artemesia tridentata	Atriplex canescens	delayed Artemesia tridentata	control	delayed Artemesia tridentata
delayed Artemesia tridentata	control	control	Artemesia tridentata	delayed Artemesia tridentata	control
control	Atriplex canescens	delayed Artemesia tridentata	Atriplex canescens	Artemesia tridentata	Artemesia tridentata
STS	FTS	STS	FTS	FTS	STS

N

Figure 3. Field plot diagram for Pioneer Study.

To determine if sagebrush seedling height varied with seeding treatment or by proximity to a saltbush seedling, we randomly selected 1 quadrat per seeding treatment plot in the fall of 1996. We measured all sagebrush seedlings within those quadrats and segregated sagebrush seedlings in the saltbush-sagebrush treatment that were within a 7.6-cm radius of a saltbush seedling.

The experimental design of the Pioneer Study was a randomized complete block with split plots and 3 replications. The main plots were direct-placed and stockpiled topsoil with seeding treatments randomly assigned within each topsoil source. Data were evaluated using analysis of variance or general linear model analysis for soils variables and for shrub density, height, and age class.

Survey Study

Fourteen, 10-year or older reclaimed sites on 8 mines in northeast, central, south-central, and southwest Wyoming were selected for study. The post-mining vegetation of these sites was dominated by fourwing saltbush-grass (5 sites) or by saltbush-sagebrush-grass (9 sites). These 14 sites and descriptions of the site are shown in Table 1.

The seed mixtures that produced the post-mining communities varied (Table 2) but fourwing saltbush and sagebrush dominated the shrub species. Seed mixtures of the saltbush sites tended to have greater grass seeding rates and lower shrub seeding rates than were used on the saltbush-sagebrush sites. The saltbush-site seed mixtures also had fewer shrub species included in the mixture. When selecting study sites, we avoided sites with steep slopes and with one exception, sites grazed by domestic livestock. All sites were revegetated by or before 1985, making the sites old enough for bond release eligibility. All measurements were made between June 29 and August 24, 1994.

Twenty, 50-m transects were systematically located across each site. The transects were oriented perpendicular to the length of the site using a compass. Transect numbers had to be reduced to 5 on the Belle Ayr site, 10 on the WyoDak site and 30 on the Pathfinder Mine site because of the small size of those areas (Table 1). Shrub density by species was determined in a 200-m^2 area by counting the number of shrubs rooted within a 4-m wide strip along the 50-m transect (Pieper, 1978). We specifically recorded shrub seedlings (plants <10 cm height). Standing-dead shrubs were also enumerated because shrubs that established and grew to the extent they left recognizable skeletons 10 or more years after seeding are indicative of seeding success. Standing-dead plants also continue to influence the developing system by affecting the immediate micro-climate. Height of standing-dead and living shrubs were measured.

Shrub densities (total live and dead) were calculated for each site. The recently enacted shrub density standard states that an average of 1 shrub/m^2 on 20% of the land affected by mining must be reestablished on the land on areas disturbed after August 1996 (Federal Register, 1996). As a method of evaluating the effectiveness of pre-1985 reclamation technology for shrub establishment, we extrapolated this standard to 0.2 shrubs/m^2 on 100% of the affected land. A one-tailed t-test ($P \leq 0.05$) was utilized to determine if individual sites were significantly less than the extrapolated standard.

Table 1. Description and locations of sampled reclaimed mine sites to assess pre-1985 shrub establishment, 1994, Wyoming

Company/ Location	Size of Sample Site	Sample Area (ha)	Seeding Year (age)	Reclamation Type	Elevation (m)	Mean Annual Precipitation [1] (mm)	Mean Annual Temperature[1] (°C)	Mean Frost Free Period[2] (days)	Soil Parent Material[3]
Northeast									
Black Thunder/Thunder Basin Coal Co., Wright		26.3	1981(13)	Fourwing	1433	280	6.8	125	Tertiary Sandstone, Clay Shale
Belle Ayr/Amax Coal West, Inc., Gillette		2.0	1981(13)	Fourwing	1433	422	6.8	125	Tertiary Sandstone, Shale
WyoDak/WyoDak Resources Development Corp., Gillette		4.5	1982(12)	Fourwing/ Big Sagebrush	1341	422	6.8	125	Tertiary Sandstone, Clay Shale
	2	6.5	1984(10)	Fourwing/ Big Sagebrush	1341	422	6.8	125	(Same)
Central									
Pathfinder/Pathfinder Mines Corp., Shirley Basin		15.8	1977(17)	Fourwing	2195	244	4.	100	Tertiary Sandstone, Clay Shale
		21.4	1982(12)	Fourwing/ Big Sagebrush	2195	244	4.1	100	(Same)
Dave Johnston/Glenrock		23.9	1984(10)	Fourwing/ Big Sagebrush	1646	328	8.8	123	Cretaceous Clay Shale
Southcentral									
Seminoe 1/Arch Minerals, Hanna		22.7	1984(10)	Fourwing	2012	261	5.5	106	Cretaceous Clay Shale
		7.7	1984(10)	Fourwing/ Big Sagebrush	2012		5	106	(Same)

Table 1 continued.

Southwest									
Bridger Coal/Bridger Coal Co., Rock Springs		6.5	1984(10)	Fourwing/ Big Sagebrush	2073	225	5.9	112	Cretaceous Clay Shale
Coal Co., Rock Springs	2	33.2	1984(10)	Fourwing/ Big Sagebrush	2073	225	5.9	112	(Same)
Kemmerer Coal/Pittsburg & Midway Coal Mining Co. Kemmerer		36.4	1980(14)	Fourwing	2225	274	3.5	71	Carboniferous Limestones, Clay Shale, Loamstones, Redbed Sandstones
	2	13.0	1981(13)	Fourwing/ Big Sagebrush	2225	274	3.5	71	(Same)
	3	37.6	1981(13)	Fourwing/ Big Sagebrush	2225	274	3.5	71	(Same)

(1) Owenby and Ezell (1992)
(2) Martner (1986)
(3) Young and Singleton (1977)

Table 2. Reclamation information for study sties at 8 Wyoming mines by location. The information was obtained from records at the individual mines.

Location Year(s) (#)	Seeding Seeded	Method	Seeding rates (kg/ha) [2] All Grasses	All Forbs	ATCA1	ARTR	CHNA	EULA	Other Shrubs
Black Thunder	1981	D & B	21.3		1.1				
Belle Ayr	1981	D	28.0	5.3	3.4		0.1	1.1	ROWO=1.1, SAVE=0.1
WyoDak #1	1982	D & B	15.	0.6	1.1	0.6	3.4	2.2	ATGA=3.4, ARCA=0.6
WyoDak #2	1984	D & B	16.	0.6	5.6	0.6	3.4		ATGA=3.4
Pathfinder #1	1977	D	?	?	2.2				--
Pathfinder #2	1982	D	15.	2.2	2.2	1.1 (Blk)		1.1	
Dave Johnson	1981	D & B	20.2	1.	0.6	0.6		0.6	CHVI=2.2, ARCA=0.6
Seminoe #1	1984	?	16.8		1.7		0.6	0.6	SAVE=0.6, ATCO=1.7
Seminoe #2	1984	D	16.8		1.7	0.3	0.6	0.6	SAVE=0.6, ATCO=0.6
Bridger #1	1981	D & B	19.5	2.8			0.6	1.7	
Bridger #1	1984[4]	D & B	24.4	6.7	2.2		1.1	1.1	ATGA=2.2, AFRF=1.
Bridger #1	1984[4]	D & B				3.9	1.1	2.2	ATGA=5.6, ARFR=3.4 ATCO=0.6
Bridger #2	1980	B	13.4	0.6					
Bridger #2	1981[4]	B	19.5	2.8			0.6	1.7	--
Bridger #2	1984[4]	B	29.	5.6		2.2			ATGA=1.1
Bridger #2	1984[4]	B				3.9		2.2	ATGA=5.6, ARFR=3.4 ATCO=0.6

Table 2 Continued.

Location Year(s)	Seeding		Seeding rates (kg/ha) [2]						
(#)	Seeded	Method	All Grasses	All Forbs	ATCA1	ARTR	CHNA	EULA	Other Shrubs
Kemmerer #1	1980	D	15.7		2.2	0.3			
Kemmerer #2	1980	D	15.7		2.2	0.3			
Kemmerer #3	1981	D	15.7		2.2	0.3			

[1] D = drilled, B = broadcast
[2] Indicates pure live seed unless otherwise stated
[3] Plant symbols: ARCA = Artemisia cana, ARFR = Artemisia frigida, ARTR = Artemisia tridentata, ATCA = Atriplex canescens, ATCO = Atriplex confertifolia, CHNA = Chrysothamnus nauseosus, CHVI = Chrysothamnus viscidiflorus, EULA = Eurotia lanata, ROWO = Rosa woodsii, SAVE = Sarcubatus vermiculatus.
[4]The original reclamation sites were overseeded in these subsequent years

Percent aerial cover of shrub species was obtained using the line-intercept method (Canfield 1941). Along each transect, species canopy cover was recorded in centimeters and divided by the transect total (5000 cm). Gaps in the shrub canopy of 4 cm or less were considered to be continuous canopy.

Shrub canopy cover and density by species were converted to relative cover and relative density. Relative cover was calculated by dividing the absolute cover of each shrub species by total cover of all shrub species. Likewise, relative shrub density was calculated by dividing the absolute density of each species by total shrub density for all species. Shannon-Weiner diversity indices were calculated for each sample site (Shannon and Weaver, 1949). Higher diversity indices indicate greater plant community diversity. Antelope and sage grouse habitat requirements were used to assess wildlife habitat quality of shrub communities on reclaimed mined land since both are abundant sagebrush community wildlife species, economically important game species, they represent a mammal and bird species having uniquely different habitat requirements, and detailed habitat requirements of both species are documented in published literature (Hulet et al. 1984, Postovit 1981, Roberson 1984, Braun et al. 1977, Dobkin 1995, and Yoakum 1984). Although other wildlife species are associated with these reclaimed study sites, the absence of published detailed habitat specification for these species limits the evaluation of these sites adequacy for wildlife habitat.

E. Results and Discussion

Establishment Study

Topsoil baseline chemical and nutrient parameters showed the direct-placed topsoil to have significantly higher concentrations for most of the parameters measured (Table 3). Stockpiling the topsoil did not have as much effect on the edaphic properties as reported by others (DePuit 1988, White et al. 1989). This reduced response to stockpiling may be partly due to the topsoil stockpile being seeded to perennial grasses after it was salvaged and stockpiled. This practice generally enhances soil permeability, enhances soil biological functions through the contribution of organic matter to the soil system, and generally improves soil physical properties.

Sagebrush seedling densities exhibited a three-way interaction between topsoil management, mulch type and grass competition for all dates evaluated (Table 4-6). Sagebrush seedling densities responded differently in spring 1992, fall 1992 and spring 1993 compared to fall 1993 and 1994. The large increase in sagebrush seedling densities exhibited between spring 1993 and fall 1993 was due to the wet and cool spring and summer of 1993 (Table 7). Even though 1993 climatic conditions greatly increased the sagebrush seedling density, the density observed in 1992 on the direct-placed topsoil-no competition, mulched treatments (Table 4) exceeded the recently established shrub density standard (1 shrub/m^2) for Wyoming (Federal Register, 1996). This emphasizes the importance of good cultural practices especially since these seedling densities were achieved during a below average (87%) precipitation year. Direct-placed topsoil treatments resulted in 40% more sagebrush seedlings than the stockpiled topsoil treatment, and in 1992 and the spring 1993, the differences were generally 1 to 2 orders of magnitude greater for the direct-placed topsoil. The 1992 soil moisture content of the surface