SEED GERMINATION AND VIABILITY OF WYOMING SAGEBRUSH IN NORTHERN NEVADA

Carlos A. Busso, Mónica Mazzola and Barry L. Perryman

SUMMARY

*Seed size and germination behavior affect performance of early seedlings. The purpose of this study was to investigate relationships between seed size and germination percentage, germination rate, time course of germination and seed viability in Wyoming big sagebrush (*Artemisia tridentata *ssp.* wyomingensis *Beetle and Young). Working hypotheses were: 1) for single seeds, germination percentages and rates are positively related to seed weight; and 2) some TTC-unstained seeds are not unviable, but dormant. Seed collection was conducted at Battle Mountain and Eden Valley sites in northern Nevada during November 2002 and 2003. Individual sagebrush seeds were placed into weight classes, using 0.05mg·seed^{-1} increments, ranging from ≤0.15 to >0.40mg·seed^{-1}. Seeds were incubated in darkness at 15ºC, seedlings with radicles ≥1.0mm were removed daily during the first 10 days, and every other day thereafter until day 32. The study terminated on day 37. Triphenyl tetrazolium chloride (TTC) test was used to determine viability of ungerminated seeds, and Evan's blue to sort out ungerminated seeds into either dead or dormant categories. Results supported both hypotheses. Maximum germination at both sites, years and all seed weight ranges was reached in 5 to 6 days after imbibition. This suggests that moist soil for several consecutive days would help provide optimum field germination in this species. Use of the vital stain Evan's blue demonstrated that, albeit in a small percentage (≤5%), some TTC-unstained seeds were not dead but only dormant with germination potential given appropriate conditions. When sagebrush is used for restoration, relatively heavy seeds should be used because they have the greatest germination potential.*

Introduction

Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis* Beetle and Young) has a wide ecological amplitude (Beetle and Johnson, 1982) in the western United States. It helps prevent erosion, provides wildlife habitat and forage, and improves rangeland aesthetics (Vale, 1974). Knowledge about the ecophysiology of Wyoming sagebrush will contribute to a more efficient use of this species in degraded rangeland restoration activities of the Intermountain West Region of North America. Western rangelands cannot be restored without native plant materials. Whenever there is a desire to reestablish big sagebrush in areas that have been severely disturbed, knowledge of its germination ecology is essential. Despite a growing recent interest in seed size variation (Rockwood, 1985) and the large number of studies on effects of seed size and shrub performance (Hou and Romo, 1998), few studies have focused on individual seed weight as an explanatory

KEY WORDS / *Artemisia tridentata* ssp. *wyomingensis* / Rangeland Restoration / Seed Weight Versus Seed Germination and Viability /

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RESUMEN

*El tamaño de la semilla y las características de la germinación afectan el desempeño de las plántulas en estadios tempranos. El objetivo de este estudio fue investigar las relaciones entre tamaño de semilla y porcentaje de germinación, tasa de germinación, variación de la germinación con el tiempo y viabilidad de las semillas en artemisa (*Artemisia tridentata *ssp.* wyomingensis *Beetle y Young). Las hipótesis de trabajo fueron: 1) para semillas individuales, las tasas y porcentajes de germinación están positivamente relacionados al peso de las semillas; y 2) algunas semillas no teñidas con el TTC no están muertas sino durmientes. Las semillas fueron recolectadas en Battle Mountain y Eden Valley, al norte de Nevada, EEUU, en noviembre 2002 y 2003. Las semillas individuales fueron clasificadas por peso en clases de 0,05mg·semilla^{-1}, variando de ≤0,15 a >0,40mg·semilla^{-1}, y fueron incubadas en oscuridad a 15ºC; las plántulas con radículas ≥1,0mm se removieron diariamente durante los primeros 10 días, y luego cada 2 días hasta el día 32. El estudio concluyó el día 37. La prueba del cloruro de trifenil tetrazolio (TTC) se usó para determinar la viabilidad de las semillas no germinadas, y el azul de Evans para separar semillas no germinadas en durmientes o muertas. Los resultados apoyaron ambas hipótesis. La germinación máxima en ambos sitios, años y todos los rangos de semillas se alcanzó 5-6 días luego de la imbibición. Esto sugiere que un suelo húmedo por varios días consecutivos ayudaría a proveer una germinación óptima en campo. El uso del azul de Evans demostró que aunque en pequeño porcentaje (≤5%), algunas semillas no teñidas por TTC no estaban muertas sino durmientes, con potencial para germinar dadas condiciones apropiadas. Cuando este arbusto es usado para restaurar pastizales degradados se deberían usar semillas relativamente pesadas, que tienen el mayor potencial para germinar.*

RESUMO

*O tamanho da semente e as características da germinação afetam o desempenho das plântulas em estados prematuros. O objetivo deste estudo foi investigar as relações entre tamanho de semente e porcentagem de germinação, taxa de germinação, variação da germinação com o tempo e viabilidade das sementes em "Artemisa" (*Artemisia tridentata *ssp.* wyomingensis *Beetle e Young). As hipóteses de trabalho foram: 1) para sementes individuais, as taxas e porcentagens de germinação estão positivamente relacionadas ao peso das sementes; e 2) algumas sementes não tingidas com o TTC não estão mortas senão dormentes. As sementes foram recolhidas em Battle Mountain e Eden Valley, ao norte de Nevada, EEUU, em novembro 2002 e 2003. As sementes individuais foram classificadas por peso em classes de 0,05mg·semente^{-1}, variando de ≤0,15 a >0,40mg·semente^{-1}, e foram incubadas no escuro a 15ºC; as plântulas com radículas ≥1,0mm se removeram diariamente durante os primeiros 10 dias, e logo cada 2 dias até o dia 32. O estudo concluiu no dia 37. A prova do cloreto de trifenil tetrazólio (TTC) se usou para determinar a viabilidade das sementes não germinadas, e o azul de Evans para separar sementes não germinadas em dormentes ou mortas. Os resultados apoiaram ambas hipóteses. A germinação máxima em ambos locais, anos e todas as faixas de sementes se alcançou 5-6 dias após a embebição. Isto sugere que um solo úmido por vários dias consecutivos ajudaria a prover uma germinação ótima em campo. O uso do azul de Evans demonstrou que embora em pequena porcentagem (≤5%), algumas sementes não tingidas por TTC não estavam mortas senão dormentes, com potencial para germinar dadas as condições apropriadas. Quando este arbusto é usado para restaurar pastagens degradadas se deveriam usar sementes relativamente pesadas, que têm o maior potencial para germinar.*

variable in germination experiments.

Usually, seed size and seedling vigor are positively correlated (Morse and Schmitt, 1985). Large and medium size seeds of various shrub species germinate faster and in higher numbers than small seeds (Springfield, 1973). Total germination and germination rate influence dynamics of seedling populations in natural habitats. The greater the germination rate for any given species, the shorter the germination time (Scott *et al.*, 1984). Post-germination growth in shrub species has been negatively correlated with time-to-germination (Hou and Romo, 1998). Seed size and germination behavior can affect early seedling performance (Banovetz and Scheiner, 1994) and seed size is, typically, positively correlated with seedling vigor (Morse and Schmitt, 1985). Although high seedling vigor may not necessarily translate into superior performance in adult plants (Harper and Obeid, 1967), it often enhances seedling survival under natural conditions (Morse and Schmitt, 1985).

Temperature influences on seed germination is one of the basic parameters governing the periodicity of germination and plant establishment in wildland seedbeds. Evans and Young (1986) reported that total germination of Wyoming sagebrush was 100% under 15ºC conditions. However, sagebrush seeds had low germination in some trials (McDonough and Harniss, 1974; Harniss and McDonough, 1976; Young *et al.*, 1991). It has been reported that seed weight is an important variable for germination of some shrub species (Hou and Romo, 1998). However, information on seed weight variation on several germination parameters is lacking in Wyoming sagebrush.

The triphenyl tetrazolium chloride (TTC) assay has long been used to evaluate seed viability (Moore, 1962; Peters, 2000). This technique, however, is unable to distinguish between dead and viable, but dormant, seeds that may still have germination potential. We evaluated visually seed metabolic activity with TTC and with a vital stain, Evan's blue, in an attempt to minimize this limitation.

The goal was to examine the current unexplored role of seed weight in germination success and timing in Wyoming sagebrush, and assess the efficacy of the TTC technique. This shrub species was chosen because it is an excellent candidate for rangeland restoration, and information on its germination ecology is incomplete. Working hypothesis were: 1) for single seeds, germination percentages and germination rates are positively related to seed weight; and 2) some TTC-unstained seeds are viable, but dormant, retaining their potential capacity for germination. Specific objectives were to determine whether seed weight influences germination percentage and viability, and germination rates; and to classify seeds in germinated, ungerminated but viable, dead and dormant in populations of Wyoming sagebrush.

Study Sites

Seed collection was conducted during 2002 and 2003 at two sites, Izzenhood Ranch Site and Eden Valley Site. Mature Wyoming sagebrush stands were sampled near Battle Mountain, Lander County, Nevada (116º58'N, 40°57'W), and Winnemucca, Humboldt County, Nevada (117º23'N, 41º12'W). The first site was in the Bureau of Land Management (BLM) Elko District, approximately 40km N/NW of the town of Battle Mountain, NV. The second site was in the BLM Winnemucca District, between the Hot Springs Range and Osgood Mountains, approximately 38km N/NW of the town of Golconda, NV.

At Izzenhood Ranch, the study area consisted of approximately 23ha of BLM land with 0-2% slope. Elevation was between 1350-1740m. Enko-Shabliss-Orvada was the predominant soil series association (very fine sandy loam and fine sandy loam). Vegetation composition included Wyoming big sagebrush, Thurber needlegrass (*Stipa thurberiana* Piper) and Indian ricegrass (*Achanatherum hymenoides* [Roem. and Schult.]) (USDA-NRCS, 1992); cheatgrass (*Bromus tectorum* L.) and tumblemustard (*Sisymbrium altissimum* L.) dominated this site. Mean annual precipitation is 203-254mm (USDA-NRCS-PRISM, 1998). At Eden Valley site, the research area consisted of approximately 42ha of BLM land on a 0-8% east-facing hillslope with elevation between 1500-1740m. Hunnton-Zevadez-Enko was the predominant soil series association (very fine sandy loam and fine sandy loam). The vegetation composition for the site included Wyoming big sagebrush, Sandberg bluegrass (*Poa secunda* Presl.), and Bottlebrush squirreltail (*Elymus elymoides* [Raf.] Swezey) (USDA-NRCS, 1992). As in the first site, cheatgrass and tumblemustard were dominant. Average annual precipitation is between 254 and 305mm (USDA-NRCS, PRISM, 1998).

Within each site and year, stands were selected for similar size of Wyoming big sagebrush plants, similar cover and topography, and absence of excessive grazing or other disturbances. Sites were also selected to minimize supplemental moisture or subsurface runoff due to microsite influences to reduce variations in demography between sites (Bonham *et al.*, 1991). Climatic data, as monthly values of temperature (June-November) and precipitation (March-November) for the periods 2002 and 2003 were obtained from National Weather Service climate stations located in Battle Mountain and Paradise Valley, the closest to the sampled sites. Temperature and precipitation values were taken beginning in June or March, respectively, because flowerstalks or initiation of leaf growth were reported for this species during these months in Nevada (Everett *et al.*, 1980). Seed collections were initiated in November. Although climatic conditions at individual sites differ from those at the weather stations, the direction and magnitude of major climatic variations should be similar throughout the area.

Materials and Methods

Field methods

A minimum of 100 randomly selected, similar-size sagebrush plants were sampled from each location. At the Battle Mountain site, sampling was conducted along a transect of at least 10km because of the patchy distribution of sagebrush. At Eden Valley, sampling was conducted along a 5km transect.

Laboratory methods

Inflorescences were air-dried and stored at 4°C until use. Seeds (achenes) were then hand picked using a magnifying glass, and stored in paper bags at 4°C until tested (Welch *et al.*, 1996). Seed size was measured as dry weight. Mean monthly temperatures were taken from June onwards since Wyoming sagebrush flower stalks can appear as early as June (Everett *et al.*, 1980). The seed stalks are forming then, but they are not mature with functional seeds that early.

Based on seed weight distribution of each population, seeds were classified into different weight-classes. Thereafter, effects of seed weight on germination percentage and coefficient of velocity, and seed viability were determined. About 3700 seeds were individually weighed. They were classified into 4 to 5 seed size classes each year at the two sites ranging from ≤0.15 to >0.40mg·seed^{-1}. Before initiating the germination study, a stratification treatment was imposed on all seeds that consisted of maintaining seeds in a cold room (4°C) for 10 days. Thereafter, seeds were dusted with Captan (N-[(trichloromethyl)-thio]-4-cyclohexene-1,2-dicarboximide) prior to the germination test to prevent fungi development (Hou and Romo, 1998). Seeds were germinated in 9cm petri dishes on moistened, double layers of N° 1 Whatman filter paper. There were 20 seeds per dish and 5 dishes per treatment for each of the 4 individual collections. A total of 2000 seeds were monitored during the study. Filter paper was kept moist. Petri dishes were randomized and stacked into plastic bags closed with rubber bands to retard water loss (Meyer and Monsen, 1992). Dish position within the plastic bags was systematically changed while inspecting for germinated seeds. Inspection was conducted daily for the first 10 days and every other day thereafter to the 32nd day; five days later the experiment was terminated. After counting, germinated seeds were removed from petri dishes on each observation date. Tests were conducted at 15°C in dark germinators (Meyer and Monsen, 1992). Big sagebrush seeds germinate in the dark without pretreatment (Young *et al.*, 1991). Use of continuous 15°C for 24h was comparable to the moderate seedbed temperature definition of Young *et al.* (1991). This category of seedbed temperature mimics spring germination conditions, based on several years of microenvironmental monitoring in field seedbeds in the Great Basin big sagebrush zone (Evans and Young, 1972). Seeds were considered germinated when the radicle was ≥1mm (Meyer and Monsen, 1992).

Viability of ungerminated seeds was determined by triphenyl tetrazolium chloride (TTC) test (Peters, 2000). Seeds that remained unstained after incubation with the TTC solution were tested using the Evan's blue (0.25% w/v) stain. Evan's blue does not penetrate intact, semi-permeable membranes (Gaff and Okong'O-Ogola, 1971). Longitudinal sections of unstained-TTC seeds were soaked in Evans's blue for 15 to 20min at room temperature (25°C). Excess dye was rinsed from the sections, mounted in water and examined under a microscope. Seed sections killed by boiling water were evenly stained, dark blue (Busso *et al.*, 1989). Experimental material with such an appearance was considered dead. TTC-unstained seeds which did not stain with Evan's blue were considered dormant, and those that stained evenly were considered dead.

The coefficient of velocity (CV) was calculated as $CV=100(\Sigma N_i/\Sigma T_iN_i)$ where N is the number of seeds germinated on day I and T is the number of days from seeding (Scott *et al.*, 1984). This coefficient increases as more seeds germinate and with shorter germination times.

Data analysis

Treatments (2 sites × 2 years × 4 to 6 weight-classes) were arranged in a completely randomized design within the germination chamber. Differences in germination percentages between sites in 2003, and between years in Battle Mountain, were tested using two-way analysis of variance (ANOVA; sites or years × seed size ranges). Regression analysis was used to study the relationship between seed weight and germination percentage within each year. With this purpose, the lowest and highest values were obtained for each of the 20 seed weight ranges coming from both sites and years. As reported by Hou and Romo (1998), the middle point within each seed weight range was obtained to scale

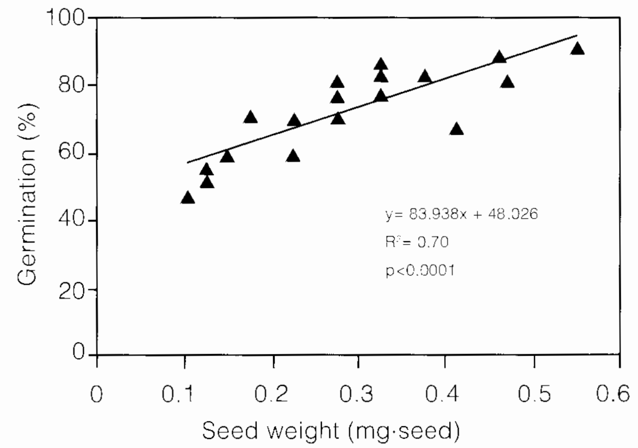

Figure 1. Predicted regression line for maximum germination of *Artemisia tridentata* spp. *wyomingensis* with different seed weights. Each symbol is the mean of n=5. Data from individual regression lines for 2002 and 2003 were combined because these regression lines were not statistically different ($p>0.05$) following the procedure of Neter *et al.* (1985).

the independent variable. Regression lines for both years were compared using the procedure described by Neter *et al* (1985), and the data pooled if they were not significantly different ($p>0.05$).

Percentage germination of each class category of viability within each seed weight range was compared using two-way ANOVA within each site and year. Variation of germination percentage through time within each seed weight range was analyzed using repeated measures ANOVA. Because of different seed weight ranges between Eden Valley 2002 and the other 3 site/year combination, coefficients of velocity were analyzed separately for these sites using one-way ANOVA (seed weight range comparison) for Eden Valley 2002 and two-way ANOVA (site x seed weight range) for Eden Valley 2003, and Battle Mountain 2002 and 2003. Fisher's (protected) least significant difference test was utilized for mean separation when F tests indicated that a variable was significant ($P<0.05$, Steel and Torrie, 1980). All percentage values were transformed to arcsine values before statistical analysis, but untransformed values are presented in figures, tables and text.

Results and Discussion

Seed size ranges

Greater seed sizes showed greater ($p<0.05$) germination percentages at both sites and years (Figure 1 and Table I). Seed weight (X) and germination percentage (Y) were significantly ($p<0.05$) related in 2002 ($Y=50.4 + 64.9$ X; $R^2=0.54$) and 2003 ($Y=47.7 + 92.0$ X; $R^2=0.83$). Aiken and Springer (1995) in *Panicum virgatum* also reported that total percent germination increased linearly with increases in seed weight. This is somewhat inconsistent with results of Hou and Romo (1998) who reported that total germination of silver sagebrush (*Artemisia cana* Pursh) initially increased with seed weight, but then declined at weights greater than about 0.57mg·seed^{-1}. Other studies have found that seed weight had little or no effect on seed germination (Vaughton and Ramsey, 1998).

The greater the seed size ranges, the greater were the coefficients of velocity (Table II), indicating that greater seed sizes will have higher germination with shorter germination times (Scott *et al.*, 1984). However, Giles (1990) observed that plants of wild barley (*Hordeum vulgare* spp. *spontaneum*) emerging from small seeds were observed to germinate faster than plants from large seeds. Maximum germination at both sites, years and in all seed size ranges was reached between 5 to 6 days after imbibition (Table III). Germination studies on this species do not necessarily need to go beyond this time limit to obtain maximum germination, saving time, labor and money. In *A. tridentata* spp. *wyomingensis*, McDonough and Harniss (1974) reported that 3 to 6 days were required at all tested temperatures, except 10ºC and 10º-2ºC, to reach one-half of the final germination percentage, although an 8h photoperiod was used at those temperatures. Meyer and Monsen (1992) also showed that Wyoming big sagebrush germinated to 50% in less than 7 days. The present results may partially explain why sagebrush establishment is an episodic event: given the prolific seed production in sagebrush, the data suggest that moist soil during several consecutive days would provide maximum germination in *A. tridentata*

TABLE I

MEAN VALUES FOR PERCENT GERMINATION OF DIFFERENT SEED SIZE RANGES FOR VARIOUS CATEGORIES OF SEED VIABILITY OF SEEDS COLLECTED AT TWO SITES IN NORTHERN NEVADA DURING 2002 AND 2003*

Site and year	Seed size range	% of seed in each viability category			
	mg/seed	Germinated	TTC (viable)	Dead	Dormant
Eden Valley 2002	≤0.15	47 ±4.6 c,a	34 ±2.9 ab,b	16 ±2.9 a,c	3 ±2.0 a,c
	>0.15-0.20	71 ±5.3 a,a	23 ±6.0 b,b	4 ±1.9 a,c	2 ±2.0 a,c
	>0.20-0.25	59 ±7.6 b,a	38 ±7.2 a,b	3 ±2.0 a,c	0 b,c
	>0.25	68 ±5.4 ab,a	25 ±4.5 b,b	2 ±1.2 b,c	5 ±1.6 b,c
Eden Valley 2003	≤0.20	54 ±2.4 c,a	22 ±4.6 ab,b	23 ±4.1 a,b	1 ±1.0 a,c
	>0.20-0.25	70 ±2.7 b,a	21 ±3.7 ab,b	7 ±2.5 b,c	2 ±1.2 a,c
	>0.25-0.30	71 ±6.2 b,a	28 ±6.2 a,b	1 ±1.0 b,c	0 a,c
	>0.30-0.35	86 ±3.7 a,a	12 ±2.5 b,b	1 ±1.0 b,b	1 ±1.0 a,b
	>0.35	88 ±4.6 a,a	12 ±4.6 b,b	0 b,b	0 a,b
Battle Mountain 2002	≤0.20	59 ±7.3 c,a	12 ±3.7 a,bc	24 ±4.8 a,b	5 ±2.7 a,c
	>0.20-0.25	70 ±2.2 b,a	18 ±4.6 a,b	11 ±3.3 b,bc	1 ±1.0 a,c
	>0.25-0.30	76 ±4.3 ab,a	13 ±4.1 a,b	10 ±1.6 b,b	1 ±1.0 a,b
	>0.30-0.35	76 ±4.3 ab,a	16 ±4.3 a,b	5 ±1.6 b,bc	3 ±1.2 a,c
	>0.35	81 ±4.3 a,a	17 ±4.6 a,b	0 b,c	2 ±1.2 a,c
Battle Mountain 2003	≤0.20	52 ±4.4 d,a	3 ±2.0 b,b	45 ±4.2 a,a	0 a,b
	>0.20-0.25	70 ±4.2 c,a	13 ±2.5 a,b	17 ±4.4 b,b	0 a,c
	>0.25-0.30	80 ±2.2 b,a	13 ±2.0 a,b	7 ±2.0 c,bc	0 a,c
	>0.30-0.35	83 ±3.0 b,a	13 ±3.4 a,b	4 ±1.9 c,bc	0 a,c
	>0.40	91 ±1.9 a,a	9 ±1.9 ab,b	0 c,b	0 a,b

* Mean ±SE, n=5.

Within columns, different letters to the left of the comma indicate significant differences ($p<0.05$) among seed size ranges within each study site and year. Within rows, different letters to the right of the comma indicate significant differences ($p<0.05$) among seed viability categories within each study site and year.

spp. *wyomingensis*, a process that may produce greater shrub establishment. Infrequent patterns of sagebrush seedling establishment have been attributed, at least in part, to periods of unfavourable weather (Lommasson, 1949), but little supporting evidence has been available. Maier *et al.* (2001) concluded that climatic conditions supporting widespread pulses of big sagebrush [*A. tridentata* spp. *wyomingensis*, *A. tridentata* spp. *tridentata* Beetle and Johnson and *A. tridentata* spp. *vaseyana* (Rydb.)] establishment on native sites should be examined in greater depth. They reported strong evidence that precipitation variables significantly affect high levels of seedling recruitment on native, undisturbed big sagebrush sites throughout Wyoming. Additionally, successful recruitment of big sagebrush is partially dependent on seed size and viability (McDonough and Harniss, 1974; Geritz, 1995). Greater seed sizes can contribute to better sagebrush seedling establishment. Seed viability in the present study was higher ($p<0.05$) in the greater than in the smaller seed weight ranges at both locations and years (Table I).

The greater the seeds, the higher the germination percentages at both sites in 2002 and 2003 (Figure 1 and Table I). It has long been demonstrated that large seeds of various shrub species germinate in higher numbers than small seeds (Springfield, 1973; Hou and Romo, 1998). The size of the seed is also known to affect the fitness of the plant growing from it, larger seeds often have higher fitness (Giles, 1990). Initial seed size can also affect time of germination (Tabla III), seedling growth rate and the number of seeds produced in herbaceous species (Giles, 1990). However, small seeds can also contribute to plant fitness by exploiting safe sites that by chance are left without any larger seeds (Geritz, 1995). If there was no variation among safe sites, then small seeds would never have the opportunity to occupy a safe site, and only the largest possible seeds would yield established plants. Investment of resources in small seeds would be lost and hence be selected against. Nevertheless, if seed dispersal is random so that small seeds have a chance to reach safe sites that remain unoccupied by larger seeds, then production of small seeds would be beneficial if the competitive disadvantage is outweighed by a larger seed number and hence a higher efficiency for colonizing unoccupied safe sites. Small seed size tends to increase the chances of a seed's being incorporated in the pool of buried seeds (Grime, 1979), and also increases seed longevity (Harper, 1977).

By the time this study was conducted, seeds collected in 2002 had been stored under laboratory conditions for over 1½ years. Seed storage in the laboratory, however, is not the same as seed storage in soil in the field, where the seeds are subject to fluctuation in soil moisture and temperature, seed predators, and fungi (ie. Louda, 1989). This may help to explain why Young and Evans (1989) found a short viability of big sagebrush seeds in the soil. They found that for 6 months out of the year there was no detectable reserve of seeds in the soil. Mined-land reclamation specialists in the Powder River Basin of Wyoming have routinely observed new sagebrush seedlings over a period of 2-3 years after seeding (Schuman *et al.*, 1998). It must be rec-

TABLE II
MEAN VALUES FOR COEFFICIENTS OF VELOCITY, AN INDEX FOR THE RATE OF GERMINATION, OF DIFFERENT SEED SIZE RANGES FOR SEEDS COLLECTED IN EDEN VALLEY AND BATTLE MOUNTAIN IN NORTHERN NEVADA DURING 2002 AND 2003

	Seed size range mg/seed			
	>0.25	>0.20-0.25	>0.15-0.20	≤0.15
Eden Valley 2002	5.84 ±0.07 a	5.81 ±0.06 ab	5.62 ±0.04 bc	5.46 ±0.10 c
	>0.30-0.35	>0.20-0.25	>0.25-0.30	≤0.20
Eden Valley 2003, and Battle Mountain 2002 and 2003	6.00 ±0.04 a	5.89 ±0.05 b	5.88 ±0.05 b	5.79 ±0.09 b

* Mean ±SE, n=5.
Within the same row, means followed by different letters are statistically significant ($p<0.05$).

TABLE III
TIME COURSE OF GERMINATION PERCENTAGE AFTER SEED IMBIBITION IN DIFFERENT SEED SIZE CATEGORIES ON SEEDS COLLECTED AT TWO SITES IN NORTHERN NEVADA DURING 2002 AND 2003*

	Range mg/seed	Days after imbibition 2	4	5	14	37
Eden Valley 2002	≤0.15	3 ±2.0 a	18 ±3.4 ab	30 ±5.7 bc	44 ±4.0 c	47 ±4.6 c
	>0.15-0.20	2 ±1.2 a	40 ±3.9 b	57 ±7.7 bc	71 ±5.3 c	71 ±5.3 c
	>0.20-0.25	11 ±6.0 a	46 ±8.9 bc	50 ±7.6 c	58 ±8.5 c	59 ±7.6 c
	>0.25	30 ±5.5 a	48 ±6.8 abc	56 ±4.8 bcd	67 ±5.8 d	68 ±5.4 d
	≤0.20	9 ±4.6 a	29 ±7.3 bc	40 ±6.5 bcd	53 ±2.6 d	54 ±2.4 d
	>0.20-0.25	11 ±3.3 a	48 ±5.6 bc	54 ±2.9 cd	69 ±2.4 e	70 ±2.7 e
Eden Valley 2003	>0.25-0.30	15 ±5.0 a	43 ±7.2 bc	56 ±4.3 cd	70 ±5.7 ef	71 ±6.2 ef
	>0.30-0.35	40 ±5.9 a	67 ±6.4 bc	75 ±3.2 cd	85 ±3.5 e	86 ±3.7 c
	>0.35	30 ±4.5 a	68 ±2.0 b	84 ±4.0 c	88 ±4.6 c	88 ±4.6 c
	≤0.20	10 ±3.2 a	43 ±8.1 bc	49 ±8.0 cd	57 ±7.0 cd	59 ±7.3 d
	>0.20-0.25	13 ±1.2 a	53 ±5.6 bc	61 ±2.9 cd	70 ±2.2 d	70 ±2.2 d
Battle Mountain 2002	>0.25-0.30	27 ±3.4 a	55 ±6.5 bc	60 ±5.2 bcd	76 ±4.3 e	76 ±4.3 e
	>0.30-0.35	27 ±4.1 a	61 ±7.5 bc	70 ±5.7 cd	75 ±4.2 cd	76 ±4.3 cd
	>0.35	13 ±3.4 a	51 ±7.5 b	66 ±6.6 bc	81 ±4.3 d	81 ±4.3 d
	≤0.20	19 ±3.3 a	41 ±4.3 bc	46 ±4.3 bc	51 ±4.8 c	52 ±4.4 c
	>0.20-0.25	31 ±2.9 a	60 ±4.2 bc	66 ±3.3 c	67 ±3.4 c	69 ±4.3 c
Battle Mountain 2003	>0.25-0.30	47 ±6.2 a	71 ±4.3 bc	76 ±3.3 c	80 ±2.2 c	80 ±2.2 c
	>0.30-0.35	49 ±3.3 a	69 ±4.3 bc	78 ±3.7 cd	81 ±1.9 d	82 ±2.5 d
	>0.35-0.40	46 ±8.1 a	72 ±5.4 bc	77 ±4.9 bcd	83 ±5.1 d	83 ±5.1 d
	>0.40	46 ±4.8 a	80 ±5.9 c	86 ±2.9 cd	91 ±1.9 d	91 ±1.9 d

* Each value is the mean (±SE) of n=5. Within the same row, means followed by different letters are statistically significant ($p<0.05$).

ognized, however, that there may not be any germination pattern that characterizes any one subspecies of big sagebrush (Meyer and Monsen, 1992). Attempting to characterize the germination response of a native species that occurs over a wide range of habitats from one or a few collections often results in accounts that are usually conflicting (Evans and Young, 1986). These differences appear to represent discrete samples along a more or less continuous spectrum of variation among field populations.

Sites and years

Within each of the seed weight classes, germination percentage was similar ($p>0.05$) at both sites and years. This may be due partially because both sites had a similar ($p>0.05$) precipitation during June to November (Battle Mountain: 64.0mm in 2002 and 86.11mm in 2003; Eden Valley: 73.91mm in 2002, and 66.04mm in 2003), and also a similar ($p>0.05$) mean monthly temperature during the same period (15.5 ±0.3°C). June to November precipitation and temperature values were taken because flowerstalks have been reported for this species during these months in Nevada (Everett *et al.*, 1980). This result agrees with that reported by Harniss and McDonough (1976), that Wyoming big sagebrush did not show a significant difference in year-to-year germination between three consecutive years near Dubois, Idaho. Evans and Young (1986) also found no differences between average germination for big sagebrush seeds produced at different locations during two consecutive years, although these authors recognized that year-to-year variations in environmental conditions would have a big influence on seed germination.

The number of seed weight ranges was greater at both sites during 2003 than during 2002 (Tables I and III). This can partially be attributed to greater precipitation in 2003 (140.72mm in Eden Valley; 183.39mm in Battle Mountain) than 2002 (95.5mm in Eden Valley; 113.28mm in Battle Mountain) during the period from March to November. A previous study (Busso and Perryman, unpublished data) using simple linear regression demonstrated that monthly precipitation (March to November) was an important predictor of mean seed size ($p=0.079$; $r^2=0.85$). Geritz (1995) also reported that the range of seed sizes can increase as the total amount of resources available to plants also increase. In dry life zones, there is an indication that heavier seeds may be selected for (Rockwood, 1985). It is important that source-identified site-matched seedlots be used when seeding this species for disturbed land or fire rehabilitation. Nonadapted seeds may misread germination cues and germinate at inappropriate times, seedlings may fail to emerge or persist, and the results may be stand failure (Meyer and Monsen, 1990).

Seed viability

Greatest ($p<0.05$) seed mortality occurred in the lower seed weight ranges in both locations and years (Table I). Larger seed weight ranges showed a greater viability percentage (germinated + viable) than smaller seeds at both locations and years (Table I). This finding agrees with that of Bell *et al.* (1995) in species native to the southwest of Western Australia that represented a range of plant families, life-history strategies, fire-response syndromes, seed-storage types and seed weights.

Ungerminated, TTC-stained seeds have previously been classed as dormant (Meyer and Monsen, 1992). In addition, ungerminated, TTC-unstained seeds have been considered nonviable (Evetts and Burnside, 1972). Use of the vital stain Evan's blue demonstrated that, albeit in a small percentage, some TTC-unstained seeds were not dead but dormant (Table I), with germination potential given appropriate conditions. It is suggested that viability should be divided into 4 classes: germinated, ungerminated but viable (TTC-stained), and ungerminated but either dormant or dead. A distinction must be made between ungerminated, TTC-stained seeds (viable) and ungerminated, TTC-unstained seeds (either dormant or dead). TTC-stained seeds have high respiratory activity, and that is why they undergo enzymatic reduction of the tetrazolium salt to insoluble, red formazan in living tissues (Smith, 1951). TTC-unstained seeds have such a low respiratory activity that they are unable to make this metabolic conversion and remain unstained. This would alter the results of Hou and Romo (1998) in silver sagebrush that found 63% viability (TTC) of non-germinated seeds in the heaviest class for that species, and suggested seeds were dormant when the test only indicated high respiratory activity.

Conclusions

The results demonstrate that germination percentages and coefficients of velocity were positively related to seed weight. Maximum germination at both study sites, years and all seed weight ranges was reached in 5 to 6 days after imbibition, suggesting that moist soil for several consecutive days would help provide optimum field germination in Wyoming sagebrush. Ungerminated, TTC-stained seeds have been previously considered as dormant. This is wrong since for staining these seeds must have a high respiratory activity. Ungerminated, TTC-unstained seeds have also often been considered as dead, when they can really be either dead or dormant. Dormant seeds can still have germination potential given appropriate conditions. A clear separation between either dead or dormant seeds was made using the vital stain Evan's blue in our study. When sagebrush is used for restoration, relatively heavy seeds should be used since they have the greatest potential for germinating. Mature seeds collected at the field should be allowed to go through a set of screen sizes from gratest to smallest, after seed threshing and cleaning have been completed in the laboratory (see Young and Young, 1999). In this manner, the greatest seeds will be retained in the upper screens, and the smallest seeds could be collected in the lower screens within the set.

REFERENCES

Aiken GE, Springer TL (1995) Seed size distribution, germination, and emergence of 6 switchgrass cultivars. *J Range Manag. 48*: 455-458.

Banovetz SJ, Scheiner SM (1994) The effects of seed mass on the seed ecology of *Coreopsis lanceolata*. *Am. Midland Naturalist* 131: 65-74.

Beetle AA, Johnson KL (1982) Sagebrush in Wyoming. University of Wyoming Agr. Exp. Sta., Laramie, Wyo. USA. 68 pp.

Bell DT, Rokich DP, McChesney CJ, Plummer JA (1995) Effects of temperature, light and gibberellic acid on the germination of seeds of 43 species native to Western Australia. *J. Veg. Sci. 6*: 797-806.

Bonham CD, Cottrell TR, Mitchell JF (1991) Inferences for life history strategies of *Artemisia tridentata* subspecies. *J. Veg. Sci. 2*: 339-344.

Busso CA, Mueller RJ, Richards, JH (1989) Effects of drought and defoliation on bud viability in two caespitose grasses. *Ann. Bot. 63*: 477-485.

Evans RA, Young JA (1972) Microsite requirements for establishment of annual rangeland weeds. *Weed Sci. 20*: 350-356.

Evans RA, Young JA (1986) Germination profiles for five populations of big sagebrush. In McArthur ED, Welch BL (Eds.) *Proc. Biology of Artemisia* and *Chrysothamnus Symposium*. General Technical Report INT-200. Intermountain Research Station, Forest Service, USDA. Ogden. UT. USA. pp.366-369.

Everett RL. Tueller PT. Davis JB. Brunner AD (1980) Plant phenology in galleta-shadscale and galleta-sagebrush associations. *J. Range Manag. 33*: 446-450.

Evetts LL. Burnside OC (1972) Germination and seedling development of common milkweed and other species. *Weed Sci. 20*: 371-378.

Gaff DF. Okong'O-Ogola O (1971) The use of non-permeating pigments for testing the survival of cells. *J. Exp. Bot. 22*: 756-758.

Geritz SAH (1995) Evolutionarily stable seed polymorphism and small-scale spatial variation in seedling density. *Am. Naturalist 146*: 685-707.

Giles BE (1990) The effects of variation in seed size on growth and reproduction in the wild barley *Hordeum vulgare* ssp. *spontaneum*. *Heredity 64*: 239-250.

Grime JP (1979) *Plant strategies and vegetation processes*. Wiley. Chichester. UK. 222 pp.

Harniss RO. McDonough WT (1976) Yearly variation in germination in three subspecies of big sagebrush. *J. Range Manag. 29*: 167-168.

Harper JL (1977) *Population biology of plants*. Academic Press. London. UK. 832 pp.

Harper JL. Obeid M (1967) Influence of seed size and depth of sowing on the establishment and growth of varieties of fiber and oil seed flax. *Crop Sci. 7*: 527-532.

Hou J. Romo JT (1998) Seed weight and germination time affect growth of 2 shrubs. *J. Range Manag. 51*: 699-703.

Lommasson T (1949) Succession in sagebrush. *J. Range Manag. 1*: 19-21.

Louda SM (1989) Predation in the dynamics of seed regeneration. In Leck. MA. Parker VT. and Simpson RL (Eds.) *Ecology of Soil Seed Banks*. Academic Press. San Diego. CA. USA. pp.25-51.

Maier AM. Perryman BL. Olson RA. Hild AL (2001) Climatic influences on recruitment of 3 subspecies of *Artemisia tridentata*. *J. Range Manag. 54*: 699-703.

McDonough WT. Harniss RO (1974) Effects of temperature on germination in three subspecies of big sagebrush. *J. Range Manag. 27*: 204-205.

Meyer SE. Monsen SB (1992) Big sagebrush germination patterns: Subspecies and population differences. *J. Range Manag. 45*: 87-93.

Moore RP (1962) Tetrazolium as a universally acceptable quality test of viable seed. *Proc. the Seed Testing Assoc. 27*: 795-805.

Morse DH. Schmitt J (1985) Propagule size. dispersal ability. and seedling performance in *Asclepias syriaca*. *Oecologia 67*: 372-379.

Neter J. Wasserman W. Kutner MH (1985) *Applied linear statistical models: regression. analysis of variance. and experimental designs*. RD Irwin. Homewood. IL. USA. 1127 pp.

Peters J (2000) *Tetrazolium testing handbook*. 2nd ed. Association of Official Seed Analysts. 50 pp.

Rockwood LL (1985) Seed weight as a function of life form. elevation and life zone in neotropical forests. *Biotropica 17*: 32-39.

Schuman GE. Booth DT. Cockrell JR (1998) Cultural methods for establishing Wyoming big sagebrush on mined lands. *J. Range Manag. 51*: 223-230.

Scott SJ. Jones RA. Williams WA (1984) Review of data analisis methods for seed germination. *Crop Sci. 24*: 1192-1199.

Smith FE (1951) Tetrazolium Salt. *Science 113*: 751-754.

Springfield HW (1973) Large seeds of winterfat germinate better. *J. Range Manag. 26*: 153-154.

Steel GD. Torrie JH (1980) *Principles and procedures of statistics. A biometrical approach*. 2nd ed. McGraw-Hill. New York. USA. 633 pp.

USDA-NRCS (1992) *Soil Survey of Humboldt County. Nevada. East Part*. Vol. 1 & 2. USDA-Soil Conservation Service. Washington. DC. USA. 2025 pp.

USDA-NRCS-PRISM (1998) *PRISM Climate Mapping Project – Precipitation West Region*. USDA-NRCS. Washington. DC. USA. Available in CD.

Vale TR (1974) Sagebrush conversion projects: An element of contemporary environmental change in the western United States. *Biodivers. Cons. 6*: 274-284.

Vaughton G. Ramsey M (1998) Sources and consequences of seed mass variation in *Banksia marginata* (Proteaceae). *J. Ecol. 86*: 563-573.

Young JA. Evans RA (1989) Dispersal and germination of big sagebrush (*Artemisia tridentata*) seeds. *Weed Sci. 37*: 201-206.

Young JA. Palmquist DE. Evans RA (1991) Temperature profiles for germination of big sagebrush seeds from native stands. *J. Range Manag. 44*: 385-390.

Young. JA. Young ChG (1999) *Collecting. Processing and Germinating Seeds of Wildland Plants*. Timber Press. Portland. OR. USA. 236 pp.

Welch BL. Briggs SF. Johansen JH (1996) *Big sagebrush seed storage*. Research note. Intermountain Research Station. USDA Forest Service. Nº INT-RN-430. Provo. UT. USA. 3 pp.