Sustainable Agriculture Research; Vol. 3, No. 2; 2014
ISSN 1927-050X E-ISSN 1927-0518
Published by Canadian Center of Science and Education

Effect of Air-Borne Salinity on the Growth and Appearance of the Tropical Perennial Strandline Plant, *Commelina erecta* subsp. maritima (C.V. Morton) C.V. Morton

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Received: July 16, 2013 Accepted: February 12, 2014 Online Published: March 31, 2014

doi:10.5539/sar.v3n2p77 URL: http://dx.doi.org/10.5539/sar.v3n2p77

Abstract

Selection of salt spray tolerant plants with good physical appearance is of concern to ornamental growers in coastal communities. *Commelina erecta* subsp. *maritima* (C.V. Morton) C.V. Morton is a seashore plant that is widely distributed along the coast of West Africa. Therefore, the effect of salt spray was examined on the plant in a greenhouse experiment to determine its responses to various levels of air-borne salinity and to have an insight in the ecophysiological adaptations underlying these responses. It was also aimed to determine if varying levels of salt spray differentially damaged the plant. Filtered seawater was used to spray potted plants at: two sprays per week (2SS), four sprays per week (4SS) or six sprays per week (6SS) while in the control treatment (CSS), plants were sprayed with deionized water. Plants sprayed with seawater did not differ significantly ($p > 0.05$) in percentage necrotic leaf area compared to the control. All the plants survived but growth was inhibited by salt spray. Salt spray caused a significant ($p < 0.05$) reduction in leaf size and total chlorophyll content. Salt was accumulated in the shoot of salt-sprayed plants which led to ion toxicity. Salt sprays led to reduction in amount of essential nutrients in plant parts. *C. erecta* subsp. *maritima* adjusted osmotically to salt stress and increased stem succulence for ion dilution. The growth of the plant was negatively affected by salt sprays but it showed no significant necrotic damage, hence it is suitable for use as a landscaping plant in coastal beaches.

Keywords: commelinaceae, salt spray, water status, ion toxicity, necrosis, landscape

1. Introduction

Plants that tolerate coastal conditions have become increasingly important in coastal communities where drift materials are deposited by spring tides or storms as strandline. Salt tolerance in strandline plants has been mostly limited to examinations of tolerance to soil salinity, which has been widely documented such as in *Beta maritima* (Koyro, 2000) and *Cakile maritima* (Debez et al., 2004; Megdiche et al., 2007), but their response to air-borne salinity is has received relatively little attention. Unlike the salt marsh, where plant species are exposed to tidal inundation and thus to high salinity (Flowers & Colmer, 2008; De Vos et al., 2010), the strandline is out of reach of mean high tide and only rarely flooded with seawater. Thus, salt exposure at the strandline is mainly composed of salt sprays (Boyce, 1954; Rozema et al., 1985; Griffiths et al., 2006; Griffiths, 2006; De Vos et al., 2010).

Salt spray is formed by seawater droplets breaking in the zone of heavy surf and the small droplets are blown landward by wind (Boyce, 1954). The salts may enter the aerial organs of the plants, especially where small surface injuries are present (Boyce, 1954). In this way, it can disrupt the water balance of plants (Munns, 1993), and cause necrosis or loss of leaves and lead to growth reduction (Sykes & Wilson, 1988; Tominaga & Ueki, 1991). Salt spray has been reported to cause growth reduction in many coastal plants (Cheplick & Demetri, 1999; Morant-Manceau et al., 2004; Scheiber et al., 2008). Shorter stem, fewer leaves and inhibition of root development were observed in *Triplasis purpurea* (Cheplick & Demetri, 1999); *Leymus mollis* (Gagne & Houle, 2002) and *Myrica pensylvanica* (Griffiths & Orians, 2003) sprayed with seawater. Recently, Kekere and Bamidele (2012) stated that *Diodia maritima* seedlings sprayed with seawater had reduced number of leaves and lateral branches, reduction in root growth, number of branches, leaf size and biomass. Leaf size was also reduced in Crambe maritima sprayed with seawater (De Vos et al., 2010) which they reported to provide reduced area for water loss through transpiration. They also reported reduced chlorophyll content and attributed it to chloride and sodium ions from the salt spray. Salt spray can lead to water stress in plants. Crambe maritima for example had higher water content (De Vos et al., 2010) under salt spray. It has been reported that increased succulence in the presence of salt is an adaptive mechanism for ion dilution (Debez et al., 2004). Research has demonstrated that salt spray disrupts the water balance of plants (Munns, 1993; Touchette et al., 2009) and they can adjust osmotically to water stress through reduction in water potential (Griffiths & Orians, 2003; Griffiths, 2006; Touchette et al., 2009). Plant species growing in the vicinity of the tide line have adapted to salt spray in various ways (Rozema et al., 1985; De Vos et al., 2010). Since salt spray is an important natural selective abiotic factor on coastal plant communities (Boyce, 1954; Barbour et al., 1985; Rozema et al., 1985; De Vos et al., 2010) and *C. erecta* subsp. *maritima* grows close to the ocean, it is expected to be adapted to salt spray. Therefore, the effect of salt spray was examined on *C. erecta* subsp. *maritima* in a greenhouse experiment to determine its growth responses to various levels of airborne salinity and to have an insight into the ecophysiological adaptations underlying these responses.

Many coastal plant species have shown necrotic damage due to salt sprays. When exposed to salt spray, necrotic damage was found in several coastal forage grasses (Marcum, 1999); *Pinus rigida* (Griffiths & Orians, 2004), *Solidago puberula*, *Solidago rugosa*, *Gaylussacia baccata* and *Quercus ilicifolia* (Griffiths & Orians, 2003), *Deschampsia caespitosa* and *Melica californica* (Hunter & Wu, 2005), *Miscanthus sinensis* and *Pennisetum Alopecuroides* (Scheiber et al., 2008) and *Diodia maritima* (Kekere & Bamidele, 2012). This necrotic damage can result in a decrease in net photosynthesis that might be expressed in reduced growth (Griffiths & Orians, 2004). However, the necrotic levels of some plants are not affected by salt spray. some coastal plants have shown been reports on plants that were not affected by salt sprays. Griffiths and Orians (2003) reported that *Myrica pensylvanica* showed high resistance to necrotic damage caused by salt spray and attributed it to thick cuticle on leaf surface that limits salt entry and re-growth of new leaves. However, foliar salt spray from seawater may be an important factor to consider when selecting taxon for use in coastal locations (Scheiber et al., 2008; Conolly et al., 2010). Landscaping and gardening projects in coastal regions called for selection of plants that have the ability to cope with seawater sprays considering the high level of the death of sea side horticultural plants in Africa and other parts of the world (Scheiber et al., 2008; Conolly et al., 2010). Landscape value is largely determined by the physical appearance of individual plants, and plants with necrotic tissue are not attractive in gardens and landscapes (Bernstein et al., 1972). Plants showing damage due to salt stress are inherently of less value than plants without such damage, regardless of the concentration of ions causing the necrosis (Conolly et al., 2010).

Commelina erecta subsp. *maritima* commonly called white mouth dayflower is the subspecies of *Commelina erecta* that thrives in strandline habitats. It belongs to the family Commelinaceae. It is a fleshy, creeping, almost glabrous herb with blue flowers. It is perennial and monocotyledonous with succulent stem and swollen nodes often mucilaginous. The root system is adventitious and the stem is rhizomatous and branched. The leaves are alternate, entire with sheathing bases. The inflorescence is cyme, terminal and axillary. Its flower is bisexual, actinomorphic, hypogynous, and often enclosed in boat-shaped bracts (Hutchinson et al., 1968). It is a popular sea side plant which is widely distributed along the seashore in West Africa. Only few species occupy strandline due to the severity of the environment (Lee & Ignaciuk, 1985) where salt spray is an important natural selective abiotic factor (Boyce, 1954; Barbour, DeJong, & Pavlik, 1985; Rozema et al., 1985; De Vos et al., 2010). Since *C. erecta* subsp. *maritima* is abundant and confined to the strandline and therefore may be selected for landscaping projects of coastal locations. This might provide sustainable income for landscapers who have been having some difficulties in the selection of plants that can tolerate salt spray with high aesthetic value, considering the high level of the death of sea side horticultural plants.

2. Materials and Methods

2.1 Preparation of Experimental Plants

Rooted stem cuttings of *C. erecta* subsp. *maritima* were collected from Lekki Beach in Lagos Nigeria and used to raise uniform plants in 20 × 26 cm perforated plastic pots filled with 2:1 ratio (v/v) of river sand to topsoil (Khan et al., 2000a; 2000b). The soil had a pH of 5.48, 20.42 ppm N, 3.56 ppm P, 3.56 (meg/100g) K, 2.32 (meg/100g) Ca, 2.60 (meg/100g) Mg, 8.2 (meg/100g) CEC, 3.67% C, 80.68% sand, 12.06% silt and 8.36% clay.

2.2 Experimental Location, Plant Treatment and Experimental Design

The experiment was conducted in the Greenhouse of Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba Akoko, Ondo State Nigeria (Lat. 7º 28'N, Long. 5º 44' E). In salt spray treatments, filtered seawater collected off the shore at Lekki Beach in Southern Nigeria on a single day in late July 2012 was used to spray plants twice weekly (on Mondays and Thursdays) for 12 weeks. Filtered seawater was collected using salt spray collectors arranged parallel to the coastline at about 10 m from mean seawater level (mean tide line). Each salt spray collector was made up of polypropylene filter gauze wrapped over a 30 cm long plastic tube placed vertically in a beaker. The collectors were fixed on the ground with about 20 cm of the upper part exposed. The beaker was to collect precipitation and prevent loss of trapped water (Griffiths & Orians, 2003; Doomen et al., 2006; De Vos et al., 2010; Conolly et al., 2010). The seawater had salinity of 31 ppt and pH of 8.21 with sodium and chloride accounting for approximately 86% of the ions present. The seawater was stored in a plastic keg and later measurements of salinity did not change within the short time of storage. The seawater was used to spray plants at 3 treatment levels: two sprays/week (2SS) -one spray on each of the two days), four sprays/week (4SS) -2 sprays on each of the two days; or six sprays/week (6SS) -three sprays on each of the two days, while in control spray treatment (CSS), plants were prayed with deionized water three times on each of the two days to account for any physical effect that the spraying might have on plants. Plants were sprayed at an interval of 4 hour beginning from 08:00 am in case of two and three sprays. At each spray, plants were taken outside and individual plant was sprayed with seawater at a concentration of 4 mg NaCl dm^{-2} of leaf area, which is equivalent to the accumulation rate found on plants growing close to the ocean (Griffiths & Orians, 2003). Salt deposited onto plants shoots was estimated following the method described by (Cheplick & Demetri, 1999) using five *C. erecta* subsp. *maritima* plants not used in the experiment but grown with the experimental plants. Firstly, the shoot of the plant was immersed in 150 ml of deionized water and the conductivity determined. The same shoot was sprayed once with seawater, immersed into 150 ml of deionized water, and the conductivity increase was recorded. The conductivity increase was also recorded after the same shoot was sprayed twice and thrice respectively after immersion in 150 ml of deionized water in each case. This was repeated for all the 5 plants. Salt deposition was estimated per square decimeter of leaf area surface for each of the three saltwater spray treatments at each application. The accumulated salt onto shoot for 1 spray, 2 sprays and 3 sprays equaled on average 4, 8 and 12 mg NaCl dm^{-2} leaf area day^{-1}, which fall within the levels found in the natural habitat of strandline plants (Barbour, 1978; Griffiths, 2006). Plants were sprayed using a hand-held plant mist bottle held 20 cm from the side of each shoot. Before each salt spray treatment, plastic discs were placed over the soil surface and around the base of each plant to prevent salt deposition on the soil. Also, plants were watered from the top of the soil surface at the base of the plants (about once per week) to flush out any salts that might have been deposited onto the soil during misting. This basal method of watering did not remove the salts deposited onto the shoots during the application of salt sprays. This method has been used in previous Greenhouse experiments (Barbour, 1978; Barbour et al., 1985; Cheplick & Demetri, 1999; Griffiths, 2006) suggesting that the relative level of airborne salt deposited onto the shoots was the primary cause of reduced growth in plants rather than soil salinity or combined effect of both. Salt was allowed to accumulate throughout the experiment, which is realistic in the field because in years with infrequent rain, salt spray is not washed off during the summer growing season (Cheplick & Demetri, 1999; Cheplick & White, 2002). The experiment was completely randomized with each treatment replicated 6 times.

2.3 Growth Measurement

Percentage survival and growth parameters were recorded. Shoot length was measured from the soil level to the terminal bud and leaf area was measured using a leaf area meter (LI-COR 300 model) on the first three fully expanded leaves. Stem girth was measured at about 5 cm from stem base using model 0-200 mm digital caliper. The leaves and lateral branches of individual plant were counted manually. At harvest, major roots were counted and their lengths were measured. The harvested plants were separated into leaves, stems and roots and their fresh mass was determined. Dry mass was obtained after oven-drying to constant weight at 70 ºC. Root: shoot ratio was calculated by dividing root dry mass by shoot dry mass. Total biomass was the sum of dry plans parts. The relative growth rate was calculated using the formula: RGR (relative growth rate) = (ln mass2-ln mass1)/ time.

2.4 Water Status and Chlorophyll Content

Two aspects of water status were tested at the end of the treatment period: percentage water content and plant water potential. Percentage moisture content was calculated using the formula: [(fresh mass– dry mass) / fresh mass] x 100. Plant water potential was measured using a plant moisture-stress instrument (PMS Instrument Co., Oregon, USA) on five randomly selected stems from each treatment. Water potential was taken between 06.00 and 07.00 am (Predawn) and between 12 noon and 1 pm (mid day). Total chlorophyll was extracted from the whole leaf in 80% (v/v) aqueous acetone and absorption was read with spectrophotometer at 645 and 663 nm. Chlorophyll content (mg l^{-1} fresh leaf weight) was calculated using the following formula: Total chlorophyll = $(20.2 \times D_{645} + 8.02 \times D_{663}) \times (50/1000) \times (100/5) \times ½$, where D = absorbance (Arnon, 1949).

2.5 Mineral Content

Each dried sample was ground to fine powder using Philip model blender. The powder was re-weighed and placed in a muffle furnace set at 500 ºC to determine percentage ash content. Ca^{2+}, Mg^{2+} and Fe^{2+} content were analyzed using a Perkin Elmer model 360 atomic absorption spectrophotometer after acid digestion of the ash. The Na^{+} and K^{+} concentrations were assayed by flame emission spectrophotometer. Cl^{-} was determined by silver nitrate titration while nitrogen content was obtained by Micro-Kjedahl method. Soil and plant samples were analyzed in the Central Laboratory of The National Institute for Oil Palm Research (NIFOR), Nigeria, following the standard methods of Association of Official Analytical Chemists (AOAC, 1985).

2.6 Necrotic Leaf Area

Plants were rated at 2 weeks interval based on percentage necrotic leaf area, to know the stage at which necrotic damage occurred, an aesthetically important symptom of damage. Leaf necrosis was assessed on the leaves using a grid and expressed as a proportion of the total leaf area with necrotic damage (Griffiths & Orians, 2003; Griffiths et al., 2006).

Data were subjected to single factor analysis of variance and means were separated with Turkey Honest Significant Difference (HSD) test using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) at $P < 0.05$.

3. Results

3.1 Plant Growth

Salt spray did not cause plant mortality but reduced growth (Table 1). Salt-sprayed plants had fewer leaves than did controls. The reduction in the number of leaves per plant was significant ($p < 0.05$) at all salt spray treatments compared to the control. Results of mean leaf area shoot length, root length and number of roots per plant showed similar responses as they were also significantly ($p < 0.05$) reduced in plants sprayed with seawater compared to those sprayed with deionized water. Although the values within salt spray treatments decreased with increasing level, none of these differences was significant ($p > 0.05$) when compared. The number of branches produced by plants sprayed with seawater decreased but was not significantly ($p > 0.05$) different from control. As shown in Table 1, stem girth was higher under control condition than in all the three salt treatments.

Table 1. Effect of salt spray on the growth parameters of *C. erecta* subsp. *maritima* at 12 weeks after treatment

Level of salt spray	**Survival (%)**	**Stem girth (cm)**	**Leaf Area (cm^2)**	**Number of branches $plant^{-1}$**	**Number of leaves $plant^{-1}$**	**Shoot length (cm)**	**Root Number $plant^{-1}$**	**Root Length $plant^{-1}$ (cm)**
CSS	100	0.39^{a}	4.49^{a}	24.65^{a}	129.02^{a}	70.65^{a}	69.00^{a}	44.00^{a}
2SS	100	0.24^{b}	2.25^{b}	19.21^{a}	82.42^{b}	45.94^{b}	53.38^{b}	30.50^{b}
4SS	100	0.29^{b}	2.19^{b}	18.00^{a}	72.25^{b}	47.11^{b}	53.30^{b}	29.50^{b}
6SS	100	0.26^{b}	2.09^{b}	14.97^{ab}	62.85^{b}	44.52^{b}	58.40^{b}	33.00^{b}

Each value is a mean of 6 replicates. For each parameter, means with the same letter (in superscript) in the same column are not significantly different at $P > 0.05$ (Turkey HSD). CSS = control spray (deionized water sprays), 2SS = two salt sprays per week, 4SS = four salt sprays per week, 6SS = six salt sprays per week.

3.2 Biomass and Chlorophyll Content

Fresh and dry mass of plant parts were significantly lower ($p < 0.05$) under salt sprays than in control except for the root where there was no difference (Table 2). The relative growth rate (RGR) showed results similar to the whole-plant biomass (Table 3). Both decreased with increasing level of salt spray with a significant difference when CSS was compared with 2SS, 4SS and 6SS. Similarly, total chlorophyll content of leaves had significantly ($p < 0.05$) lower values in salt-treated plants than in control spray treatment. Root: shoot ratio was significantly ($p < 0.05$) higher in plants sprayed with seawater than those sprayed with deionized water, showing that the shoot was more affected than the root zone (Table 3).

Table 2. Effect of salt spray on the fresh and dry mass of plant parts in *C. erecta* subsp. *maritima* at 12 weeks after treatment

Parameter	**Plant part**	**Level of salt spray**			
		CSS	**2SS**	**4SS**	**6SS**
Fresh mass (g)	**Leaf**	6.90^a	3.21^b	2.67^b	1.25^b
	Stem	19.84^b	13.95^a	11.24^a	9.86^a
	Root	18.62^a	13.93^a	12.20^a	10.38^a
Dry mass (g)	**Leaf**	5.10^b	2.56^a	2.11^a	1.01^a
	Stem	10.03^b	5.94^a	4.70^a	3.75^a
	Root	14.87^a	11.14^a	9.76^a	7.82^a

Each value is a mean of 6 replicates. Means with the same letter (in superscript) in the same row are not significantly different at $P > 0.05$ (Turkey HSD). CSS = control spray (deionized water sprays), 2SS = two salt sprays per week, 4SS = four salt sprays per week, 6SS = six salt sprays per week.

Table 3. Effect of salt spray on the biomass, root: shoot, relative growth rate (RGR) and leaf total chlorophyll of *C. erecta* subsp. *maritima* at 12 weeks after treatment

Level of salt spray	**Total biomass ($g\ plant^{-1}$)**	**Root: Shoot**	**RGR ($gg^{-1}d^{-1}$)**	**Total chlorophyll (mgg^{-1} fresh leaves)**
CSS	30.00^a	0.98^b	0.1811^a	3.59^a
2SS	19.64^b	1.31^a	0.0761^b	2.37^b
4SS	16.57^b	1.43^a	0.0741^b	2.17^b
6SS	10.58^{bc}	1.22^a	0.0687^b	2.10^b

RGR = relative growth rate. Each value is a mean of 6 replicates. Means with the same letter (in superscript) in the same column are not significantly different at $P > 0.05$ (Turkey HSD). CSS = control spray (deionized water sprays), 2SS = two salt sprays per week, 4SS = four salt sprays per week, 6SS = six salt sprays per week.

3.3 Mineral Content

The concentration of Na^+ and Cl^- in plant parts was affected by salt sprays (Figures 1). Na^+ and Cl^- increased significantly ($p < 0.05$) with increasing level of seawater spray treatment. Na^+ and Cl^- were accumulated in the shoot (leaf and stem) but not in the root as a result of salt deposition on plant surface following salt spray treatments. The value of percentage ash content in control spray was significantly ($p < 0.05$) lower than those in the parts of salt-sprayed plants except in the root (Figure 2). The ash content increased significantly as the level of salt spray increased, in which Na^+ and Cl^- ions are the major contributors. Their concentrations were however higher in the leaf than in the stem. With the exception of K^+ in the root, essential elements including K^+, Ca^{2+}, Mg^{2+} and Fe^{2+} at 2SS, 4SS and 6SS were lower in values than at CSS (Table 4). Na^+: K^+ ratio values were higher in the parts of plants subjected to salt spray compared to control except in the root (Figure 3). The highest value was obtained at 6SS while the lowest was at CSS.

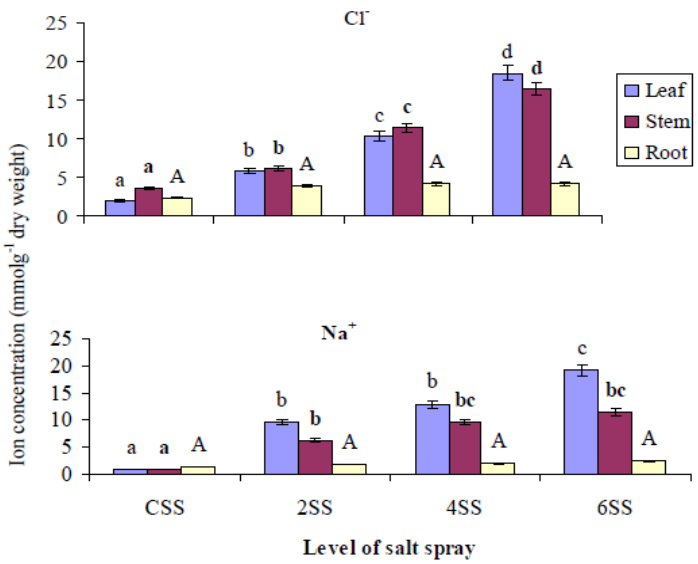

Figure 1. Effect of salt spray on Na^+ and Cl^- concentration (mmolg^{-1} dry weight) in the leaf, stem and root of *C. erecta* subsp. *maritima* at 12 weeks after treatment. For each plant part, bars with the same letter are not significantly different at P > 0.05 (Turkey HSD). CSS = control spray (deionized water sprays), 2SS = two salt sprays per week, 4SS = four salt sprays per week, 6SS = six salt sprays per week

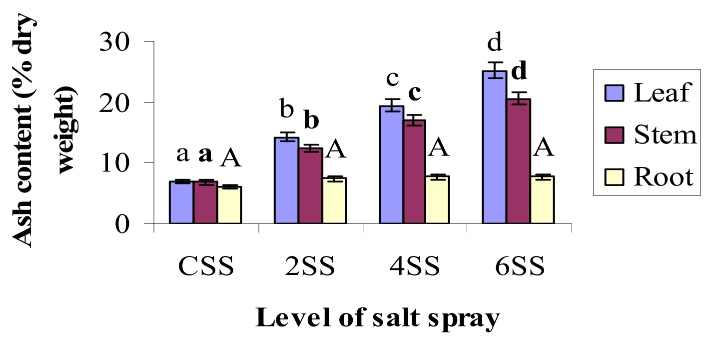

Figure 2. Effect of salt spray on the ash content (% dry weight) in leaf, stem and root of *C. erecta* subsp. *maritima* at 12 weeks after treatment. Each bar represents mean ± standard error of 6 replicates. For each plant part, bars with the same letter are not significantly different at P > 0.05 (Turkey HSD). CSS = control spray (deionized water sprays), 2SS = two salt sprays per week, 4SS = four salt sprays per week, 6SS = six salt sprays per week

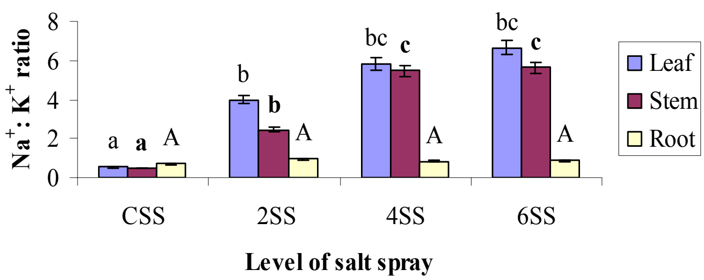

Figure 3. Effect of salt spray on Na: K ratio of leaf, stem and root of *C. erecta* subsp. *maritima* at 12 weeks after treatment. Each bar represents mean ± standard error of 6 replicates. For each plant part, bars with the same letter are not significantly different at P > 0.05 (Turkey HSD). CSS = control spray (deionized water sprays), 2SS = two salt sprays per week, 4SS = four salt sprays per week, 6SS = six salt sprays per week

Table 4. Effect of salt spray on nutrient content (mmolg^{-1} dry weight) in the leaf, stem and root of *C. erecta* subsp. *maritima* at 12 weeks after treatment

Plant part	**Level of salt spray**	**Tissue elemental concentration (mmolg^{-1} dry weight)** Ca	Mg	K	Fe	N
Leaf	CSS	10.24 $\pm$0.13^{a}	1.28 $\pm$0.04^{a}	3.60 $\pm$0.07^{a}	1.24 $\pm$0.02^{a}	7.14 $\pm$0.66^{a}
	2SS	7.16 $\pm$0.17^{ab}	1.24 $\pm$0.02^{a}	2.60 $\pm$0.07^{a}	0.22 $\pm$0.02^{b}	4.07 $\pm$0.46^{b}
	4SS	7.44 $\pm$0.16^{ab}	1.00 $\pm$0.02^{a}	2.08 $\pm$0.04^{a}	0.20 $\pm$0.01^{b}	4.35 $\pm$0.37^{b}
	6SS	7.80 $\pm$0.13^{ab}	0.88 $\pm$0.01^{a}	2.04 $\pm$0.05^{a}	0.14 $\pm$0.02^{b}	3.28 $\pm$0.21^{b}
Stem	CSS	10.64 $\pm$0.71^{a}	2.08 $\pm$0.02^{a}	4.16 $\pm$0.10^{a}	0.50 $\pm$0.01^{a}	12.22$\pm$ 0.62^{ab}
	2SS	5.36 $\pm$0.24^{ab}	2.56 $\pm$0.02^{a}	3.32 $\pm$0.11^{a}	0.08 $\pm$0.01^{b}	7.81 $\pm$0.55^{ab}
	4SS	7.44 $\pm$0. 26^{ab}	2.24 $\pm$0.03^{a}	3.44 $\pm$0.11^{a}	0.05 $\pm$0.01^{b}	7.30 $\pm$0.54^{ab}
	6SS	2.56 $\pm$0.21^{b}	0.60 $\pm$0.01^{b}	2.52 $\pm$0.12^{ab}	0.01 $\pm$0.002^{a}	7.18 $\pm$0.55^{ab}
Root	CSS	6.32 $\pm$0.22^{a}	4.20 $\pm$0.02^{a}	2.24 $\pm$0.02^{a}	0.62 $\pm$0.01^{a}	5.90 $\pm$0.04^{a}
	2SS	5.20 $\pm$0.22^{a}	3.08 $\pm$0.01^{a}	4.48 $\pm$0.01^{a}	0.60 $\pm$0.01^{a}	5.89 $\pm$0.04^{a}
	4SS	4.72 $\pm$0.14^{a}	3.56 $\pm$0.03^{a}	4.52 $\pm$0.01^{a}	0.52 $\pm$0.01^{a}	5.43 $\pm$0.03^{a}
	6SS	5.24 $\pm$0.16^{a}	3.04 $\pm$0.03^{a}	4.92 $\pm$0.01^{a}	0.62 $\pm$0.01^{a}	5.33 $\pm$0.04^{a}

Each value is a mean of 3 replicates. For each plant part and element, means with the same letter (in superscript) in the same row are not significantly different at P > 0.05 (Turkey HSD). CSS = control spray (deionized water sprays), 2SS = two salt sprays per week, 4SS = four salt sprays per week, 6SS = six salt sprays per week.

3.4 Water Status

Although not significantly different from the control treatment, seawater spray led to an increase in percentage moisture content of only the stem while that of the leaf and root were not affected (Figure 4). Both predawn and mid day plant water potentials became significantly more negative ($p < 0.05$) with increasing seawater spray (Figure 5). Mid-day plant water potential was more negative than that of the predawn. Necrosis increased as number of sprays increased but plants treated with seawater did not differ significantly ($p > 0.05$) from the control (Figure 6).

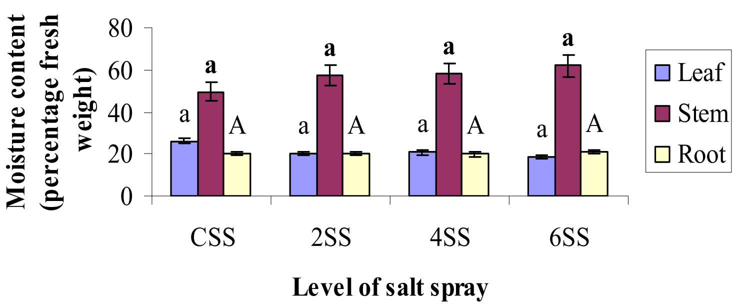

Figure 4. Effect of salt spray on the moisture content (percentage fresh weight) of *C. erecta* subsp. *maritima* at 12 weeks after treatment. Each bar represents mean $\pm$ standard error of 6 replicates. For each plant part, bars with the same letter are not significantly different at P > 0.05 (Turkey HSD). CSS = control spray (deionized water sprays), 2SS = two salt sprays per week, 4SS = four salt sprays per week, 6SS = six salt sprays per week

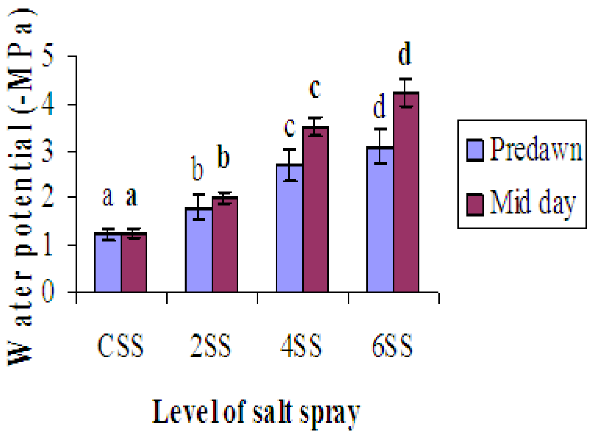

Figure 5. Effect of salt spray on the water potential (-MPa) of *C. erecta* subsp. *maritima* at 12 weeks after treatment. Each bar represents mean ± standard error of 5 replicates. For each plant part, bars with the same letter are not significantly different at P > 0.05 (Turkey HSD). CSS = control spray (deionized water sprays), 2SS = two salt sprays per week, 4SS = four salt sprays per week, 6SS = six salt sprays per week

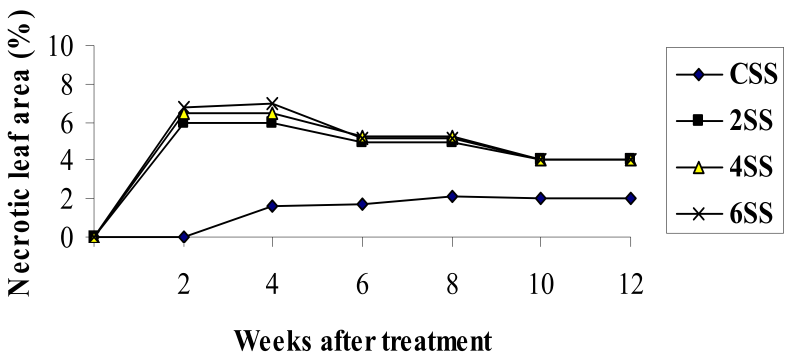

Figure 6. Effect of seawater sprays on the necrotic leaf area (%) of *C. erecta* subsp. *maritima* at 2 weeks interval for 12 weeks. Each value is a mean of 3 replicates. CSS = control spray (deionized water sprays), 2SS = two salt sprays per week, 4SS = four salt sprays per week, 6SS = six salt sprays per week

4. Discussion

Beach plants grow in very sandy soil which is porous with high leaching rate, thus, salt does not accumulate in the root zone (Lee & Ignaciuk, 1985; Griffiths & Orians, 2003). Growth reduction in *C. erecta* subsp. *maritima* in this investigation supports the findings that salt spray caused growth reduction in *Leymus mollis* (Gagne & Houle, 2002) and *M. pensylvanica* (Griffiths & Orians, 2003). Reduction in root elongation has been reported in many coastal plant species (Griffiths & Orians, 2004; Griffiths et al., 2006). Salt penetrate leaves through lesions or via stomata and is consequently translocated to other plant parts (Boyce, 1954; Barbour, 1978) which could affect root development. Shoot length reduction in this study agrees with the report that salt spray inhibits shoot elongation in coastal plant species (Griffiths et al., 2006). Similarly, Scheiber et al. (2008) reported shorter plants when *Miscanthus sinensis* and *Pennisetum alopecuroides* were exposed to increasing concentrations of salt sprays. It was however suggested that reduced plant size could be one adaptation through which the characteristic dwarf stature of strand vegetation is maintained (Griffiths & Orians, 2003). Reduction in number of branches agrees with the previous work of (Cheplick & Demetri, 1999) who recorded reduced number of tillers in *Triplasis purpurea* sprayed with salt relative to control plants. Number of branches was also reported to be reduced in *Diodia maritima* by seawater sprays (Kekere & Bamidele, 2012). Reduction in leaf number in plants sprayed with seawater supported by earlier reports in *Scaevola sericea* seedlings by Goldstein et al. (1996) and De Vos et al. (2010) on Crambe maritima. Reduced leaf number in this study (Table 1) was due to early leaf senescence and subsequent defoliation. This was because only the leaves counted were those born by the plant at the end of the experiment while those already defoliated were not accounted for. Leaf area reduction is in conformity with the results reported for *Pinus rigida* (Griffiths & Orians, 2004) and *Diodia maritima* (Kekere & Bamidele, 2012) following exposure to salt sprays. Leaf length was also reduced in Crambe maritima seedlings after 13 weeks spraying with salt water (De Vos et al., 2010). Reduced leaf size was as a result of reduction in leaf area expansion and hence reduction of light interception. However, reduction in leaf area provides reduced area for water loss through transpiration and changes in water use efficiency in plants, which have been identified to be adaptive mechanisms under water stress (Morant-Manceau et al., 2004). Reduced photosynthetic leaf area (Table 1) was attributed to inhibition of leaf expansion due primarily to chloride and sodium ions from the salt spray. This leads to reduction of light interception for photosynthetic activities with consequential effect on growth. However, leaf area reduction is in contrast with that of Touchette et al. (2009) on *Spartina alterniflora*. Reduction in chlorophyll content (Table 3) was due to the observed necrotic spots (Figure 6). When there are necrotic spots on the leaf, total photosynthesis and carbohydrate stored in the root decrease, leading to decreased growth (Conolly et al., 2010). Likewise, necrotic spots in needles of *P. rigida* seedlings were reported to decrease net photosynthesis that was expressed in reduced growth and long-term survivorship (Griffiths & Orians, 2004). Chlorophyll reduction might also be due to nutrient deficiency and ion toxicity caused by Na^+ and Cl^- ions (Debez et al., 2004). Application of NaCl to foliage of *Thuja occidentalis* and *Picea glauca* induced fragmented cuticles, disrupted stomata, collapsed cell walls, coarsely granulated cytoplasm, disintegrated chloroplasts and nuclei, and disorganized phloem (Kozlowski, 1997).

Moisture content increased in stem in the presence of salt. Likewise, leaf succulence increased in salt sprayed seedlings of Crambe maritima as compared with the control (De Vos et al., 2010). It has been reported that increased succulence in the presence of salt is an adaptive mechanism for ion dilution (Debez et al., 2004). Moisture content in leaf and root obtained in this research agrees with the report of Griffiths and Orians (2003) that leaf water content of *Solidago puberula*, *Solidago rugosa*, *Gaylussacia baccata*, *Myrica pensylvanica*, *Pinus rigida* and *Quercus ilicifolia* was not significantly different compared with control for each. Research has demonstrated that salt spray disrupts the water balance of plants (Munns, 1993; Touchette et al., 2009). The results in the study agrees with that of *Solidago puberula*, *Solidago rugosa*, *Gaylussacia baccata*, *Myrica pensylvanica*, *Pinus rigida* and *Quercus ilicifolia which* showed a significant decrease in predawn plant water potential in response to salt spray, indicating that the plants became more water-stressed with increasing salt spray (Griffiths & Orians, 2003). Reduction in plant water potential indicates that they adjust osmotically in response to increases in salt stress (Griffiths, 2006; Touchette et al., 2009).

Reduction in fresh and dry mass of the shoot agrees with the results recorded for *Triplasis purpurea* (Cheplick & Demetri, 1999). Shoot and whole-plant biomass were also reported to decrease as salt spray increased in *Miscanthus sinensis* and *Pennisetum Alopecuroides* (Scheiber et al., 2008). Root mass was not affected in this study which conforms to that of *Crambe maritima* (De Vos et al., 2010). This was as a result of preventing salt deposition onto the soil which suggests that the relative level of airborne salt deposited onto the shoots was the primary cause of reduced growth. Total biomass reduction can be attributed to the negative influence of salt spray on growth parameters. Although the RGR values of salt-treated plants decreased, they did not significantly differ from the control treatment, which is in line with the findings of De Vos et al. (2010) on Crambe maritima. Increase in root: shoot ratio revealed that the shoot that had direct contact with salt was more negatively affected than the root due to the effect of sodium and chloride ions. This resulted in accumulation of salt to the shoot following foliar spray of plants with seawater. Elevated level of percentage ash content (Figure 2) was as a result Na^+ and Cl^- deposition on the shoot following salt spray (Figure 1).

High concentrations of Na^+ and Cl^- in the shoot (leaf and stem) had negative effects on the growth due to ion toxicity. Na^+ toxicity for example has been linked with disruption of metabolic activities, development of water stress, reduction of turgor and induction of oxidative cell damage (Rozema, Bijwaard, Prast, & Broekman, 1985). Salt spray reduced uptake of essential nutrients many of which act as cofactors in metabolic pathways, help in the maintenance of membrane stability and are integral part of chlorophyll structure (De Vos et al., 2010). Increase in Na^+: K^+ ratio was due to Na^+ interfering with K^+ uptake which is essential for growth. Selection and use of K^+ under Na^+ salinization, as well as the maintenance of high K^+/Na^+ ratio is essential for salt tolerance (Cromer et al., 1985). Na^+ may have a direct toxic effect, by interfering with the function of potassium as a cofactor in various metabolic reactions, indicating the existence of displacement of K^+ by Na^+ since both most likely share the same position in transport system. All these must have been responsible for the growth reduction in the plant.

Many plant species have shown necrotic damage due to salt sprays, for example, Marcum (1999) reported necrotic leaf area in several coastal forage and turf grasses. Seawater sprays likewise resulted in leaf necrotic damage in *Pinus rigida* (Griffiths & Orians, 2004), *Solidago puberula*, *Solidago rugosa*, *Gaylussacia baccata* and *Quercus ilicifolia* (Griffiths & Orians, 2003), *Deschampsia caespitosa* and *Melica californica* (Hunter & Wu, 2005) and *Miscanthus sinensis* and *Pennisetum Alopecuroides* (Scheiber et al., 2008). Similarly, Kekere and Bamidele (2012) reported necrotic damage to seedlings of *Diodia maritima* by seawater sprays. The damage could result in a decrease in net photosynthesis that might be expressed in reduced growth (Griffiths & Orians, 2004). However, there have been reports on plants that were not affected by salt sprays. It was reported that necrotic levels were not affected by salt spray in *Myrica pensylvanica* seedlings in a greenhouse experiment (Griffiths & Orians, 2003). They attributed low necrosis probably because the leaf surface has thick cuticle to limit salt entry or since the plant was grown in the protective environment of the greenhouse, the leaves had few stomata to reduce entry points to salt spray. Necrosis was not significantly affected at the end of the experiment because of leaf senescence and re-growth of new leaves. Interestingly, landscape value is largely determined by the physical appearance of individual plants. Plants with necrotic tissue are not attractive in gardens and landscapes (Bernstein et al., 1972). Plants showing damage due to salt stress are inherently of less value than plants without such damage, regardless of reduced plant size or the concentration of ions causing the necrosis (Conolly et al., 2010).

5. Conclusion

In conclusion, the growth of *C. erecta* subsp. *maritima* was negatively affected by salt spray but it can cope with the levels that it encounters in its natural habitat. Also, since landscape value is largely determined by the physical appearance of individual plants and plants with necrotic tissue are not attractive in gardens and landscapes, *C. erecta* subsp. *maritima* is therefore a suitable selection for seaside landscapes. However, the observed necrosis was low probably because the leaf surface has thick cuticle to prevent salt entry or since the plant was grown in the protective environment of the greenhouse, the leaves had few stomata to reduce entry points to salt spray. Research is therefore needed on the anatomy of the plant under the influence of salt spray.

Acknowledgements

Appreciations go to Dr O. F. Olotuah and Dr. O. A. Obembe of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria for their painstaking efforts in proofreading the manuscript and offer of valuable suggestions. I am grateful to the technologists in research laboratories of the National Institute for Oil Palm Research (NIFOR) Nigeria including Mr Okerenwogba M. B., Mr Joseph Jack Udoh and Mr Omoruyi Efe for their support during the laboratory analyses of soil and plant samples.

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