

Consequences of salinity and freezing stress for two populations of *Quercus virginiana* Mill. (Fagaceae) grown in a common garden¹

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KURTZ, C. M. (USDA Forest Service, 1992 Folwell Avenue N., St. Paul, MN 55108 and Department of Forest Resources, University of Minnesota, 1530 Cleveland Ave. N., St. Paul, MN 55108), J. A. SAVAGE, I.-Y. HUANG, AND J. CAVENDER-BARES (Department of Ecology, Evolution and Behavior, University of Minnesota, 1987 Upper Buford Circle, St. Paul, MN 55108). Consequences of salinity and freezing stress for two populations of *Quercus virginiana* Mill. (Fagaceae) grown in a common garden. J. Torrey Bot. Soc. 140: 145–156. 2013.—Climate change is of increasing concern in coastal forests where rising sea levels could lead to dramatic shifts in ecosystem composition. To investigate how inundation may impact coastal ecosystems, we examined the sensitivity of *Quercus virginiana* Mill., a dominant tree in the southeastern U.S., to increased soil salinity and examined whether high salinity could increase its susceptibility to freezing damage (–10 °C). In a greenhouse, we examined the effect of three salt treatments (0–6 ppt) on acorn development and sapling physiology. We examined samples collected from two populations: inland Florida (FL) and coastal North Carolina (NC). We found that higher salt concentrations did not affect acorn germination, but did retard emergence. In the sapling stage, high salinity was more detrimental to plants from the FL population, causing greater declines in photosynthetic rates, water use efficiency, and dark quantum yield. FL plants also demonstrated a lower freezing tolerance than NC plants but freezing temperatures did not exacerbate effects of salt stress. Our data demonstrate important population-level differences in the salt and freezing tolerance of *Q. virginiana*. Since salt tolerance is important to the recruitment, growth, and survival of coastal *Quercus* species, this research can help with future conservation and management of this important species.

Key words: chlorophyll fluorescence, freeze tolerance, *Quercus virginiana*, salt tolerance.

Sea levels are expected to rise 1 to 3 mm per year in the 21st century due to a reduction in the water stored in both ice caps and glaciers, and the thermal expansion of water (Douglas 1991, Roemmich 1992, Meehl et al. 2005). Inundations of coastal ecosystems such as those from extreme storm surges are also predicted to increase due to climate change causing sea water (35 ppt NaCl) to infiltrate the groundwater supply (Oude Essink 2001). Since tree zonation is strongly determined by species' salt tolerance (Oosting 1945, Williams

et al. 1998), successional patterns and species distributions are likely to be altered by rising sea levels (Delaune et al. 1987) and higher salt concentrations in the groundwater are predicted to reduce regeneration in coastal ecosystems (Williams et al. 1999). As a result, sea level rise from tidal inundation may devastate coastal ecosystems unless species either adapt to more saline conditions or migrate inland (Nicholls et al. 1999).

Quercus virginiana Mill. (live oak) is a critical ecological component of coastal and maritime barrier island forest ecosystems in the southeastern U.S. (Helm et al. 1991) as well as in interior hammock forests (Cavender-Bares et al. 2004, Spector and Putz 2006, Cavender-Bares and Pahlich 2009). This species plays an important ecological role in these forests, providing shade, nest sites, and food for wildlife, including the threatened Florida Scrub Jay, *Aphelocoma coerulescens* (Woolfenden 1973). Due to their dense wood, long life-span, and hurricane resistance (Smith et al.

¹ This work was supported, in part by a grant from the Dean's office of the Graduate School at the UMN and by NSF IOS: 0843665 to Dr. J. Cavender-Bares. The authors thank Dr. K. Cavender-Bares and E. Warner for assistance with the freezing treatments and R. Montgomery, W.K. Moser and J. Stanovick for comments on the manuscript.

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Received for publication September 12, 2012, and in revised form April 2, 2013.

1997), live oaks are also thought to be important in preventing coastal erosion (Spector and Putz 2006). Although the dominance of *Q. virginiana* in coastal areas suggests it has high resistance to salt spray and soil salinity, the ability of this species to tolerate salt water inundation is currently unknown.

When plants are exposed to saline conditions, they undergo a series of physiological responses that result from both osmotic stress from lowered soil water potential and salt toxicity from accumulation of salt in their tissue (Hasegawa and Bressan 2000, Munns 2002, Zhu 2002). These responses include a reduction in leaf chlorophyll and carotenoid concentration, an increase in turgor pressure (Parida and Das 2005), and decline in leaf function (Alaoui-Sosse et al. 1998, Naidoo et al. 2002, Gebauer et al. 2004, Lopez-Climent et al. 2008), as well as a decrease in growth and an increase in mortality (Conner et al. 1998). High salinity can also threaten plants through decreased seed viability (Allen et al. 1998) and increased seedling mortality (Williams et al. 1999). Evidence suggests that *Quercus* species are especially susceptible to salt water inundation and multiple species (*Q. michauxii* Nutt. [swamp chestnut oak], *Q. nigra* L. [water oak], and *Q. nuttallii* Palmer [Nuttall oak]) show decreased photosynthetic activity and stomatal conductance under saline conditions (McLeod et al. 1999).

These effects are further complicated by the potential interaction between salt stress and short-term freezing events, which occur at higher frequencies in the northern part of the range of *Q. virginiana* (Cavender-Bares et al. 2011). There is evidence that freezing tolerance is important in determining species' northern range limits (Sakai and Weiser 1973, Larcher and Bauer 1981, Savage and Cavender-Bares in press) and that high xylem tension increases the extent of vascular failure after freeze-thaw events (Ball et al. 2006, Willson and Jackson 2006). As a result, the combined effect of high salinity levels and freezing temperatures could prevent species from occupying more northern habitats and limit the northern distribution of many coastal species. To date, few studies have investigated the combined effects of salt and freezing on coastal plant species (Stuart et al. 2007).

To investigate the interaction of salt and freezing tolerance in *Q. virginiana*, we compared two populations, one from inland FL

and one from coastal NC. These populations have habitats differing in both minimum winter temperature and salt exposure. Previous research indicates that maternal lines from these same regions have genetically-based differences in freezing tolerance that correspond to latitude (Cavender-Bares 2007, Cavender-Bares et al. 2011), a trend which has also been observed in other *Quercus* species (Aranda et al. 2005). In *Q. virginiana* and other evergreen or semi-evergreen *Quercus* species, including *Q. ilex* and *Q. suber*, chilling and freezing stress cause reduced quantum yield of photosynthesis (Cavender-Bares et al. 1999, Cavender-Bares et al. 2005) and reduced stomatal conductance, as well as enhanced non-photochemical quenching (Corcuera et al. 2005). Cold acclimation through seasonal exposure to cold temperatures increases frost resistance in these species, as well as in *Q. virginiana* (Cavender-Bares 2005, 2007) with individuals grown at cold winter sites showing greater frost resistance than those grown at warmer winter sites (Molla et al. 2006).

Our overall objectives were to test the sensitivity of *Q. virginiana* to short-term salt exposure at early life stages, determine whether there is genetically-based variation between populations in salt tolerance, and examine the combined effects of salt and freezing for different populations. We tested the following hypotheses: (1) that germination is not inhibited by salt exposure at concentrations similar to those found in coastal environments but is inhibited by higher salt concentrations, (2) that increased salt concentrations should cause a decline in physiological function over time in salt-exposed saplings, (3) that there should be an interaction between salt and freezing stress, such that individuals exposed to both stress factors would show the greatest loss of photosynthetic function, (4) that saplings from coastal NC populations should have higher salt tolerance than saplings from inland FL populations when grown under common conditions, and (5) that the more northern population (NC) should have a higher freezing tolerance than the southern population (FL).

Materials and Methods. GERMINATION EXPERIMENT. The species was identified based on Nixon et al. (1997). Acorns collected from Florida and North Carolina (Table 1) were stored in a refrigerator (6 °C) for approximately 18 months, cleaned with tap water, and

Table 1. *Quercus virginiana* acorn collection locations (latitude and longitude) for the germination experiment.

Location	Latitude (°N)	Longitude (°W)
1 - Carolina Beach, NC	34.5523	-77.39727
2 - San Felasco Hammock State Preserve, FL	29.74800	-82.45534
3 - University of Florida, Gainesville, FL	28.37610	-82.19961
4 - Hugh Taylor Birch State Park, FL	26.13009	-81.48314
5 - Big Shoals State Park, FL	30.23543	-82.56802

submerged for several hours. The viable acorns (those that sank) were used for the germination experiment. Of the 132 viable acorns, 117 were from FL and 15 were from NC. The germination study was conducted at the species level (the data were not partitioned by location of origin) because of inconsistent viability.

Acorns were planted in a greenhouse (26 °C, 30% relative humidity) in early spring. Four to five acorns were planted at a depth approximately two times their length in square pots (8.26 cm × 8.26 cm × 12.7 cm) containing BM2 Professional germinating mix (Berger Horticulture, Saint-Modeste (Quebec Canada)). Each pot was watered daily with 60 mL of water containing 0, 2, or 6 ppt salt (Instant Ocean sea salt, nitrate and phosphate free). The acorns were checked weekly for germination (when the radicle emerged from the seed coat) and then subsequently for emergence (when the shoot first became visible above the soil). After three weeks, germinated acorns (a total of 31) were planted individually in 25.4 cm deep containers that contained the same soil mix used in the germination trials.

The 2 ppt treatment was chosen to reflect the tolerance of other species in coastal soils and the 6 ppt salinity treatment was chosen to examine the impact of a salinity level above the tolerance threshold for many woody species, including four coastal *Quercus* species (McLeod et al. 1999). Six ppt is lower than the average salinity of sea water and may be lower than salinity levels experienced during inundation.

SAPLING EXPERIMENT. Two-year old saplings from six maternal lines (three from FL and three from NC) were grown from acorns collected from inland forests of FL in Gainesville (28.3760994 °N, -82.1996075 °W) and from coastal NC, collected at Cedar Point (34.6891976 °N, -77.09354 °W) and south Wilmington (34.2363754 °N, -77.965565 °W). The Gainesville population is located 46.8 km

inland, while the Cedar Point and Wilmington populations are 3.1 km and 5.2 km inland, respectively. Saplings were grown in a greenhouse where in the summer minimum temperatures were between 22 and 26 °C and the minimum winter temperature was 4 °C (Koehler et al. 2012). These conditions are similar to those experienced in North Carolina (Hutchins 1947). Saplings were kept in 6.25 L tree pots containing a 50:50 (sand: top soil) mixture, fertilized with one tablespoon of slow release fertilizer (Osmocote). There were 29 saplings from the FL population and 30 from the coastal NC population, with ten saplings assigned at random to each salinity treatment (0, 2, or 6 ppt), except the 0 ppt FL population (nine saplings). The saplings were watered with 60 mL of 0, 2, or 6 ppt salt water daily, beginning in March 2007. After three weeks, water was increased to 120 mL of salt water daily because the first pre-dawn measurement (see below) showed low water potential even for the 0 ppt treatment.

To compare the response of saplings to different salt concentrations, photosynthetic rate (*A*), stomatal conductance (*g_s*), pre-dawn water potential (Ψ_{PD}), chlorophyll fluorescence (maximum quantum yield, F_v/F_m), and relative chlorophyll content were measured after two, four, and seven weeks of salt treatments, on April 6, 18, and May 11. These traits were measured on a subset of six saplings per treatment combination (salt and population treatments) with the exception of chlorophyll fluorescence and relative chlorophyll content, which were measured on the full sampling set. Measurements were taken on fully expanded leaves near the top of the plant unless otherwise mentioned. *A* and *g_s* were measured with a LI-COR 6400 (LI-COR Portable Photosynthesis System, Lincoln, Nebraska, USA) under a photosynthetic photon flux density of 1200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ on a fully expanded leaf near the top of each plant between 08:00 and 11:30 with the leaf temper-

ature set at 30 °C. Instantaneous water use efficiency (WUE) was inferred from A/g_s and also by calculating the slope of the relationship between A and g_s each week of measurement. Ψ_{PD} was measured with a Scholander pressure chamber (Soil Moisture Equipment Corporation, Santa Barbara, California, USA) before dawn on a leaf near the bottom of each plant. Chlorophyll fluorescence was measured with a Mini-PAM (WALZ, Effeltrich, Germany) once before dawn and once between 8:30 and 11:00 AM, on a fully expanded leaf near the top of each sapling. Maximum quantum yield, F_v/F_m was calculated as $(F_m - F_o)/F_m$. Non-photochemical quenching (NPQ) was calculated as $(F_m - F_m')/F_m'$ (Stern-Volmer relationship).

Relative chlorophyll content was measured weekly with a Minolta SPAD-502 meter (Spectrum Technologies Inc., Plainfield, Illinois, USA). Measurements were taken on two leaves on each plant, one fully expanded leaf near the top (young leaf) and one near the bottom (older leaf). The Minolta SPAD values were converted to chlorophyll concentration using the following equation: $y = 5.53x - 62.9$, where y = chlorophyll content (mg m^{-2}) and x = SPAD value (Cavender-Bares et al. 2004). Measurements of A , g_s , chlorophyll fluorescence, and chlorophyll content were consistently measured on the same leaves throughout the experiment.

COMBINED SALT AND FREEZING EXPERIMENT. After ten weeks of salt treatment, 30 NC and 29 FL saplings from each salt treatment were sampled for a set of freezing tests. On each plant, stem segments containing at least five leaves were cut and their bases were inserted into rose tubes containing water with the same salinity as the watering treatment. The plants were then put in the freezer with the tubes in a large water reservoir to ensure that the leaves would freeze but not the tubes. To regulate the temperature, a programmable Polystat temperature controller (Cole-Palmer, Sydney, Australia) was used. The samples were cooled gradually over 1.75 hours to a temperature of -10 °C. They were kept at this temperature for four hours and then warmed to room temperature over 50 min. This temperature was selected because it was effective at differentiating population-level differences in freezing tolerance (Cavender-Bares 2007) and was within the range of expected minimum

temperatures at the southern edge of the species range (Cavender-Bares and Holbrook 2001). F_v/F_m was measured before freezing and 30 minutes, 2 hr, 14 hr, and 24 hr after freezing. For the non-freezing control, two randomly chosen saplings from each salt treatment of each population were cut, kept in rose tubes of the same salinity water as the previous treatment, and kept at room temperature. All samples were kept dark in a cardboard box and F_v/F_m was measured in the dark, consistently using the same leaves and the same location on each leaf.

STATISTICAL ANALYSES. We examined the main fixed effects of salinity, time, and their two-way interactions on seedling germination rates using repeated measures MANOVA. Gas exchange, chlorophyll fluorescence, and predawn water potential data for each week were analyzed using a least squares regression model considering the fixed factors of salinity and population and their interactions. Significant differences between individual treatment combinations were determined using Tukey's HSD ($\alpha = 0.05$). We completed a least squares regression analysis of A on g_s because the slope of this line gives an integrated measurement of WUE over time (Savage and Cavender-Bares 2011). We used repeated measures MANOVA to examine changes in leaf chlorophyll concentration over time. For the freezing experiment, dark quantum yield (F_v/F_m) was compared before and after the treatment with a least squares regression model considering salt, population, freezing effects, and their interactions. We examined whether there was any change in post-freezing values over a 24 hr period using a repeated measures MANOVA (considering salt, population, and salt by population effects). Gas exchange and predawn water potential data were log transformed, and chlorophyll fluorescence data were either Johnson Su or Johnson Si transformed to meet assumptions of normality and equal variance. All statistical analyses were completed in JMP, Version 9 (SAS Institute Inc., Cary, North Carolina, USA, 2010).

Results. GERMINATION EXPERIMENT. All acorns with the same salt treatment were pooled together irrespective of source location, as discussed previously, and the effects of different salt concentrations were compared at

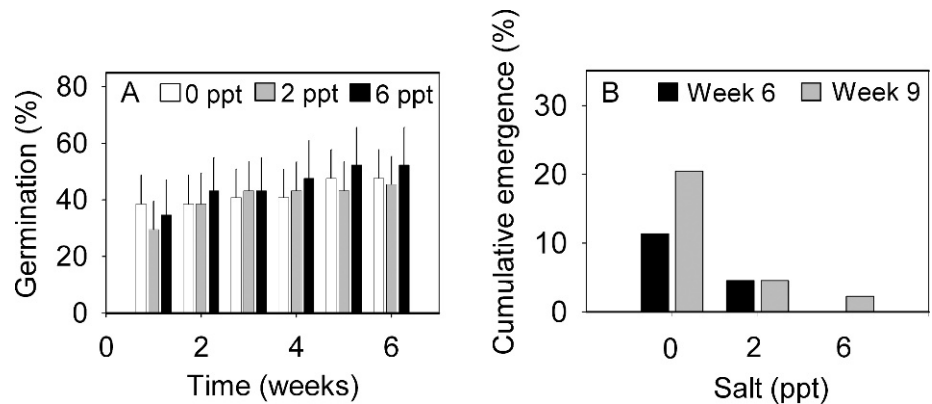


FIG. 1. Impact of three salinity treatments (0, 2, and 6 ppt NaCl) on acorns and seedlings of *Q. virginiana* grown in a greenhouse. (A) Germination percentage for each week of treatment. Error bars are $\pm$ one standard error. (B) Cumulative emergence in weeks six and nine of the salt treatment.

the species level. Germination rates across salt treatments increased over time (MANOVA, $F = 3.58$, d.f. = 4, 20, $P = 0.02$) but did not differ among salt treatments ($F = 0.02$, d.f. = 2, 23, $P = 0.98$, Fig. 1A). In contrast, seedling emergence was inhibited by salt exposure (Fig. 1B). After 11 weeks, the acorns in the 6 ppt salt treatment had the lowest emergence (2.3%) followed by the 2 ppt treatment (4.5%). The 0 ppt salt treatment had the greatest emergence (20.5%). We also compared germination of three replicate pots of five acorns collected at locations 2 and 5 (Table 1). In the sixth week, the percent germination from location 2 (0 ppt = 80%, 2 ppt = 73% and 6 ppt = 93%) was nearly two times that of location 5 (0 ppt = 33%, 2 ppt = 47% and 6 ppt = 47%) and the difference between these two locations was statistically significant (ANOVA, $F = 33.9$, d.f. = 17, $P < 0.0001$). This is consistent with the larger average size of acorns collected at location 2 (data not shown).

SAPLING PHOTOSYNTHESIS. After two weeks of salt treatment, the average photosynthetic capacity (A) of the salt treated plants (both in 2 ppt and 6 ppt) was lower than in the non-treated plants (Fig. 2A). There was a significant salt effect on A during this week ($P = 0.01$ and during week seven ($P = 0.008$, Table 2). Throughout the experiment, the FL population had lower average A in the high salt treatment (6 ppt) than the NC population but this difference was not statistically significant. Stomatal conductance (g_s) responded more slowly to the salt treatments than A and

did not demonstrate a significant salt effect until week seven ($P = 0.05$, Table 2 and 3). At this point, the average g_s ($\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) in the 0 ppt treatment was 0.054 ± 0.007 (SE), while the average g_s in the 2 and 6 ppt treatments were 0.045 ± 0.009 (SE) and 0.028 ± 0.004 (SE), respectively. Meanwhile, WUE demonstrated a significant population ($P = 0.007$) and salt effect ($P = 0.03$) in week four but these effects were not sustained in week 7 (Table 2 and Fig. 2). We also examined a more integrated measure of WUE, the slope of the relationship between A and g_s . This relationship was significant for both the FL and NC populations after two and seven weeks (Table 3). On average, NC plants had a greater integrated WUE than FL plants although this difference was only marginally significant (paired t -test, $t = -2.42$, $P = 0.07$).

SAPLING PRE-DAWN WATER POTENTIAL. There was no significant difference in the pre-dawn water potential (Ψ_{PD}) of the plants grown under different salinity treatments after two weeks of salt treatment (Table 2 and Fig. 2). However, in week four and seven, Ψ_{PD} varied significantly with salt concentration ($P = 0.0005$ and $P < 0.0001$, respectively, Table 2) leading to significantly lower water potential in the 6 ppt than the 0 ppt for each population.

SAPLING CHLOROPHYLL CONTENT. On average, NC plants had higher chlorophyll content in their leaves than FL plants ($P < 0.0001$, Table 4 and Fig. 3). There was no significant effect of salt on chlorophyll content but there was an interaction between leaf location (top

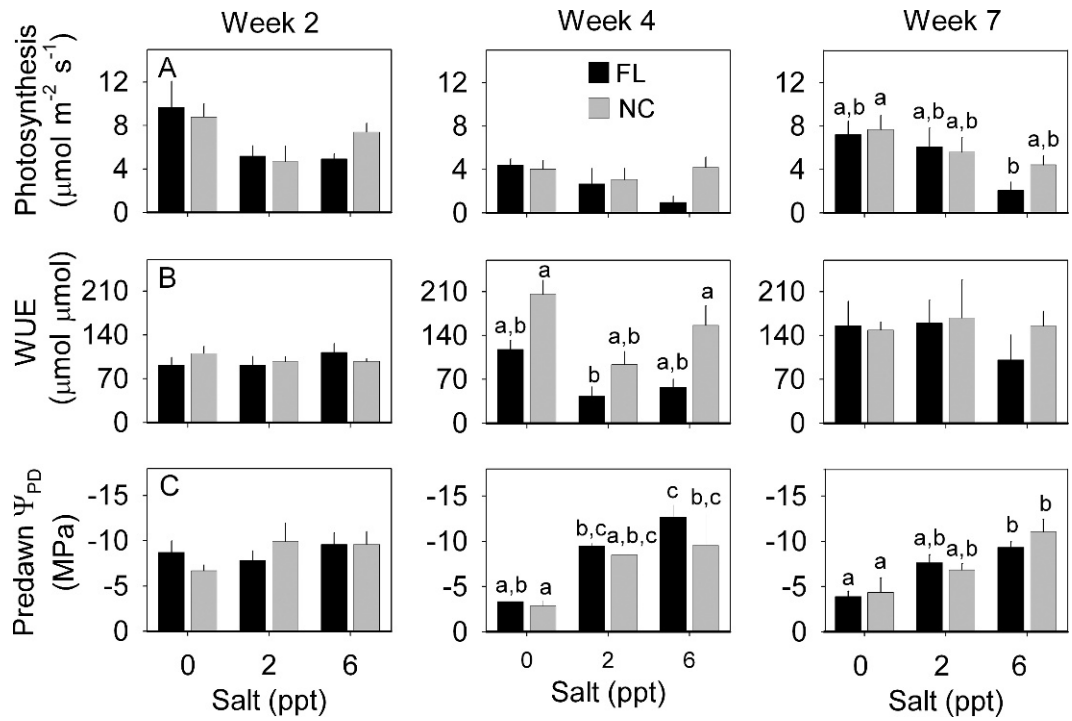


FIG. 2. The effect of three salt treatments (0, 2, and 6 ppt NaCl) on *Q. virginiana* saplings from two different populations (Florida, FL, and North Carolina, NC) after 2, 4, and 7 weeks of treatment. (A) Leaf photosynthetic capacity (A) on a leaf area basis. (B) Instantaneous water use efficiency (WUE) estimated as A divided by stomatal conductance. (C) Predawn water potential (Ψ_{PD}). Error bars are $\pm$ one standard error. Significantly different values are noted with letters (Tukey HSD, $\alpha = 0.05$).

Table 2. The effect of salt treatment and population (Pop) on photosynthetic activity (A), stomatal conductance (g_s), water-use efficiency (WUE), predawn water potential (Ψ_{PD}) and nonphotochemical quenching (NPQ) based on a LSR model. Significant values are bold and marginally significant values are italicized.

Response	Effect	DF	Week 2		Week 4		Week 7	
			<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
A	Pop	1/30	0.10	0.75	3.00	0.09	0.56	0.46
	Salt	2/30	5.28	0.01	2.24	0.12	5.63	0.008
	Pop * Salt	2/30	0.94	0.40	2.12	0.14	0.69	0.51
g_s	Pop	1/30	0.09	0.77	1.21	0.28	0.35	0.56
	Salt	2/30	2.23	0.12	2.78	0.08	3.30	0.05
	Pop * Salt	2/30	1.22	0.31	0.95	0.40	0.26	0.77
WUE	Pop	1/30	0.06	0.94	8.67	0.007	1.63	0.21
	Salt	2/30	1.34	0.26	4.12	0.03	1.26	0.30
	Pop * Salt	2/30	1.06	0.36	1.61	0.22	1.48	0.24
Ψ_{PD}	Pop	1/30	0.95	0.40	2.08	0.19	0.28	0.60
	Salt	2/30	0.02	0.89	22.58	0.0005	17.04	<0.0001
	Pop * Salt	2/30	0.91	0.42	0.57	0.58	9.76	0.48
NPQ	Pop	1/43	0.09	0.76	1.01	0.32	2.13	0.15
	Salt	2/43	1.07	0.35	2.33	0.11	1.01	0.37
	Pop * Salt	2/43	1.49	0.24	1.56	0.22	0.85	0.43

Table 3. Regression analysis of the relationship between photosynthesis and stomatal conductance (g_s) after two, four, and seven weeks of salt treatment (0, 2, and 6 ppt NaCl, $N = 36$ for each treatment). Average g_s is reported as $\text{mol H}_2\text{O m}^{-2} \text{s}^{-2}$. Significant values are bold.

	Week 2		Week 4		Week 7	
	FL	NC	FL	NC	FL	NC
Average g_s	0.086	0.073	0.045	0.033	0.042	0.039
Intercept	2.6	0.9	2.3	3.0	0.8	1.1
Slope	46.3	86.0	11.1	28.5	103.1	112.2
P	<0.001	<0.001	0.577	0.376	<0.001	<0.001
r^2	0.91	0.85	0.02	0.05	0.72	0.70

versus bottom leaves) and time ($P < 0.0001$, Table 4). This interaction was driven by the more rapid decline of the chlorophyll content in the bottom (older) leaves.

SAPLING CHLOROPHYLL FLUORESCENCE. After ten weeks, dark quantum yield (F_v/F_m) of the FL population was lower than the NC population in the high salt treatment (Fig. 4A) because of a marginally significant salt by population effect ($P = 0.08$, Table 5). There was no significant population, salt, or population by salt effects on F_v/F_m in any other week. In week seven, the NC population had a 62.9% lower NPQ than the FL population in the 6 ppt treatment, but this difference was not significant (Fig. 4B).

SAPLING FREEZING EXPERIMENT. In the absence of salt and prior to freezing, both FL and NC populations had an average dark quantum yield (F_v/F_m) greater than 0.8, which is indicative of healthy plants. Immediately following freezing at -10°C , F_v/F_m of the FL population declined to just above 0.6, and the NC population maintained values very close to 0.8 (Fig. 5). The greater decline in F_v/F_m in FL plants resulted in a significant population by salt effect ($P = 0.02$) and marginally significant population effect ($P = 0.098$, Table 6) on F_v/F_m . In the presence of high salt (6 ppt), leaves of the FL plants had low F_v/F_m (just over 0.6) prior to

freezing, which was retained immediately after freezing. There was no significant change ($\alpha = 0.05$) in the F_v/F_m values in the 24 hours following the freezing treatment in either population. We verified that the observed decrease in F_v/F_m after freezing was due to freezing stress rather than cutting stress by measuring F_v/F_m on a set of cut but unfrozen samples. These samples demonstrated no change in F_v/F_m over the time span of the experiment (data not shown).

Discussion. This study examines intraspecific variation in the salt and freezing tolerance of an ecologically important species in U.S. coastal habitats and considers how population-level variation in these traits may play a role in the survival of this species under future climatic predictions. Overall, our study suggests that *Quercus virginiana* is sensitive to short-term salt exposure, and under high salt conditions the NC population maintains a greater photosynthetic capacity (A) and a higher water-use efficiency (WUE) than FL populations, although these differences are not always significant (Fig. 2 and Table 2). We also found that the NC population exhibits a greater freezing tolerance than the FL population under the control conditions (Fig. 5). Taken together, these results suggest that there are genetically-based differences that are consistent with local adaptation of these

Table 4. The effect of salt treatment, population (Pop), time (t) and leaf location on relative leaf chlorophyll content based on a repeated measures MANOVA. Significant values are bold.

Effect	DF	F	P	Effect	DF	F	P
Pop	1/106	29.0	<0.0001	t	4/103	7.0	<0.0001
Salt	2/106	0.17	0.85	t * Pop	4/103	0.32	0.86
Leaf	1/106	8.06	0.005	t * Salt	8/206	0.58	0.79
Pop * Salt	2/106	0.11	0.90	t * Leaf	4/103	12.0	<0.0001
Pop * Leaf	1/106	0.06	0.80	t * Pop * Salt	8/206	1.14	0.34
Salt * Leaf	2/106	0.29	0.75	t * Pop * Leaf	4/103	0.48	0.75
Pop * Leaf * Salt	2/106	0.05	0.95	t * Salt * Leaf	8/206	0.27	0.97
				t * Pop * Leaf * Salt	8/206	0.99	0.45

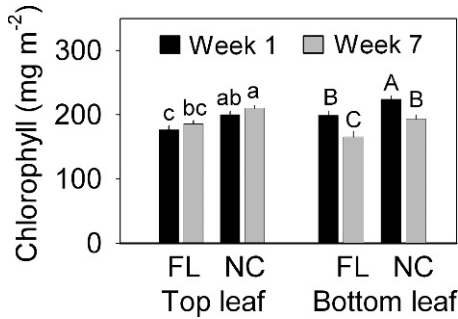


FIG. 3. Differences in leaf chlorophyll content in *Q. virginiana* saplings after one and seven weeks of salt treatment. Salt treatments are not separated because there was no effect of salt treatment on leaf chlorophyll content. One leaf was sampled from the top and one from the bottom of the plant during each sampling time. For top and bottom leaves, significantly different values are indicated with either lower case and capital letters, respectively (Tukey HSD, $\alpha = 0.05$).

populations to the salinity and climatic conditions of their home environment.

ACORN RESPONSE TO SALINITY. In general, salinity causes embryo toxicity and reduces soil osmotic potential which in turn affects water absorption (Kozłowski 1997), although studies have shown that other species demonstrate reduced germination with increasing salinity including *Atriplex triangularis* Wild. (Khan and Ungar 1984), eight families of *Taxodiaceae* (Allen et al. 1998), *Salvadora persica* L. (Ramoliya et al. 2004), and *Avena nuda* L. (Zhao et al. 2007). We found that elevated salinity (up to 6 ppt) did not reduce germination in *Q. virginiana* (Fig. 1). This was consistent with our prediction that germination is not inhibited by exposure to salt concentrations similar to those found in *Q. virginiana*'s native coastal environment. However, seedling emergence was retarded by increasing salinity (Fig. 1), suggesting that *Q. virginiana* may exhibit salt tolerance during germination, but not during shoot elongation. A higher susceptibility of seedlings to salt stress has been observed in other woody plant species (Kozłowski 1997) and indicates that early seedling survival is critical to establishment under saline conditions. However, the majority of acorns sampled were from FL populations and if there are population level differences, these data may not be indicative of the species in general. Therefore more research is needed to determine if there are population

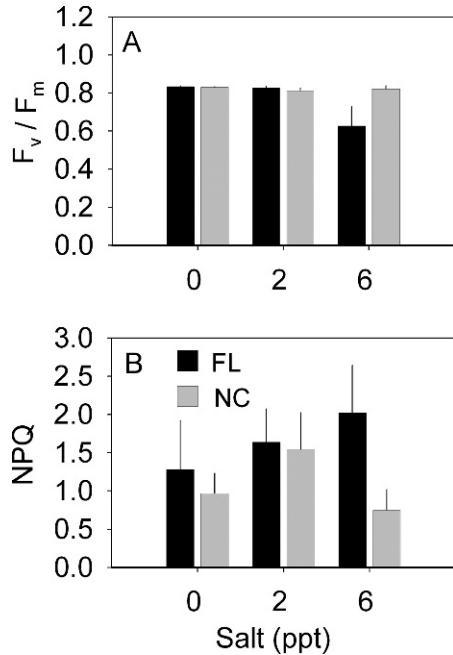


FIG. 4. The effect of three salt treatments (0, 2, and 6 ppt NaCl) on (A) maximum quantum yield (F_v/F_m) and (B) non-photochemical quenching (NPQ) in *Q. virginiana* saplings from inland FL and coastal NC populations. Measurements are after ten and seven weeks of treatment, respectively. Error bars are $\pm$ one standard error.

level differences in the susceptibility of *Q. virginiana* seedlings to salt stress and/or whether the impact of salt on seedlings limits the timing of their establishment.

Acorn size is a critical part of seedling establishment because it is primarily determined by the size of starch reserves in the seed. Seedlings from larger acorns often exhibit higher growth rates and a greater ability to overcome unfavorable environmental conditions than seedlings from smaller acorns (Aizen and Patterson 1990, Aizen and Woodcock 1996). When we compared Florida locations (2 and 5 in Table 1), we found that the location with larger acorns had a higher germination rate across salt treatments. However, further research is needed to determine if this pattern is consistent across multiple populations and whether different sizes and sources of acorns have different germination rates and salt tolerances.

SAPLING RESPONSE TO SALINITY. Both FL and NC populations were impacted by high salt concentrations and demonstrated lower A ,

Table 5. The effect of salt treatment and population (Pop) on dark adapted quantum yield (F_v/F_m) based on a LSR model. Marginally significant values are italicized.

Effect	DF	Week 2		Week 4		Week 7		Week 10	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Pop	1/53	0.04	0.84	0.09	0.76	1.11	0.30	0.72	0.40
Salt	2/53	0.24	0.79	0.35	0.71	1.29	0.28	1.61	0.21
Pop * Salt	2/53	0.40	0.67	0.14	0.86	0.42	0.82	2.65	0.08

WUE, and Ψ_{PD} in some of the high salt treatments (Fig. 2, Table 2, and Table 3). This is similar to the response observed in other shrubs (Tezara et al. 2003) and trees (Gebauer et al. 2004, Lopez-Hoffman et al. 2007) along with other *Quercus* species (McLeod et al. 1999). Our plants also exhibited yellow leaf tips, leaf mottling, marginal chlorosis and a decline in leaf chlorophyll content after prolonged exposure to salt treatments (Fig. 3), which are responses consistent with salt toxicity (Munns 2002).

Although both populations exhibited similarity in their response to the salt treatments, they appeared to differ in the severity of their response (Fig. 2). First, NC plants consistently demonstrated a higher A than FL plants under high salt conditions (6 ppt) although these differences were not statistically significant. Second, the 6 ppt treatment caused the FL population to demonstrate a decrease in F_v/F_m (Fig. 4). This response suggests that these plants experienced photo-damage and reduced photosystem activity. At the species-level, other studies have shown that coastal species tend to maintain higher F_v/F_m and lower NPQ under high salt conditions than more inland

species (Naidoo et al. 2002). The present study suggests that similar patterns can be found within populations of a single species. Since this response occurred late in the salinity treatment, it is likely more important under prolonged periods of salt water inundation.

Higher WUE is also associated with greater salt tolerance and has been observed in a variety of species including three mangrove species (Sobrado, 2000), *Hordeum vulgare* L. (Katerji, 2006) and multiple dune species (Greaver, 2007). These salt tolerant species are conservative in their water use and maintain relatively high A under saline conditions such that salinity has little effect on WUE. In this study, the NC population on average demonstrated a greater WUE than the FL population in the high salt treatment although this effect was only marginally significant (Table 3). Further, the relationship between A and g_s of *Q. virginiana* was significant after two and seven weeks of salt treatment, although the r^2 decreased after seven weeks. This suggests a reduction of A may be the result of stomatal effects in the short term but may include non-stomatal effects in the long term. Both stomatal and

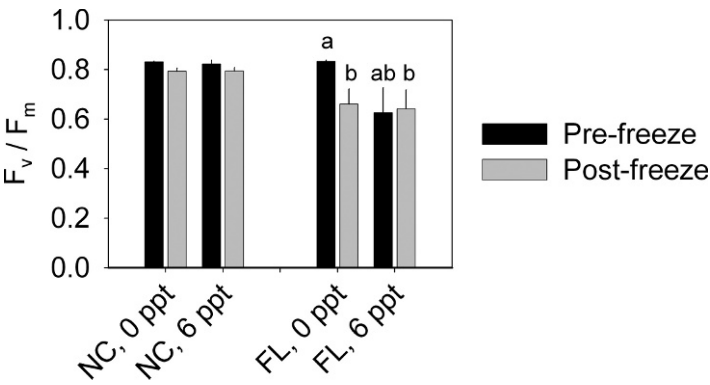


FIG. 5. Maximum quantum yield (F_v/F_m) in control (0 ppt NaCl) and high salt (6 ppt NaCl) treatments for both coastal NC populations and inland FL populations of *Q. virginiana* saplings before and after (30 min) freezing at -10°C . Error bars are $\pm$ one standard error. Pre- and post-freezing values that are significantly different are marked with letters (Tukey HSD, $\alpha = 0.05$).

Table 6. The effect of freezing, salt treatment and population (Pop) on dark adapted quantum yield (F_v/F_m) based on a MANOVA analysis. Significant values are bold and marginally significant values are italicized.

Effect	DF	F	P
Pop	1/53	2.84	<i>0.098</i>
Salt	2/53	1.17	0.318
Freezing	1/53	0.01	0.908
Pop * Salt	2/53	4.13	0.021
Freezing * Pop	1/53	0.80	0.374
Freezing * Salt	2/53	2.18	0.123
Freezing * Salt * Pop	2/53	2.27	0.113

non-stomatal factors have been previously associated with a reduction in A from salt stress (Kozłowski 1997).

Since increased salinity impedes plant water absorption, it can reduce water potential in the xylem (Parida and Das 2005). Similar to water stress, salt stress can cause plants to close their stomata and decrease their g_s . Both the Ψ_{PD} and g_s of the FL and NC populations were lower in the 6 ppt treatment after seven weeks, but did not decrease consistently over time (Fig. 2 and Table 2). Similar results were found in *Q. nuttallii* and *Q. lyrata* Walter (McLeod et al. 1999) and this may be partially explained by patchy stomatal closure (Cardon et al. 1994).

SAPLING RESPONSE TO SALINITY AND FREEZING. Both *Q. virginiana* populations demonstrated a decline in function after freezing but there was significant population level variation in their response (Fig. 5). Consistent with previous research in this system (Cavender-Bares 2007, Koehler et al. 2012), leaves of the Florida plants were more sensitive to freezing temperatures than the NC plants when they were not exposed to a high salinity treatment (Fig. 5). The higher freezing and salt tolerance of the NC population is consistent with local adaptation at the population level. Colder temperatures and higher salt concentrations in coastal NC relative to inland FL are likely to have selected for increased tolerance to these stresses. Previous studies have found evidence for local adaptation in relation to freezing tolerance. For example, ten populations of *Q. suber* L., a Mediterranean evergreen cork oak, showed a negative linear relationship between mean annual temperature at the population source and mean population F_v/F_m in the coldest month (Aranda et al. 2005).

Florida plants were also more sensitive to salt stress, as indicated by their lower predawn F_v/F_m

values prior to freezing (Fig. 5). Interestingly, their F_v/F_m was not further depressed after freezing, suggesting that salt and freezing stress do not interact in their effects on this population. In general, the interaction between freezing and salt stress is not well understood in *Quercus* species. This study provides initial evidence that salt does not exacerbate freezing stress. One possible explanation for the lack of interaction is that higher salt exposure increased the osmotic concentration inside the leaf cells and thus limited freezing injury by depressing the intracellular freezing temperature. Further research on how salinity influences minimum temperature thresholds for plant recovery could increase our understanding of how coastal forests will respond to future changes in sea levels.

It is important to note that the plants were grown under climate conditions that were more similar to those experienced in NC than those in FL, and this could impact how these plants respond to the treatments. In a previous study, we found that there were significant population-level differences in both species' freezing tolerance and their growth rate under different environmental conditions (Koehler et al. 2012). Therefore, further research is needed to determine if there could be important interactions between freezing and salt tolerance in relation to growth conditions.

Conclusions. Global climate change is causing dynamic changes in coastal forests as species are displaced by brackish waters (Delaune et al. 1987). Anticipated sea level rise and an increase in coastal storms will cause infiltration of saline water into coastal ecosystems and potentially change species distributions. We found that salt tolerance is important to the recruitment, growth and survival of a coastal *Quercus* species. As these species are increasingly exposed to brackish waters, their vigor may decrease due to reduced shoot growth, water use efficiency, and photosynthesis. Our data show important differences between populations in response to salt and freezing stress, suggesting that higher latitude coastal populations may be better adapted to salt and freezing stress. However, further research is necessary to fully understand the interaction of these combined factors. These results demonstrate that genetic variation within species is critical to consider in adaptive management strategies for future climate change.

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