STUDIES OF THE MINERAL NUTRITION OF GREASEWOOD
(SARCOPATUS VERMICULATUS)

Dissertation
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Advisor

OHIO STATE UNIVERSITY
# Table of Contents

Acknowledgements ................................................................................................. 1
Introduction ............................................................................................................ 2
Experimental methods and results ........................................................................... 9
  A. Germination experiments .............................................................................. 12
  B. Sand culture experiments ............................................................................ 22
Discussion .................................................................................................................. 49
  A. Germination experiments .............................................................................. 49
  B. Sand culture experiments ............................................................................ 52
Summary ..................................................................................................................... 65
Literature cited .........................................................................................................
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INTRODUCTION

Plants growing on soils containing large quantities of salts have interested both physiologists and ecologists for almost one hundred years. Such interest is aroused primarily because so few species are invested with the characteristics requisite to persist under such apparently adverse edaphic conditions. In spite of this interest, comparatively few investigations have been conducted to learn more of these plants and their peculiar characteristics. However, in recent years the need for arable land has become more and more acute, and as saline soils occupy vast areas and as many of them are potentially arable, it has become increasingly important that we learn more of these soils and the plants growing on them.

This study was designed as a preliminary investigation to discover some of the effects of different salt concentrations on the growth and certain physiological processes of greasewood (*Sarcobatus vermiculatus* (Hook) Torr.). This plant is a common halophyte in the Western United States and was chosen for several reasons: it often grows on marginal lands that are potentially arable; it is of some importance as a forage plant; it apparently has a wide tolerance for salinity and alkalinity, as it is found on soils differing widely in their salt content and pH; it has the succulent habit of some of the more extreme halophytes;
the seeds are readily available; and the plant is easily cultured in the greenhouse.

The literature on the effects of salt on vegetative growth is profuse, but few of the studies have been conducted on halophytes. These few studies have been limited mostly to observations and experiments on species of *Salicornia*, the most salt tolerant of all terrestrial plants. Ganong (1903), observing *Salicornia herbacea* growing at its upper tolerance limits, stated that its size varied inversely with saltiness in habitat. Terras (1934) experimented with the same species at lower salt concentrations (less than 3 per cent) and reported that it grew best when salt was present. He found the same correlation with several other halophytes. Similarly, Keller (1925) found that *Salicornia herbacea* grew best in a medium (1 per cent) degree of salinity, and Mountfort and Brandrys (1927) reported optimal development of seedlings of the same species to occur in 2.5 per cent NaCl. Halket (1915) reported that *Salicornia oliveri* and *S. ramosissima* grew better in 2-3 per cent NaCl, but *S. maritima* grew as well in the absence of salt as in 1 per cent solutions and was reduced in growth in salt concentrations above 1 per cent. Hilgard (1907) said that greasewood and several other halophytes would grow only in the presence of relatively high concentrations of salt. Stocker (1925) divided halophytes into those
that would grow only in salt-rich soils (obligate halophytes) and those that could "adapt" themselves to a non-saline soil. (facultative halophytes).

A number of researchers have mentioned that the succulent habit of many halophytes is dependent upon the presence of sodium. Batalin (1876), the first to correlate the succulence of halophytes with saline soils, stated that salt plants lost their "usual characteristics" if cultivated under ordinary greenhouse conditions. In another paper (1886) he said that *Salicornia herbacea* developed the "fleshy habit" only when grown in NaCl solutions. Among 85 species, Lesage (1890), found 54 in which increased salinity resulted in increased succulence, and 27 which were apparently unaffected by increased salinity of the substrate. Of those that were unaffected, two were typical halophytes (*Glycera maritima* and *Suaeda maritima*). Keller (1925) grew *Salicornia herbacea* in different concentrations of different salts and found that a small amount of NaCl or KCl added to the culture solution resulted in a great increase in succulence, NaCl had a greater effect that KCl, and the chlorides had a greater effect than sulphates. The water content of the tops of cotton was found to be progressively greater with an increase in NaCl or CaCl₂ content of the soil; i.e., succulence of the plant increased with an increase in salt content of the soil (Meyer, 1931).
Hayward and Long (1941) reported that the leaflets of tomato were thicker and more succulent when grown in culture solutions containing NaCl. Uphof (1941) mentions that plants have a "tendency" towards succulence when grown on saline soils. Eaton (1942) on the other hand, found no increase in the succulence of leaves of milo, cotton, tomato, alfalfa, and sugar beets with increase in the osmotic pressure of the substrate. In summary, it might be said that morphological studies have revealed that species vary widely in their succulence when grown on saline soils. Halophytes are not all succulent. Most of the Atriplex species are not succulent and they have been reported as accumulating more salt than any other plant form (Harris, 1934).

Plants differ greatly in their tolerance to salinity and alkalinity. Hilgard (1907) described Allenrolfea occidentalis and Salicornia subterminalis as two of the most salt tolerant halophytes, but they will not tolerate much variation in pH. On the other hand, Sarcabatus vermiculatus and Sporobolus airoides are very salt tolerant and will also tolerate alkaline soils. Salicornia rubra has been found in soils containing almost 7 per cent total salts (Flowers, 1934). However, it should be noted that the plants were not growing at this concentration, growth had occurred earlier in the year when the soil moisture was
higher and osmotic pressure of the soil solution subse-
quently lower. Ruhland (1915) reported growing Statice
gmelini in 10 per cent NaCl solutions, although he admitted
that the plants had "difficulty" in obtaining water from
the culture solution. Greasewood is rarely found in soils
exceeding 1 per cent total salts when the dominant salt is
NaCl (Flowers, 1934) or 3 per cent when the dominant salt
is Na$_2$SO$_4$ (Shantz and Piemeisel, 1940).

The term "salt toxicity" is often seen in the literature;
however, apart from osmotic effects, sodium per se is
probably not highly toxic to plants (Kelley 1951). Most
workers now agree that the primary effect of high soil
salinity upon plants is an osmotic one (Eaton, 1941;
Magistad and Reitmeier, 1943; Gauch and Wadleigh, 1945;
Hayward and Wadleigh, 1949). Eaton (1941), Long (1943),
and Hayward (1943), using divided root systems, have shown
that the rate of entry of water into roots is inversely
proportional to the physiological availability of the water
as measured by the osmotic pressure of the culture solution.

One cannot entirely discount the effects of the
individual ions. Some plants accumulate large quantities
of sodium and others exclude it almost entirely (Collander,
1941; Gauch and Wadleigh, 1945; Wallace, 1948). High
concentration of NaCl in the soil have been reported to
greatly reduce the amount of nitrate, calcium, and sulphate
absorbed by crop plants (Lipman, 1926; Long, 1943; Wadleigh and Gauch, 1942). The addition of sodium decreased the amount of potassium absorbed by sugar beets, tomatoes and several other crop plants (Lehr, 1941, 1942, 1947, 1951; Long, 1943).

In the course of the experiments on greasewood some difficulty was encountered in getting the seeds to germinate. Consequently a number of experiments were designed to find the best germination procedure. Obviously, one of the first avenues of investigation was to discover the effect of salt concentration and consequent osmotic pressure. Buffom (1896, 1899) was one of the first to investigate the effect of osmotic pressure on the germination of seeds and he concluded that "...the retarding effect of a salt solution on the germination of seeds is in direct proportion to its osmotic pressure when the solutions are strong." Stewart (1898) obtained similar results with a number of crop plants. Harris (1915) used soils of different salt contents and salt combinations and found that crop plants varied greatly in their germination in these media. He also found the chloride salts to be more inhibiting than the nitrate or sulfate salts. This work has been verified by a number of similar studies (Shive, 1916; Harris and Pittman, 1918; Ayers and Hayward, 1948). The work of Uhvits (1946) is of particular interest because she used different concentrations
of NaCl and parallel concentrations of mannitol to achieve osmotic pressures of 1 to 15 atmospheres. She found that alfalfa seeds were retarded more by NaCl solutions than by mannitol. This suggests a toxic effect of NaCl. More water was absorbed by the seeds from isosmotic mannitol solutions than from NaCl solutions. Very few studies have been conducted on the germination of seeds of halophytes. Gold (1939) reported that *Allenrollea occidentalis* would germinate in concentrations of NaCl and Na$_2$SO$_4$ up to 4 per cent, but that germination decreased with increasing salt concentration. Germination was greater in Na$_2$SO$_4$ than in NaCl at isosmotic concentrations. Hilton (1940) planted seeds of *Eurotia lanata* in distilled water and a graded series of NaCl solutions. Maximum germination occurred in 0.5 per cent NaCl, gradually declined up to 1.5 per cent, and then dropped rapidly to 3 per cent NaCl, above which no germination occurred.

In recent years it has come to the attention of a number of workers that germination inhibiting substances are probably present in many species of plants (Evaëri, 1949).
EXPERIMENTAL METHODS AND RESULTS

Greasewood (Sarcobatus vermiculatus (Hook) Torr.) is a spinescent, rigidly branched shrub with linear, fleshy leaves. The flowers are monoecious or dioecious, the male flowers occurring in terminal spikes and the female flowers being terminal and axillary. It usually grows from 3 to 6 ft. high, depending on soil conditions. Greasewood is widely distributed in the Western United States, occurring from North Dakota to Texas and from Kansas to California. It is usually found on moist saline flats and may be in pure stands or mixed with shadscale (Atriplex confertifolia (Torr. & Frem.) S. Wats.) or seepweed (Suaeda erecta (Wats.) A. Nels.). Because of its tolerance of highly basic soils, this plant is often regarded as an indicator of an abundance of alkali carbonates in the soil, i.e., "black alkali". In many areas it is a valuable forage plant, the succulent leaves being eaten by both sheep and cattle.

Dry fruits of greasewood were collected in November of 1949, 1950, and 1961 from the flats just west of Salt Lake City, Utah. They were stored in the laboratory in sealed bottles containing a small amount of para-dichlorobenzene as a fumigant. The fruit of greasewood (Fig. 1) is an indehiscent nutlet with a broad spreading wing (Kearney and Peebles, 1949). It is composed of two to three carpels and the single locule contains one coiled embryo surrounded
by a membraneous seed coat (Fig. 2). Endosperm is absent. From 60 to 80 per cent of the fruit collected was infected. The larva of a small beetle was found in many of them, while the embryo of many had been eaten away. As the infected fruit could be detected by the presence of a small hole in the lower side of the fruit, all infected fruit was removed for the germination experiments.
Fig. 1. The fruit of greasewood. Various stages of germination are shown.

Fig. 2. The intact seeds of greasewood can be seen on the left. The membranous seed coat is in the center and the naked embryo is shown on the right.
GERMINATION EXPERIMENTS

If properly treated, 90 to 100 per cent of the uninfected seeds of greasewood will germinate until they become 5 or 6 months old, after which their viability drops to less than 5 per cent. Two groups of germination experiments were conducted, the first during the period of high viability (seeds less than 5 months old), and the second during the period of low viability (seeds more than 6 months old).

During the period of high viability, only 50 to 75 per cent of the seeds germinate without special treatment. The first group of experiments, 1 to 8 inclusive, were conducted to discover the treatment which would result in the highest percentage of germination. The excised embryos and seeds, and the intact fruits of greasewood were subjected to different conditions and the germination noted daily. The seeds of the grass red top (Agrostis alba Auct.), and a garden variety of radish (Raphanus sativus L., var. French Breakfast) were also used in some of these experiments.

In Experiments 1 to 8 inclusive, 25 fruits, seeds, or embryos were placed on moist filter paper in a petri dish, each dish being subjected to a single treatment. Just enough culture solution (Meyer and Anderson, 1949) or leachate was added to each dish to thoroughly soak the filter paper. All the experiments were conducted at
laboratory temperatures (18 to 23°C.) unless otherwise stated. When the greasewood seeds or embryos were to be excised, the fruits were first soaked for 2 hr. in tap water. This softened the pericarp so it could easily be removed. The seed coat is merely a thin membrane and easily broken, and removed with forceps. An embryo was considered to have germinated when the hypocotyl had elongated to 50 per cent beyond its original length. The procedures and results of the first group of experiments are summarized below.

Experiment 1. Effect of removal of seed coat and pericarp. In the control set, twenty-five fruits of greasewood were soaked 2 hr. in a small amount of water, and placed on filter paper moist with culture solution. The second set was similarly soaked but the pericarps were removed prior to placing on moist filter paper. The third set was also soaked and the embryos excised and placed on moist filter paper. The results (Table 1) indicate that the presence of the seed coats inhibits germination of more than 30 per cent of the seeds. The pericarp has a further inhibitory effect upon germination of the embryo.

Table 1. The effect of removal of pericarp and seed coat on germination of greasewood seeds.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Number of seeds germinating each day</th>
<th>Total</th>
<th>Per cent germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of days since beginning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fruit</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td>13</td>
<td>52</td>
</tr>
<tr>
<td>Seed</td>
<td>9 3 1 1</td>
<td>17</td>
<td>68</td>
</tr>
<tr>
<td>Embryo</td>
<td>25</td>
<td>25</td>
<td>100</td>
</tr>
</tbody>
</table>
Experiment 2. Effect of leaching. One set of greasewood fruits was leached 48 hr. in running tap water (\(15^\circ\)C.), and placed on filter paper moist with culture solution. Another set was leached 24 hr., and a third set was not leached, although briefly rinsed in tap water to moisten. The results (Table 2) indicate that 24 hr. leaching increased the rate but not the percentage of germination. Leaching 48 hr., on the contrary, increased the number of seeds germinating but only slightly increased the rate of germination. In subsequent experiments with large masses of fruits, 2 hr. leaching appeared to be about as effective as 24 hr., although no definite data were obtained.

Table 2. The effect of leaching on the germination of greasewood seeds.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of seeds germinating each day</th>
<th>Tot.</th>
<th>Per cent germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of days since treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unleached</td>
<td>2 4 4 4 4 2</td>
<td>16</td>
<td>54</td>
</tr>
<tr>
<td>Leached</td>
<td>1 4 6 4 2</td>
<td>17</td>
<td>68</td>
</tr>
<tr>
<td>24 hr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leached</td>
<td>4 13 2 2</td>
<td>21</td>
<td>84</td>
</tr>
<tr>
<td>48 hr.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Experiment 3. Effect of leachates of pericarp, seed coat and leaves. Separate leachates of the pericarp, seed coats, and leaves of greasewood were made in the following manner. The pericarps of 75 fruits, the seed coats of approximately 400 seeds, and 1 g. of leaves were each soaked in 30 ml. of culture solution. After 12 hr. the leachate was decanted off and the pH determined (Table 3). Excised
embryos of greasewood, and the seeds of radish and red top were placed on filter paper moistened with these leachates. A control set of embryos and seeds were placed on filter paper moistened with culture solution.

The results (Table 3) of this experiment indicate that germination of the embryos of greasewood is not affected by the leachate of pericarps or seed coats in the concentration used. However, the leaf leachate slowed down the rate, and reduced the percentage of germination by 20 per cent. All of the leachates slowed down the rate of germination of both radish and red top seeds, although the effect of the leaf leachate was much more pronounced.

Table 3. The effect of leachate of pericarps, seed coats, and leaves of greasewood on germination.

<table>
<thead>
<tr>
<th>Plant and Plant part used</th>
<th>Medium</th>
<th>pH</th>
<th>Number of seeds germinating each day.</th>
<th>Percent. Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7 8 9</td>
<td></td>
</tr>
<tr>
<td>Greasewood embryos</td>
<td>Culture solution</td>
<td>5.5</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Radish seeds</td>
<td>Culture solution</td>
<td>&quot; &quot;</td>
<td>17 7 1</td>
<td>100</td>
</tr>
<tr>
<td>Red top seeds</td>
<td>Culture solution</td>
<td>&quot; &quot;</td>
<td>18 7</td>
<td>100</td>
</tr>
<tr>
<td>Greasewood embryos</td>
<td>Pericarp leachate</td>
<td>5.9</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Radish seeds</td>
<td>Pericarp leachate</td>
<td>&quot; &quot;</td>
<td>2 18 2 1</td>
<td>92</td>
</tr>
<tr>
<td>Red top seeds</td>
<td>Pericarp leachate</td>
<td>&quot; &quot;</td>
<td>2 23</td>
<td>100</td>
</tr>
<tr>
<td>Greasewood embryos</td>
<td>Seed coat leachate</td>
<td>5.8</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Radish seeds</td>
<td>Seed coat leachate</td>
<td>&quot; &quot;</td>
<td>3 9 6 2 2 1 1</td>
<td>92</td>
</tr>
<tr>
<td>Red top seeds</td>
<td>Seed coat leachate</td>
<td>&quot; &quot;</td>
<td>2 2 17 4</td>
<td>100</td>
</tr>
<tr>
<td>Greasewood embryos</td>
<td>Leaf leachate</td>
<td>5.4</td>
<td>8 9 3</td>
<td>80</td>
</tr>
<tr>
<td>Radish seeds</td>
<td>Leaf leachate</td>
<td>&quot; &quot;</td>
<td>3 5 6 2</td>
<td>64</td>
</tr>
<tr>
<td>Red top seeds</td>
<td>Leaf leachate</td>
<td>&quot; &quot;</td>
<td>1 2</td>
<td>12</td>
</tr>
</tbody>
</table>
Experiment 4. Effect of direct contact with pericarps, seed coats, and leaves. Excised embryos of greasewood, and seeds of radish and red top were placed on filter paper moist with culture solution. They were then covered with either wet pericarps, seed coats, or leaves. A control set remained uncovered. The results (Table 4) of this experiment are very similar to those of Experiment 3. The germination of greasewood embryos was not noticeably affected by direct contact with the seed coat or pericarp, but was retarded both in rate and percentage when in contact with leaves. The germination of radish seeds was only slightly retarded by contact with the pericarps or seed coats, and red top seeds were unaffected. Only 44 per cent of the radish seeds in contact with the leaves germinated and the rate of germination was slow. All but one of the red top seeds failed to germinate when in contact with the leaves.

Table 4. The effect of direct contact with the pericarps, seed coats, and leaves of greasewood on germination.

<table>
<thead>
<tr>
<th>Plant part used</th>
<th>Treatment</th>
<th>Number of seeds germinating each day</th>
<th>Percent. Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1  2  3  4  5</td>
<td></td>
</tr>
<tr>
<td>Greasewood embryo</td>
<td>None</td>
<td>25 17 7 1</td>
<td>100</td>
</tr>
<tr>
<td>Radish seeds</td>
<td>&quot;</td>
<td>18 7</td>
<td>100</td>
</tr>
<tr>
<td>Red top seeds</td>
<td>&quot;</td>
<td>12 4 6 1</td>
<td>92</td>
</tr>
<tr>
<td>Greasewood embryo</td>
<td>Pericarp</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Radish seeds</td>
<td>&quot;</td>
<td>28</td>
<td>100</td>
</tr>
<tr>
<td>Red top seeds</td>
<td>&quot;</td>
<td>16 7 1</td>
<td>96</td>
</tr>
<tr>
<td>Greasewood embryo</td>
<td>Seed coat</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Radish seeds</td>
<td>&quot;</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Red top seeds</td>
<td>&quot;</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Greasewood embryo</td>
<td>Leaves</td>
<td>4 16 2</td>
<td>88</td>
</tr>
<tr>
<td>Radish seeds</td>
<td>&quot;</td>
<td>1 4 6</td>
<td>44</td>
</tr>
<tr>
<td>Red top seeds</td>
<td>&quot;</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>
Experiment 5. Effect of pH. Three petri dishes were set up with intact fruits of greasewood. One was moistened with distilled water at pH 5.9, one was made basic to 9.5 with NaOH, and one was made acid to 1.5 with HCl. At pH 5.9, 76 per cent germinated, and at pH 1.5, 60 per cent germinated. At pH 9.5, 20 per cent germinated, and showed signs of retarded growth.

Experiment 6. Effect of different NaCl concentrations.
The intact fruits of greasewood were subjected to different concentrations of NaCl in culture solution, as shown in Table 5. In one treatment, the control, culture solution was used without NaCl.

The results (Table 5) indicate that NaCl concentration does have some effect on the germination of greasewood seeds. There was a slight reduction in the percentage germination when the NaCl concentration reached 0.300 M, and a reduction of almost 70 per cent in 0.500 M NaCl. The presence of small amounts of NaCl (up to 0.1 M) did not affect the germination significantly.

Table 5. The effect of different NaCl concentrations on germination

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of seeds germinating each day</th>
<th>Percent Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture solution</td>
<td>1 2 3 4 5 6 7 8 9</td>
<td>60</td>
</tr>
<tr>
<td>0.025 M NaCl</td>
<td>4 4 3 2 1</td>
<td>56</td>
</tr>
<tr>
<td>0.100 M NaCl</td>
<td>4 4 3 2 1</td>
<td>60</td>
</tr>
<tr>
<td>0.300 M NaCl</td>
<td>4 4 3 2 1</td>
<td>52</td>
</tr>
<tr>
<td>0.500 M NaCl</td>
<td>1 2 1 1</td>
<td>20</td>
</tr>
</tbody>
</table>
Experiment 7. Effect of storage at low temperatures.

Eight petri dishes were set up with culture solution and intact fruit of greasewood. Four of them were stored at $-3^\circ C$ and four were stored at $4^\circ C$. One plate was removed from each treatment every 7 days. The fruits stored at $4^\circ C$ started to germinate in the refrigerator after 6 days of storage and had to be eliminated from the rest of the experiment. The results obtained with the fruits stored at $-3^\circ C$. (Table 6) indicate that storage for short periods at this temperature increases the rate of germination slightly but has no great effect on the per cent of germination. Storage for longer periods at this temperature resulted in a loss in rate of germination as well as percentage germination.

Table 6. The effect of cold storage on the germination of greasewood

<table>
<thead>
<tr>
<th>No. of days of storage</th>
<th>Storage temp.</th>
<th>Germination rate each day after removal from storage</th>
<th>Percent. Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>$-3^\circ C$.</td>
<td>1 4 3 2 1</td>
<td>44</td>
</tr>
<tr>
<td>14</td>
<td>&quot;</td>
<td>3 2 2 1</td>
<td>32</td>
</tr>
<tr>
<td>21</td>
<td>&quot;</td>
<td>2 3 2 1</td>
<td>28</td>
</tr>
<tr>
<td>28</td>
<td>&quot;</td>
<td>2 2 1</td>
<td>24</td>
</tr>
</tbody>
</table>

Experiment 8. Effect of alternating temperatures.

Three petri dishes were set up with culture solution and intact fruits of greasewood. Every 12 hr. one was alternated between $4^\circ C$ and room temperature ($23^\circ C$), and one was alternated between $18^\circ C$ and room temperature. The third
dish was left at room temperature. The results of the three treatments were quite similar. The rate of germination was slightly higher in the alternating temperature treatments as was the percentage germination, but probably not significantly so.

A second group of experiments were conducted to see if there was any way to increase the percentage germination during the period of low viability of the seeds. It was desirable to begin some sand culture experiments during this period and the number of available fruits were limited. It was not known whether these seeds had lost their viability entirely, or had merely entered a period of dormancy. The techniques used were similar to those used in the previous experiments with the following exceptions. The fruits of greasewood were sterilized in a solution of 30 per cent "Clorox" and 70 per cent distilled water for 30 min., and rinsed in sterile distilled water three times before using. Twenty fruits were used in each treatment instead of twenty-five. Intact fruits of greasewood were used in all experiments.

Experiment 9. Effect of leaching. One set of fruits were leached 24 hr., one set was leached 48 hr., and one set was left unleached. The percentage of germination in all three of these treatments was exceedingly low. Two seeds germinated in both the unleached and those leached 24 hr., and only one seed germinated in the set leached 48 hr.
Experiment 10. Effect of pH. Three petri dishes were set up, one each at pH 1.5, 6.1, and 9.5. The dish at 1.5 was acidified with HCl, and the dish at 9.5 was made basic with NaOH. After 3 days, one seed germinated at pH 1.5. No other seeds germinated.

Experiment 11. Effect of different NaCl concentrations. The fruits of greasewood were subjected to different concentrations of NaCl in culture solution, and to tap and distilled water. Dish #1 was moistened with distilled water, dish #2 with tap water, dish #3 with culture solution, and dishes 4 to 11 inclusive were moistened with different concentrations of NaCl in culture solution. Although the percentage of germination was very low in all treatments, the results (Table 9) indicate that at one NaCl concentration, 0.050 M, the germination was several times greater than in any other treatment. On the strength of this evidence, 500 fruits were sterilized and placed on cheesecloth moistened with culture solution containing 0.050 M NaCl. Fifteen per cent of these germinated.
Table 9. The effect of different salt concentrations on the germination of greasewood seeds.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Daily rate of germination</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tap water</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Culture solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.025 M NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.050 M NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.075 M NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.100 M NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.200 M NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.300 M NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.400 M NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.500 M NaCl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Experiment 11. Effect of cold storage. Eight petri dishes were set up with culture solution. Four were stored at -3°C. and four were stored at -4°C. One dish was moved from each treatment to room temperature (23°C) every 7 days. One seed germinated after being kept at -4°C. for 14 days.

Experiment 12. Effect of alternating temperature. Two petri dishes were set up with culture solution. Every 12 hr. one was alternated between 4°C. and room temperature (23°C.), and one was alternated between 18°C. and room temperature, so that they remained at each temperature for 12 hours. Only one seed germinated under each treatment.
SAID CULTURE EXPERIMENTS

One sand culture experiment (#1) was conducted during the spring and summer of 1951, and the other five experiments were conducted during the winter and spring of 1952. The germination procedure was the same for all experiments. The fruits were leached with running tap water for 6 hr., and sterilized in 30 per cent "Clorox" and 70 per cent distilled water for 30 minutes. After being rinsed several times with sterile tap water, they were rolled in a "rag doll" and placed in an incubator at 30°C. At the end of 48 hr. the seeds which had germinated were removed and planted in flats of sterile granite sand. The flats were placed in a humid chamber in the greenhouse and kept moist with culture solution until the hypocotyls of the seedlings had elongated to about 1 inch (Fig. 2). They were then transplanted to the experimental plots.

The sand used in these experiments was crushed and screened at a local granite quarry where it is sold as a grit for poultry. Before being used for sand culture experiments, it was washed thoroughly in tap water to remove the finer dust particles. Several tests were run on it before it was used in these experiments. When grown in this sand, both tomatoes and soybeans showed deficiency symptoms of all of the major elements except iron. A qualitative test
of the sand for calcium was negative. The sand was soaked in
boiled distilled water for several weeks, at the end of which
time the pH of the water was 6.2.

Experiment 1. Effect of different NaCl concentrations.
This experiment consisted of eight treatments, the variable
being the amount of NaCl in the culture solution. Each plot,
or treatment, consisted of four one-gallon glazed crocks,
each containing two greasewood plants growing in granite
sand (as shown in Fig. 19). A drain hole was drilled in the
bottom of each crock, and covered with a 3 in. square of
plastic screen (Fig. 2). The bottom of the crock was then
covered with 1 in. of coarse sand (1/8 to 1/4 in. in
diameter to facilitate drainage, and filled to within 1 in.
of the top with fine sand (less than 1/8 in. in diameter).
A glass tube, fitted into the drain hole, allowed excess
solution to drain into a glass carboy.
Fig. 3. Diagramatic cross section of the one gallon crocks used in Experiments 1 and 4.

The sand was flushed several times with distilled water, and once with culture solution before the seedlings were planted. Five seedlings were transplanted into each crock and watered with culture solution until they were well established. When the seedlings had developed three pairs of leaves they were thinned to two plants per pot and the individual treatments started. At the same time the sand was covered with 1/2 in. of coarse sand to prevent algal growth on the surface.

This experiment was designed for one control plot, to be irrigated with standard culture solution, and 7 plots to be irrigated with culture solutions of different osmotic...
pressures. The osmotic pressures were varied by adding NaCl to the standard culture solution. So as not to subject the plants to sudden changes in osmotic pressure, small amounts of NaCl were added to the culture solutions every 3 days until the desired concentrations were reached. The final compositions of these solutions are summarized in Table 10.

The culture solutions were stored in painted 5 gal. carboy reservoirs, placed well above the level of the crocks and fitted with siphons. At each watering the drain hoses from the crocks were closed, and the crocks filled with culture solution from the siphons. This thorough flooding of the sand was necessary to redissolve any salt which may have crystallized on its surface. The drain hoses were then opened and the solution drained into a second set of carboys placed well below the level of the crocks. When the upper reservoirs became empty they were moved to the lower level, and replaced with the "drain" carboys. At this time the water lost by evaporation and transpiration was replenished. In this manner, each solution was used about three times before being replaced, which occurred every 5 weeks. The pH of the new solutions varied from 5 to 5.5, and only rose to 6.6 after three weeks. Therefore, no pH adjustments were deemed necessary. The experiments were set up near the center of a large greenhouse where the temperatures varied from 65 to 68°F. during the night, and 65 to 100°F. during the day. The relative humidity varied from 25 to 60 per cent.
Table 10. The composition, osmotic pressure, and pH of the culture solutions used in Experiments 1, 2, 3, and 4. In all experiments the salts mentioned above were added to a culture solution (Meyer and Anderson, 194) which was a "complete" solution except for iron. Iron was added to the sand in the form of powdered magnetite. Control plots for experiments 3 and 4 were omitted from the table but were set up the same as Treatments 1 and 21.

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Treatment number</th>
<th>Salt added</th>
<th>Millimols of salt added</th>
<th>Per cent total salts</th>
<th>Osmotic pressure (atm.)</th>
<th>Initial pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 &amp; 2</td>
<td>1 &amp; 21</td>
<td>None</td>
<td>---</td>
<td>0.13</td>
<td>0.64</td>
<td>5.5</td>
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<tr>
<td></td>
<td>2 &amp; 22</td>
<td>NaCl</td>
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<td>2.52</td>
<td>5.4</td>
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<tr>
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<td>3 &amp; 23</td>
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<td>0.50</td>
<td>4.50</td>
<td>5.5</td>
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<tr>
<td></td>
<td>4 &amp; 24</td>
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<td>148</td>
<td>1.00</td>
<td>7.99</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>5 &amp; 25</td>
<td></td>
<td>234</td>
<td>1.50</td>
<td>11.95</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>6 &amp; 26</td>
<td></td>
<td>319</td>
<td>2.00</td>
<td>14.83</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>7 &amp; 27</td>
<td></td>
<td>490</td>
<td>3.00</td>
<td>21.94</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>8 &amp; 28</td>
<td></td>
<td>661</td>
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<td>27.40</td>
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<td>832</td>
<td>5.00</td>
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</tr>
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<td>2</td>
<td>30</td>
<td></td>
<td>1004</td>
<td>6.00</td>
<td>43.67</td>
<td>5.3</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>KCl</td>
<td>16</td>
<td>0.25</td>
<td>2.18</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td></td>
<td>49</td>
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<td>33.70</td>
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</tr>
<tr>
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<td>34</td>
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<td>1.00</td>
<td>66.60</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
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<td>250</td>
<td>2.00</td>
<td>12.38</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>Na₂SO₄</td>
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<td>2.22</td>
<td>5.4</td>
</tr>
<tr>
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<td>122</td>
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<td></td>
<td>263</td>
<td>2.00</td>
<td>8.77</td>
<td>5.3</td>
</tr>
</tbody>
</table>
One difficulty was encountered in this, the first, experiment. It was originally planned to increase the osmotic pressure of the solutions by the addition of a mixture of sodium and calcium chlorides, ninety five per cent of the salt to be sodium chloride and five per cent to be calcium chloride. However, when the salt concentrations reached 0.3 M a dense white precipitate formed. A series of tests indicated that this precipitate resulted from the presence of the excess calcium, and involved the phosphate and iron ions. It thus became necessary to omit the calcium chloride.

For the duration of this experiment iron was provided in the form of a weak iron solution (50 mg. ferric chloride plus 50 mg. tartaric acid/liter); 10 ml. being poured over the sand twice a week, just prior to irrigation.

When the plants were about 4 1/2 months old, they suddenly developed a chlorotic condition. The solutions were immediately checked for any discrepancy in elemental composition or pH but none was apparent. However, the weather had been extremely hot, and the temperature of the sand around the roots rose above 100°F. As the plants appeared to be permanently injured the experiment was terminated. The plants were 137 days old. No provisions were immediately available for cryoscopic measurements, and as the dying plants had to be harvested before the leaves fell, osmotic pressure data were not obtained from the leaf
saps. The roots and shoots were oven dried at 105°C. for 48 hr. and the dry weight measured to the closest 0.1 gram. These data are summarized in Fig. 4, in which the dry weight is plotted against the osmotic pressure of the culture solutions. The results indicate a direct correlation between these two measurements.

Ash weight of the leaves was obtained in the following manner. The leaves were oven dried and ground in a Wiley Mill. The leaf powder was oxidized by a modification of the procedure outlined by Bills (1949). One gram of leaf powder was placed in a weighed crucible and moistened with 2 ml. conc. HCl. This was thoroughly stirred until the powder became black. The HCl was then evaporated off over low heat, the crucible placed in a cold muffle furnace, and heated to 500-550°C. for 4 hours. After cooling, the crucible was removed and the ash moistened with 1.5 ml. conc. HCl. Once more the HCl was evaporated off, the crucible placed in a cold furnace and heated to 500-550°C. for 3 hours. After cooling the crucible was weighed and the ash weight calculated. The ash was then prepared for analysis of sodium and potassium. It was moistened with conc. HCl and dissolved in 20 ml. distilled water. This solution was filtered and quantitatively washed into a 100 ml. volumetric flask and made up to volume. The solution was then analyzed for sodium and potassium by the
flame photometry technique (Berry, et al., 1946; Gilbert, et al., 1950; Fox, 1951).

![Graph](image)

**Fig. 4.** Experiment 1. Comparison of the total dry weight of the plants with the osmotic pressures of the culture solutions.

The ash weight, expressed as per cent of dry weight, is plotted against the osmotic pressure in Fig. 5. The results indicate that a direct correlation exists between the ash weight and the amount of NaCl in the culture solution. The results of the sodium and potassium analyses (Fig. 6) indicate
Fig. 5. Experiment 1. The relationship between the ash weight of the leaves and the osmotic pressure of the culture solutions.

da direct correlation between the amount of sodium in the culture solution and the amount of sodium in the leaves. However, the rate of leaf-sodium increase is more rapid at lower sodium concentrations of the culture solutions than at higher sodium concentrations of the culture solutions. It is also apparent (Fig. 7) that there is an inverse correlation between the sodium and potassium content of the leaves when plotted against g. NaCl/liter of culture solution. With an increase in sodium there is a corresponding decrease in potassium, although the potassium content of the culture solution was the same in all treatment.
Fig. 6. Experiment 1. The relationship between the amount of sodium in the culture solution and the amount of sodium in the leaves.

Fig. 7. Experiment. The amount of sodium and potassium found in the leaves compared to the NaCl content of the solution in which the plants were grown.
Experiment 2. Effect of different NaCl concentrations.

As so little data had been obtained from Experiment 1, it was decided to repeat it, and add two more treatments of higher salt concentrations. To simplify the daily chore of irrigating, a new irrigation system was devised.

A long metal tray was built of heavy gauge, galvanized sheet metal, and divided into 24 parallel, water tight compartments. Each compartment was 33 X 9 X 9 in. in dimensions. A 1/2 in. drain hole was drilled in the end of each compartment. The compartments were then painted with black Tygon paint according to instructions from the manufacturers (U.S. Stoneware Co., Inc.). Each drain hole was fitted with a one-hole stopper and covered with plastic screen (Figs. 8 and 9). The compartment was then filled with granite sand, 1 in. of coarse sand being placed on the bottom to facilitate drainage. Each compartment held about 125 lb. of sand, to which was added 57 g. of magnetite (ferrous-ferric oxide), making a 0.1 per cent mixture. Magnetite is a slowly soluble form of iron, providing a constant supply of iron to the roots (Chapman, 1939).

Figure 9 is a diagram of a single compartment and irrigation system. At each watering, the drains of all 24 compartments were closed and air pressure released into the carboy reservoirs to force the solutions up through the perforated plastic tubes. When the solutions thoroughly
Fig. 8. A general view of the irrigation method used in Experiments 2, 3, and 5. The pipes shown are part of the air supply system, consisting of a surge tank (empty oxygen cylinder), solenoid, air line filter, and a distributing line with outlets to each carboy. The painted carboys are the solution reservoirs.

covered the sand, the air pressure was shut off and the solutions drained back into the carboy reservoirs by gravity. The plants were watered once or twice a day, depending on weather conditions.

Twenty seedlings were transplanted to each compartment, but were thinned to sixteen seedlings when well established. The culture solutions used were the same as those used in Experiment 1 (Table 10), except for the iron complex, which was omitted. Twice a week, water lost by evaporation and
Fig. 9. Diagramatic cross section of a single plot of the type used in Experiments 2, 3, and 5.

Transpiration was replenished. Solutions were replaced every 3 weeks with fresh solutions.

When the plants were 181 days old the experiment was terminated. Figure 10 is a photograph of the experiment just prior to termination. Several measurements of the leaves, stems and roots were taken as criteria of growth. As the leaves of greasewood are very easily broken, especially when succulent, no measurements were made until the termination of the experiment.

The length of thirty leaves from each plot was taken by measuring ten leaves near the tops of the plants, ten leaves near the middle of the plants and ten leaves near the bottom of the plants. This was necessary because of the great
Fig. 10. Experiment 2, in which graded concentrations of NaCl were added to the culture solutions. Total salt concentrations are shown in per cent.

Variation occurring between leaves on different parts of the plant. The measurements were randomized by measuring one or two leaves from each plant in the plot. The linear shaped leaves usually occurred in clusters of three, and the center leaf was measured, as it was always the longest. The leaves were measured to the closest millimeter. The leaf thickness was measured in a similar manner, the dimension measured being the vertical thickness mid-way between the apex and the base of the leaf. A small micrometer was used and measurements made to the closest 0.01 millimeter.

The results of these leaf measurements can be seen in Fig. 11, where they are plotted against the osmotic pressure of the culture solution. In general, the length of the leaves decreased, while the thickness of the leaves increased with an increase in osmotic pressure of the culture solution. In observing the plants this was quite noticeable. All of the leaves of the plants growing in higher salt concentrations were succulent, while only the bottom leaves of those plants
Fig. 11. Experiment 2. Comparison of leaf length and leaf thickness of plants grown in culture solutions of different osmotic pressures.

Growing in lower salt concentrations were succulent, the rest being linear. The wet weight of the leaves was measured to the closest 0.1 g. and the leaves immediately sealed in glass bottles and placed in a deep-freeze (-20°C).

As some of the plants had a single long stem and some of them had many shorter stems, the stems were individually measured.

The length of the stems was measured to the closest millimeter and the number of stems 2 cm. long or longer recorded. In Fig. 12 the length of the stems is plotted against the osmotic pressure of the solutions, and an inverse correlation is indicated; however, the lowest concentration of NaCl resulted in an increase in stem length
Fig. 12. Experiment 2. Relation between the average stem length and the osmotic pressure of the culture solutions in which the plants were grown.

over that of the control plot. As the roots of the plants of each plot were usually intermingled, they were weighed as a single unit, to the closest 0.1 gram. The stems and roots were oven dried at 105°C. for 48 hr. and the dry weight measured to the closest 0.1 gram. In Fig. 13 the total dry weights of the plants are plotted against the osmotic pressures of the culture solutions. As the osmotic pressure of the solutions increased slightly, the dry weight also increased; however, the dry weight of both roots and stems decreased with further increase in osmotic pressure. The dry weight of the leaves was added to the dry weight of the stems and the root-shoot ratio calculated. No correlation between dry weight and the osmotic pressure of the culture solution was apparent.
Fig. 13. Experiment 2. The relation between the dry weight and the osmotic pressure of the culture solutions in which the plants were grown.

The osmotic pressures of the culture solutions and of the leaf saps were obtained by the freezing point depression method (Harris and Gortner, 1914), using a Beckman thermometer. Fifteen grams of frozen leaves were ground with a mortar and wooden handled pestle, both of which had been stored at -20°C. In this manner the leaves could be thoroughly ground without sand and ground "dry", as they remained frozen for at least 5 min. in the pre-cooled mortar. The ground leaves were then placed in a gooch crucible which had a thin layer of glass wool over the perforated bottom. The crucible was placed in a gooch crucible holder in the top of a vacuum flask. In about 30 min. the ground leaves had thoroughly thawed and a vacuum of 0.3 to 0.5 atm. was applied and the leaf sap collected in a calibrated centrifuge
tube inside the vacuum flask. Five to seven milliliters of leaf sap could easily be obtained from 15 g. of leaves. The method proved quite reproducible as indicated by a number of measurements made on one sample of leaves over a 2 week period. There was no great difference in these measurements. The pH of the culture solutions and the leaf sap was measured with a glass electrode (Tables 10 and 12).

The Beckman thermometer used for the freezing point depression measurements required a minimum of 4 to 5 ml. of leaf extract. However, some of the plots produced less than 1 g. of leaves, not nearly enough for the needed amount of leaf extract. No other thermometers or extraction apparatus was immediately available in the laboratory in Salt Lake City. Therefore, the leaves were shipped in the frozen state to Ohio State University, where it was hoped, the rest of the osmotic pressure measurements could be obtained. Although many precautions were taken to insure the safe transit of the leaf materials, they were thawed and even mixed when opened. Needless to say, no more data were obtained from these leaves.

The results of the osmotic data measured in Salt Lake City can be seen in Fig. 14, in which the osmotic pressures of the leaf extracts are plotted against the osmotic pressures of the culture solutions. The osmotic pressure of the leaf sap increased very rapidly with a slight increase in the osmotic pressure of the culture solution.
Fig. 14. Comparison of the osmotic pressures of the leaf extract from plants grown in NaCl, KCl, and Na₂SO₄ solutions.

Experiment 3. Effect of different KCl concentrations.

The conditions and procedures of this experiment were the same as for Experiment 2 with two exceptions; the added salt was KCl instead of NaCl, and there were only five treatments instead of ten. The composition and osmotic pressures of the culture solutions can be seen in Table 10. One plot, the 0.04 per cent total salts treatment, developed a leak in the metal compartment and had to be watered by hand. In observing the plots it was obvious that this treatment was not equal with other treatments. As this was also borne out in the measurement data, this treatment was omitted from further consideration. The plants were 182 days old when the experiment was terminated. Fig. 15 is a photograph of the plants taken at this time.
Fig. 15. Experiment 3, in which graded concentrations of KCl were added to the culture solutions. Total salt concentrations are shown in per cent.

The measurements were taken in the same manner as in Experiment 2. In Fig. 16 the length and thickness of the leaves is plotted against the osmotic pressure of the culture solutions. The leaf length increased with a slight rise in osmotic pressure but decreased below that of the control at higher pressures. The leaf diameter followed a similar pattern but did not decrease much at higher concentrations. A slight increase in osmotic pressure of the culture solutions doubled the amount of stem tissue, but further increase in pressure greatly reduced the amount of stem tissue developed (Fig. 17). In a similar manner, the dry weight of the plants was almost double that of the controls at lower osmotic pressures of the culture solutions but was less than 50 per
Fig. 16. Experiment 3. Comparison of leaf thickness and leaf length with the osmotic pressure of the culture solutions in which the plants were grown.

Fig. 17. Experiment 3. Relation between the average stem length and the osmotic pressure of the culture solutions in which the plants were grown.
cent of the controls at higher pressures (Fig. 18). There was no apparent correlation between root/shoot ratio and osmotic pressure of the culture media. The osmotic pressure of the leaf extract rose very rapidly with an increase in that of the solution.

![Graph showing dry weight of plants compared with osmotic pressure of culture solutions](image)

**Fig. 18.** Experiment 3. Dry weight of the plants compared with the osmotic pressure of the culture solutions in which they were grown.

**Experiment 4. Effect of different Na\(_2\)SO\(_4\) concentrations.**

This experiment was set up the same as Experiment 1. Each plot consisted of four one-gallon crocks, each containing two greasewood plants. There were four treatments, one control and three plots with different amounts of Na\(_2\)SO\(_4\) added to the culture solution. Table 10 is a summary of the composition and osmotic pressure of these solutions. Fig. 19 is a photograph of the experiment when the plants were 149 days old, when the experiment was terminated.
Fig. 19. Experiment 4, in which graded concentrations of Na₂SO₄ were added to the culture solutions. Total salt concentrations are shown in per cent.

The measurements were made in the same manner as in Experiments 2 and 3. In Fig. 20, the leaf length and leaf thickness are plotted against osmotic pressure of the culture media. Neither the leaf length or thickness is correlated with osmotic pressure. The stem length was greater in the two median osmotic pressures than in either the control or higher pressure. (Fig. 21). As seen in Fig. 22, the total dry weights at all three higher osmotic pressures was greater than that of the control. However, at 8.8 atm. osmotic pressure the dry weight was less than at either 3.3 or 5.2 atm. Fig. 14 indicates a direct correlation between the osmotic pressure of the leaf saps and that of the culture solutions, but the increase in osmotic pressure of the leaf sap is not nearly as great as in the NaCl and KCl treatments.
Fig. 20. Experiment 4. Comparison between the leaf length and leaf thickness and the osmotic pressure of the culture solutions in which the plants were grown.

Fig. 21. Experiment 4. Relation between the average stem length and the osmotic pressure of the culture solutions in which the plants were grown.
Fig. 22. Experiment 4. Relation between the average dry weight and the osmotic pressure of the culture solutions in which the plants were grown.

Experiment 5. Effect of different proportions of NaCl and KCl on the absorption of sodium and potassium. With the exception of the solutions used, this experiment was set up the same as Experiments 2 and 3. There were seven treatments, each with different proportions of NaCl and KCl added to the culture solution. The osmotic pressures of the solutions were approximately the same in all treatments.

Table II summarizes the compositions of the solutions.

Table II. Experiment 5. The amount of NaCl and KCl added to the culture solution in each treatment.

<table>
<thead>
<tr>
<th>Treatment No.</th>
<th>Amount of each salt added to each treatment (g./L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>51  52  53  54  55  56  57</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.157 0.964 0.771 0.578 0.386 0.193 0.000</td>
</tr>
<tr>
<td>KCl</td>
<td>0.000 0.193 0.386 0.578 0.771 0.964 1.157</td>
</tr>
</tbody>
</table>
Fig. 23. Experiment 5. Different proportions of KCl and NaCl were added to the culture solutions. The approximate KCl/NaCl ratios are shown.

Fig. 23 is a photograph of the experiment the day it was terminated. The plants were 99 days old. Observation revealed no apparent morphological differences so no such measurements were made. The dry weights of the roots and shoots were measured and the root/shoot ratios calculated. However, there was no apparent correlation between these measurements and the different treatments. The leaves were ashed by the method described for Experiment 1, but there was no obvious correlation between ash weight and NaCl-KCl treatment. The ash was then analyzed for sodium and potassium by the method described for Experiment 1.
The sodium content of the leaves increased with an increase in NaCl in the culture medium (Fig. 24). In a similar manner, the potassium content of the leaves increased with an increase in KCl in the culture medium. However, when the KCl and NaCl were of the same concentration, the amount of sodium in the leaves was almost twice as great as the amount of potassium present. Even when there was twice as much KCl as NaCl in the culture medium, the amount of sodium still exceeded the amount of potassium in the leaves.

Fig. 24. Experiment 5. Comparison of sodium and potassium content of the leaves of plants grown in solutions containing different proportions of NaCl and KCl.
DISCUSSION

Germination Experiments

It is evident from the results of Experiment 1 that both the seed coat and pericarp of the greasewood fruit delay germination of the embryo (Table 1). Experiment 2 was set up on the assumption that this inhibition might result from the presence of a water soluble inhibitor as reported by Hilton (1940) for *Eruotia lanata* and by Stout and Tolman (1940) for sugar beet. Leaching the fruits did increase the rate of germination but there was no great increase in total germination (Table 2). However, when large numbers of fruits were placed in a rag doll for germination, it was found that total germination was greatly increased if the rag doll containing the fruits was rinsed daily in tap water. Experiment 3 was designed to help clarify these conflicting observations. It is obvious (Table 3) that the concentrations of the leachates used did not inhibit the germination of greasewood embryos, although they did slow down the rate of germination of radish and red top seeds. The leaf leachate reduced both the rate of germination and total germination of greasewood embryos, and greatly affected the germination of both radish and red top. Beadle (1952) found that the bracteoles surrounding the seeds of *Atriplex* spp. contained enough salt to be inhibitory.
The dried leaves of greasewood are 40-55 per cent salt (Fig. 6), and the leaf leachate may have had an osmotic pressure high enough to be inhibitive. The effect of salt concentration was observed in Experiment 6, and the results indicate a linear decline in germination with increase in salt concentration. This is in agreement with most investigators (see introduction, page 7), but does not agree with the results obtained by Schratz (1934) and Chapman (1936) with another halophyte, Aster tripolium. The germination of this species was delayed by high salt concentrations but the total germination was the same in all solutions tested.

The pH was obviously not a limiting factor as indicated by Experiment 5 and Table 4, although it must be pointed out that the solutions used were not buffered. An attempt to increase germination by several temperature treatments failed. It is apparent that greasewood seeds will germinate over a wide range of pH, salt concentrations and temperature.

The second series of experiments, set up during the period of low viability, was designed to discover whether the loss of viability was permanent or temporary. In addition to the experiments mentioned, in which the fruit were left intact, another series using naked embryos was conducted, the results were essentially the same as with intact fruits.

All evidence indicates that most of the seeds had died, or at least were no longer viable. As a rule, the seeds of desert shrubs remain viable for a number of years (Beadle, 1952).
On the strength of observation alone, and in spite of the evidence of the above experiments, the writer is of the opinion that more thorough experimentation will reveal the presence of a germination inhibitor in the pericarp and leaves, although it may be only a concentration of salt. Inhibition by the seed coat is apparently merely mechanical as practically all of the viable embryos would germinate when the moisture swollen seed coat was merely ruptured but not removed.
Sand Culture Experiments

The plan of this study was to grow greasewood with culture solutions containing different concentrations of either NaCl, KCl or Na₂SO₄, measure the growth and osmotic pressure of the leaf sap at each concentration, and compare the effects of the different salts on the plants.

Average dry weight and average stem length per plant were used as criteria of growth. These two criteria confirm each other. In all three salt treatments, the lowest concentration of added salt resulted in an increase in dry weight and stem length over the control treatment (Fig. 4, 12, 13, 17, 18, 21 and 22). This agrees with the results obtained by Lehr (1941, 1942), Hartwell et al. (1908, 1913, 1919), and Sayre and Vittum (1947) for certain crop plants. It should be noted that these investigators obtained their best results when the K content of the soil was low. In the experiments with greasewood the K content of the culture solution could not be considered as being low. At the lowest NaCl and KCl concentrations, the growth in the KCl was much greater than in the NaCl (Figs. 13 and 18). In higher isomotic pressures, growth is more nearly comparable between these two salt treatments, and decreases linearly with increase in osmotic pressure. These results agree with those obtained by other investigators for crop plants, although Halket (1915) reported that two species of Salicornia grew best at 2-3 per cent NaCl.
The plants treated with Na₂SO₄ cannot be compared directly with the other salt treatments as they were of different age when harvested. In spite of being younger, the plants at 5 atm. osmotic pressure produced more growth than plants at isosmotic pressures in the NaCl or KCl treatments. The plants in the 5 atm. osmotic pressure treatment (Na₂SO₄) grew more than at any other pressure, while greatest growth in the KCl and NaCl treatments occurred at about 2 atm. osmotic pressure.

This confirms numerous reports in the literature which maintain that sulfates are less "toxic" than chlorides (Keller, 1925; Hayward and Long, 1942; Wall and Hartman, 1942; Magistad et al., 1943). The effects of the chloride ion has been investigated several times. Hayward and Long (1941) reported that tomatoes absorbed much more chloride than sulfate, and Jatis (1938) and Steiner (1934) stated that chloride accounts for over 50 per cent of the high osmotic pressures of the leaf extracts of plants growing on saline soils. Harris (1934) measured the osmotic pressure and chloride and sulfate contents of the leaf sap of greasewood collected from many sites in Northern Utah. Fig. 25 is a graph made from his data. It indicates that chloride is absorbed in much greater amount than sulfate by greasewood.
Fig. 25. A comparison of the sulfate and chloride content of greasewood leaves and the osmotic pressure of their leaf sap. Data from Harris (1934), pp. 113-116.

The acidity of leaf sap has been reported to increase with chloride content (Pettinger, 1932) but not with sulfate content (Eaton, 1942). Table 12 gives the pH values of the leaf extract of greasewood grown in NaCl, KCl, and Na₂SO₄ culture solutions. It is readily seen that they do not differ greatly from each other.

**Table 12.** The pH of leaf extracts from Experiments 2, 3, and 4.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NaCl</th>
<th>KCl</th>
<th>Na₂SO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>21 22 23 24 25 26</td>
<td>31 32 33 34 35</td>
<td>41 42 43 44</td>
</tr>
<tr>
<td>pH</td>
<td>6.06.1 6.36.16.25.96.05.95.95.95.75.85.86.06.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Haas (1945) suggests that the result of an increase in chloride content and subsequent increase in acidity is an effect on certain enzyme systems. Gold (1951) reported that chlorides depress enzyme activity much more strongly than sulfates. In 1930, Garner et al. grew tobacco on soils of different KCl contents and found that increased chloride upset the organic acid balance in the leaves, and their starch content was greatly increased. Baslavskaja (1936) observed that the chlorophyll content of potato leaves decreased with an increase in chloride ion content, which consequently reduced the carbohydrate content. Such conflicting evidence with different species leads one to conclude that species vary greatly in their reaction to high chloride ion content, although there is little doubt that they do affect enzyme systems.

In their native habitat, the leaves of greasewood are quite succulent, being almost cylindrical in form. With the exception of a few lower leaves, the leaves of greasewood grown in culture solution without added salt or in low concentrations of salt, were relatively long and terete or angular in cross section (Fig. 11, 16 and 20). As the salt concentration increased, the leaves became shorter in length and thicker in cross section. In the higher NaCl concentrations all of the leaves were very succulent. There is no obvious explanation as to why the lowermost
leaves in all treatments were succulent unless the age of
the leaves is a factor. The cause of succulence in plants
remains unknown, but the dependence of halophytes upon Na
for the expression of the succulent habit has been reported
by many investigators (Batalin, 1876; Lesage, 1890; Keller,
1925; Uphof, 1941). In Fig. 26 and 27 the effects of NaCl
and KCl on leaf length and leaf diameter are compared. The
correlations are quite close, although plants in the po-
tassium series seem to have slightly longer and thicker
leaves in most treatments. This does not agree with Keller's
(1925) results with Salicornia, as he found NaCl to have a
greater effect than KCl. The sodium sulfate treatments did
not have nearly as great an effect upon succulence as the
NaCl and KCl treatments. However, comparison is hardly
valid as the plants treated with Na$_2$SO$_4$ were much younger
at time of measurement.

The plants growing in the NaCl solutions of 27.4, 36.3,
and 43.7 atm. osmotic pressure were greatly stunted in growth
(Fig. 12 and 13) with little or no apparent elongation of the
internodes. Plants have been reported as living in soil
solutions of osmotic pressures above 40 atmos., but it is
not reported that the plants were growing at these pressures
(Ruhland, 1915; Flowers, 1934). Therefore, it is significant
to point out that three weeks before the termination of
Experiment 2, most of the plants growing in the NaCl solution
at 43.7 atm. grew a new set of leaves. Magistad and
Fig. 26. A comparison of leaf lengths of plants grown in solutions containing NaCl and KCl.

Fig. 27. A comparison of the thickness of leaves from plants grown in solutions containing NaCl and KCl.
Reiteimeier (1943) found that no vegetation occurred in soils whose soil solutions reached 45 atm. osmotic pressure. They also reported that the soil solution around the roots of Salicornia and Allenrolfes, two pioneer halophytes, to be between 30 and 38 atmospheres. The osmotic pressure of the soil solution in the root zone of greasewood growing in the field rarely exceeds 10-12 atmospheres (Flowers, 1934; Richards et al, 1947).

One of the criteria of salt tolerance of plants is their capacity to develop rather high osmotic pressures of tissue fluids by accumulating salt ions (Hayward and Wadleigh, 1949). This is apparently true of greasewood, as the magnitude of rate of increase of osmotic pressure is greater for the leaf extracts than for the corresponding culture solutions (Fig. 14). The data of Harris (1934) (Fig. 25) and the sodium content of the leaves (Fig. 6) indicate that this increase is probably due to an increase of sodium and chloride ions. At no time was the sodium content of the leaves, or the osmotic pressure of the leaf extract below that of the corresponding culture solution. In general, this agrees with our concepts of the properties of tissue fluids of halophytes. However, Keller (1925) stated that "luxuriant" plants of Salicornia herbacea did not raise the osmotic pressures of their tissue fluids until growth was suppressed by the high osmotic pressure of the culture media.
He maintained that Salicornia can "regulate" the salinity of its cell sap and may contain less NaCl than the soil it is growing in. It is obvious that the results obtained from greasewood do not agree with his conclusions concerning Salicornia.

Unfortunately, an accident prevented the writer from obtaining osmotic pressure data from the four treatments at the highest NaCl concentration. However, an attempt is made in Fig. 28 to extrapolate the osmotic pressure curve for leaf sap from Experiment 2 on the strength of evidence provided by the ash weight of the leaves from Experiment 1. There is no evidence that such a correlation exists, except that the increase in ash weight means an increase in salt content, and an increase in salt content implies an increase in osmotic pressure of the tissue fluids. The data of Harris (1934) (Fig. 25) indicates that the accumulation of chloride ions is correlated with an increase in osmotic pressure of the leaf extract. Similarly, Fig. 6 indicates an increase of sodium content with an increase in osmotic pressure of the culture media. However, this experiment will have to be repeated before definite conclusions can be reached.
Fig. 28. A comparison of the ash weight and osmotic pressure of the leaf extract of plants growing in solutions of different osmotic pressures. The dashed line is extrapolated.
It is generally known that most plants accumulate potassium to a greater degree than any other ion, and tend to exclude sodium. The "success" of halophytes has often been attributed to their capacity to accumulate and tolerate within their tissues, large quantities of NaCl.

The ash weight of the leaves of plants grown in NaCl can be seen in Fig. 5. The extremely high value of over 55 per cent of the dry weight is found in leaves of plants growing in a 4 per cent salt. Most plants do not exceed 10 per cent, although sugar beet has been reported as high as 21 per cent. Fig. 5 also indicates that the ash weight, indicative of ion accumulation, is very high (above 45 per cent) in NaCl concentrations well below 1 per cent. An examination of Fig. 6 will reveal that much of this increase in ash weight results from increased sodium content, which may account for as much as 18 per cent of the dry weight. From Harris' (1934) data (Fig. 25), we might conclude that large quantities of chloride were also absorbed. Steiner (1934) reported that the sodium exceeded the chloride in Spartina glabra by about 50 per cent. Fig. 7 indicates that sodium accumulation increased rapidly with the addition of relatively small amounts of NaCl, but the amount of sodium accumulated gradually leveled off at the higher NaCl concentrations. It is unfortunate that the leaf tissues of Experiment 2 could not be analyzed as they would have
yielded information at even higher salt concentrations. It may be that the amount of sodium that can be accumulated by greasewood is limited, and in these higher concentrations (5 and 6 per cent NaCl), the sodium in the culture solution may exceed that in the plant, as Keller (1925) reported for *Salicornia herbacea*.

The increase in accumulation of sodium with increase of NaCl in the culture medium was accompanied by a corresponding decrease in the accumulation of potassium. Lehr (1941, 1942) and Long (1943) obtained similar results with sugar beets. The amount of K found in the leaves is still not low when compared to agricultural crops, being about 1.5 per cent of the dry weight. However, this is only about 17 per cent of the amount found in the leaves of the control plants, indicating over 80 per cent reduction in the amount of K absorbed.

Although sodium is not one of the essential elements for plant growth, it is generally known that certain crops produce greater yields when sodium is present in the soil. Hartwell (1907), Mullison (1942), and Lehr (1941, 1942, 1947, 1951) have indicated in numerous experiments that sodium will not only replace most of the potassium in some crops, e.g., sugar beet, alfalfa, barley, and wheat but will even result in production of greater yields than equal concentrations of potassium alone. As most of the crops which
react in this manner are also those plants most tolerant to saline soils, it was suspected that greasewood might react in a similar manner. Figures 4, 11, 12, and 13 show that the addition of small amount of NaCl (0.02 M) did result in increased growth, but further increase in salt concentration resulted in decreased growth.

Experiment 5 was designed with culture solutions containing different proportions of NaCl and KCl at a total concentration of approximately 0.02 M. However, neither the appearance of the plants, dry weight measurements, nor the ash weight measurements showed any correlation with NaCl-KCl proportions. The amount of potassium absorbed by the leaves was inversely proportional to the amount of sodium absorbed by the leaves and directly proportional to the amount of potassium in the culture media (fig. 24). With a decrease in the KCl/NaCl ratio (of the culture solution) the rate of decline in amount of potassium accumulated is of about the same magnitude as the rate of increase in the amount of sodium accumulated. This indicates that these ions approximately complement each other. Apparently the complementary action is not entirely equitable. With a decrease in KCl/NaCl ratio there is a slight decrease in the total ions (\(\text{Na} + \text{K}\)) accumulated, and when the KCl/NaCl ratio is 1, the accumulated sodium is almost double the accumulated potassium.
The significance of these observations is not entirely clear in the light of present knowledge of potassium-sodium relationships. Van Itallie (1938) mentions that it is the KCl/NaCl ratio rather than the total concentration which is the determining factor regulating cation absorption. Significantly, he also deplores the lack of knowledge concerning these processes.
Experiments were conducted in which seeds of greasewood were placed in petri dishes on moist filter paper, subjected to various treatments, and the rate of germination observed. The seeds germinate readily, if the pericarp and seed coat are first removed, until they become 5 or 6 months old. At this time their viability drops from about 75 per cent to less than 5 per cent.

It is apparent that the seed coat mechanically inhibits germination. When the seeds are soaked in water the membranous seed coat becomes very turgid. Germination can be increased by 30 per cent by puncturing this swollen seed coat. The pericarp and leaves probably contain a chemical inhibitor of germination, although it may be only accumulated salt. Germination was not retarded in NaCl concentrations up to 0.3 M. However, the leaves of greasewood are often 40 to 50 per cent salt on the dry weight basis, which may be sufficient to inhibit germination when the leaves come in contact with the seeds.

Neither cold temperature pretreatment nor alternating temperatures increased germination. Germination occurred in solutions of pH 1.5, 5.9, and 9.5, although it was somewhat retarded at the latter value. Neither cold temperature, alternating temperatures, salinity, nor leaching treatments
increased germination of seeds that had apparently lost their viability, i.e., that were older than 6 months.

Five sand culture experiments were conducted in which greasewood plants were subjected to thirty-six individual treatments. Different amounts of either NaCl, KCl or Na₂SO₄ were added to a standard complete culture solution. Each plot of sixteen plants was irrigated daily with one of these solutions. The NaCl solutions were graded from 0.25 to 6.0 per cent total salts, and the KCl and Na₂SO₄ solutions were graded from 0.2 to 2 per cent total salts.

Dry weights and stem length were measured as criteria of growth. The addition of small amounts equivalent to (0.02 M) of NaCl or KCl resulted in maximum growth of greasewood. With further increase in chloride salt concentrations the decline in growth was linear. In the Na₂SO₄ experiments, maximum growth occurred at a much higher salt concentration (0.25 M). It is apparent that chloride salts inhibit growth much more than sulfate salts. There was no difference in the pH of the leaf sap from plants grown in either chloride or sulfate salt solutions. The salt concentrations in which maximum growth occurred are approximately the same as the salt concentrations of soils usually supporting greasewood.

The thickness and length of the leaves were measured as criteria of succulence. The higher the salt concentration of the medium, the shorter and thicker were the leaves.
This effect was much more consistent in plants growing in chloride salt solutions than those growing in sulfate salt solutions. There was no apparent difference in the effect of sodium and potassium on succulence.

The osmotic pressures of the leaf extracts and culture solutions were measured cryoscopically. The osmotic pressure of a leaf extract always exceeded that of the culture solution in which the plant was growing by 15 to 25 atmospheres. The osmotic pressures of the leaf saps of plants growing in the KCl solutions always exceeded those of plants growing in NaCl solutions, at isosmotic pressures, by about 5 atmospheres. When the osmotic pressure of the culture solutions was 2 atm., the osmotic pressures of the leaf saps of plants growing in Na$_2$SO$_4$ and KCl solutions were the same. However, in culture solutions of osmotic pressures higher than 2 atm. the osmotic pressures of leaf saps of plants growing in KCl solutions always exceeded those of plants growing in Na$_2$SO$_4$ solutions. Growth of new leaves occurred on all plants including those in the NaCl culture solution of 45.7 atm. osmotic pressure, the highest salt concentration used.

Ash weight of the plants growing in NaCl solutions was measured and found to be extremely high, exceeding 55 per cent of the dry weight when the culture solution contained 4 per cent salt. Even when the culture solution contained
The plants growing in culture solutions of high NaCl content accumulated more sodium than plants growing in culture solutions of lower NaCl content; the correlation, however, was not linear. It appears that there is a limit to the amount of sodium which can be accumulated in greasewood. The leaves of plants growing in culture solutions of low NaCl content contained much less potassium than the leaves of plants growing in culture solution to which no NaCl had been added.

In one experiment, NaCl and KCl were both added to seven different culture solutions but in different proportions, the osmotic pressure being about the same (2.25 atm.) in all solutions. With a decrease in KCl/NaCl ratio there was an increase in the amount of sodium absorbed and a corresponding decrease in the amount of potassium absorbed. While this correlation was inverse, the ions did not exactly complement each other. The amount of sodium absorbed exceeded the amount
of potassium absorbed when the KCl/NaCl ratio was two, and the amount of sodium was double the amount of potassium absorbed when the KCl/NaCl ratio was unity.
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