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Characterization of a Sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) Die-Off on the Hanford Site

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Summary

The Hanford Site contains one of the few remaining contiguous areas of shrub-steppe habitat left in Washington State. This habitat is home to many native plant and wildlife species, some of which are threatened with extinction or are unique to the Site. The importance of the Hanford Site increases as other lands surrounding the Site are developed, and these native species and habitats are lost. Stands of Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) on the Site are a particularly important component of shrub-steppe habitat, because a number of wildlife require big sagebrush for food and cover.

Since 1993, researchers and field biologists have made anecdotal observations of dying and declining sagebrush in stands of shrubs near the 100 Areas. This study was initiated to delineate and document the general boundary where sagebrush stands appear to be declining.

We mapped the areal extent of the die-off using a global positioning system and found that the central portion of the die-off encompasses 280 hectares. Shrub stand defoliation was estimated to be near or greater than 80% in this area. The remainder of the die-off area exhibits varying mixtures of completely defoliated, partially defoliated, and healthy-looking stands. Declining sagebrush stands comprise a total of 1776 hectares.

Within the die-off area and at the perimeter, we measured sagebrush cover and categorized shrubs according to the percent of the canopy with live foliage within six belt transects. Reproductive capability and viability of shrubs also were evaluated based on inflorescence number, seed production estimates, and seed germination.

Preliminary results indicate that seed weight and seed count decline as shrub defoliation increases. Germination rates were variable, but general trends across transects were noted based on the degree of stand decline. These observations indicate that it may be important to consider the die-off with regard to habitat restoration and mitigation efforts on site. Possible causes and pathways of shrub death have not yet been investigated; however, based on results of this study, the sagebrush die-off appears to result from natural causes. Information from this study provides a baseline for future monitoring and will help determine whether the die-off area continues to expand.

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1.0 Introduction

The U.S. Department of Energy's (DOE) Hanford Site occupies 1450 km^2 within the semiarid Pasco Basin of the Columbia Plateau in southeastern Washington (Figure 1.1). The Site is located within the Columbia Basin ecoregion, and the most prevalent vegetation at the site is shrub-steppe. The most common shrub communities on the Hanford Site include big sagebrush (*Artemisia tridentata*).

Since 1945, when the Hanford Site was established, shrub-steppe vegetation within Site boundaries has been largely unaltered and protected from human intrusion. As a result, the Site represents one of the few large contiguous sagebrush stands in the state. Sagebrush-obligate species and other native wildlife populations depend on this habitat as other sagebrush stands on lands surrounding Hanford decline.

In spring 1993, site biologists began to notice a die-off of sagebrush stands just south of the 100-D/DR Area (Weiss 1996; Mike Sackschewsky, PNNL, personal communication). Biologists have since observed the spread of the die-off to the surrounding sagebrush community, including stands across the Columbia River (Rhett Zufelt, PNNL, personal communication). A significant portion (1776 hectares) of the Wyoming big sagebrush community on the Hanford plateau is now affected by an unknown influence, causing an observable decline in the above ground vegetative portion of the sagebrush.

In 1993, Pacific Northwest National Laboratory (PNNL) began to characterize the sagebrush die-off on the Hanford Site, but the phenomenon was not formally documented until the current study was conducted beginning in fall 1996. The primary objective of the study was to establish a general boundary to delineate where the sagebrush stands appeared to be declining. This information will provide a baseline for future monitoring and help determine whether the die-off area is expanding or recovering. The second objective was to assess seed production and seed viability of sagebrush within the die-off area. Because big sagebrush does not reproduce vegetatively, evaluation of seed production and germination success is a first step in assessing the sagebrush stand's reproductive potential with respect to the die-off.

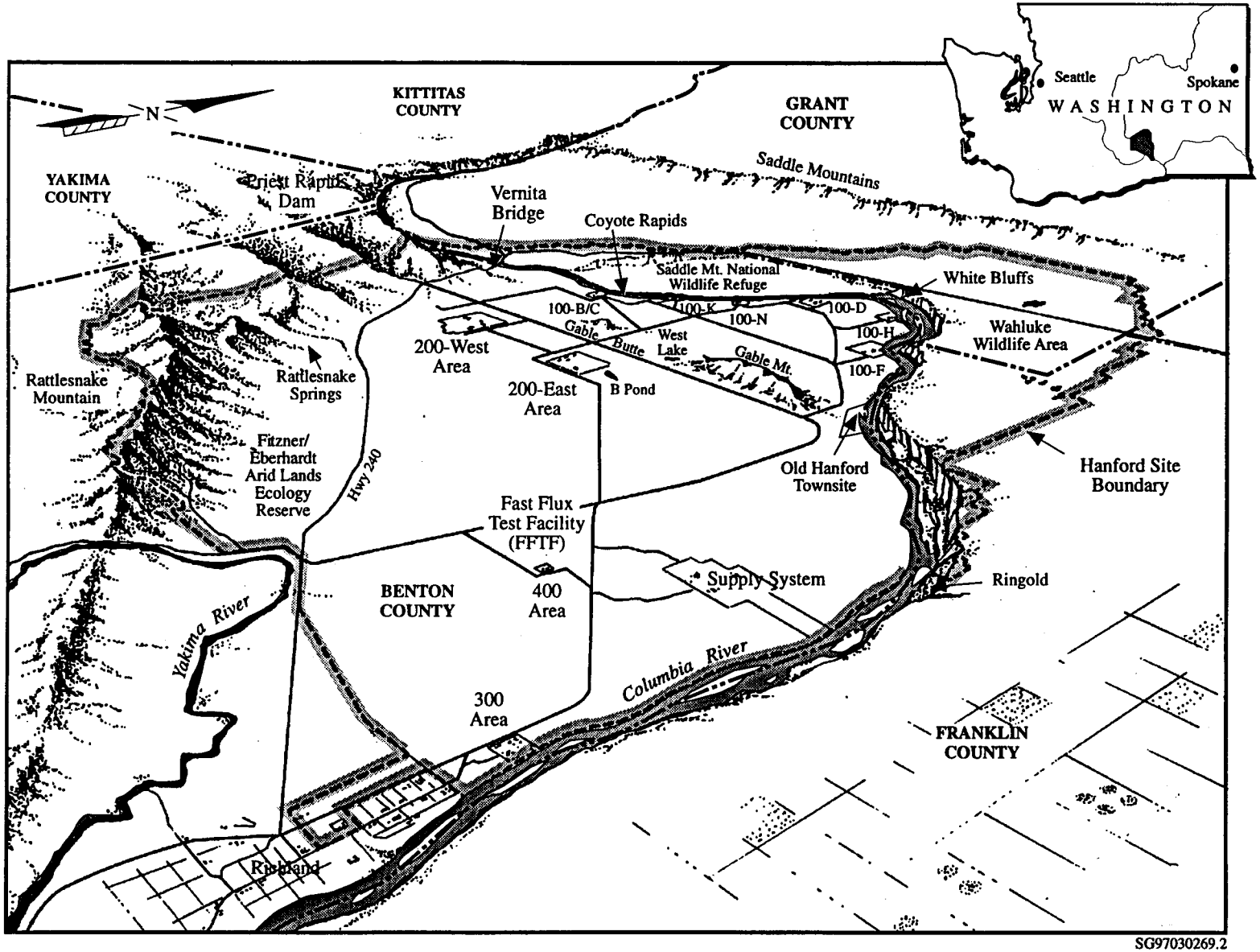

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Figure 1.1. Location of the Hanford Site in Southeastern Washington

2.0 Methods

2.1 Mapping

The extent of the die-off was mapped using a Global Positioning System (GPS). The GPS data were transferred to a Geographical Information System [(GIS); USA-CERL GRASS 4.1], which was used to produce a final map of the die-off area. Sagebrush stands that exhibited noticeable shrub decline were included within the die-off area boundary. A qualitative approach was used to determine noticeably affected stands and individuals in the field through visual observation; however, these preliminary data are not sufficient to quantify the die-off nor compare it to a normally declining stand.

In addition to mapping the extent of the die-off, six 25-m x 5-m belt transects were established to examine the phenomenon in more detail. One belt transect lies outside the delineated die-off area, with the other five located within the declining stands. Two 100-m line transects also were established for seed collection. Line Transects A and B correspond to a border of belt transects 1 and 2. Each sagebrush within the sampling area was measured and assigned to one of the following four categories:

- I - no foliage
- II - less than 50% of branches with foliage
- III - 50-90% branches with foliage
- IV - greater than 90% branches with foliage.

Transect location, shrub location, average canopy dimension (height, longest diameter, perpendicular diameter), shrub category, and total number of inflorescences were recorded for each sagebrush shrub in the transect.

2.2 Seed Collection

A 100-m line transect was established at two of the six belt transects to determine sagebrush seed production. Line Transects A and B correspond to a border of Belt Transects 1 and 2. Line Transect B represented a moderately affected sagebrush stand (18% shrubs without foliage) while Line Transect A ran through a more seriously affected stand (95% shrubs without foliage). Average canopy dimensions, total inflorescence number and shrub category were recorded for each sagebrush along the transect.

On December 3, 1996, five inflorescences were taken from each sagebrush along the transect and placed in a paper bag, one bag per shrub. Care was taken to harvest the inflorescences without shaking out the seeds. Samples were left to air dry at room temperature for 8 days. The weight of the combined air-dried inflorescence stems was measured for each set of five inflorescences. The inflorescence chaff was separated from the stems by hand. A subsample of the chaff was weighed out, then the seeds were manually separated from the chaff. The pure seed weight and quantity were recorded for each subsample. The subsample seed number, seed weight, inflorescence weights, and total inflorescence count per shrub were used to extrapolate seed number and seed weight per inflorescence.

2.3 Germination Test

Seeds were stored in paper bags in the laboratory until tested. The test was conducted 96 days after harvest. Thirty seeds from each shrub subsample were manually separated from the inflorescence chaff, except for those subsamples that yielded fewer than 30 seeds.

Seeds were germinated on moist filter paper in petri dishes placed on a south-facing window during mid-March 1997. The window was coated with an ultraviolet (UV)-protection filter. The number of germinating seeds was recorded daily for 10 days, and the percent germination was calculated for each sample for that time period. During the 10-day test, seeds were exposed to temperatures ranging from 21°C to 28°C, and seeds and filter paper were moistened as necessary. Seeds harvested in November 1996 from healthy sagebrush stands outside the die-off area south of Gable Mountain were germinated using the same methods.

3.0 Results

3.1 Mapping

The mapped area where sagebrush is noticeably declining encompasses about 1776 hectares. The central portion of the die-off area, where shrub defoliation was estimated to be near or greater than 80%, encompasses 280 hectares. Sagebrush stands south of the core die-off area have not declined to the same extent as the core area (Figure 3.1). These stands exhibited varying mixtures of completely defoliated, partially defoliated, and healthy-looking sagebrush. Along the southern border of the die-off, the sagebrush community supports generally healthy-looking sagebrush intermixed with a few affected individual sagebrush. We located five other die-off areas isolated from the main area: two locations northwest of the Hanford Townsite and three across the Columbia River from the 100K and 100N reactors (Figure 3.1).

3.2 Seeds and Transects

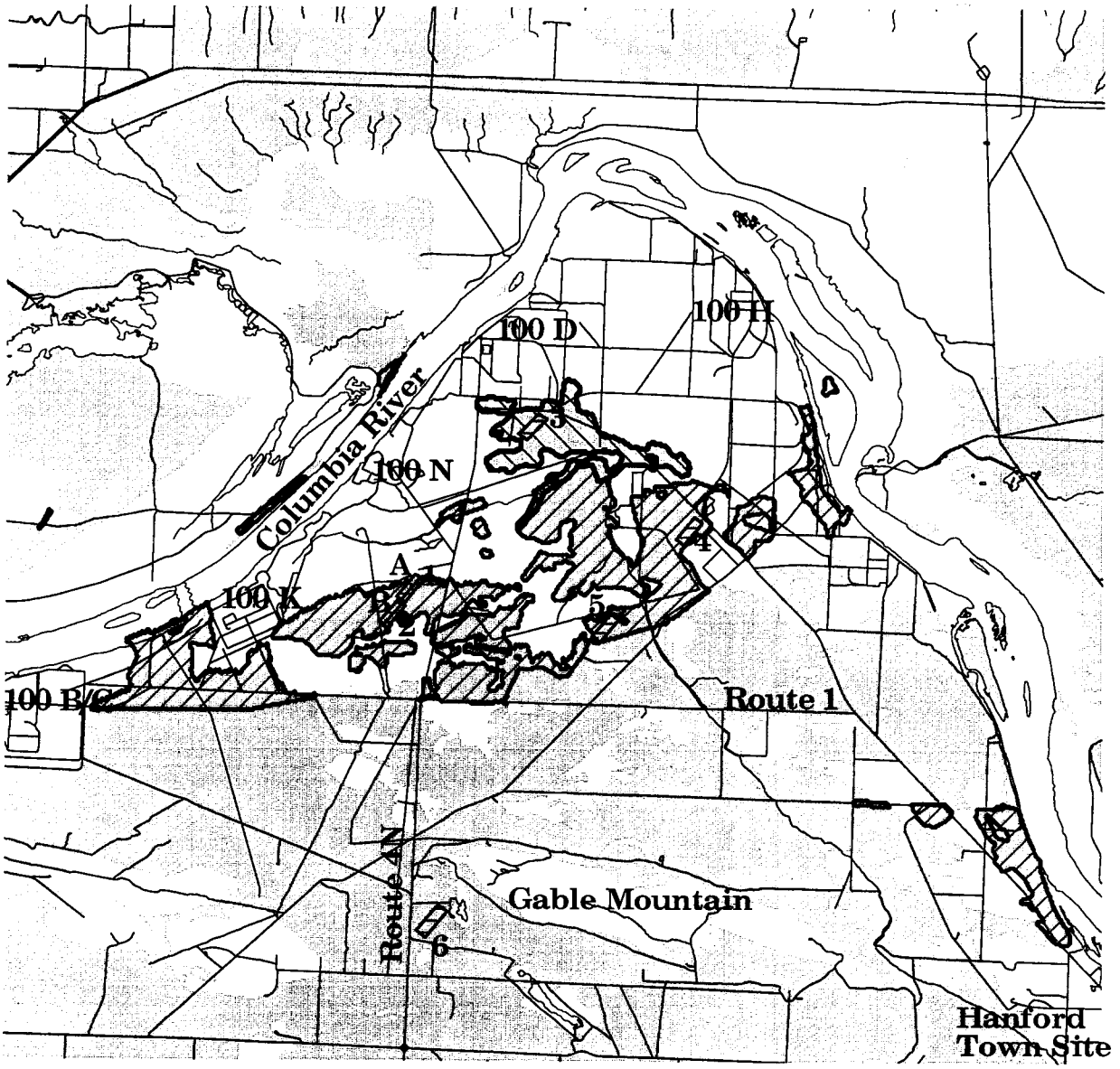
The six belt transects showed a broad range in percentage of shrubs that appear to be affected (categories I, II, III, IV; Table 3.1). The percentage of shrubs without foliage (category I) in the belt transects varied from 15% to 95% (Table 3.2). The two belt transects (1, 3) located in the core die-off area exhibited the greatest percentages of sagebrush without foliage, 95% and 81%, respectively. Belt transects 2, 4, and 5 were located within the die-off area but outside the core area. These transects exhibited 18%, 15%, and 48% shrubs without foliage, respectively. The percentage of sagebrush in category I for belt transect 6, located outside the die-off area next to Gable Mountain, was 18%. This value was within the range of those percentages found in the die-off area.

These data, along with those listed in Table 3.3 illustrate the patchy nature of the die-off within the declining stands. Table 3.4 lists the shrub densities of the six belt transects, which range from 1,700 to 5,000 shrubs per hectare, with an average transect density of 3,400 shrubs per hectare.

3.3 Seed Values and Germination Percentages

For both line transects, the average pure seed weight per inflorescence decreased as apparent sagebrush health decreased (Table 3.5). The number of seeds per inflorescence also decreased as shrub health decreased (Table 3.5). The quantity of seed weight values and seed count values for each category were insufficient to determine if this decrease in seed weight is statistically significant. No seed weight or number values were obtained from category I shrubs because these shrubs bore no inflorescences.

Table 3.6 lists seed germination results from the shrubs in Line Transect A, located in the core die-off area, and Line Transect B, located outside the core area. Line Transect A germination rates varied from 13% to 32%. Line Transect B germination rates were relatively constant, ranging from 31% to 35%. The average percent germination for the seed samples collected outside the die-off area was 49%. Germination results for these samples are listed in Table 3.7.



Sagebrush Die-off as of 1997
Hanford Site, WA

Scale
5 0 Kilometers

Legend
- *Artemisia tridentata* - Big Sagebrush
- Core Die-Off Area (>80% dead)
- Affected Area (Patches, 100% dead to 100% alive)
- Roads (Paved and Unpaved)
- Hydrological Features
- Belt Transect
- Line Transect (A & B)

Ecology Group
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National Laboratory

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Figure 3.1. Core Area Sagebrush Stands

Table 3.1. Shrub Categories

Shrub Category	Percent of Shrub Affected
I	No foliage
II	< 50% branches with foliage
III	50 - 90% branches with foliage
IV	>90% branches with foliage

Table 3.2. Percentage of Shrubs Measured on Belt Transects 1-6 in Category I (no foliage)

Belt Transect (25 m x 5 m)	Category I Percentages
Transect 1	95%
Transect 2	18%
Transect 3	81%
Transect 4	48%
Transect 5	15%
Transect 6	18%

Table 3.3. Number of Shrubs in Each Category on Belt Transects 1-6

Belt Transect	Category I	Category II	Category III	Category IV
Transect 1	20	0	0	1
Transect 2	6	7	6	14
Transect 3	25	1	2	3
Transect 4	24	13	6	7
Transect 5	9	7	19	27
Transect 6	10	2	12	30

Table 3.4. Shrub Density Measured on Belt Transects 1-6

Belt Transect (25 m x 5 m)	Density (shrubs per hectare)
Transect 1	1700
Transect 2	2600
Transect 3	2500
Transect 4	4000
Transect 5	5000
Transect 6	4400

Table 3.5. Average Infloresence Number per Shrub, Average Pure Seed Weight per Inflorescence, Average Pure Seed Number per Inflorescence, and Average Pure Seed Number per Gram for Transects A and B, Categories II - IV

Category	Inflorescence Number per Shrub, Average	Seed Weight, mg, per Inflorescence	Seed Number per Inflorescence	Seed Number per Gram
Transect A				
Category II (n = 3)	4	2.1 ± 2.5 mg	6.7 ± 2.2	4290 ± 9500
Category III (n=1)	9	2.4 ± n.c. mg	15.7 ± n.c.	6,450 ± n.c.
Category IV (n = 6)	71	2.9 ± 1.7 mg	28.3 ± 17.5	10,000 ± 4840
Transect B				
Category II (n = 1)	51	5.1 ± n.c. mg	44.8 ± n.c.	8,750 ± n.c.
Category III (n = 3)	93	8.5 ± 9.5 mg	53.9 ± 55.0	6,360 ± 1480
Category IV (n = 11)	137	14.4 ± 19.1 mg	133 ± 159	8,060 ± 8570

n = Number of values per category.
n.c. = Not calculable.

Table 3.6. Percent Germination of Sagebrush Seeds in Categories II - IV for Line Transects A and B

Category	Transect A: Percent Germination Success, %	Transect B: Percent Germination Success, %
II (<50% branches with foliage)	32	33
III (50-90% branches with foliage)	13	31
IV (>90% branches with foliage)	19	35

Table 3.7. Percent Germination of Sagebrush Seeds Collected Outside Die-Off Area

Control	Percent Germination Success
A	50
B	43
C	40
D	50
E	43
F	67
Average	49

4.0 Discussion

At this time, the cause of the Hanford Site die-off is unknown. Clues to possible causes may be obtained from other die-offs in the western United States. Shrub die-offs are not uncommon occurrences in the arid lands of the western United States. Such episodes have been reported in Nevada, Wyoming, Idaho, and British Columbia (Dobrowolski and Ewing 1990). Currently, in Washington, the state Department of Fish and Wildlife also is investigating a sagebrush decline on their land holdings in eastern Washington (Peggy Bartel, Washington Department of Fish and Wildlife, personal communication).

A review of literature concerning documented shrub die-offs gives perspective to an evaluation of the die-off on the Hanford Site with respect to the total affected area and cause. In recent years, "Large areas in the western United States have been affected by a high mortality of shrubs...within Utah alone, 1 million acres were affected" (Weber et al. 1989). Nelson et al. (1989) noted "extensive, unexplained, rather rapid death of wildland shrubs occurred between 1977 and 1986 across Great Basin Country and adjacent areas." Shrubs included shadscale (*Atriplex confertifolia*), winterfat (*Ceratoides lanata*), budsage (*Artemisia spinescens*), Wyoming big sagebrush and narrow-leaved low rabbitbrush (*Chrysothamnus viscidiflorus ssp. viscidiflorus*).

Relevant studies related to shrub die-offs found severe rootlet mortality, root rot, and vascular shoot-wilt indicative of disease induced by fungal pathogens (Nelson et al. 1989). Rootlet mortality, soil salinity, and anaerobiosis could be the primary factors that predisposed shrubs to disease development in Utah (Nelson et al. 1989). Weber et al. (1989) also suggested increased soil salinity and moisture predispose the shrubs to pathogenic root-rot organisms.

Seed production serves as an index of plant reproductive energy expenditure. General parameters for measuring seed production are the number of seeds produced and seed weight. Seed viability is another variable that may be included, but may be influenced by factors independent of the amount of energy the plant expends. Seed production generally decreases when woody, perennial plants are stressed, because the plant diverts reproductive energy to vegetative maintenance (Larcher 1995). During die-off conditions, the amount of photosynthesizing canopy declines, so shrubs must cope with reduced energy acquisition while maintaining vegetative and reproductive growth.

Results from this study qualitatively demonstrate that seed production declines as vegetative growth declines. The trends in seed weight and seed count per inflorescence suggest that healthier shrubs invest more energy in seed development than less healthy shrubs. Whether this difference in weights and counts is detrimental to shrub reproduction is unknown. However, the trends are interesting and merit further data collection.

Germination results do not show general trends across categories. However, when comparing results from one transect to another, it can be seen that germination rates in the core area transect (Transect A) were lower that those reported from the transect outside the core area (Transect B). With such a small data set, a direct correlation cannot be made between reduced germination rates and declining shrub health. The number of replicates per category was variable because seeds were collected on a transect basis instead of being collected from a standard number of shrubs from each category within the general transect area. The variable number of samples per category may have influenced the germination results.

An interesting observation is the difference in data between Line Transects A and B. Although both transects are within the die-off area, Line Transect B was located outside the core die-off area, in an area that exhibited substantially less decline in shrub health. Differences between areas can be seen in the percent of shrubs without foliage, 95% in Line Transect A versus 18% in Line Transect B. Also note that differences occur in seed germination, seed weights, and seed number, between the two transects. For the transect located within the core area (Line Transect A), germination success, seed weights, and seed number were lower than those found in the transect located outside the core area (Line Transect B). This may indicate that the degree of shrub decline has an impact on the amount of energy invested in seed production, as would be expected.

The information collected from Line Transects A and B on seed production were additions to our original scope but hopefully will provide the impetus for further study and more extensive data collection. Declines in seed production and seed viability imply that the potential is low for these sagebrush stands to regenerate or 'self-restore' the shrub component of the habitat. It is also important to note that timeliness of seed collection may influence both seed count data and germination results. Walton (1984) found that most viable sagebrush seed is dispersed during the first seven days after the inflorescence ripens. Therefore, it is suggested that further seed data collection note the seed stage (soft dough, hard dough) at the time of harvest for each shrub.

Continued mapping and monitoring are needed as new stands of affected sagebrush are found, particularly on the Wahluke Slope and the Columbia River islands to enable proper resource management. Additional characterization of the sagebrush stands within the die-off area also would be beneficial. The affected sagebrush stands were dispersed over a range of soil types, spatial locations, and topographic features. Additional research focusing on these variations in relation to the die-off pattern may provide insight both to possible causes of shrub mortality and potential restoration/mitigation planning. The establishment of more belt transects would provide further detail on the patchy nature of the die-off, determine if categories used in this study were effective in classifying shrubs, and determine if the die-off is significantly different from natural decline within sagebrush stands.

5.0 Conclusions

This preliminary study was meant to characterize the sagebrush die-off on the Hanford Site. Over the past 4 years, the die-off has spread from an undocumented several hectare area just south of the 100-D Area to the delineated 1776 hectares. Currently, no reason exists to believe the die-off is caused by anything other than natural pathogens.

Further characterization and investigation into this phenomenon could provide answers to the cause of the die-off or at least establish potential pathways for the spread of the die-off. The establishment of potential exposure pathways is especially important for future DOE environmental restoration and mitigation efforts. Exposure pathway information could aid in minimizing the possibility of the die-off spreading to newly revegetated areas. The structure and integrity of the shrub-steppe is vital in maintaining the unique wildlife populations that exist in this region, and sagebrush is a fundamental component of this habitat type.

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