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Effects of temperature and salinity on germination of non-pelleted and pelleted guayule (*Parthenium* argentatum A. Gray) seeds

Paul L. Sanchez[a], Mei-kuang Chen[b], Mohammad Pessarakli[c], Hank J. Hill[d], Michael A. Gore[e], Matthew A. Jenks[a,*]

[a] USDA-ARS, U.S. Arid-Land Agricultural Research Center, 21881 North Cardon Lane, Maricopa, AZ 85138, USA
[b] Department of Teaching, Learning and Sociocultural Studies, The University of Arizona, 1430 E. Second Street, Tucson, AZ 85721, USA
[c] School of Plant Sciences, The University of Arizona, Forbes Bldg., Rm 303, P.O. Box 210036, Tucson, AZ 85721, USA
[d] Seed Dynamics Inc., Salinas, CA 93901, USA
[e] 310 Bradfield Hall, Cornell University, Ithaca, NY 14853, USA

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ABSTRACT

Guayule (*Parthenium argentatum* A. Gray) is an important domestic source of natural rubber. Commercial field plots are currently established using greenhouse grown seedlings that are hand sown as plugs. However, this practice is expensive and laborious. Direct sowing of guayule seed in the field would reduce time and cost significantly, and yet the effects of seed pelleting, temperature, salinity level, and their interactions on guayule seed germination are not well established. To test germination requirements, non-pelleted (control) and pelleted seeds were planted in solutions having electrical conductivity (EC) of 0, 2, 4, 6, 8, and 10 mS/cm at 10, 20, 30, and 40 °C for 7 days. After 7 days, the non-germinated seeds were transferred to distilled water plates in a 20 °C environment. Seed pelleting, temperature, salinity, and their interactions significantly affected guayule germination. The optimal conditions for seed germination (i.e., highest germination rates) were found to be 20 °C and EC 0–2 mS/cm, regardless of pelleting. Both temperature and salinity delayed germination and decreased viability. Germination was inhibited at both 10 and 40 °C. Salinity effects on seeds decreased as germination temperature became optimal. Lowest germination percentages were observed at EC 6–10 mS/cm and at 30 and 40 °C. Germination percentages increased for treatments after seeds were transferred to optimal conditions. Importantly, pelleted guayule seeds exhibited higher germination than non-pelleted seeds in all treatments. Our results provide important new insights that can help guide the selection of optimal seasonal and soil conditions for field establishment with new direct seeding methods.

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1. Introduction

Germination is characterized by the elongating embryonic root penetrating the seed coat or pericarp (Jones et al., 2013). Temperature (Batlla et al., 2009; Baskin and Baskin, 1998) and salinity (Keifer and Ungar, 2002; Stumpf et al., 1986) are two of the more important abiotic factors affecting germination (Sartorato and Pignata, 2008; Gardarin et al., 2010). As a general response, temperature extremes (Roberts, 1988) and salinity (Iqbal et al., 2006; Gulzar and Khan, 2001) can inhibit germination and induce dormancy. High salinity can delay both imbibition and germination rates as well as reduces root growth (Uhvits, 1946; Simon, 1984; Werner and Finkelstein, 1995). Negative effects of salinity on germination have been attributed to both osmotic stress and ion toxicity (Khan and Rizvi, 1994; Khan and Ungar, 1998; Song et al., 2005). The inhibitory effect of salinity on seed germination has been reported to increase as temperature increases in many plant species including *Prosopis juliflora* (El-Keblawy and Al-Rawai, 2005), *Sarcobatus vermiculatus* (Khan et al., 2002), and *Atriplex cordobensis* (Aiazzi et al., 2002).

Guayule (*Parthenium argentatum* A. Gray), a xerophytic shrub of the family Asteraceae, is a valuable source of commercial rubber and latex. In addition, it has commercial promise as a future source of antibiotic resins and biofuels. Natural populations of guayule can be found in the semiarid Chihuahuan Desert region of north central Mexico and in the Big Bend area of Southwest Texas (Lloyd, 1911; West et al., 1991). Mature plants of guayule can withstand temperature fluctuations of −18 to 49 °C (Estilai and Ray, 1991); however, there is a narrower temperature range for germination (Chandra, 1991). Germination and early seedling growth in guayule is relatively sensitive to high salinity (Miyamoto et al., 1982).

Abbreviations: EC, electrical conductivity; mS/cm, MilliSiemen per centimeter.

* Corresponding author. Matthew A. Jenks.
E-mail addresses: paul.sanchez@ars.usda.gov (P.L. Sanchez), kuang@email.arizona.edu (M.-k. Chen), pessarak@email.arizona.edu (M. Pessarakli), hankh@seeddynamics.com (H.J. Hill), mag87@cornell.edu (M.A. Gore), matt.jenks@ars.usda.gov (M.A. Jenks).

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Transplanting seedlings established in greenhouse-grown cell packs is state-of-the-art practice for field establishment of guayule; however, this practice has a significant cost to commercial production. The estimated cost of the seedling transplant method for field establishment was US $1600 ha^{-1} in the United States (Bucks et al., 1986; Foster et al., 1999; Foster and Coffelt, 2005). Direct seeding using standard planting equipment should reduce the cost of stand establishment (Bucks et al., 1986), but effective agronomic methods for direct seeding have not yet been developed. Optimal temperature and soil condition (such as salinity levels) requirements for acceptable stand establishment need to be elucidated.

Parts of the desert Southwestern United States, a current target region for commercial guayule production, exhibit soil temperatures ranging from below 10 °C to above 30 °C during the year (http://ag.arizona.edu.met). Salinity, another environmental concern which occurs naturally in irrigation water, is nearly ubiquitous across the Southwestern United States (Chhabra, 1996). Seed pelleting technologies have been beneficial for stand establishment of small-seeded species (Taylor et al., 1998), such as lettuce, in the desert Southwestern United States. Pelleting increases seed size and improves seed shape allowing for more effective use of mechanical planters (Hill, 1999). Pesticides, herbicides, and growth regulators – all seed amendments that enhance stand establishment, can also be applied via the seed pellet.

Limited studies of direct seeding in commercial guayule production (Dissanayake et al., 2008; Foster and Moore, 1992; L. Johnson, personal communication) indicate the need for further research. Such additional research would explore in greater depth the effects of environmental conditions and lead to an optimization of field establishment of commercial guayule plots. In the present study, the effects of temperature and salinity were evaluated and the performance of non-pelleted and pelleted seeds on germination and initial seedling establishment of guayule compared.

2. Materials and methods

2.1. Seed source and growing conditions

Seeds were sown in growth chambers with 12:12 light and dark conditions, 40–60% relative humidity and with varying temperature settings of 10, 20, 30, and 40 °C. Light intensity inside the growth chambers ranges from 1867 to 1883 Foot-candles (Fc) viewed using Extech Instruments Easy View Digital light Meter (Model EA31). YULEX-B line seeds (Yulex Corporation, Chandler, AZ) were chosen based on high viability, using tetrazolium test (Association of Seed Analysts, 2004). Bekaardt et al. (2010) and Bedane et al. (2006) studied the importance of seed quality for germination and seedling survival of guayule. The seeds of the guayule YULEX-B line used in this study were harvested in May 2010. Seeds were air dried and packed in seed cotton drawstring bags. These bags were then placed in buckets and stored in a climate controlled seed room at 25–30 °C and 10–30% relative humidity. Only filled seeds of YULEX-B line were used. Jorge et al. (2007) showed that filled guayule seeds were of high quality. Seed pelleting was performed by Seed Dynamics, Inc., Salinas, CA. Seeds were pelleted with an experimental formulation to a diameter of approximately 4 mm using inert materials such as sand, diatomaceous earth and binders. Different EC levels (0, 2, 4, 6, 8, and 10 mS/cm) were prepared using sodium chloride (NaCl) (Sigma–Aldrich, S9888). The EC solutions were prepared separately in 100 ml labeled beakers. Electric conductivity (mS/cm) was measured using a Milwaukee (MW) 802 pH/EC/TDS meter.

Three preliminary experiments were conducted inside the selected growth chambers as preparation for this study. The first experiment was conducted to determine the number of observation days to be used. Filled YULEX-B line raw seeds were placed at 100 seeds each in four Petri dishes lined with filter paper containing 15 ml distilled water. Germination at 20 °C was observed daily for 14 days. Results showed that a period of 7 days was sufficient for completion of seed germination, the same findings as those of Miyamoto et al. (1982). The second experiment was conducted to determine the temperature regimes to be used in our study. Viable seeds of YULEX-B line were exposed for 7 days to temperatures of 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, and 40 °C. At each temperature, there were 10 seeds in each of four Petri dishes lined with filter paper containing 15 ml of water. Results indicated that temperatures of 10, 20, 30, and 40 °C could be used in our study. Furthermore, these temperatures were representative of the annual maximum temperatures observed in Maricopa, Arizona (http://ag.arizona.edu.met), the location of our study. The third experiment was conducted to determine the seed density to be used in our study. We placed 10, 20, 30, 50, and 100 viable seeds in each of four separate Petri dishes lined with filter paper containing 15 ml of distilled water at 20 °C. After 7 days we found 100% germination in all seed densities used. We selected 30 as the number of seeds to be used in our study.

2.2. Seed germination

The seed treatments studied were all the combinations of the following:(1) non-pelleted vs. pelleted, (2) germination in temperatures of 10, 20, 30 and 40 °C, and (3) imbibition in EC solutions of 0, 2, 4, 6, 8, and 10 mS/cm. The experiment was laid out in a three factor factorial in completely randomized design with four replications. A seed was considered to have germinated when the emerging radicle was visible (Fig. 1a). The number of germinated seeds was counted daily over 7 days. Salt solutions were pipetted and replaced with 15 ml of freshly prepared solution every 2 days. The EC meter was used to check and maintain stable salt concentration. Measurements of leaf length, stem length, and root length were made using a dissecting microscope. Photos were taken using Auto-Montage Pro software and an Auto-Montage Microscope (Syncroscopy, MD, USA). Viability of the seeds was determined using the tetrazolium test (Association of Seed Analysts, 2004). Non-germinated seeds were transferred to distilled water plates in a 20 °C environment. The number of these non-germinated seeds that germinated was recorded daily for 7 days. Measurements of leaf length, stem length, and root length were made using a dissecting microscope. Seedling length is the sum of leaf length, stem length, and root length.

2.3. Plant parameters measured

Plant parameters recorded were the final germination percentage and mean time to germination. The final germination percentage was calculated as the total number of seeds that germinated in 7 days divided by the total number of seeds (30) multiplied by 100. Mean time to germination (MTG), was calculated as:

$$\mathrm{MTG} = \sum \frac{n_i \times d_i}{N_i}$$

where n is the number of seeds germinating up to day i, d is the incubation period in days, and N is the total number of seeds germinating in the treatment (Gimenéz Luque et al., 2013).

2.4. Statistical analysis

All data were expressed as mean ± standard error (s.e.), and each mean value was calculated from four replicates. Data were analyzed using a three-way analysis of variance (ANOVA) and multiple regression to test the effects of seed pelleting, temperature, salinity,

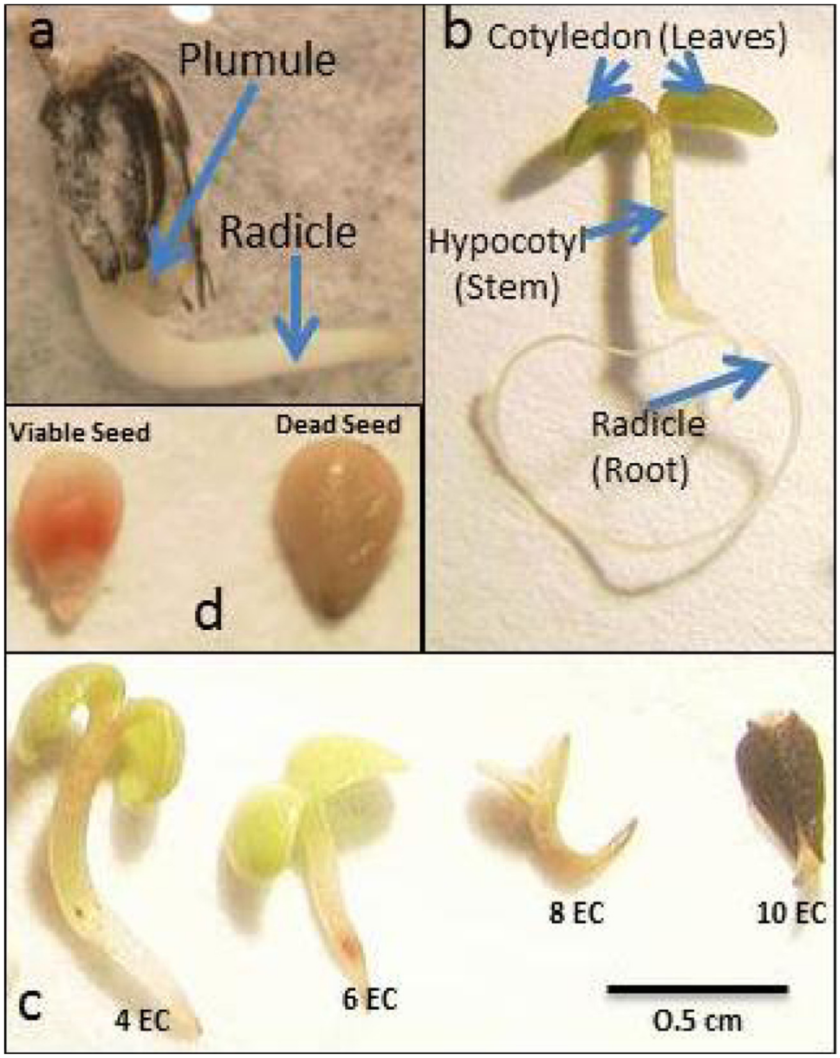

Fig. 1. Seeds and seedlings of guayule grown under different temperature and salt conditions: (a) normal germinated seed; (b) healthy seedling; (c) salt-stressed germinated seed and seedlings; and (d) viable and dead seeds after tetrazolium test.

and their interactions on seed germination and seedling elongation at 7 days and after temperature inhibition of seed germination. For the significant main effects, post hoc test (Tukey HSD) was carried out to compare the means of outcome variables at different levels of temperature and salinity. In multiple regression, all the treatment variables (type of seed, temperature, and salinity) and the interaction terms (type of seed × temperature, type of seed × salinity, temperature × salinity, and type of seed × temperature × salinity) were included in the initials models, and in the final models only the significant interactions (all main effects were retained in the model in spite of the significant levels of the results) were included. All statistical analyses were performed using IBM SPSS Statistics 21 (IBM Corp., 2012).

3. Results

3.1. Initiation of germination and mean time to germination

Our data show that non-pelleted and pelleted seeds of the YULEX-B line used in this study did not germinate at 10 and 40 °C (Table 1). In addition, the mean time to germination was significantly longer at 30 °C than at 20 °C (Table 1), thus suggesting that 20 °C is a more favorable temperature for germination. Mean time to germination generally increased as salinity level increased, indicating that salt stress delayed germination. The negative influence of salinity on mean time to germination increased significantly as temperature increased (F ratio = 169.9, df = 15, $p < 0.0001$). At 30 °C non-pelleted seeds did not germinate in EC 6–10 mS/cm.

3.2. Final seed germination at day 7

Percent seed germination was significantly affected by seed pelleting (F ratio = 21.38, df = 1, $p < 0.001$), temperature (F ratio = 8781.37, df = 3, $p < 0.001$), salinity (F ratio = 165.38, df = 5, $p < 0.001$), and their two-way and three-way interactions (F ratio = 24.29, df = 3, F ratio = 20.98, df = 5, F ratio = 116.56, df = 15, $p < 0.001$ for all two-way interactions; F ratio = 42.26, df = 15, $p \leq 0.001$ for three-way interaction). The optimum temperature for seed germination was 20 °C, with 100% of the control and pelleted seeds germinating at 0–10 EC and 0–2 EC, respectively (Fig. 2). Only 72% of the pelleted seeds germinated at an EC of 10 mS/cm. Regression analysis showed a significant decrease in seed germination with an increase in the interaction of temperature and salinity ($R^2 = 0.95^{**}$, $\beta = -2.2$, percent of germination = .85 × type of seed − .30 × temperature + 1.67 × salinity − 1.06 × interaction between type of seed and temperature − 2.2 × interaction between temperature and salinity + 1.9 × three way interaction among type of seed, temperature, and salinity). The negative effect of salinity

Table 1
Effects of temperature and salinity on mean time to germination of non-pelleted (control) and pelleted seeds of guayule. EC is electrical conductivity (mS/cm).

Salinity (EC)	Mean time to germination (days)							
	Non-pelleted seeds				Pelleted seeds			
	Temperature				Temperature			
	10 °C[a]	20 °C[a]	30 °C[a]	40 °C[a]	10 °C[a]	20 °C[a]	30 °C[a]	40 °C[a]
0[b]	0.0 aA	2.0 bA	4.3 cA	0.0 aA	0.0 aA	2.1 bA	4.5 cA	0.0 aA
2[b]	0.0 aA	2.0 bA	4.4 cA	0.0 aA	0.0 aA	2.1 bA	4.9 cA	0.0 aA
4[b]	0.0 aA	2.3 bA	4.8 cA	0.0 aA	0.0 aA	2.1 bA	5.0 cA	0.0 aA
6[b]	0.0 aB	2.3 bB	0.0 cB	0.0 aB	0.0 aB	2.1 bB	5.2 cB	0.0 aB
8[b]	0.0 aB	2.5 bB	0.0 cB	0.0 aB	0.0 aB	2.1 bB	5.3 cB	0.0 aB
10[b]	0.0 aB	2.5 bB	0.0 cB	0.0 aB	0.0 aB	2.1 bB	5.8 cB	0.0 aB

[a] For temperature (across columns), mean numbers followed by different low case letters are significantly different using Tukey HSD test at 5% level.
[b] For salinity (across rows), mean numbers followed by different upper case letters are significantly different using Tukey HSD test at 5% level.

Table 2
Effects of 7 day temperature and salinity treatments on seedling length (cm) of non-pelleted and pelleted seeds of guayule treated with 20 and 30 °C, and a 7 day treatment without salt at 20 °C (removing temperature inhibition) of seeds pre-treated with 10 and 40 °C and different salinity levels. EC is electrical conductivity (mS/cm).

Salinity (EC)	After 7 day treatment with salt[a]				After 7 day treatment without salt at 20 °C[d]			
	Non-pelleted seeds		Pelleted seeds		Non-pelleted seeds		Pelleted seeds	
	Temperature				Temperature			
	20 °C[b]	30 °C[b]	20 °C[b]	30 °C[b]	10 °C[e]	40 °C[e]	10 °C[e]	40 °C[e]
0[c,f]	2.81 aA	2.77 bA	1.95 aA	1.48 bA	2.04 aA	1.58 bA	1.94 aA	1.47 bA
2[c,f]	1.04 aB	0.57 bB	1.78 aB	1.26 bB	1.91 aB	1.25 bB	1.77 aB	0.98 bB
4[c,f]	0.81 aB	0.43 bB	1.63 aB	1.24 bB	1.81 aC	1.08 bC	1.62 aC	0.94 bC
6[c,f]	0.21 aC	0.0 bC	1.04 aC	0.84 bC	1.64 aD	0.0 bD	1.01 aD	0.91 bD
8[c,f]	0.22 aC	0.0 bC	0.93 aC	0.80 bC	1.54 aD	0.0 bD	0.92 aD	0.81 bD
10[c,f]	0.10 aC	0.0 bC	0.69 aC	0.70 bC	1.39 aE	0.0 bE	0.77 aE	0.71 bE

[a] For this variable, mean numbers followed by different low case letters are significantly different using Tukey HSD test at 5% level.
[b] For temperature (across columns), mean numbers followed by different low case letters are significantly different using Tukey HSD test at 5% level.
[c] For salinity (across rows), mean numbers followed by different upper case letters are significantly different using Tukey HSD test at 5% level.
[d] For this variable, mean numbers followed by different low case letters are significantly different using Tukey HSD test at 5% level.
[e] For temperature (across columns), mean numbers followed by different low case letters are significantly different using Tukey HSD test at 5% level.
[f] For salinity (across rows), mean numbers followed by different upper case letters are significantly different using Tukey HSD test at 5% level.

on seed germination was more severe at 30 °C. Non-pelleted seeds planted in EC of 6–10 mS/cm did not germinate at 30 °C. In contrast, pelleted seeds performed better than non-pelleted seeds at an EC of 4 mS/cm or greater (Fig. 2).

3.3. Seedling elongation

Total seedling length was significantly affected by pelleting (F ratio = 72.4, df = 1, $p < 0.0001$), temperature (F ratio = 464.4, df = 3, $p < 0.0001$), salinity (F ratio = 117.7, df = 5, $p < 0.0001$), and their two-way and three-way interactions (F ratio = 24.2, df = 3, F ratio = 33.5, df = 5, F ratio = 40.2, df = 15, $p < 0.0001$ for all two-way interactions; F ratio = 11.5, df = 15, $p < 0.0001$ for three-way interaction). Seedlings were significantly longer at 20 °C than those at 30 °C (Table 2). Seedling lengths significantly decreased with increase in salinity. At the optimum conditions for germination in this study (20 °C and EC 0 mS/cm), seedlings arising from non-pelleted seeds (2.81 ± 0.15 cm) grew longer than those of pelleted seeds (1.95 ± 0.08 cm) (Table 2). Seedling length, consisting of cotyledons, a hypocotyl, and tap root (Fig. 1b), decreased with an increase in salinity level (Fig. 1c). The main effect on the seedlings was on taproot length, which decreased with increasing salinity levels ($R^2 = 0.53^{**}$, $\beta = -0.69$, root length = .15 × type of seed − .17 × temperature − .69 × salinity). The effect of salinity on seedling elongation was greater at 30 °C. In addition, some of the radicles were darker at 30 °C.

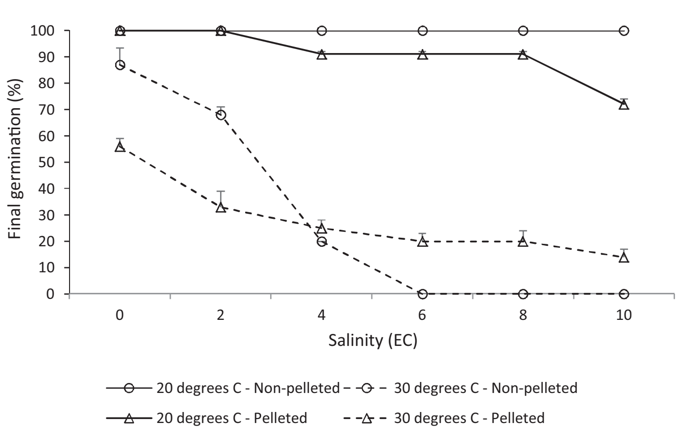

Fig. 2. Final germination (%) of non-pelleted (○) and pelleted (△) guayule seeds at 7 days. Seeds were treated with different levels of salinity at two temperatures (20 and 30 °C). Differences in germination among treatments are statistically different at the $p < 0.001$ level. The bars indicate standard error.

3.4. Temperature inhibition of seed germination and total seedling length of seeds that were inhibited by 10 and 40 °C

Guayule non-pelleted and pelleted seeds planted in solutions of 0, 2, 4, 6, 8, and 10 mS/cm at 10 and 40 °C that did not germinate were transferred to distilled water plates in a 20 °C environment. Guayule seeds inhibited by a low temperature of 10 °C germinated after they were transferred to optimum conditions (20 °C, 0 mS/cm). These seeds germinated in 2 days. The seeds inhibited by a high temperature of 40 °C also germinated after they were transferred to optimum conditions. These seeds germinated in 3 days.

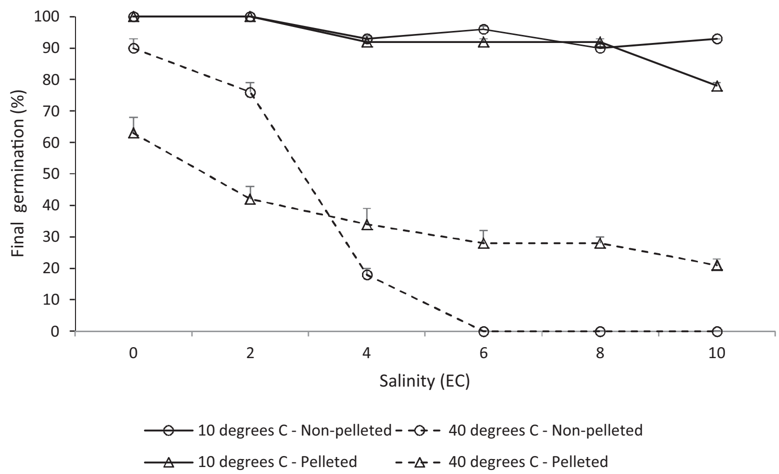

Fig. 3. Final germination (%) of non-pelleted (◯) and pelleted (Δ) guayule seeds pre-treated with different levels of salinity at two temperature regimes (10 and 40 °C). Temperature inhibited seeds were transferred to distilled water plates in a 20 °C environment. Differences in percent germination among treatments are statistically different at the $p < 0.0001$ level. The bars indicate standard error.

Final germination of the seeds inhibited by supra-optimal temperatures of 10 and 40 °C was not significantly affected by seed pelleting (F ratio = 1.6, df = 1, $p = 0.21$), but was significantly affected by temperature (F ratio = 4770.4, df = 1, $p < 0.0001$), salinity (F ratio = 235.0, df = 5, $p < 0.0001$), and their two-way and three-way interactions (F ratio = 24.2, df = 1, F ratio = 38.8, df = 5, F ratio = 114.8, df = 5, $p < 0.0001$ for all two-way interactions; F ratio = 52.4, df = 5, $p < 0.0001$ for three-way interaction). Final germination of high temperature (40 °C) inhibited seeds decreased as salinity increased (Fig. 3). Regression analysis showed a decrease in percent germination with an increase in salinity and temperature pre-treatments ($R^2 = 0.93^{**}$, $\beta = -1.03$, recovery rate = .16 × type of seed – .26 × temperature + .20 × salinity – .42 × interaction between type of seed and temperature – .34 × interaction between type of seed and salinity – 1.03 × interaction between temperature and salinity + .72 × three way interaction among type of seed, temperature, and salinity). Less than 50% of the seeds pre-treated with EC 4–10 mS/cm at 40 °C germinated.

The effects of seed pelleting, temperature, salinity, and their interactions on seed mortality were similar to their effects on final germination. For seeds imbibed with EC 0 mS/cm at 40 °C, seed mortality was 10% and 37% for non-pelleted and pelleted seeds, respectively (Fig. 3). However, seed mortality of pelleted seeds was significantly less than that of non-pelleted seeds at EC 4–10 mS/cm. A 100% mortality of non-pelleted seeds was found at high temperatures (30 and 40 °C) combined with high salinity levels (EC 6–10 mS/cm). Seed mortality was confirmed using tetrazolium tests (Fig. 1d).

Total seedling length was not significantly affected by seed pelleting (F ratio = 2.47, df = 1, $p = 0.12$), but significantly affected by temperature (F ratio = 1518.45, df = 1, $p < 0.0001$), salinity (F ratio = 345.01, df = 5, $p < 0.0001$), and their two-way and three-way interactions (F ratio = 355.71, df = 1, F ratio = 9.42, df = 5, F ratio = 9.49, df = 5, $p < 0.0001$ for all two-way interactions; F ratio = 79.73, df = 5, $p < 0.0001$ for three-way interaction). The 7 day seed exposure to 10 and 40 °C, and different salinity levels affected seedling lengths of YULEX-B line even after the inhibited seeds were transferred to distilled water at 20 °C. Temperature and salinity significantly decreased seedling lengths (Table 2). Seedlings from seeds inhibited by 40 °C were significantly shorter than those seeds inhibited by 10 °C. Increase in salinity significantly decreased the seedling length of those seeds inhibited by 40 °C ($R^2 = 0.92^{**}$, $\beta = -0.81$, seedling length = .02 × type of seed – .41 × temperature – .15 × salinity – .21 × interaction between type of seed and temperature – .74 × interaction between seed and salinity – .81 × interaction between temperature and salinity + 1.11 × three way interaction among type of seed, temperature, and salinity).

4. Discussion

Currently, limited information is available about the conditions needed to promote commercially viable germination and seedling establishment for field sown seeds of guayule. The study presented here reveals how seed pelleting, temperature, salinity, and their interactions can significantly affect germination and initial seedling establishment of guayule. Of the temperature treatments studied, a temperature of planting seeds at 20 °C produced the highest germination percentage. Chandra (1991) found that germination of seeds for the accession 11591 decreased as temperatures dropped below 19 °C or rose above 30 °C. They found maximum germination at 20–30 °C. The genotype they used appeared to perform better at higher temperatures than the YULEX-B line tested in this study. Our findings indicate that the time of seed sowing to optimize germination should target planting when soil temperatures in the field are closer to 20 °C. For planting in the Maricopa, AZ test site, the optimal time for sowing is most likely to occur from March to April or September to October.

Increasing planting temperature (40 °C) delayed seed germination for the guayule germplasm YULEX-B line. Seed germination occurred between 2 and 3 days at an optimum temperature of 20 °C, between 4 and 7 days at 30 °C, and germination was completely inhibited at 40 °C. Although germination was also inhibited at 10 °C, the mechanism for guayule response to high temperature likely differs from its response to low temperatures. Plants can sense cold temperature by membrane fluidity, whereas high temperature response may also include involvement of heat shock proteins (Jones et al., 2013). High temperature also increased seed and seedling mortality. Understanding the mechanisms of guayule's response to supra-optimal cold and heat may help in targeting best planting dates, and guide breeding programs seeking to develop new varieties with broader thermal range tolerances for germination and seedling establishment in the field.

Salinity is an important factor to consider for a successful field establishment using direct seeding of guayule, especially in the regions being targeted for commercial production in the Southwestern United States, as salinity is increasingly problematic in these arid land agricultural soils. High salinity delayed germination of YULEX-B line, and inhibited germination at the highest levels tested. Similarly, Miyamoto et al. (1982) found that increasing salt concentrations slowed germination rate of several guayule lines. Understanding the mechanism of guayule's response to high salinity can help in developing methods to minimize its effects on seed germination, and guide breeding programs seeking to develop new varieties with tolerance to salinity. Several studies showed how germination is inhibited under high salt concentration and that the seeds can germinate after the salt stress is removed (Keifer and Ungar, 1997; Khan and Ungar, 1997; Khan et al., 2000; Khan et al., 2001). We observed a similar response in seeds that were inhibited by high salt levels of EC 4–8 mS/cm. The degree to which the seed and seedling mortality we observed for YULEX-B line was due to osmotic effects of ion toxicity (Khan and Ungar, 1997) is still uncertain.

Guayule seed germination was also impacted by the interaction between temperature and salinity. We found seed germination inhibition, as well as seed and seedling mortality increased with increasing salinity at both 30 and 40 °C treatments. We found that these seeds germinated when the temperature was optimum (20 °C), and the seeds rinsed with distilled water to remove salt. However, the overall growth of seedlings from seeds planted in the highest temperatures and salinity was low. Resulting seedlings had shorter roots and smaller and discolored leaves. The reduction in root development may be due to osmotic or toxic effects of NaCl, and/or unbalanced nutrient uptake by the seedlings (Bor et al., 2003). Rejili et al. (2009) found that the inhibitory effect on germination of *Lotus creticus* at high salinity was greater at higher temperatures of 35 °C, and El-Keblawy and Al-Rawai (2005) found similar effects in the invasive shrub *P. juliflora*. In some arid regions, high germination rates of seeds are observed during the rainy season when soil salts are flushed away by precipitation (El-Keblawy, 2004). In the wild, the desert forb (*Zygophyllum simplex*), germinates only when temperature and salinity are low. These conditions are provided for during the monsoon rains or during the winter season (Khan and Ungar, 1997). As a case in point, however, total rainfall in the low elevation deserts of Southern Arizona is low, and soil temperatures reach more than 30 °C even during the summer monsoon season. Hence, planting time will be a critical concern as methods for commercial planting of guayule using direct seeding approaches are applied. The appropriateness of seed pelleting is also recommended by the results of this study, as the non-pelleted guayule seeds are more sensitive to increasing temperature and salt concentrations than pelleted seeds.

5. Conclusions

Temperature and salinity are two of the most important factors for successful germination and seedling establishment of the high quality guayule YULEX-B line. The results of this study of temperature and salinity were used to determine the best time to plant YULEX-B line in Maricopa, Arizona. Currently an ongoing field experiment is aimed at comparing the performance of pelleted and non-pelleted YULEX-B line seeds. Future studies will be conducted to compare the germination performance of YULEX-B line with other guayule lines.

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