SEED GERMINATION CHARACTERISTICS OF *CHRYSOTHAMNUS NAUSEOSUS* SSP. *VIRIDULUS* (ASTEREAE, ASTERACEAE)

M. A. Khan[1], N. Sankhla[2], D. J. Weber[3], and E. D. McArthur[4]

ABSTRACT.—Rubber rabbitbrush (*Chrysothamnus nauseosus* [Pallas] Britt. ssp. *viridulus*) may prove to be a source of high-quality cis-isoprene rubber, but its establishment is limited by a lack of information on seed germination. Consequently, seeds were germinated at alternating temperatures (5–15, 5–25, 15–25, and 20–30 C) in light and dark as well as constant temperatures (15–40 C with 5-C increments) to determine temperature response. Seeds were also germinated in solutions of polyethylene glycol 6000 (0 to −5 bar), salinity regimes (1, 17, 51, and 86 mM) at all the above-mentioned temperatures to determine salinity and temperature interaction. The hormones GA_3 (0, 2.9, 29.0, and 58.0 um) and kinetin (0, 4.7, 23.5, and 47.0 um) were used to study their effect on overcoming salt- and temperature-induced germination inhibition. Seeds of *C. nauseosus* ssp. *viridulus* were very sensitive to low temperature. Best germination was achieved at 25 and 30 C, but these seeds also germinated at a higher temperature (35 C). The seeds of rabbitbrush germinated at both constant and alternating temperatures. Light appears to play little or no role in controlling germination of the seeds of rubber rabbitbrush. However, seeds of rabbitbrush were sensitive to salinity, and seed germination was progressively inhibited by increase in salt concentration, although a few seeds still germinated at the highest saline level. Progressively higher concentrations of polyethylene glycol also progressively inhibited germination. Suppression of seed germination induced by high salt concentrations and high temperatures can be partially alleviated by the application of either GA_3 or kinetin.

Chrysothamnus nauseosus (Pallas) Britt. ssp. *viridulus* (Hall) Hall & Clements is the largest and most robust of the approximately 20 subspecies of *C. nauseosus* (rubber rabbitbrush) (Hall and Clements 1923, Anderson 1966). Anderson (1986a) has reduced *C. nauseosus* ssp. *viridulus* to a variety of *C. nauseosus* ssp. *consimilis*. In this report we have chosen to maintain it at the subspecies level because several characteristics such as higher rubber content (Ostler et al. 1985), size, habitat, and distribution distinguish it from ssp. *consimilis*.

Rubber rabbitbrush as a species occurs widely west of the one hundredth meridian in North America, barely extending from the United States north into Canada and south into Mexico. Rubber rabbitbrush is usually 30–230 cm in height, having several erect stems from the base and with moderately flexible leafy branchlets (McArthur et al. 1979a, McMinn 1980). Subspecies *viridulus*, however, may grow to 3 m in height in the alkaline valleys of west central Nevada and eastern California.

The several subspecies of this perennial shrub show considerable variation in distribution and adaptation (Hall and Clements 1923, McArthur et al. 1979a, Anderson 1986b), in phenolic plant chemistry and palatability to browsing animals (Hanks et al. 1975), in relation to gall-forming insects (McArthur et al. 1979b, Wangberg 1981, McArthur 1986), in seed-germination characteristics (McArthur, Jorgensen, and Weber, unpublished), and in rubber and resin content (Hall and Goodspeed 1919, Ostler et al. 1986, Weber, McArthur, and Hagerhorst, unpublished). The potential of this taxon for rubber production was first suggested by Hall and Goodspeed (1919) and has recently been rated as promising by Ostler et al. (1986). Results of nuclear magnetic resonance analyses demonstrate that Chrysil, the rubber from rabbitbrush, is a high-quality cis-isoprene molecule type rubber. It may be that the rubber production from guayule (*Parthenium argentatum*) and rubber rabbitbrush, both composite family shrubs, could be processed through the same industrial plant. Rabbitbrush is adapted to a wide range of soils including those that are alkaline and to temper-

[1]Department of Botany, University of Karachi, Karachi 32 Pakistan.
[2]Department of Botany, University of Jodhpur, Jodhpur, India.
[3]Department of Botany and Range Science, Brigham Young University, Provo, Utah 84602.
[4]USDA Intermountain Research Station, Forest Service, Provo, Utah 84601.

TABLE 1. Effect of NaCl and alternating temperatures on the percent of germination of *Chrysothamnus nauseosus* ssp. *viridulus* seeds in light.

NaCl	Alternating temperatures (C)			
(mM)	5–15	5–25	15–25*	20–30*
0	53.3^{a}	67.8^{a}	90.0^{a}	94.4^{a}
17	49.2^{a}	46.6^{b}	80.0^{b}	79.8^{b}
51*	35.1^{a}	34.0^{bc}	40.2^{c}	53.2^{c}
86*	12.0^{b}	12.0^{c}	35.9^{c}	18.62^{3}

^{abcd}Values with the same letter in a column are not significantly different as determined by the Duncan multiple-range test at α = .05.

*Two-way analysis of variance indicates (1) NaCl inhibitory above 17 mM, and (2) alternating temperatures (15–25 and 20–30 C) are stimulatory.

TABLE 2. Effect of NaCl and alternating temperatures on the percent of germination of *Chrysothamnus nauseosus* ssp. *viridulus* seeds in dark.

NaCl	Alternating temperatures (C)			
(mM)	5–15	5–25	15–25*	20–30*
0	54.5^{a}	54.5^{a}	91.8^{a}	96.3^{a}
17	57.2^{a}	53.2^{a}	83.8^{a}	73.2^{b}
51**	33.3^{b}	26.6^{b}	42.6^{c}	54.5^{c}
86**	16.0^{b}	9.3^{c}	34.6^{c}	27.9^{d}

^{abcd}Values with the same letter in a column are not significantly different as determined by the Duncan multiple-range test at α = .05.

*Two-way analysis of variance indicates (1) NaCl inhibitory above 17 mM, and (2) alternating temperatures (15–25 and 20–30 C) are stimulatory.

ate climates, whereas guayule grows in frost-free or nearly frost-free areas (Johnson and Hinman 1980). Rubber rabbitbrush accessions have up to 6.5% rubber content (Hall and Goodspeed 1919, Ostler et al. 1986, Weber, McArthur, Hagerhorst, unpublished) and will resprout when tops are harvested (Young et al. 1984).

Additional information will be required on germination of rubber rabbitbrush seed if it is to be used as a commercial crop for rubber production. Obtaining young rabbitbrush plants by rooting fresh cuttings is difficult (Everett et al. 1978). Transplanting nursery seedlings is a reliable method, but direct seeding should be more economical. However, direct seeding methods have received little study. Stevens et al. (1981) reported that *C. nauseosus* seed germination declines from 80 to 14% from the second through the fifth year of warehouse storage. In another report, Stevens et al. (1986) demonstrated the importance of proper seed placement in the soil for germination and seedling vigor. Deitschman et al. (1974) reported that eight collections of *C. nauseosus* had an average of 63% seed viability. Sabo et al. (1979) reported a maximum germination of 76% for *C. nauseosus* ssp. *consimilis*.

Our objective was to investigate effects of simulated environmental factors and growth regulators on germination characteristics of rabbitbrush accession with high rubber yield potential to obtain more information on seed physiology. This information could be useful in growing plants from seeds.

MATERIALS AND METHODS

Seeds of *Chrysothamnus nauseosus* (Pallas) Britt. ssp. *viridulus* were collected in the fall of 1984 from plants growing at Palmetto, Esmeralda Co., Nevada (collected by McArthur, Weber, and Sanderson). This population has large plants up to 3 m in height. Seeds were separated from inflorescences and stored at 4 C in paper bags. Germination tests were carried out in 9-cm-diameter glass petri dishes containing Whatman No. 1 filter paper moistened with 5 ml of distilled water or other test solutions. Three replicates of 25 randomly selected seeds each were used for each treatment. Seeds were considered to be germinated with the emergence of the radicle.

To determine the effect of temperature on germination, we used growth chambers to obtain alternating regimes of 5–15, 5–25, 15–25, and 20–30 C based on a 24-hour cycle, where the higher temperature (15, 25, and 30 C) coincided with a 12-hour light period, and the lower temperature (5, 15, and 20 C) coincided with the dark period. Seeds were also germinated under constant temperatures ranging in 5-C increments from 15 to 40 C. The petri dishes were randomized at each temperature regime. We studied the light requirement by comparing germination in petri dishes in the dark (covered in a box) with germination in petri dishes in the light. Seeds were germinated in distilled water, 17, 51, and 86 mM NaCl solution under the above-mentioned temperature regimes.

Water stress was imposed by adding polyethylene glycol (PEG-6000) to distilled water to give a wide range of osmotic potential (from 0 to −0.5 mpa) (Michel and Kaufman 1973). Several concentrations of GA_3 (0, 2.9, 29.0, and 58.0 um), and kinetin (0, 4.7, 23.5, and 47.0 um) were applied. We recorded germination every day for three days and calculated the rate of germination using an index of

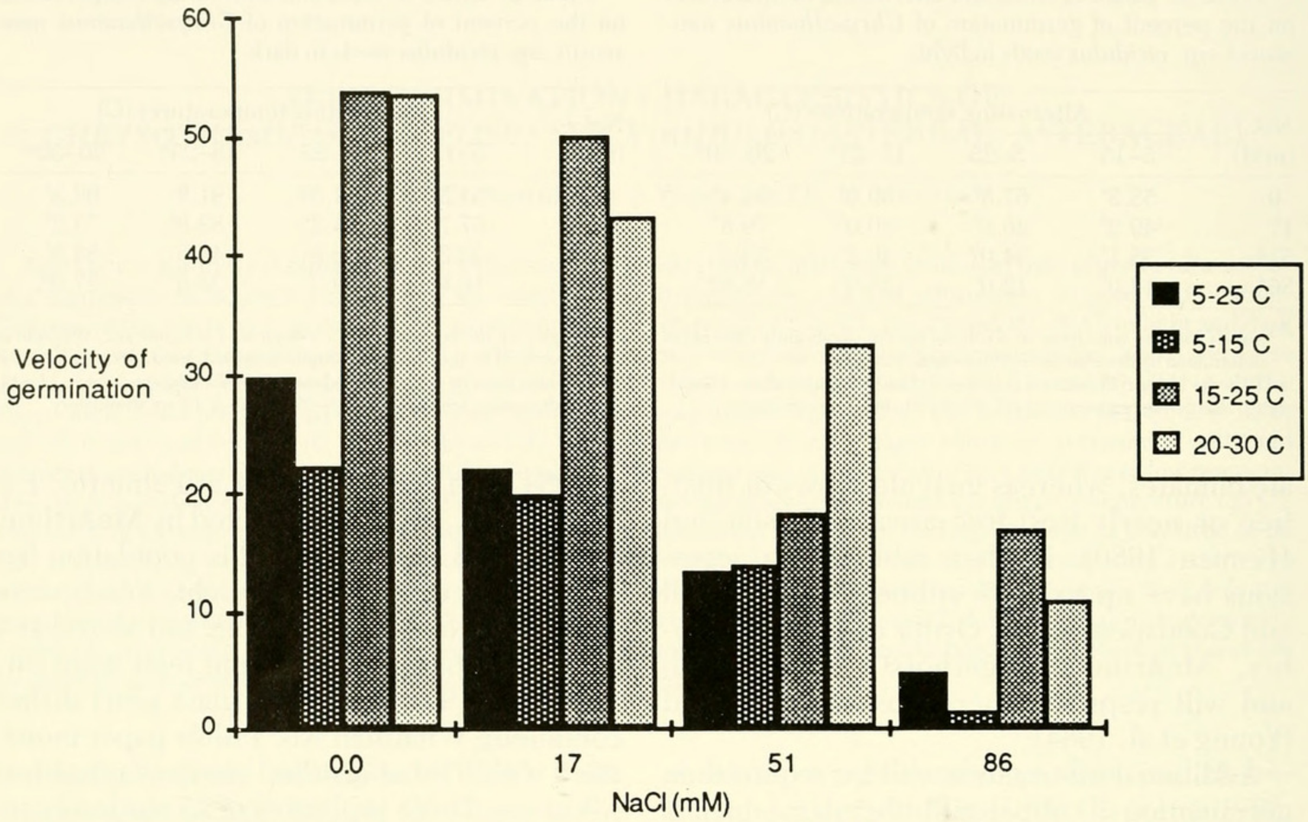

Fig. 1. Rate of germination (velocity of germination) of seeds of *Chrysothamnus nauseosus* ssp. *viridulus* at alternating temperatures and three concentrations of NaCl.

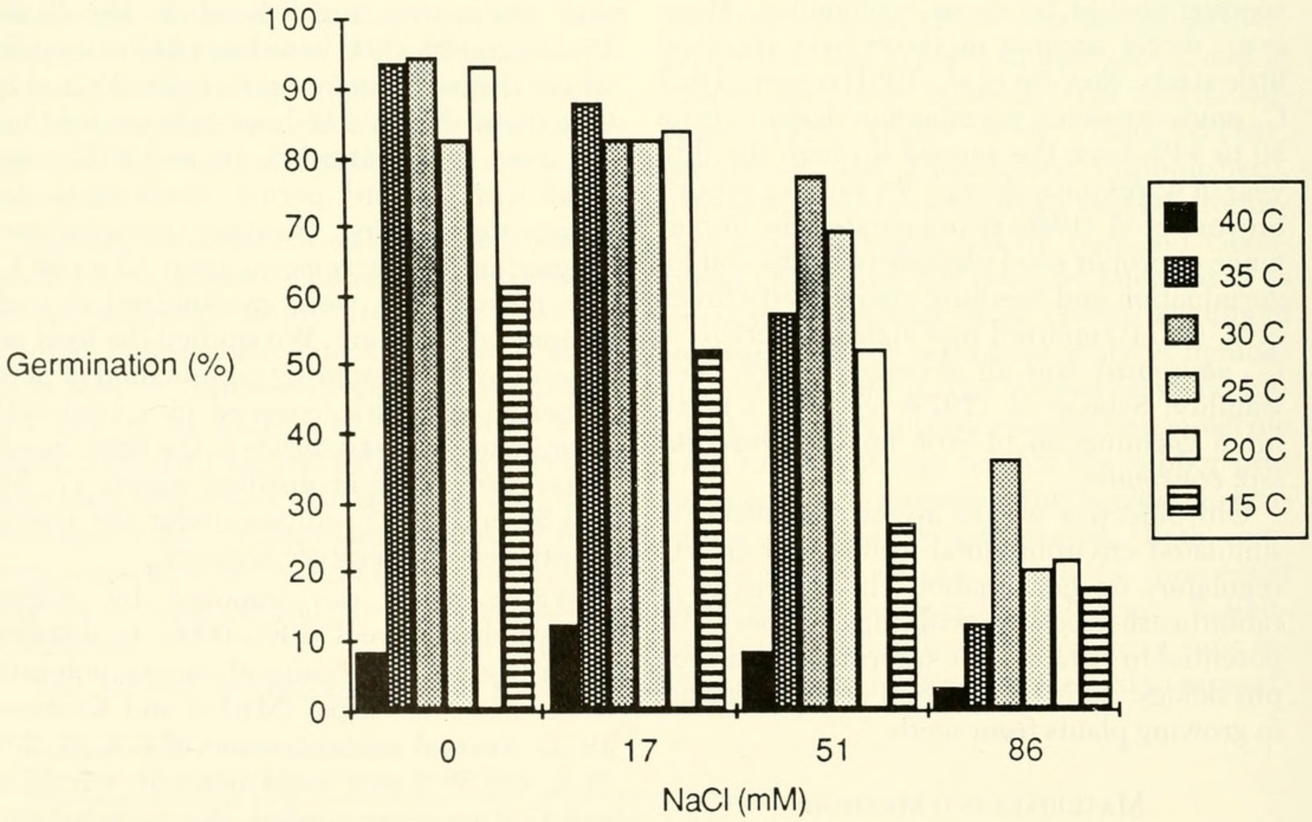

Fig. 2. Percent germination of seeds of *Chrysothamnus nauseosus* ssp. *viridulus* at constant temperatures and three concentrations of NaCl.

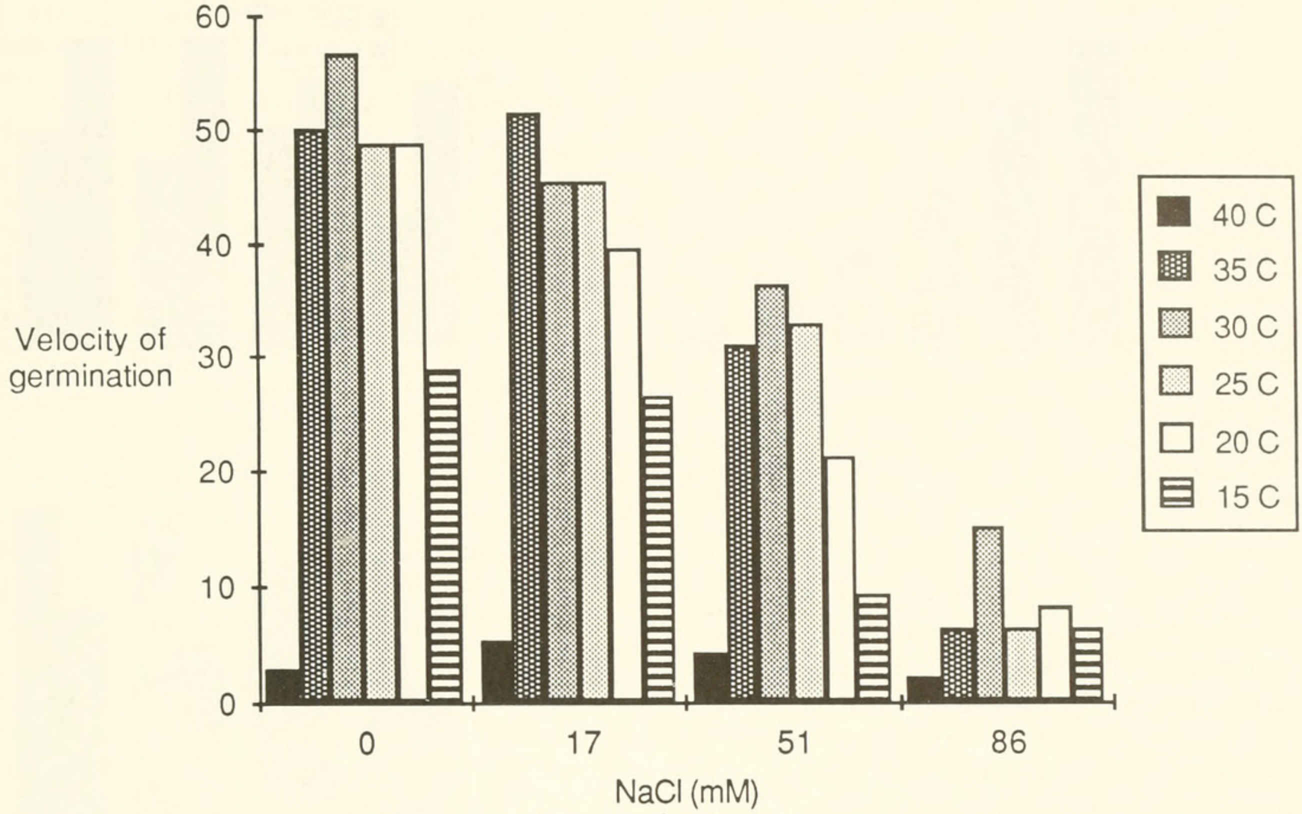

Fig. 3. Rate of germination (velocity of germination) of seeds of *Chrysothamnus nauseosus* ssp. *viridulus* at constant temperatures and three concentrations of NaCl.

germination velocity = G/t, where G = percentage of seed germinated at one-day intervals, t = total germination period (Khan and Ungar 1984).

Percentage germination data were transformed (Arcsin percent) before statistical analyses. The treatments were compared with Duncan's multiple range test.

Results and Discussion

LIGHT AND TEMPERATURE EFFECTS.—Rabbitbrush seeds were germinated in light and dark conditions at constant and alternating temperatures. There were no differences in germination response between seeds germinated in light or in dark (Tables 1, 2), suggesting that the seeds were insensitive to light. Alternating temperature regimes of 20–30 and 15–25 C yielded maximum germination. Substantially less germination occurred in the 5–15 and 5–25 C temperature treatments (Tables 1, 2). Rate of germination estimated by using an index of germination velocity indicated that rates of germination at 15–25 and 20–30 C were twice as high as those of 5–15 and 5–25 C treatment (Fig. 1). These results indicate that low night temperatures inhibit both the rate and the final germination percentages. Sabo et al. (1979) reported that seeds of *C. nauseosus* ssp. *consimilis* germinated well with a peak percentage of 76% at alternating temperatures of 13–27.5 C. Alternating temperatures of 13–27.5 C (8 h) and 23–27.5 C (16 h) gave the best range responses, with germination times increasing slightly at the cooler temperature (Sabo et al. 1979).

Rabbitbrush seed germinated substantially at constant temperatures including 35 C (α = .05) (Fig. 2). Sabo et al. (1979) reported that germination of *C. nauseosus* ssp. *consimilis* was inhibited at a temperature above 27.5 C. The rate of germination of *C. nauseosus* ssp. *viridulus* was maximum at 30 C and decreased with either an increase or decrease in temperature (Fig. 3). At 40 and 15 C, the velocity of germination was significantly reduced.

SALINITY EFFECT.—NaCl salinity significantly inhibited (α = .05) the rabbitbrush seed germination at a concentration of 86 mM (Tables 1, 2). Rates of germination were significantly reduced (α = .05) at higher salinity (51 and 86 mM NaCl) (Table 1, Fig. 3). No interaction of light, temperature, and salinity was observed to affect seed germination. This spe-

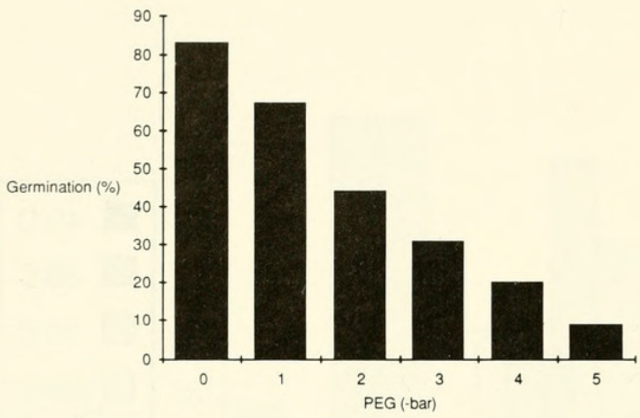

Fig. 4. Percent germination of seeds of *Chrysothamnus nauseosus* ssp. *viridulus* at different levels of moisture tension.

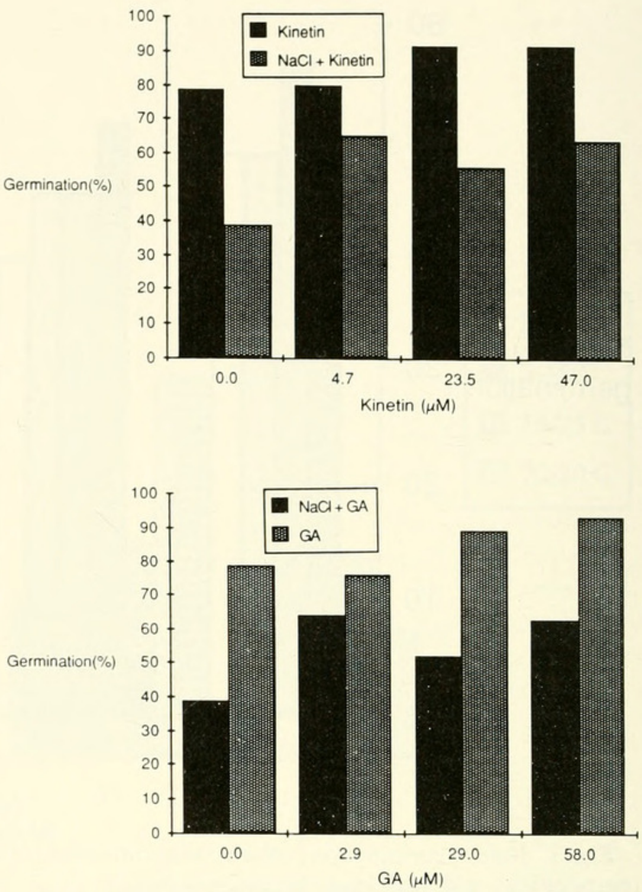

Fig. 5. Effect of kinetin and gibberellic acid on the germination of seeds of *Chrysothamnus nauseosus* ssp. *viridulus* in relation to three concentrations of NaCl.

cies has been reported to be highly salt tolerant (Sabo et al. 1979, Roundy et al. 1981), but our study indicated that it behaved like typical glycophytes at the germination stage. In natural field situations, seed germinates and seedlings could become established when precipitation dilutes natural high-salt concentrations. Once established, plants can tolerate relatively harsh conditions. In natural stands, *C. nauseosus* ssp. *viridulus* grows in alkaline valleys (Hall and Clements 1923).

Moisture Stress

Rabbitbrush seed germination was progressively reduced as the moisture tension increased to −5 bar (Fig. 4). At −5 bar treatment, less than 10% of seeds germinated compared to 85% in control. Seeds of *C. nauseosus* ssp. *consimilis* showed 34% germination at −7 bar treatment (Sabo et al. 1979).

Plant Growth Substances

Various concentrations of kinetin and GA_3 promoted germination of *C. nauseosus* ssp. *viridulus* as compared to nontreated control. NaCl (>51 mM) significantly (α = .05) inhibited germination. This salt-induced inhibition was reduced by the inclusion of various concentrations of kinetin and GA_3 in the medium (Fig. 5).

Inhibition of seed germination induced by high salt concentration could be alleviated by application of GA_3 (Ungar and Binet 1975, Boucaud and Ungar 1976, Ungar 1977, Ungar 1984, Khan and Ungar 1985) and cytokinins (Boothby and Wright 1962, Odegbaro and Smith 1969, Kaufmann and Ross 1970, Ross and Hegarty 1980, Bozcuk 1981, Khan and Ungar 1985).

High salt concentrations induce dormancy in seeds of many plant species (Hydecker 1977). Boucaud and Ungar (1976) found that salinity depresses seed cytokinin levels but not the concentration of GA_3. However, dormancy induced by salinity, which may be similar to that caused by emergence-restricting seed coats, was broken by an application of GA_3 but not by kinetin (Ungar 1977). Khan and Ungar (1985) reported that GA_3 and kinetin can break salt-induced dormancy in *Atriplex triangularis*, suggesting that when exposed to salt stress, seeds of various species behave differently in response to exogenous application of growth substances.

Inhibition of germination caused by high temperature (40 C) can also be partially alleviated by addition of GA_3 and kinetin in the medium (Table 3). In addition, GA_3 and kinetin also partially alleviated the inhibitory effects of salt and higher temperature on germination.

TABLE 3. Velocity of germination and germination percentage at 40 C with NaCl, Ga_3, and kinetin.

Treatment	Velocity of germination	% germination
Control	15.0	27.9
NaCl (51 mM)	10.0	16.0
GA_3 (20 ppm)	21.7	43.9
Kinetins (10 ppm)	24.3	47.9
Salt + GA_3	19.0	36.0
Salt + kinetins	27.3	47.9

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