Gibberellin A_3 Treatment of Seeds, Container Volume, Substrate pH, and Nitrogen Source and Rate Influence Growth of Containerized Seabeach Amaranth (*Amaranthus pumilus*)[1]

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Abstract

Seeds of seabeach amaranth (*Amaranthus pumilus* Raf.), a species federally listed as 'threatened,' were stratified (moist-chilled) for 90 days at 4C (39F) or treated with a solution of the potassium (K) salt (K-salt) of gibberellin A_3 (K-GA_3) at 1000 mg·liter^{-1} (ppm) for 24 hr. After treatment, both groups of seeds were sown in containers of two volumes, 139 or 635 cm^3 (9 or 39 in^3) with a substrate of peat:pine bark (1:1, v/v) amended with one of two rates of pulverized dolomitic lime [2.24 or 4.48 kg·m^{-3} (3.8 or 7.6 lb·yd^{-3})]. Containers were maintained in a greenhouse. After seedling emergence, seedlings were fertilized with a 20N-4.4P-16.6K (20N-10$P_2 0_5$-20$K_2$0) acidic, water soluble fertilizer or a 15N-2.2P-12.5K (15N-5$P_2 0_5$-15$K_2$0) basic, water soluble fertilizer applied thrice weekly at nitrogen (N) application rates (NARs) of 75, 150, 225, or 300 mg·liter^{-1}. The study was terminated 8 weeks after seeds were sown and data recorded. Regardless of fertilizer, acidic or basic, top dry weight and leaf area of seabeach amaranth increased linearly with increasing NAR. Maximum top dry weight and leaf area occurred with N at 300 mg·liter^{-1}, whereas root dry weight was unaffected by NAR. Both fertilizers increased electrical conductivity (EC) linearly with increasing NAR, and EC values of 1.15 to 1.18 dS·m^{-1} were adequate for maximum top growth or leaf area. Substrate pH decreased linearly with increasing NAR 21, 43, and 57 days after initiation. Top and root dry weights and leaf area were greatest for seedlings derived from seeds treated with K-GA_3. Large containers yielded top and root dry weights and leaf area 61, 33, and 57% greater, respectively, than smaller containers. Top N concentration increased linearly with increasing NAR for acidic and basic fertilizers with N concentrations of 58.4 and 50.4 mg·g^{-1}, respectively, at maximum top dry weight. Although top nutrient content of N increased linearly with NAR, top N content was unaffected by either rate of lime or type of fertilizer.

Index words: beach restoration, Amaranthaceae, recovery plans, threatened species, dune species, mineral nutrition, sexual propagation.

Significance to the Nursery Industry

Seabeach amaranth, a summer annual native to the beaches and barrier islands of the Atlantic Coast, is currently listed as 'threatened', and various state and federal agencies are interested in restoring the plant to areas where it once grew. Restoration, however, will require seedling transplants that are currently unavailable and also protocols for production of containerized seedlings. Results of this study demonstrate containerized seedlings of seabeach amaranth can be produced successfully with N at 300 mg·liter^{-1} provided by an acidic or basic fertilizer having a 4.5N:1P:3.8K or 6.8N:1P:5.7 ratio, respectively, with a corresponding electrical conductivity of 1.15 to 1.18 dS·m^{-1}.

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Introduction

Seabeach amaranth [*Amaranthus pumilus* Raf. (Amaranthaceae Juss.)] is a summer annual native to beaches and barrier islands of the Atlantic Coast and once ranged from Massachusetts to South Carolina (25). However, by 1990 it no longer occurred in six of the nine states of its original range, with the remaining populations occurring in New York, North Carolina, and South Carolina (25). Disappearance of the species from a large portion of its historic range and vulnerability of the plant to various threats, both natural and human, resulted in seabeach amaranth being listed as 'threatened' by the U.S. Fish and Wildlife Service (23). The threatened status of the plant resulted in development of a recovery plan by Weakley et al. (25).

Loss of seabeach amaranth from many areas where it was once endemic has raised concerns as it plays an important role in the initial stages of the development of sand dunes by trapping and binding sand on the beach (24, 25). Weakley et al. (25) described the plant as 'a classic example of a fugitive species' — 'an inferior competitor which is always excluded locally under interspecific competition, but which persists in newly disturbed habits by virtue of its high dispersal ability; a species of temporary habitats (12).' The plant is also regarded by ecologists as an indicator species that reflects the vitality and vigor of a beach ecosystem. Thus, various state and federal agencies are interested in restoring seabeach amaranth to areas where it once grew. Beach restoration and sand renourishment projects have also created a demand for seedling transplants of the species, but seedling stock is unavailable. Therefore, to reestablish the plant in locations where it was once endemic and to meet the demand for transplants will require protocols for propagation and culture. One approach would involve production of containerized seedlings that can be planted in suitable beach environments. Countless studies have been published regarding production of various bedding plants, and much of this research may be applicable to seabeach amaranth (7). If production protocols are developed for the species, they may provide opportunities for growers to produce and sell plants to federal, state, and private agencies for recovery efforts.

Seeds of seabeach amaranth are relatively easy to germinate provided one is aware of previous research (1, 3). Freshly harvested seeds of the species are physiologically dormant (have embryo dormancy) and require a period of stratification (moist-chilling) to break (release) dormancy (1, 3). Stratification of 84 to 120 days is necessary to remove physiological dormancy completely followed by germination at high temperatures (e.g., 8/16-hr thermoperiod of 30/20C (86/68F) with light (e.g., a daily 16-hr photoperiod) to achieve maximum germination. A recent report by Norden et al. (18) noted the need for lengthy stratification to remove embryo dormancy can be eliminated by treatment of the seeds with the potassium (K) salt (K-salt) of gibberellin A_3 (K-GA_3). Treatment of the seeds for 24 hr with a solution of K-GA_3 at 1000 mg·liter^{-1} (ppm) will remove physiological dormancy without the need for stratification. Treatment of the seeds with K-GA_3 also reduces sensitivity of the seeds to light and appears to broaden the range of temperatures at which germination will occur. Although Norden et al. (18) reported K-GA_3 treatment will remove physiological seed dormancy of seabeach amaranth, they did not observe subsequent growth of seedlings to determine if this treatment has any deleterious effects on seedling growth.

Although protocols for seed germination have been published, little if any quantitative information has been published regarding culture. Skaradek and Murray (21) reported successful greenhouse culture of seedlings of seabeach amaranth in '5 cm^2' (0.8 in^2) containers with 'a mixture of half peat and half sand thoroughly moistened to saturation.' The seedlings were later transplanted to the field and grown in a loamy soil overlaid with 5 to 8 cm (2 to 3 in) of sand. The plants grew in the field to maturity producing seeds. Unfortunately, the report of Skaradek and Murray (21) is not very detailed and lacks such information as the volume of the containers in which the seedlings were grown, fertilization of the seedlings, or substrate pH. Therefore, the following research was conducted to develop protocols for containerized production of seedling transplants of seabeach amaranth. To develop such protocols various factors were investigated including the influence of K-GA_3 treatment of seeds on subsequent seedling growth, container volume, substrate pH, and nitrogen (N) source and rate.

Materials and Methods

The study was a 2 × 2 × 2 × 4 × 2 factorial with six replications in a split-plot design. The main plots were two container volumes, two rates of lime substrate amendment, and two fertilizers with differing sources of N with four rates of each fertilizer. The sub-plot was two treatments for removing seed embryo dormancy: stratification at 4C (39F) for 90 days, or treatment with K-GA_3 at 1000 mg·liter^{-1} for 24 hr prior to sowing.

Containers included 635-cm^3 (39-in^3) Regal 45G plastic pots (Kord Products, Inc., Brampton, Ontario, Canada) or a 2 × 2 cell square [individual cell volume = 139 cm^3 (9 in^3) from modified Traymaster Rosepot flats, (Mackenzie Nursery Supply, Inc., Perry, OH). The substrate was peat:pine bark (1:1, v/v) amended with one of two rates of pulverized dolomitic lime [2.24 or 4.48 kg·m^{-3} (3.8 or 7.6 lb·yd^{-3}), also referred to as low or high rates, respectively)]. The containers were filled with the appropriate substrate, tapped twice on a bench to settle the substrate, and moistened with tap water.

Seeds of an Oak Island, NC, population of seabeach amaranth collected in September 2003 and placed in dry storage at 4C (39F) in November 2003 were removed from storage February 2, 2005, and graded. The seeds were graded under a dissecting scope to remove abnormal or damaged seeds and any debris not removed by previous cleaning prior to storage. From the graded seeds, two lots consisting of 650 seeds per lot were removed from the graded seeds. One lot was mixed with 200 mL of moist sand [dry sand:tap water (10:1, v/v)] and the seed/sand mixture was placed in a non-vented 3.8-liter (1-gal) polyethylene food storage bag. The sand had been sieved dry through a 16-mesh [1.59 mm (0.06 in)] screen and the fine separate retained for this study. The polyethylene bag was sealed with a twist tie and the bag was placed in the dark at 4C (39F) for 90 days to allow for seed stratification. The other lot was returned to dry storage at 4C (39F) for 89 days. On May 1, 2005, this lot of seeds was removed from dry storage at 4C (39F). The seeds were placed in a 125-mL Erlenmyer flask containing 50 mL of a solution of K-GA_3 at 1000 mg·liter^{-1}. The solution was prepared by dissolving K-GA_3 in distilled water (pH of distilled water = 6.3). The flask was wrapped with aluminum foil to exclude light and was placed on a rotary shaker (100 revolutions per minute) for 24 hr at 21C (70F). Following treatment of both seed lots, seeds were sown (the experiment was initiated) May 2, 2005, in containers of two volumes.

Prior to seeding, the 635-cm^3 (39-in^3) containers were partitioned with a wooden stake placed horizontally in the center of the pot; one half was for K-GA_3 treated seeds and the other for stratified seeds. Three seeds of seabeach amaranth were then sown (covered to the minimum diameter of the seeds) in both container volumes, in each respective partition of the 635-cm^3 (39-in^3) container and two of four designated cells in the 2 × 2 cell squares. The two designated cells in each 2 × 2 square were diagonally opposite; in one cell stratified seeds were sown, and in the other cell seeds treated with K-GA_3 were sown.

Following sowing, containers were moved to the Department of Horticultural Science Greenhouses and maintained under natural photoperiod and irradiance with days/nights of 28 ± 2/19 ± 2C (82 ± 4/66 ± 4F). A pressure compensated Chapin E0W60 emitter (Chapin Watermatics, Inc., Watertown, NY) was placed on each side of the partition in the large 635-cm^3 (39-in^3) containers. In the 2 × 2 cell square containers, an emitter was placed in each of the cells in which seeds were sown. Two fertilizers were selected based on the sources of N: Peter's 20N-4.4P-16.6K (20N-10P_2O_5-20K_2O) Professional Water Soluble Peat-lite Special (Scotts-Sierra Hort. Products Co., Marysville, OH) with N derived from ammonium nitrate and potassium nitrate (an acidic fertilizer) and Peter's 15N-2.2P-12.5K (15N-5P_2O_5-15K_2O) Excel Cal-Mag Water Soluble Fertilizer (Scotts-Sierra Hort. Products Co.) with N derived from ammonium nitrate, calcium nitrate, potassium nitrate, urea phosphate, and magnesium nitrate (a basic fertilizer). Micronutrients were present in both fertilizers and no source of sulfur (S) was added other than as an anion. Each fertilizer was applied at N application rates (NARs) of 75, 150, 225, or 300 mg·liter^{-1} via a D16 Dosatron (Dosatron, Clearwater, FL) using a 1:100 ratio of fertilizer:tap water to maintain a leaching fraction (volume leached ÷ volume applied) > 0.2. To simplify discussion of the effects of rate of fertilization, only the N rate will be listed, but the reader should be cognizant a 4.5N:1P:3.8K or 6.8N:1P:5.7K ratio, for the acid or basic fertilizer, respectively, was maintained at all rates of N.

Containers were fertigated thrice weekly starting May 16, 2005, except for when substrate was too moist. No other irrigation was required. Prior to fertigation, containers were misted daily with tap water to prevent seed washout. Four weeks after sowing, seedlings were thinned to one seedling per cell (2 × 2 cell square) or per partition in the 635-cm^3 (39-in^3) plastic pots. Substrate solution was collected 21, 30, 36, 43, 50, and 57 days after initiation of the experiment via the pour-through nutrient extraction technique (27). Solution pH and electrical conductivity (EC) of the extracted solutions were measured with an Accumet Benchtop pH and Electrical Conductivity Meter (Fisher Scientific Co. LLC., Pittsburgh, PA).

During the study, the substrate was preventatively treated with a Banrot 40WP (etridiozole and thiophanate-methyl) drench for damping off at a rate of 0.95 g·liter^{-1} twice at 3-week intervals. In previous studies, fungus gnats (*Orfelia* Costa sp.) were observed on seedlings of seabeach amaranth where larvae would enter young stems and feed on the vascular tissue. Therefore, all containers were drenched with Gnatrol (*Bacillus thuringiensis* subsp. *israelensis*) (Valent BioSciences Corp., Libertyville, IL) weekly at a rate of 10.4 mL·liter^{-1}. Plants were also treated as needed with spray applications of Merit® 2F {Imidacloprid, 1-[(6-Chloro-3-pyridinyl)methy1]-*N*-nitro-2-imidazolidinimin} (Bayer Environ. Sci., Res. Triangle Park, NC) at a rate of 0.33 mL·liter^{-1} for adult fungus gnats.

On June 29, 2005, 8 weeks after initiation (8 weeks after sowing of seeds), the study was terminated and various data recorded including survival. Survival was used as a quantitative index to determine the percentage of seedlings still alive at the conclusion of the study. Plants were separated into roots, stems, and leaves and leaf area was measured for replications 1 to 4 using a LI-COR LI-3100 Leaf Area Meter (LI-COR Biosciences, Lincoln, NE). Leaf, stem, and root dry weights were also recorded following drying at 60C (140F) until weights were stable. Top dry weight (leaf + stem dry weight) and root:top ratio [RTR (root dry weight ÷ top dry weight)] were calculated from these data.

After recording dry weights, stems and leaves of each plant were combined (tops) and ground using a Foss Tecator Cyclotec™ 1093 sample mill (Analytical Instruments, LLC, Golden Valley, MN) to pass a 0.5-mm (0.02-in) sieve. Tops were analyzed for N, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), S, sodium (Na), manganese (Mn), zinc (Zn), copper (Cu), iron (Fe), and boron (B) by the North Carolina Department of Agriculture and Consumer Services, Raleigh. Nitrogen concentrations were determined by oxygen combustion with an elemental analyzer (NA 1500, CE Elantech Instruments, Milan, Italy). All other mineral nutrient concentrations were determined by EPA method 200.7 with an ICP spectrophotometer (Optima 3300 DV ICP Emission Spectrometer; Perkin Elmer Corp., Wellesley, MA) following open-vessel nitric acid (HNO_3) digestion in a microwave digestion system (CEM Corp., Matthews, NC). Mineral nutrient contents were calculated based on percentage concentration of a nutrient divided by 100 and multiplied by top dry weight.

All data were subjected to analysis of variance procedures (ANOVA) and regression analysis, where appropriate (SAS Inst. Inc., Cary, NC). All three-way and four-way interactions were nonsignificant, and significant two-way interactions are presented in tables. Treatment means were separated by the F test, $P = 0.05$, where appropriate. When significant ($P = 0.05$), simple linear and polynomial curves were fitted to the N rate data. The maximum of the polynomial curve was calculated as the zero point in a first-order derivative of the independent variable.

Results and Discussion

pH. As anticipated, substrate pH was affected by NAR, rate of lime and, surprisingly, container volume. However, there were no significant interactions (Tables 1 and 2). Substrate pH decreased linearly with increasing NAR 21, 43, and 57 days after initiation (Table 1). The high rate of lime produced significantly higher substrate pH at all sample dates (Table 2). In addition, pH in the larger containers was significantly greater than the smaller containers at the high

Table 1. Effect of nitrogen application rate (NAR) on substrate pH of containerized seabeach amaranth.[z]

NAR (mg·liter^{-1})	Days after initiation (DAI)					
	21	30	36	43	50	57
75	5.84 ± 0.57	6.07 ± 0.54	5.93 ± 0.47	6.08 ± 0.45	5.92 ± 0.42	6.04 ± 0.42
150	5.79 ± 0.56	6.00 ± 0.56	5.96 ± 0.48	6.03 ± 0.46	5.78 ± 0.43	5.96 ± 0.45
225	5.62 ± 0.55	5.84 ± 0.60	5.91 ± 0.47	5.88 ± 0.60	5.79 ± 0.41	5.79 ± 0.58
300	5.52 ± 0.55	5.81 ± 0.56	5.78 ± 0.46	5.77 ± 0.49	5.71 ± 0.38	5.81 ± 0.40
Significance						
Linear[y]	*	NS	NS	*	NS	*
Quadratic	NS	NS	NS	NS	NS	NS

[z]Data are means of 192 observations ± 1 SE.

[y]Regression equations are: 21 DAI, y = 6.0 – 0.002x, R^2 = 0.98; 43 DAI, y = 6.2 – 0.001x, R^2 = 0.98; 57 DAI, y = 6.2 – 0.00x, R^2 = 0.92.

NS,* Nonsignificant or significant at $P \leq 0.05$, respectively.

Table 2. Effect of rate of lime substrate amendment and container volume on substrate pH of containerized seabeach amaranth.[z]

Lime rate (kg·m^{-3})	Days after initiation: 21, Container volume (cm^3) 139	21, 630	30, 139	30, 630	36, 139	36, 630
2.24	5.23 ± 0.06	5.25 ± 0.07	5.46 ± 0.04	5.39 ± 0.07	5.54 ± 0.05	5.49 ± 0.06
4.48	6.08 ± 0.07	6.22 ± 0.07	6.35 ± 0.07	6.51 ± 0.06	6.19 ± 0.06	6.36 ± 0.05
Significance	*	*	*	*	*	*

Lime rate (kg·m^{-3})	Days after initiation: 43, Container volume (cm^3) 139	43, 630	50, 139	50, 630	57, 139	57, 630
2.24	5.55 ± 0.05	5.47 ± 0.07	5.48 ± 0.06	5.55 ± 0.06	5.58 ± 0.06	5.52 ± 0.07
4.48	6.29 ± 0.07	6.44 ± 0.03	5.98 ± 0.06	6.19 ± 0.06	6.15 ± 0.07	6.35 ± 0.06
Significance	*	*	*	*	*	*

[z]Data are means of 96 observations ± 1 SE.

* Significant at $P \leq 0.05$.

lime rate after 30, 36, 50, and 57 days (data not presented). There was no difference in substrate pH between the container volumes at the low rate of lime. Most substrate pH values were within the recommended range for soilless media (5.4 to 6.0) (7).

Electrical conductivity. EC was affected by NAR, fertilizer, and the NAR × fertilizer interaction (Table 3). EC was affected similarly at all sample times, therefore, EC values were averaged over all sample times. Both fertilizers increased EC linearly with increasing NAR. A N rate of 150 to 300 mg·liter^{-1} resulted in a similar EC for both fertilizers whereas N at 75 mg·liter^{-1} resulted in a higher EC for plants receiving the basic fertilizer. Maximum top growth occurred with N at 300 mg·liter^{-1} (Table 4); therefore, EC values of 1.15 to 1.18 dS·m^{-1} should be adequate for maximum growth. Recommended EC values for cock's comb [*Celosia* L. sp. (Amaranthaceae)] range from 1.0 to 2.6 dS·m^{-1} (26). Likewise, optimal EC values for 'Gloria Scarlet' silver cock's comb (*Celosia argentea* L. 'Gloria Scarlet') were 1.1 to 1.5 dS·m^{-1} (11).

Growth. Stem and leaf dry weights responded similarly to all treatments; therefore, only top dry weight data are presented. Top dry weight of seabeach amaranth was affected by all treatments, but there were no significant interactions and only the main effects are presented. Top dry weight of seabeach amaranth increased linearly with increasing NAR, and maximum top dry weight occurred with N at 300 mg·liter^{-1} (Table 4). At 300 mg·liter^{-1}, top dry weight increased 106% compared to top dry weight with N at 75 mg·liter^{-1}. While N at 300 mg·liter^{-1} is high, N recommendations for annuals can be as high as 255 mg·liter^{-1} when applied with every irrigation (17). Similar to top dry weight, leaf area increased linearly with increasing NAR resulting in maximum leaf area at 300 mg·liter^{-1} which was 131% greater than leaf area at 75 mg·liter^{-1}. In contrast, root dry weight was unaffected by NAR.

RTR decreased linearly with increasing NAR for both fertilizers, acidic and basic (Table 4). This was not surprising as increasing NARs typically reduce RTR (8). As plants transition from N deficient to adequate N, they typically allocate more photosynthate to top growth (8). With the acidic fertilizer, RTR decreased 59% from 75 to 300 mg·liter^{-1}, compared to 29% for the basic fertilizer.

Top and root dry weights, leaf area, and survival were affected significantly by fertilizer (excluding leaf area), container volume (excluding survival), seed treatment, and

Table 3. Effect of nitrogen application rate (NAR) on substrate EC of containerized seabeach amaranth grown with an acidic or basic fertilizer (fert.).[z]

NAR (mg·liter^{-1})	Acidic fert.[y] (dS·m^{-1})	Basic fert.[x] (dS·m^{-1})
75	0.397 ± 0.025	0.624 ± 0.027
150	0.608 ± 0.042	0.677 ± 0.038
225	0.912 ± 0.023	0.966 ± 0.020
300	1.150 ± 0.050	1.180 ± 0.055
Significance		
Linear[w]	***	***
Quadratic	NS	NS

[z]Data are means of 48 observations ± 1 SE.

[y]Acid fert. = 20N-4.4P-16.6K (20N-10P_2O_5-20K_2O) with N derived from ammonium nitrate and potassium nitrate.

[x]Basic fert. = 15N-2.2P-12.5K (15N-5P_2O_5-15K_2O) with N derived from ammonium nitrate, calcium nitrate, potassium nitrate, urea phosphate, and magnesium nitrate.

[w]Regression equations are: acidic fert., y = 0.13 + 0.003x, R^2 = 0.99; basic fert., y = 0.37 + 0.003x, R^2 = 0.97.

NS, *** Nonsignificant or significant at $P \leq 0.001$, respectively.

Table 4. Effect of nitrogen application rate (NAR) on top and root dry weight, root:top ratio (RTR), and leaf area of containerized seabeach amaranth.[z]

NAR ($mg \cdot liter^{-1}$)	Top dry weight (g)	Root dry weight (g)	RTR[y] Acidic fert.[x]	RTR[y] Basic fert.[w]	Leaf area (cm^2)
75	0.93 ± 0.60	0.16 ± 0.09	0.22 ± 0.09	0.14 ± 0.05	92.2 ± 50.5
150	1.32 ± 0.66	0.19 ± 0.11	0.15 ± 0.08	0.15 ± 0.05	130.3 ± 58.9
225	1.57 ± 1.12	0.17 ± 0.13	0.12 ± 0.12	0.09 ± 0.05	162.5 ± 111.9
300	1.92 ± 1.13	0.19 ± 0.11	0.09 ± 0.04	0.10 ± 0.03	213.2 ± 107.5
Significance[v]					
Linear	***	NS	***	***	***
Quadratic	***	NS	NS	NS	***

[z]Data for top dry weight, root dry weight, and leaf area are means of 96 observations ± 1 SE. Data for RTR are means of 48 observations 1 ± SE.

[y]RTR = root dry weight ÷ top dry weight.

[x]Acid fertilizer (fert.) = 20N-4.4P-8.2K ($20N\text{-}10P_20_5\text{-}20K_20$) with N derived from ammonium nitrate and potassium nitrate.

[w]Basic fert. = 15N-2.2P-12.3K ($15N\text{-}5P_20_5\text{-}15\ K_20$) with N derived from ammonium nitrate, calcium nitrate, potassium nitrate, urea phosphate, and magnesium nitrate.

[v]Regression equations are: top dry weight (linear), $y = 0.63 + 0.004x$, $R^2 = 0.99$; top dry weight (quadratic), $y = 0.58 + 0.0049x - 0.0000018x^2$, $R^2 = 0.99$; RTR (acidic fert.), $y = 0.25 - 0.0006x$, $R^2 = 0.97$; RTR (basic fert.), $y = 0.17 - 0.0002x$, $R^2 = 0.79$; leaf area (linear), $y = 50.7 + 0.527x$, $R^2 = 0.99$, leaf area (quadratic), $y = 66.42 + 0.32x + 0.00056x^2$, $R^2 = 0.99$.

NS, *, **, *** Nonsignificant or significant at $P \le 0.05$, 0.01, or 0.001, respectively.

lime rate, and all interactions were nonsignificant (Table 5). The basic fertilizer, larger container volume [635 cm^3 (39 in^3)], K-GA_3 treated seeds, and the 4.48 $kg \cdot m^{-3}$ (7.6 $lb \cdot yd^{-3}$) lime amendment resulted in increased top and root dry weights compared to the acidic fertilizer, smaller container volume [139 cm^3 (9 in^3)], stratified seeds, and 2.24 $kg \cdot m^{-3}$ (3.8 $lb \cdot yd^{-3}$) limestone amendment, respectively. Top and root dry weights of seabeach amaranth were 17 and 19% larger with the basic fertilizer compared to the acidic. However, both fertilizers yielded similar leaf areas. Contrary to these results, survival was higher (5%) with the acidic fertilizer than the basic (Table 5).

Table 5. Effect of fertilizer, container volume, seed treatment, and rate of substrate lime amendment on top and root dry weight, leaf area, and survival of containerized seabeach amaranth.[z]

	Top dry weight (g)	Root dry weight (g)	Leaf area (cm^2)	Survival (%)
Fertilizer[y]				
Acidic	1.32 ± 0.95	0.16 ± 0.11	147.6 ± 102.0	96 ± 20
Basic	1.55 ± 0.99	0.19 ± 0.12	151.4 ± 92.2	91 ± 28
Significance	*	*	NS	*
Volume (cm^3)				
139	1.10 ± 0.61	0.15 ± 0.09	116.6 ± 61.7	92 ± 28
630	1.77 ± 1.15	0.20 ± 0.13	182.5 ± 113.7	95 ± 21
Significance	*	*	*	NS
Seed treatment				
K-GA_3[x]	1.59 ± 1.02	0.20 ± 0.11	165.3 ± 104.8	97 ± 16
Stratified[w]	1.28 ± 0.91	0.16 ± 0.11	133.7 ± 86.2	90 ± 31
Significance	*	*	*	*
Rate of lime ($kg \cdot m^{-3}$)				
2.24	1.23 ± 0.99	0.15 ± 0.11	136.0 ± 105.1	90 ± 31
4.48	1.63 ± 0.92	0.20 ± 0.11	163.1 ± 86.6	97 ± 16
Significance	*	*	*	*

[z]Data are means of 192 observations ± 1 SE.

[y]Acid fertilizer (fert.) = 20N-4.4P-16.6K ($20N\text{-}10P_20_5\text{-}20K_20$) with N derived from ammonium nitrate and potassium nitrate; basic fert. = 15N-2.2P-12.5K ($15N\text{-}5P_20_5\text{-}15K_20$) with N derived from ammonium nitrate, calcium nitrate, potassium nitrate, urea phosphate, and magnesium nitrate.

[x]K-GA_3 = seeds treated with K-GA_3 at 1000 $mg \cdot liter^{-1}$ for 24 hr prior to sowing.

[w]Stratified = seeds stratified (moist-chilled) for 90 days prior to sowing.

NS, * Nonsignificant or significant at $P \le 0.05$, respectively.

Seabeach amaranth grown in the large containers had top and root dry weights and leaf area 61, 33, and 57% greater, respectively, compared to the smaller containers (Table 5). Likewise, plants of 'Sweet Charlie' strawberry (*Fragaria* × *ananassa* Duch. 'Sweet Charlie') were larger when propagated in 150- or 300-cm^3 (9.2- or 18.3-in^3) containers compared to 75-cm^3 (4.6-in^3) containers.

Top and root dry weights, and leaf area of plants grown from K-GA_3 treated seeds were 24, 25, and 24% larger, respectively, than plants grown from stratified seeds (Table 5). Plants from K-GA_3 treated seeds also had 7% higher survival than plants from stratified seeds. Why the K-GA_3 treated seeds produced larger plants is not completely understood, and one hypothesis is the K-GA_3 treatment affected the metabolic rate of the embryos. A second and more likely explanation is the more rapid germination of the K-GA_3 treated seeds resulted in a time advantage over seedlings resulting from stratified seeds. Research by Norden et al. (18), indicated the higher the K-GA_3 treatment, the faster seeds of seabeach amaranth will germinate.

Plants of seabeach amaranth grown at the high rate of lime had top and root dry weights 33% greater than those grown at the low rate. This is not surprising as the pH of dune soil from North Carolina beaches ranges from 7.4 to 7.9 (19). Leaf area was also 20% greater with the high rate of lime, whereas survival was 7% greater at the higher rate.

Mineral nutrient concentrations and contents. Nitrogen concentration in tops increased linearly with increasing NAR for acidic and basic fertilizers with N concentrations of 58.4 and 50.4 $mg \cdot g^{-1}$, respectively, at maximum top dry weight (Tables 4 and 6). Mills and Jones (16) reported a range of foliar N concentrations of 37.1 to 41.3 $mg \cdot g^{-1}$ for silver cock's comb (*Celosia argentea* L.). However, it is not possible to determine if these N concentrations represented values at maximum growth. Only top P concentrations of seabeach amaranth grown with the acidic fertilizer increased linearly with increasing NAR, whereas foliar P concentrations of amaranth grown with the basic fertilizer and foliar K concentrations of amaranth grown with either fertilizer were unaffected by NAR. This is contrary to recent reports with herbaceous and woody perennial plants where foliar P and K concentrations increased quadratically or linearly with increasing NAR (6, 10).

Top Ca concentration of seabeach amaranth grown with the acidic fertilizer decreased linearly with increasing NAR, while top Ca concentration of plants grown with the basic fertilizer increased linearly with increasing NAR (Table 6). Cabrera and Devereaux (4) reported a decrease in foliar Ca concentration where NH_4-N was a significant fraction of the N supply as NH_4 competes with Ca for uptake. The major component of the basic fertilizer was NO_3-N, and it would not have competed with Ca uptake. In addition, the basic fertilizer also contained Ca. Even though the basic fertilizer also contained Mg, top Mg concentrations were unaffected by NAR or fertilizer (Table 6).

Sulfur is a major component of enzymes and proteins associated with growth, therefore S concentrations might be expected to increase with NAR (14, 15). However, top S concentrations of seabeach amaranth decreased linearly with increasing NAR for both fertilizers (Table 6). These results are similar to those of Cabrera and Devereaux (4) who reported increasing NAR decreased foliar S concentrations.

Both fertilizers linearly decreased concentration of Na in tops of seabeach amaranth (Table 6). The Na levels reported herein are considerably higher than levels reported for silver cock's comb (16). The higher Na levels are most likely attributed to seabeach amaranth being a halophyte (21), but no research appears to have been reported regarding the salt-exclusion mechanism in seabeach amaranth. There are several reported salt-exclusion mechanisms occurring in grasses such as members of subfamily Chloridoideae which possess salt-excreting 'microhairs' (5), as well as vacuolar Na^+/H^+ antiporters that sequester Na into vacuoles to prevent toxic salt accumulation in the cytoplasm (20). Most halophytes tightly regulate Na uptake at salinity levels below or equivalent to seawater, thus, a decreasing top Na concentration could be due to dilution caused by increasing growth (9). Foliar Mn, Zn, and B concentrations of seabeach amaranth were affected by NAR only when grown with the acidic fertilizer (Table 6). Both top Mn and Zn concentrations decreased linearly with increasing NAR, while foliar B concentrations increased linearly with increasing NAR. Foliar Fe (mean = 122.8 $mg \cdot g^{-1}$ ± 5.5) and Cu (mean = 9.7 $mg \cdot g^{-1}$ ± 0.9) concentrations were unaffected by NAR or fertilizer.

Lime rate significantly affected top mineral nutrient concentrations (Table 7). At the low lime rate, top N, P, and Mn concentrations of seabeach amaranth were significantly higher than plants grown at the high lime treatment. In contrast, top Ca, Mg, and Na concentrations of seabeach amaranth grown at the high lime rate were significantly higher compared to the low rate. In addition, top K (Table 7), and top S, Fe, Cu, Zn, and B concentrations were unaffected by lime rate (data not presented).

Mineral nutrient contents of tops were affected by all main effects, but all interactions were nonsignificant. Top nutrient contents of N, P, K, Ca, Mg, S, Na, Fe, Mn, Zn, and B increased linearly with increasing NAR (data not presented), whereas Cu was the only nutrient unaffected by NAR (data not presented). As previously mentioned, top concentrations of Ca, Mn, and Zn for only the acidic fertilizer, as well as S for both fertilizers, decreased with increasing NAR. Conversely, the content of all nutrients increased with NAR. The decreasing top concentrations were probably due to dilution as the plants increased in dry weight.

Top nutrient contents of Ca, Mg, and Mn were affected by rate of lime, and Ca and Mg were affected by fertilizer (Table 8). Top Ca and Mg contents were significantly higher at the high lime rate, which can be attributed to a doubling of the input of dolomitic limestone at the high lime treatment. However, top Mn content of amaranth when grown with the high rate of limestone decreased compared to plants grown with the low lime rate probably due to decreasing Mn availability with increasing pH. The basic fertilizer yielded significantly higher top Ca and Mg content than the acidic fertilizer which can be attributed to the nutrient sources of the basic fertilizer. However, top N, P, K, S, Na, Zn, Cu, Fe, and B contents were unaffected by either rate of limestone or fertilizer (data not presented).

In summary, seabeach amaranth can be produced successfully in containerized production with maximum top growth occurring with N at 300 $mg \cdot liter^{-1}$ provided by an acidic or basic fertilizer having a 4.5N:1P:3.8K or 6.8N:1P:5.6K ratio, respectively. Limiting fertilizer inputs to the lowest nutrient concentrations consistent with adequate growth is an important consideration for growers. It should be implemented,

Table 6. Effect of nitrogen application rate (NAR) and type of fertilizer (fert.) on top mineral nutrient concentrations of containerized seabeach amaranth.[z]

NAR (mg·liter^{-1})	N Acidic fert.[y]	N Basic fert.[x]	P Acidic fert.	P Basic fert.	K Acidic fert.	K Basic fert.
	mg·g^{-1}					
75	36.1 ± 3.3	44.0 ± 4.7	6.9 ± 0.6	7.0 ± 1.0	32.2 ± 2.7	30.5 ± 2.7
150	48.1 ± 3.9	41.2 ± 6.7	9.3 ± 0.7	6.1 ± 1.9	30.4 ± 2.1	31.0 ± 2.9
225	51.8 ± 6.4	50.9 ± 5.0	10.7 ± 1.7	8.0 ± 1.3	32.8 ± 5.2	33.2 ± 2.1
300	58.4 ± 4.6	50.4 ± 5.2	11.7 ± 0.6	7.3 ± 0.5	33.3 ± 2.8	31.0 ± 1.9
Significance						
Linear[w]	***	**	***	NS	NS	NS
Quadratic	NS	NS	NS	NS	NS	NS

NAR (mg·liter^{-1})	Ca Acidic fert.	Ca Basic fert.	Mg Acidic fert.	Mg Basic fert.	S Acidic fert.	S Basic fert.	Na Acidic fert.	Na Basic fert.
	mg·g^{-1}							
75	14.2 ± 1.6	11.9 ± 1.0	9.7 ± 1.1	8.9 ± 0.7	5.6 ± 0.6	4.4 ± 0.4	7.3 ± 0.7	7.5 ± 0.4
150	11.1 ± 0.8	13.1 ± 2.2	9.0 ± 0.5	9.1 ± 1.4	4.5 ± 0.4	4.0 ± 0.5	7.4 ± 0.9	7.3 ± 1.0
225	10.9 ± 2.0	13.0 ± 1.9	8.9 ± 0.9	9.7 ± 1.0	4.1 ± 0.8	3.7 ± 0.2	6.7 ± 0.7	6.2 ± 0.7
300	9.3 ± 1.5	13.6 ± 1.1	8.9 ± 1.0	9.5 ± 1.0	3.7 ± 0.2	3.3 ± 0.3	5.5 ± 0.5	5.8 ± 0.4
Significance								
Linear[w]	***	*	NS	NS	***	***	***	***
Quadratic	NS	NS	NS	NS	NS	NS	NS	NS

NAR (mg·liter^{-1})	Mn Acidic fert.	Mn Basic fert.	Zn Acidic fert.	Zn Basic fert.	Cu Acidic fert.	Cu Basic fert.	Fe Acidic fert.	Fe Basic fert.	B Acidic fert.	B Basic fert.
	µg·g^{-1}									
75	275 ± 97	211 ± 69	147 ± 18	124 ± 54	10 ± 2	13 ± 11	110 ± 24	117 ± 11	33 ± 6	33 ± 5
150	225 ± 68	210 ± 93	109 ± 22	108 ± 7	9 ± 2	9 ± 4	126 ± 29	139 ± 64	36 ± 3	37 ± 6
225	205 ± 55	177 ± 68	113 ± 31	99 ± 14	8 ± 4	7 ± 2	133 ± 50	114 ± 30	41 ± 8	39 ± 3
300	180 ± 67	181 ± 71	96 ± 20	117 ± 61	8 ± 3	13 ± 17	141 ± 72	103 ± 38	40 ± 5	37 ± 6
Significance										
Linear[w]	*	NS	**	NS	NS	NS	NS	NS	*	NS
Quadratic	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Data are means of 48 observations ± 1 SE.

[y]Acid fert. = 20N-4.4P-16.6K (20N-10P_2O_5-20K_2O) with N derived from ammonium nitrate and potassium nitrate.

[x]Basic fert. = 15N-2.2P-12.5K (15N-5P_2O_5-15K_2O) with N derived from ammonium nitrate, calcium nitrate, potassium nitrate, urea phosphate, and magnesium nitrate.

[w]Regression equations are: N (acidic), $y = 30.9 + 0.094x$, $R^2 = 0.97$; N (basic), $y = 39.4 + 0.039x$, $R^2 = 0.78$; P (acidic), $y = 5.7 + 0.021x$, $R^2 = 0.98$; Ca (acidic), $y = 15.1 – 0.020x$, $R^2 = 0.94$; Ca (basic), $y = 11.7 + 0.007x$, $R^2 = 0.90$; S (acidic), $y = 6.0 – 0.008x$, $R^2 = 0.96$; S (basic), $y = 4.8 – 0.005x$, $R^2 = 0.99$; Na (acidic), $y = 8.3 – 0.008x$, $R^2 = 0.90$; Na (basic), $y = 8.3 – 0.008x$, $R^2 = 0.97$; Mn (acidic), $y = 297.4 – 0.405x$, $R^2 = 0.98$; Zn (acidic), $y = 153.4 – 0.199x$, $R^2 = 0.89$; B (acidic), $y = 31.4 + 0.032x$, $R^2 = 0.90$.

NS, *, **, *** Nonsignificant or significant at $P \le 0.05$, 0.01, or 0.001, respectively.

Table 7. Effect of rate of lime on top nutrient concentration of containerized seabeach amaranth.[z]

Lime rate (kg·m^{-3})	N	P	K	Ca	Mg	Mn	Na
	mg·g^{-1}						
2.24	49.8 ± 8.2	8.7 ± 2.2	31.5 ± 3.6	11.5 ± 2.0	8.8 ± 1.0	0.3 ± 0.05	6.5 ± 1.0
4.48	45.4 ± 7.6	8.0 ± 2.1	32.1 ± 2.4	12.8 ± 2.1	9.6 ± 0.9	0.1 ± 0.03	6.9 ± 1.0
Significance	*	*	NS	*	*	*	*

[z]Data are means of 192 observations ± 1 SE.

NS, * Nonsignificant or significant at $P \le 0.05$, respectively.

Table 8. **Effects of rate of lime and type of fertilizer on top nutrient content of containerized seabeach amaranth.**[z]

	Ca	Mg	Mn
	mg		µg
Lime rate (kg·m^{-3})			
2.24	16.4 ± 8.4	12.9 ± 7.0	393 ± 200
4.48	21.3 ± 8.4	16.0 ± 5.8	239 ± 92
Significance	*	*	*
Fertilizer[y]			
Acidic	15.2 ± 7.4	12.7 ± 6.9	305 ± 188
Basic	22.5 ± 8.4	16.2 ± 5.8	327 ± 159
Significance	*	*	NS

[z]Data are means of 192 observations ± 1 SE.

[y]Acidic fertilizer (fert.) = 20N-4.4P-16.6K (20N-10-P_20_5-20K_20) with N derived from ammonium nitrate and potassium nitrate; basic fert. = 15N-2.2P-12.5K (15N-5P_20_5-15K_20) with N derived from ammonium nitrate, calcium nitrate, potassium nitrate, urea phosphate, and magnesium nitrate.

NS, * Nonsignificant or significant at $P \leq 0.05$, respectively.

whenever possible, because it is a cost-saving technique that can significantly reduce the levels of nutrient runoff from nurseries (22). Growth data indicated larger plants can be grown with a basic fertilizer, a larger container, and a high rate of lime (Table 5). Further, results herein indicate seed treatment with K-GA_3 does not appear to cause any undesirable morphological effects, and results in larger plants.

Currently, the ideal plant size for coastal restoration plantings of seabeach amaranth is unknown. Large plants can be produced quickly, but it is possible that plants produced in small containers and at a lower N rate may be better suited for beach plantings since they would be less susceptible to physical injury. This situation mirrors results reported by Liptay et al. (13) where 'TH-318' tomato [*Solanum lycopersicum* L. var. *lycopersicum* (syns. *Lycopersicon lycopersicum* Karst., *Lycopersicon esculentum* Miller) 'TH-318'] transplants produced using the float system at a high N rate (350 mg·liter^{-1}) exhibited reduced survival in comparison to transplants produced at lower rates of N (100 to 200 mg·liter^{-1}).

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