Effects of salinity and sand burial on germination and establishment of *Blutaparon portulacoides* (St.Hil.) Mears (Amaranthaceae) on backshore of southern Brazil

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Abstract

The effect of different salinities on the germination was tested during 40 days in laboratory, using six concentrations of NaCl (0, 45, 80, 130, 170 and 215 mM NaCl) and three concentrations of seawater (25, 30 and 35%). In addition, the survival and growth of seedlings were tested in five constant salinity regimes (0, 45, 80, 130 and 170 mM NaCl) as well as in five regimes of simulating seawater overwash. The effect of sand burial on the germination was tested during 40 days in the laboratory, using five different depth (0, 1, 2, 4 and 6 cm). Additionally, 100 seedlings (2-weeks old) were planted along the backshore and their survival and sand deposition observed at 5-day intervals, during 30 days. The results showed that total germination declines with increased salinity concentration, the same was observed for lag time. The effects of salinity on seedling growth showed that height and distance between nodes were a more sensitive indicator of salinity stress, showing a gradual reduction in growth with increase of NaCl. Seedlings showed a gradual decrease in survival and dry biomass in relation to the frequency of flooding with seawater. The germination of B. portulacoides seeds decresed significantly at depths greater than 2 cm, and was inhibited at 6 cm. Seedlings showed high mortality at even low sand burial. The results obtained in laboratory on germination and growth of B. portulacoides in salinities above those normal in its natural habitat could explain why it is a dominant species at the backshore. However, sand deposition is a major environmental factor that determines the establishment of new seedlinas.

Key words: salt tolerance, seedling growth, dune species, Atlantic coast

Introduction

The zone above the high spring tideline on the backshore of beaches may be one of the most physically stressful places in which a plant can begin life (Hesp, 1991). Here seed, seedling, plant growth, and survival can be affected mainly by sand movement (Maun and Lapierre, 1984; Harris and Davy, 1987; Zhang and Maun, 1990a) and by the salinity of the substrate (Barbour, 1970; Seneca, 1972). Thus, plants of backshore and foredunes necessarily must withstand episodes of burial with sand (Harris and Davy, 1987) and drastic fluctuations of soil salinity (Barbour and De Jong, 1977; Rozema *et al.*, 1983). Seed germination and seedling establishment under various salinities has been

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investigated in many halophytes (Ungar, 1962; Bazzaz, 1973; Marañon et al., 1989) and in dunes species (Sykes and Wilson, 1989; Greipsson and Davy, 1994). According to Harper and Benton (1966), burial of seeds may be beneficial because it maintains adequate moisture around the seeds and protects the seeds and emerged seedlings from desiccation. On the other hand, excessive burial by sand affects seed germination, seedling emergence (Maun and Lapierre, 1986; Zhang and Maun, 1990b), seedling establishment (Harris and Davy, 1987; Zhang and Maun, 1990a; 1990b), plant morphology (Maun and Riach, 1981), biomass (Maun and Lapierre, 1984; Harris and Davy, 1987; Zhang and Maun, 1992), and the balance between sexual and vegetative reproduction (Barbour et al., 1985). On the backshore areas of southern Brazil, substantial salinity until 80 mM NaCl was found in the interstitial soil solution (Cordazzo, 1999), while Cordazzo and Seeliger (1993) reported a mean sand accretion of 8.6 cm/month. The zonation of these coastal dunes was controlled mainly by sand movement and additionally, by other environmental factors (Cordazzo and Seeliger, 1993). Despite its widespread distribution and importance in coastal places of southern Brazil, a pioneer species of coastal dunes, Blutaparon portulacoides (St.Hil.) Mears has hitherto received little attention in relation to the effects of salinity and sand burial on germination and seedling establishment. Thus, the purpose of this study was (i) to determine the effects of salinity and sand burial on germination and seedling emergence in Blutaparon portulacoides; (ii) to examine the effects of salinity on survival and growth of seedlings; (iii) to investigate if the average potential elongation surpassed the average of depth from which seedlings emerged.

Material and Methods

Six treatments, expressed as percentages of NaCl by weight, were used: 0, 0.25, 0.5, 0.75, 1.0, and 1.25% (0, 45,

80, 130, 170 and 215 mM NaCl.L-1), to determine the germination response of seeds to various levels of NaCl. Seed germination experiments were carried out on two layers of filter paper in 9cm diameter Petri dishes with 5 mL of test solution added to each dish. A set of four dishes with 25 seeds (selected randomly) per dish was used to test the effect of each salinity. All dishes were sealed with Nescofilm to reduce evaporation, and the solution was changed completely every week. Petri dishes were randomly distributed in temperature-controlled incubators (15-30C; 12-12 hours) for 40 days. Germination was checked every day and defined as the protrusion of the radicle or plumule.

To determine the effect of high salinity on seed germination, four replicates of 25 seeds per each concentration, were soaked in three different concentrations of seawater 25‰, 30‰ and 35‰; approximately 340, 430 and 600 mM NaCl.L-1, which correspond to a range of salinity of coastal water in southern Brazil. After 30 days, germination was counted and seeds that had not germinated were transfered to Petri dishes with distilled water for 40days, using the same methodology discribed previously.

To evaluate quantitatively the effect of salinity upon the growth of seedlings of B. portulacoides, 10 seedlings of similar size and age (7-days old) were transplanted to individual 9 cm diameter plastic pots filled with 4:1 sand and potting compost mixture. Seedling growth was tested under five salinity treatments: 0, 45, 80, 130, and 170 mM NaCl.L-1. Solutions were prepared by adding the appropriate amounts of NaCl to Hoagland's nutrient solution. Application of 30 mL of the solution was sufficient to wet the substrate completely. To prevent salt build up in the substrate, pots were flushed with an equal volume of distilled water prior to treatment. After 60 days, all plants were harvested and gently washed. The dry mass of leaves, roots and rhizomes were measured after 48 hours in an oven at 80 °C. Also, the height, leaf

number, number of nodes, distance between nodes and number of branches were recorded.

Seedlings of B. portulacoides are subject to occasional flooding by seawater during exceptional high tides. One hundred and twenty five seedlings (7days old) were planted in individual plastic pots, as described above, and cultivated in a glasshouse for 12 weeks. Plants were divided into five groups which received differential flooding with 50 mL of seawater (600 mM NaCl); once a week, once per 2 weeks, once per 3 weeks, one per 4 weeks, and control (no seawater). All pots were flushed with an equal volume of distilled water prior to treatment and additionally pots received 20 mL of Hoagland's nutrient solution every 2 weeks. At the end of the experiments height, dry mass of leaves and survival were measured to determine seedling response to overwash effect.

To investigate the effects of sand burial, seven hundred seeds of similar mass $(0.9 \pm 0.04 \text{ mg})$ were chosen and soaked in distilled water for 12 hours for imbibition. Subsequently, seeds were buried on a 2 cm sand bed in 12 cm diameter plastic pots, at depths of 0, 1, 2, 4, 6, 8 and 10 cm with previously washed in distilled water sand. Four replicates of 25 seeds were used for each depth of burial. Seedling emergence was monitored daily, during 40 days and, at the end of experiment, sand in each pot was washed out to retrieve both the ungerminated seeds and unemerged seedlings.

Ten dark-germinated seedlings (oneday old) were transplanted into test tubes (15 cm long and 2.5 cm diameter) with a double layer of wet filter paper at the botton. Test tubes were sealed with Nescofilm to avoid contamination and evaporation and were subsequently transferred to darkness in the glasshouse. At the same time, 10 seedlings of similar age were burried on a 2 cm sand bed in 18cm diameter plastic pots at 10 cm sand depth. All pots were randomly arranged in a glasshouse over a period of 25 days. Thus, at the end of the experiment it was possible to determine if the average potential elongation surpassed the average depth of emerged seedlings. Potential elongation was defined as the final length of seedling grown in the absence of a physical barrier.

Along the backshore 10 plots (50 x 50cm) were demarcated by installing wooden stakes at each corner. In each plot, 10 (2-week old) seedlings were planted and their survival estimated by observation at 5-day intervals. At each observation, during 30 days, the surviving seedlings were counted and sand movement (deposition or erosion) in each plot was measured.

All data on percentage germination, growth, and seedling emergence were arcsin-square root transformed for normalization before analysis of variance (ANOVA). When the ANOVA null hypothesis was rejected, Tukey's multiple range test was used to compare means at the 5% level (Sokal and Rohlf, 1981). However, the data shown in graphs and tables are untransformed.

Results

Germination in *B. portulacoides* did not gradually decline with increased salinity but remained relatively unaffected up to a NaCl concentration of 80 mM, after which it decreased significantly (Table 1) to 50% at 215 mM NaCl. The time for the first germination and the time for 50% of germination were also affected negatively by salinity greater than 80 mM NaCl (Table 1).

Table 1. Summary comparison of the effect of salinity on the germination of *Blutaparon portulacoides*, where, **L** is the time in days to the start of germination, **G%** is the total germination, and **t(50)** is the median germination time (time to 50 % of germination).

Salinity (mM NaCl L-1)	L	G%	t(50)
0	1	93 (a)	4.7
45	1	92 (ab)	3.8
80	1	90 (ab)	4.7
130	2	73 (bc)	7.6
170	2	63 (c)	14.5
215	3	50 (c)	27.0
		F = 10.51; p<0.000	1

Means with the same letter in the column are not statistically different according Tukey's multiple range test at 5% significance level.



Figure 1. Survivorship curve (dotted line) and cumulative sand accretion (continuous line) of *B. portulacoides*, for a period of 30 days after seedling planted in southern Brazilian backshore (n = 100).

Seeds of B. portulacoides did not germinate in the three seawater concentration tested (25%, 30% and 35%) within 30 days. However, when seeds that had not germinated in the three seawater concentration were transfered to distilled water, they showed germination of 75%, 73%, 72%, respectively, without significant differences between treatments. Analysis of variance of the data indicated no significant differences in germination between the three concentrations of seawater, after transfer to distilled water (F = 2.41; p = 0.144; d.f.= 2), but t(50) was reduced to 1 day.

Seedlings displayed symptoms of salinity stress mainly in the higher concentrations of NaCl. The tips of older leaves became yellowish. At low NaCl concentrations, seedlings of B. portulacoides did not show the typical symptoms of salinity stress. Only at 170 mM NaCl, did seedlings showed some chlorosis in older leaves. However, the analysis of variance of growth data detected a significant adverse effect in seedlings grown in concentrations greater than 80 mM NaCl (Table 2). Height of seedlings and distance between nodes were a more sensitive indicator of salinity stress than other variables (Table 2), showing a gradual reduction in growth with increase of NaCl concentration.

Seedlings showed a gradual decrease in survival and dry biomass in relation to the frequency of flooding with seawater at 30‰ of salinity (Table 3). Final height of plants was a more sensitive indicator of the effect of differential flooding conditions with seawater (Table 3), showing a gradual decline with increasing frequency of overwash.

The percent germination in *B. portula-coides* was low (Table 4) with 23% at the surface, and decrease significantly at depths greater than 2 cm. No germination was observed at depths greater than 6 cm. The highest seedling emergence of 100 and 98% occurried from 0 and 1 cm burial depths. The emergence fell significantly to 50% at 2 cm and 27% at 4 cm. No seedlings of *B. portulacoides* emerged from burial

 Table 2. Effect of salinity on the growth of Blutaparon portulacoides seedlings cultivated in a glasshouse for 60 days (n = 10 plants per each salinity treatment).

Variables	SALINITY (mM NaCl)					
	0	45	80	130	170	F
Height (cm)	20.9 ± 2.2	11.2 ± 0.8	7.0 ± 0.5	5.4 ± 0.5	4.7 ± 0.8	31.6
	(a)	(b)	(bc)	(c)	(c)	p < 0.0001
Leaf dry mass (mg)	270.8 ± 16.2	231.9 ± 13.2	200.7 ± 11.8	174.6 ± 11.9	161.9 ± 9.2	12.5p
	(a)	(ab)	(bc)	(c)	(c)	< 0.001
Rhizome dry mass (mg)	246.6 ± 25.2	266.7 ± 19.1	130.9 ± 11.2	95.4 ± 8.8	78.0 ± 13.1	27.7p
	(a)	(a)	(b)	(b)	(b)	< 0.05
Root dry mass (mg)	707.0 ± 45.7	691.8 ± 49.1	427.2 ± 27.8	301.5 ± 17.1	278.5 ± 20.3	35.9p
	(a)	(a)	(b)	(bc)	(c)	< 0.0001
No. of leaves	29.6 ± 2.6	25.8 ± 2.0	14.3 ± 1.0	12.2 ± 0.9	12.0 ± 1.3	22.6p
	(a)	(a)	(b)	(b)	(b)	< 0.001
No. of nodes	7.2 ± 0.3	6.6 ± 0.3	5.5 ± 0.1	5.0 ± 0.3	4.3 ± 0.3	15.4p
	(a)	(ab)	(bc)	(c)	(c)	< 0.001
Distance between nodes (cm)	2.9 ± 0.4	1.6 ± 0.1	1.1± 0.1	1.0± 0.1	1.0 ± 0.1	33.9p
	(a)	(b)	(bc)	(c)	(c)	< 0.001
No. of branches	2.9 ± 0.4	2.9 ± 0.2	1.7 ± 0.2	1.1±0.1	1.1 ± 0.1	12.5p
	(a)	(a)	(b)	(b)	(b)	< 0.05

Means with the same letter in the row are not statiscally different according to Tukey's multiple range test at 5% significance level.

Table 3. Perfomance of *Blutaparon portulacoides* seedlings when subjected to different conditions of overwash with seawater (600 mM NaCl) for 12 weeks. Means ± SE are based on 25 seedlings planted in individual pots for each treatment.

OVERWASH	INITIAL HEIGHT (cm)	FINAL HEIGHT (cm)	AERIAL BIOMASS (mg)	SURVIVAL (%)
Once per week	1.02 ± 0.06	1.82 ± 0.20 (a)	58.3 ± 6.7 (a)	66.6
Once per 2 weeks	0.96 ± 0.02	4.22 ± 0.57 (b)	135.5 ± 27.1 (bc)	93.3
Once per 3 weeks	0.98 ± 0.02	5.26 ± 0.33 (bc)	160.0 ± 26.0 (bc)	100.0
Once per 4 weeks	0.97 ± 0.02	5.29 ± 0.43 (bc)	191.5 ± 34.7 (c)	100.0
Control (no seawater)	0.99 ± 0.02	6.45 ± 0.83 (c)	201.8 ± 25.8 (c)	
	F = 0.68 (ns)	F = 9.99; p < 0.001	F = 4.43; p < 0.001	100.0

Means with the same letter in the column are not statistically different according to Tukey's multiple range test at 5% significance level. (ns = not significant)

Table 4. Percentage of germinated seeds (G%), seedling emergence of germinated seeds (E%) and time of emergence (te) of B. portule	а-
coides seeds at different depths of sand burial in a glasshouse.	

DEPTH (cm)	G%	E%	te (days)
0	23 (a)	100 (a)	3
1	17 (a)	98 (a)	14
2	8 (b)	50 (b)	20
4	4 (c)	27 (c)	30
6	0 (d)	0 (d)	
8	0 (d)	0 (d)	
10	0 (d)	0 (d)	
	F = 14.8; p < 0.001	F = 22.4; p < 0.001	

Values in each column followed by different letters are statistically different (P < 0.05) according to Tukey's multiple range test.

depths greater than 6 cm. Seedlings began to emerge at 3, 14, 20 and 30 days after sowing, respectively for 0, 1, 2, and 4 cm depths (Table 4).

The average potential elongation of seedlings of B. portulacoides was 4.2 cm, greathes than the average depth from which they could emerge (4.0 cm) but the difference was not significant (F =2.14; p = 0.193). However, only 27% of seedlings emerged at 4 cm depth (Table 4), while 100% of germinated seedlings emerged without sand deposition. Seedlings of B. portulacoides showed high mortality at even low sand burial (Figure 1), probably because of their prostate growth forms. Less than 20% of seedlings survived at 1 cm sand accretion. A significative correlation (Spearman r = -0.98; P < 0.0001) between seedlings survival and sand burial was found. A few seedlings observed died althrough they had not been buried. An additional factor, probably desiccation, may have reduced seedling survival, as some seedlings were found to be dry.

Discussion

The precise salinity concentrations causing delay and reduction in the proportion of seeds germinating depends upon the salt tolerance of each individual species (Ungar, 1982; Martinez et al., 1992). The results here reported demonstrated that Blutaparon portulacoides exhibit germination behaviour similar to those studies, with halophytes (Ungar, 1978; Rozema, 1975) and glycophytes (Bazzaz, 1973; Redman, 1974; Rozema, 1975). Germination of B. portulacoides seeds showed semihalophyte behaviour, according to Waisel (1972), with little effect on germination below 80 mM NaCl.L-1 and a strong decline in seed germination at higher salinity. A reduction in cumulative germination, as well as the increase in the time requirement for 50% germination at high salinities may be attributed to low water imbibition induced by low external osmotic potentials (Ungar and Hogan, 1970; Seneca and Cooper, 1971; Waisel, 1972; Rozema, 1975; Greipsson and Davy, 1994), enforcement dormancy by high salinity (Davy and Figueroa, 1993; Redondo *et al.*, 2004) or to specific toxic ion effects (Seneca, 1972; Waisel, 1972; Redmann, 1974; Ungar, 1978).

Germination in distilled water of previously ungerminated, salt-treated seeds indicated that the inhibitory effect of NaCl on germination is mainly due to osmotic effects. Thus, seed viability was not affected by high NaCl concentrations per se. The rapidity of germination in B. portulacoides suggests some sort of stimulation by NaCl, which was also reported by Woodell (1985) in some coastal plants. The rapid germination of seeds after salt pre-treatments has been attributed to weakening of the testa by solutions of low osmotic potential (Ungar, 1978) and was also reported by Cordazzo (1999) for Spartina ciliata. The ability of coastal species seed to remain dormant when subject to high salinity is probably of adaptive value, since seeds are often dispersed by the sea and germination would be a disadvantage or lethal under such conditions (Ignaciuk and Lee, 1980; Greipsson and Davy, 1994). An additional importance of this dormancy response to high salt stress in seeds of coastal species is that it permits seed survival during dry periods, when the salinity hazard rises (Ungar, 1978) and during periods of temporary flooding with highly saline waters (Ungar and Hogan, 1970). However, after rainfall has leached or diluted the excess salts, seeds will still be capable of germinating, as reported by Hocking (1982) for seeds of Cakile maritima, and by Cordazzo (1999) for seeds of Spartina ci*liata*. Another important effect of the release of dormancy with the alleviation of salt stress is that it determines the salinity level at the period of seedling development, which is probably one of the most sensitive periods in the life of coastal plants (Ungar, 1982).

Although seedlings of *B. portulacoides* showed a significant reduction in growth at the highest salinity, the species tolerated salinities up to 130 mM NaCl. L-1, which was higher than the natural salt concentrations in the native soils (Cordazzo, 1985). Saline conditions affect plant growth in a variety of ways, such as inducing poor physical soil conditions (Waisel, 1972), decreasing water uptake (Bernstein, 1961), causing toxic accumulation of sodium and chloride (Houle et al., 2001), and reducing nutrient availability (Schat, 1982). Generally, when salinity increases and water potentials around plant roots decreases, the turgor of plant cells is reduced, and the growth ceased at least temporarily, and the plant may wilt (Dainty, 1979; Waisel, 1972), but the turgor of plant cells can be adjusted by osmoregulation to meet environmental changes in halophytes (Dainty, 1979). Plant miniaturization in response to salinity, a phenomenon also observed by Gallagher (1979) in salt marsh plants from Georgia (USA), occurred in B. portulacoides; it was manifested as a reduction in height, number of nodes and internode length.

Different growth strategies and adaptations were observed in coastal plants. B. portulacoides develops succulence as its main adaptation to growth in saline soils (Hueck, 1955; Pfadenhauer, 1978). The foliar succulence is low in the first phases of development, but increases during the ontogenesis (Pfadenhauer, 1979). The progressive increase of salinity did not cause a significant increase in succulence in seedlings cultivated in five concentrations between 0 and 600 mM NaCl (Farias, 1985), but an increase in succulence was observed in older leaves, which became yellowish and fell (Pfadenhauer, 1979). Another feature of this C4 plant (Farias, 1985) is that the density of stomata decreases with increase of salinity (Dillenburg, 1986).

According to Rozema (1976), the zonation of dune and beach plains vegetation may partially depend on the salt resistance of individual plant species. The results here presented about germination and growth of *B. portulacoides* in salinities above those registered in natural conditions could explain why it is a dominant species at the backshore, subject to overwash with seawater during exceptional high tides.

Zhang and Maun (1990b) pointed out that sand accretion may have three important effects on seedling establishment: (i) the seeds may be forced to remain dormant, (ii) the seeds may germinate but the seedlings are unable to emerge from sand, and (iii) the seedlings may emerge but are unable to survive a post-emergence burial. The first hypothesis on the enforcement of seed dormancy was strongly supported in this study; a significant decrease in germination was obtained in this species with increase of burial depth, and yet at the end of experiment ungerminated seeds were still viable. The mechanism of enforced dormancy may be low soil temperature or reduced temperature fluctuation (Fenner, 1985; Zhang and Maun, 1990a; Cordazzo, 1994) and lower oxygen availability and CO2 accumulation at greater depths (Zhang and Maun, 1990b; Li et al., 2006). The enforced dormancy usually would be a selective advantage because it would increase the number of seeds in the seed bank and maintain the potential to produce seedlings in the following years if they were exposed by sand erosion (Fenner, 1985; Zhang and Maun, 1990a). Also avoids inevitable mortality by not germinating at depth (Fenner, 1985). Several studies (Maun and Lapierre, 1986: Zhang and Maun, 1990b) showed that germination of seeds at the surface (0 cm) was lower than in shallow burial, because the excessive temperature fluctuations, light and desiccation may have prevented them for germinating (Maun and Riach, 1981; Li et al., 2006). The apparent contradiction in *B. portulacoides* seeds, which has high germination at surface, may be explained by its positively photoblastic seeds characteristics (Cordazzo, 1994). Thus, germination is restricted only at sand surface or shallow depths (< 2 cm).

The total seedling emergence and time of emergence decreased with sowing

depth, and a maximum percent emergence ocurred from 0 to 2 cm. Similar with the results reported by Tyndall et al. (1986) with four mid-Atlantic beach species (Cakile edentula, Uniola paniculata, Spartina patens and Triplasis purpurea) and by Cordazzo (1994) with southern Brazilian coastal dune species (Panicum racemosum, Spartina ciliata and Cakile maritima), B. portulacoides showed that the mean length of buried seedlings was less than its potential elongation in darkness condition. This is probably because the physical (mechanical) resistance of sand, particularly when moist and compacted, creates more difficulty for seed emergence (Tyndall, 1985).

The results for survival of seedlings artificially planted and a significant negative correlation found with sand accretion, support Watkinson *et al.* (1979) in their assertion that "establishment from seed is risky and rare on the mobile sand areas". Complete burial by sand usually decreases the survival chances of individual plants, because of it deprives leaves of sunlight, decreases aeration (that suppress photosynthesis and carbohydrate production) and exposes plants to soil microfauna (Yu *et al.*, 2004; Perumal and Maun, 2006).

In conclusion, sand burial is a major factor that determines the survival and establishment of *B. portulacoides* seedlings on backshore areas of southern Brazil. Since the establishment of new plants occurrs primarily through vegetative rhizome fragments (Bernardi and Seeliger, 1989; Cordazzo, 1994; Cordazzo and Seeliger, 2003), seedling establishment may not play a significant role in maintaining its populations. However, some seedling may survive in more stable places and help to increase or maintain genetic diversity.

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