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# Salt Exclusion and Mycorrhizal Symbiosis Increase Tolerance to NaCl and $CaCl_2$ Salinity in 'Siam Queen' Basil

**Carolyn F. Scagel[1] and David R. Bryla**
*U.S. Department of Agriculture, Agricultural Research Service, Horticultural Crops Research Unit, 3420 NW Orchard Avenue, Corvallis, OR 97330*

**Jungmin Lee**
*U.S. Department of Agriculture, Agricultural Research Service, Horticultural Crops Research Unit Worksite, 29603 U of I Lane, Parma, ID 83660*

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***Abstract*. A study was conducted to evaluate the effects of salinity on growth and nutrient uptake in basil (*Ocimum basilicum* L. 'Siam Queen'). Plants were fertilized with a complete nutrient solution and exposed to no, low, or moderate levels of salinity using NaCl or $CaCl_2$. The plants in control and moderate salinity treatments were also inoculated or not with the arbuscular mycorrhizal fungus (AMF), *Rhizophagus irregularis* (Blaszk., Wubet, Renker, & Buscot) C. Walker & A. Schler., to determine whether AMF mitigate the effects of salinity stress. Electrical conductivity (EC) of leachate collected from salinity treatments reached levels ≥8 $dS \cdot m^{-1}$ but had no effect on plant growth in the first 41 days of treatment. However, by 75 days, plants exposed to low and moderate levels of NaCl and $CaCl_2$ had 20% to 38% less dry weight (DW) than controls. Reductions in DW were similar between NaCl and $CaCl_2$ and was greater in roots than in shoots. Both NaCl and $CaCl_2$ salinity reduced stomatal conductance ($g_S$) within 25 days, but hastened flowering by 2–3 days, and nearly doubled the DW of flowers at 75 days. Salinity from NaCl increased uptake of Na and reduced uptake of Ca, whereas $CaCl_2$ salinity increased uptake of Ca and reduced uptake of Mg and Mn. Both salts also increased relative uptake of N, Cu, and Zn, and reduced relative uptake of S and Fe. In general, Na was concentrated in roots and excluded from shoots, whereas Cl was concentrated primarily in leaves. Both salts reduced root colonization by AMF. However, AMF increased $g_S$ by 10% with NaCl and 22% with $CaCl_2$, and increased shoot DW by 22% and 43%, respectively. Other than Ca and Cl, AMF did not enhance nutrient uptake under NaCl or $CaCl_2$ salinity. 'Siam Queen' basil was moderately tolerant to salinity, due at least in part to exclusion of Na from the shoots, and inoculation with AMF increased tolerance to both NaCl and $CaCl_2$ salinity. Differences in basil tolerance to NaCl and $CaCl_2$ indicate plants may have different mechanisms for dealing with salinity and sensitivity is not solely a function of EC. This highlights the importance of understanding the source of salinity in irrigation waters and soil for predicting damage.***

Saline groundwater, recycled irrigation water from agricultural runoff, and wastewater captured from municipal and industrial effluents are being used more frequently to irrigate crops worldwide (Garrido et al., 2014). These water sources are often poor in quality and frequently contain salt levels that are detrimental to many plant species. To manage this water profitably, more information is needed on how plants respond to different salts and to identify salinity thresholds that restrict growth and quality in various crops (Shannon and Grieve, 1999). Most salinity research in horticulture has focused on NaCl. However, $CaCl_2$ is also prevalent in many sources of irrigation water, as well as soils, soilless substrates, fertilizers, and pesticides (Grattan and Grieve, 1999). Depending on the composition and concentration of salts in the water or growing substrate, ion toxicities or nutritional deficiencies may arise in plants because of a predominance of specific ions and competition effects among ions (Shannon and Grieve, 1999).

Salinity reduces production and quality in many crops by 1) intensifying plant water stress, as a direct consequence of lower $\psi_S$ in the soil or growing substrate, 2) increasing accumulation of ions to toxic levels, and 3) causing nutrient imbalances within the plants (Marschner, 2002; Munns and Tester, 2008; Parida and Das, 2005). Toxicity effects are commonly seen at high levels of salinity and usually occur rapidly. Nutritional disorders, on the other hand, are typically seen at low to moderate levels of salinity and are often subtle and accumulate over a longer period of time (Grattan and Grieve, 1999). In this latter case, salinity can alter nutrient availability and modify the transport or partitioning of nutrients within the plant. For example, NaCl may reduce Ca availability in the growing substrate and limits Ca transport and mobility within the plant (Grattan and Grieve, 1999). Depending on the source of salts, salinity can also reduce P uptake by limiting the availability of phosphate ions in the soil (Bano and Fatima, 2009), or it can compete with specific ions at nutrient uptake sites within the roots (Shannon and Grieve, 1999). For example, high concentrations of $Na^+$ can reduce plant uptake of $K^+$ ions, whereas high concentrations of $Cl^-$ may reduce uptake of nitrate ($NO_3$) and sulfate ions ($SO_4^{2-}$), and high concentrations of $Ca^{2+}$ often reduces uptake of $Mg^{2+}$. It is also possible that the physiological effects of salinity on the plant may increase the plants requirement for specific nutrients and thus alter crop fertilizer requirements (Grattan and Grieve, 1999).

Basil (*Ocimum* L.) is a herbaceous crop that has culinary, medicinal, and industrial uses (Vieira and Simon, 2000). There are about 30 species of *Ocimum* (Vieira et al., 2003) and one of the most commonly cultivated species of basil is *O. basilicum* L. (Bernstein et al., 2010; Lee and Scagel, 2009; Scagel and Lee, 2012). The crop is grown for fresh and processed markets and is produced commercially worldwide in a broad range of growing systems, including home gardens, commercial fields, soilless substrates, and hydroponic facilities (The Herb Society of America, 2003). Within each of these systems, salinity can occur quickly and uniformly (e.g., solution culture), or it can occur heterogeneously over space and time. Duration and timing of plant exposure to salinity can have different impacts on crop productivity and quality (Bazihizina et al., 2012). Basil has been shown previously to be moderately tolerant to salinity (Attia et al., 2011; Barbieri et al., 2012; Omer et al., 2008; Prasad et al., 2007). However, none of these studies lasted longer than 40 d after germination, and some commercial production cycles of basil last up to 110 d depending on production system and end use (Succop and Newman, 2004). Furthermore, although high levels of $CaCl_2$ are common in many growing systems only one study has examined the effects of $CaCl_2$ salinity in basil (Zahedi et al., 2011), and this study only assessed effects on germination and young seedling vitality.

Like many crops, basil forms beneficial associations with AMF, which colonize the roots and improve nutrient uptake and quality in the plants (Scagel and Lee, 2012). Mycorrhizae have been shown to increase, decrease, and have no effect on salinity tolerance of different crop plants (Hameed et al., 2014). Lack of consistent plant response to mycorrhizal fungi in a saline environment may be

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[1]Corresponding author. E-mail: carolyn.scagel@ars.usda.gov.

a result of differences in the timing and level of salinity, salinity source (e.g., salts of Na or Ca), crop species, and growing environment. Hajbagheri and Enteshari (2011) found that AMF increased root length and shoot DW of an unnamed basil (*O. basilicum*) cultivar in the presence of NaCl, but the study was extremely short term (72 h of salt solution application), and nutrient status of the plants was not determined. Elhindi et al. (2016) improved growth of sweet basil (*O. basilicum* 'Nano Compatt') and mitigated the effects of NaCl salinity on nutrient uptake by inoculating plants with an isolate of AMF, *Glomus deserticola*, collected from high (≈10 dS·m$^{-1}$) soil, but salt treatments were only applied once during the 70-d experiment.

Based on the results of these previous findings, the objectives of the present study were to evaluate the long-term response of basil to low and moderate levels of NaCl or $CaCl_2$ and if inoculation with AMF improves growth and nutrient uptake under moderate salinity conditions. The experiment was conducted in a greenhouse using basil (*O. basilicum* 'Thai Siam Queen'), a purported salinity-tolerant basil cultivar (Omer et al., 2008) in which nutrient and phenolic composition is responsive to inoculation with AMF (Scagel and Lee, 2012).

## Materials and Methods

*Plant material and growth conditions.* Plants of *O. basilicum* 'Thai Siam Queen' were propagated from seed (Botanical Interests, Inc., Broomfield, CO) in 102-cell plug trays (40 mL/cell; Oasis Grower Solutions, Ken, OH) filled with soilless substrate (Black Gold Seedling Mix, SunGro Horticulture, Agawam, MA) plus or minus AMF inoculum (*Rhizophagus irregularis* syn. *Glomus intraradices* Schenck & Sm.). The inoculum was produced, as described previously (Scagel and Lee, 2012), and was mixed with 25 parts of the soilless substrate. One seed was placed into each plug tray cell and covered with a fine layer of substrate. Trays were then placed in a mist chamber with supplemental light (18 h·d$^{-1}$; Lumigrow ES330; Limigrow, Inc., Novato, CA) with full range of photosynthetically active radiation and a setting of 10 for red and blue wavelengths until the first set of true leaves had expanded. Afterward, the trays were moved to a growth chamber (75% relative humidity; 23 °C; 18 h/ 6 h day/night, F34/35U, Koninklijke-Philips, Amsterdam, The Netherlands) and watered with deionized water (no fertilizer) as needed until transplanting. After 20 d in the growth chamber, the seedlings were transplanted into 2.5-L containers (Poly-Tainer NS300; Nursery Supplies, Orange, CA) filled with an inert, calcined, nonswelling illite and silica clay (Turface; Profile Products, LLC, Buffalo Grove, IL) and placed in the greenhouse. Calcined clay was used for the potting medium to reduce confounding factors such as the availability of soil nutrients at different salinity levels and to facilitate root harvest (Grattan and Grieve, 1999; Tavakkoli et al., 2010). When the plants developed four full sets of leaves, they were pruned back to three nodes to encourage branching. A total of 90 noninoculated and 54 inoculated seedlings were transplanted.

Each planted container was placed inside an 7.6-L bucket (to collect leachate) at 6 d after transplanting and then fertigated daily with a Hoagland's nutrient solution (mg·L$^{-1}$: 40 N, 1.5 P, 28 K, 4.3 Mg, 6.5 S, 10 Ca; µg·L$^{-1}$: 52 B, 52 Cu, 225 Fe, 100 Mn, 20 Mo, and 100 Zn; Hoagland and Arnon, 1950). Supplemental lighting was supplied by 330-W light-emitting diode lamps as described above. Photosynthetically active radiation (400–700 nm) was measured at a fixed location on an adjacent bench in the greenhouse, using a quantum light sensor (LI-190SA; LI-COR Biosciences, Lincoln, NE) and air temperature and relative humidity were measured using a shielded temperature/relative humidity sensor (LI-1400-104; LI-COR Biosciences). Readings from each sensor were recorded hourly using a data logger (LI-1400; LI-COR Biosciences). Photosynthetic photon flux density reached a maximum of 1060 µmol·m$^{-2}$·s$^{-1}$ and a total of 25.3 mol·m$^{-2}$·d$^{-1}$ during the experiment, and temperature and relative humidity ranged from 13.8 to 31.6 °C and 26.6% to 77.4%, respectively.

*Experimental design.* Salinity treatments were initiated 36 d after transplanting and included noninoculated plants grown at five salinity rates (no salt control and low and moderate levels of NaCl or $CaCl_2$) and AMF-inoculated plants grown at three salinity rates (no salt control and moderate levels of NaCl or $CaCl_2$). Treatments were arranged in a randomized complete block design with 18 replicates per treatment and a total of 144 plants. Each treatment was applied using a double drip-line injection system (Aragues et al., 1999), whereby fertilizer solution was added using one drip line and NaCl or $CaCl_2$ mixed with fertilizer solution was added using two other lines [see Bryla and Scagel (2014) for details]. The system consisted of three injector pumps (DZ14MZ2; Dosatron, Clearwater, FL) connected in series. The first pump injected a concentrated fertilizer solution into a mainline at a 1:50 (v/v) ratio. The mainline was then divided into three secondary lines, including one line that supplied diluted fertilizer solution directly to each plant and one line each that was attached to injector pumps. The pumps were used to inject 2 M solutions of NaCl or $CaCl_2$. Salt solutions were injected at a 1:100 (v/v) ratio for the first 6 weeks of treatment. However, EC of the leachate was less than expected. Therefore, injection ratios were increased to a 1:75 (v/v) ratio for the following 2 weeks and then increased to a 1:50 (v/v) ratio for the remainder of the experiment. Pressure-compensating drip emitters with flow rates of 4 and 8 L·h$^{-1}$ (The Toro Company, Bloomington, MN) were used to provide each plant with the same volume of fertilizer solution and one of three concentrations of salt (Table 1). Plants were fertigated once or twice a day (142 mL/application) for a total of 59 applications between 0 and 41 d, and 46 applications during the remaining 34 d of treatment. Deionized water was used to make the stock solutions, and tap water was used to irrigate and inject the solutions. Drawdown volumes from stock tank solutions and emitter output volumes were used to calculate daily salt and Cl application rates.

*Measurements.* The pH and EC were measured weekly on samples of the five salinity solutions and on leachate from each of the eight treatments. The salinity solutions were collected from four replicates each using 20 test lines on the system, and leachate was collected from five replicates per treatment using a pour-through extraction method (Torres et al., 2010). To collect the leachate, 125 mL of distilled water was poured evenly by hand over the surface of the substrate (30 min after the plants were fertigated) and allowed to drain freely for 30 min into saucers placed under each container. Volumes of each sample were recorded and measured for pH and EC using a combination pH/conductivity meter with probes at 25 °C (SevenGo Pro, Mettler-Toledo Inc., Columbus, OH).

Each plant was evaluated weekly for leaf necrosis (sometimes described as tip burn or leaf scorch). Leaf symptoms of salinity damage (tip burn and marginal leaf necrosis) was similar among all treatments ($P > 0.1$) and occurred in <5% of the leaves by 75 d (data not shown). Stomatal conductance was also measured weekly on five plants per treatment, using a leaf porometer (SC-1; Decagon Devices, Inc., Pullman, WA). The measurements were taken on three fully expanded leaves per plant between 1100 and 1600 HR.

Half of the plants in each treatment (9 plants) were harvested destructively after 41 d of treatment, and the other half were harvested after 75 d of treatment. Plants were cut off at the substrate surface and divided into stems, leaves, and flowers (75 d only). The root system was gently pulled out of the

Table 1. Salt concentration, electrical conductivity (EC), and pH of five salinity treatments applied to 'Siam Queen' basil plants.

| | 0–41 d of treatment | | | 41–75 d of treatment | | |
|---|---|---|---|---|---|---|
| Salinity treatment[z] | Concn (mM)[y] | EC (dS·m$^{-1}$)[x] | pH[x] | Concn (mM)[y] | EC (dS·m$^{-1}$)[x] | pH[x] |
| Control | 0 | 0.4 d | 7.0 a | 0 | 0.4 d | 7.1 a |
| NaCl (low) | 57 | 1.3 c | 6.9 ab | 91 | 2.1 c | 7.0 ab |
| NaCl (moderate) | 115 | 2.2 b | 6.9 ab | 181 | 3.6 b | 7.0 ab |
| $CaCl_2$ (low) | 57 | 2.3 b | 6.8 b | 91 | 3.8 b | 6.9 b |
| $CaCl_2$ (moderate) | 115 | 4.0 a | 6.8 b | 181 | 6.9 a | 6.9 b |

[z]Each treatment was mixed in a standard Hoagland's nutrient solution (Hoagland and Arnon, 1950).
[y]Concentration values based on stock concentrations, injection ratios, application duration, and emitter output.
[x]Average values of 40 (41 d) and 24 (75 d) replications. Means followed by a different letter within a column are significantly different at $P \leq 0.05$.

containers, carefully shaken and rinsed with water, and cleaned with tweezers to remove any remaining debris. The roots were then rinsed with distilled $H_2O$, subsampled for clearing and staining for AMF assessment, and fresh weight recorded. Root colonization by AMF was quantified, as previously described (Scagel and Lee, 2012). Plant tissue samples (5 per treatment) were oven-dried at 60 °C for at least 4 d and weighed. Dried plant parts were ground to pass through a 40-mesh (425 μm) screen and analyzed for C and N using a combustion analyzer (TruSpec CN; Leco Corp., St. Joseph, MI; Scagel et al., 2007) and for P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn, and Na using inductively coupled plasma-optical emission spectroscopy (Optima 3000DV; Perkin Elmer, Wellesley, MA; Scagel et al., 2007) following microwave digestion in 70% (v/v) nitric acid (Gavlak et al., 2005). Concentrations of Cl were analyzed using an ion selective electrode (perfectION comb Cl, Mettler Toledo, Schwezenback, Switzerland) following extraction in nitric acid (Rieger and Litvin, 1998). Total uptake of Ca, Na, and Cl was calculated as the sum of the nutrient content from each plant organ. Uptake of other nutrients was calculated relative to control treatments to adjust for any treatment effects on plant size (Chapin and Van Cleve, 1989). For example, uptake by noninoculated plants in low and moderate salt treatments was calculated as a percentage of noninoculated controls; and uptake by inoculated plants in moderate salt treatments was calculated as a percentage of noninoculated plants for each salt treatment.

*Statistical analyses.* All data were analyzed using the Statistica analytical software system (Version 12; StatSoft Inc., Tulsa, OK). The data were checked for normality using the Komogorov–Smirnov test, and tested for homogeneity of variance using Levene's test. Biomass allocation and root colonization data were arcsine transformed before analyses to meet assumptions of homogeneity of variance and presented as back-transformed means. Differences in treatment EC and pH from 0 to 41d and from 42 to 75d were assessed using one-way analysis for variance (ANOVA) with five salinity treatments (control and low and moderate levels of NaCl or $CaCl_2$). Differences in leachate EC and pH was assessed using two-way ANOVA in a complete factorial design with eight treatments (the five salinity treatments without AMF and three of the treatments, including the control and moderate levels of NaCl or $CaCl_2$, with AMF) and seven measurement dates. All other data were analyzed separately for each harvest. Sodium uptake was analyzed using Kruskal–Wallis ANOVA and Median Test, and differences among means were assessed at $P \leq 0.05$. All other data were analyzed using ANOVA to answer the following questions: 1) how does salinity source and rate affect growth and nutrient composition of the plants (one-way ANOVA on five treatments, including the control and low and moderate levels of NaCl or $CaCl_2$); and 2) how do AMF alter the response of basil to salinity (two-way ANOVA on three salinity treatments with and without AMF, including the control and moderate levels of NaCl or $CaCl_2$)? Means from ANOVA were separated using Fisher's least significant difference test for planned comparisons between the control and other treatments and using Tukey's honestly significant difference test for unplanned comparisons among the treatment ($P \leq 0.05$). Relationships between selected variables were assessed using best subsets regression with Mallows $C_p$ technique as the criterion for choosing the best subset of predictor effects from linear and quadratic models (Mallows, 1973). Correlations between variables were assessed using Pearson correlation coefficient (r).

## Results

*EC and pH of the nutrient solutions and the pour-through leachates.* The EC of nutrient solutions for each salinity treatment ranged from 0.4 to 4.0 $dS \cdot m^{-1}$ at 0–41 d, and from 0.4 to 6.9 $dS \cdot m^{-1}$ at 42–75 d (Table 1). The concentration of the salts was identical between NaCl and $CaCl_2$ treatments at both the low and moderate levels. However, EC was nearly twice as high with $CaCl_2$ due to the greater amount of Cl (Fig. 1A). On average, a 1.0 $dS \cdot m^{-1}$ increase in nutrient solution EC resulted in an $\approx$1.4 $dS \cdot m^{-1}$ increase in leachate EC during the first 41 d of the experiment and a 1.6 $dS \cdot m^{-1}$ increase between 42 and 75 d (Fig. 1B). Leachate EC increased over time and was eventually >4 $dS \cdot cm^{-1}$ in each salinity treatment (Fig. 1C). Inoculation with AMF had no effect on leachate EC during the experiment (Fig. 1B and C).

The pH of the nutrient solutions decreased slightly with salinity and was 0.2 units lower in the $CaCl_2$ treatments than in the control (Table 1). The pH of the leachate also decreased over time and, by the end of the experiment, was <5.0 with moderate levels of NaCl and with low and moderate levels of $CaCl_2$ (Fig. 1D). Inoculation with AMF, on the other hand, reduced pH of the control treatment but had little or no effect on pH of the salinity treatments (Fig. 1D).

*Mycorrhizal colonization.* Mycorrhizal colonization was reduced by salinity at 41 and 75 d but was unaffected by the source of salinity on either date (Table 2). In general, the percentage of roots colonized by AMF increased over time in the control treatment ($P \leq 0.01$) and declined in the salinity treatments ($P \leq 0.05$). There was no evidence of mycorrhizal colonization in the noninoculated plants (data not shown).

*Flowering, plant growth, and biomass allocation.* The plants started flowering within 30–34 d of treatment. On average, plants in the salinity treatments flowered 2–3 d earlier than those in the control treatments ($P \leq 0.05$). Total DW of plants was similar among treatments at 41 d (Fig. 2A), but was reduced by salinity and increased by AMF at 75 d (Fig. 2B). By 75 d, low levels of NaCl and $CaCl_2$ reduced DW of nonmycorrhizal plants by 20% and 28%, respectively, whereas moderate levels reduced DW by 34% and 38%, respectively. Inoculation with AMF, on the other hand, increased total DW by 11% in control plants, by 22% in plants treated with a moderate level of NaCl, and by 43% in plants treated with a moderate level of $CaCl_2$.

Salinity influenced biomass allocation at 75 d (Fig. 2C). In general, plants allocated more biomass to flowers and leaves and less biomass to roots when they were exposed to low or moderate levels of NaCl and $CaCl_2$ ($P \leq 0.01$). On average, control plants allocated 32% of the total biomass to flowers and leaves and 30% to roots, whereas those in the salinity treatments allocated 44% to flowers and leaves and only 17% to roots. AMF had less influence on biomass allocation than salinity (Fig. 2C). In the absence of salinity treatment biomass allocation was similar between the mycorrhizal and nonmycorrhizal plants. However, in the presence of a moderate level of $CaCl_2$, plants with AMF allocated 9% more biomass to stems and 10% less biomass to flowers and leaves than those without AMF ($P \leq 0.01$) and in the presence of a moderate level of NaCl, plants with AMF allocated 6% more biomass to stems and 5% less to roots ($P \leq 0.05$).

*Stomatal conductance.* Stomatal conductance decreased over time and differed among treatments within 27 d (data not shown). On average, $g_s$ declined with salinity, and was greater in mycorrhizal plants than in nonmycorrhizal plants treated with NaCl or $CaCl_2$ (Table 3). Nonmycorrhizal plants treated with a moderate level of $CaCl_2$ had the lowest $g_s$ among the salinity treatments.

*Uptake and allocation of Na, Ca, and Cl.* Not surprisingly, NaCl and $CaCl_2$ salinity increased the content of Na, Ca, and Cl in plants. Plants treated with low or moderate levels of NaCl contained an average of six times more Na than the controls and 17–18 times more Na than the $CaCl_2$ treatments (Fig. 3A and B). Similarly, plants treated with low or moderate levels of $CaCl_2$ contained an average of 1.6 times more Ca than the controls and 2.1 times more Ca than the NaCl treatments (Fig. 3C and D) and plants treated with either salt contained an average of 8–16 times more Cl than the control treatments (Fig. 3E and F). In general, the total content of Na in plants increased with the level of NaCl salinity at 41 and 75 d but was unaffected by AMF on either date. In contrast to Na, Ca content only increased with the level of $CaCl_2$ at 41 d. In addition, AMF increased Ca content in the control treatment at 41 d and in the moderate $CaCl_2$ treatment at 75 d. Total Cl content was unaffected by the level of NaCl or $CaCl_2$ salinity (low vs. moderate) but was up to 38% greater with AMF in both of the salinity treatments on each date. Interestingly, Cl content was also greater in plants treated with $CaCl_2$ than with NaCl, even when EC was comparable between the treatments and the same amount of Cl was applied (i.e., low $CaCl_2$ vs. moderate NaCl; Fig. 1A and C).

The patterns of Na, Ca, and Cl allocation were similar between 41 and 75 d, except plants had no flowers at 41 d (41 d data not shown). In most cases, the majority of Na in

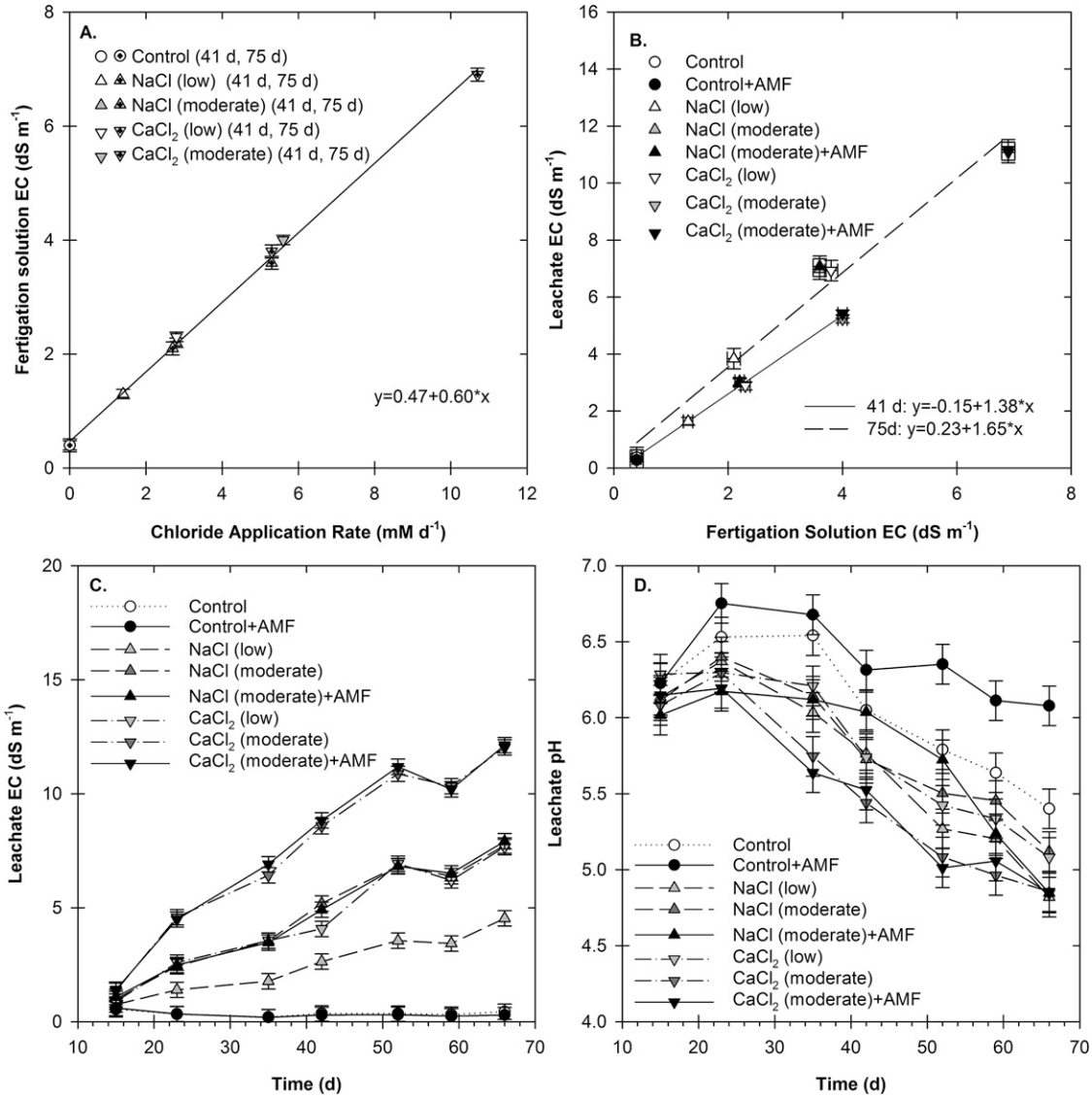


Fig. 1. (**A**) Chloride application rate, (**B**) electrical conductivity (EC) in fertigation solution, and (**C**) EC and (**D**) pH in leachate from containers of 'Siam Queen' basil grown in nutrient solution containing no additional salt (control) or low and moderate levels of NaCl or $CaCl_2$. Plants in the no salt and moderate salt concentration treatments were also inoculated or not with the arbuscular mycorrhizal fungus (+AMF), *Rhizophagus irregularis*). Symbols represent the mean of (**A**, **B**) 40 (41 d) and 24 (75 d) replicates and (**C**, **D**) five replicates and error bars represent the least significant difference between the means at $P \leq 0.05$ [least significant difference ($LSD_{0.05}$)].

Table 2. Effects of a moderate level of NaCl and $CaCl_2$ salinity on the percentage of root length colonized by arbuscular mycorrhizal fungi in 'Siam Queen' basil.

| | Mycorrhizal colonization (%) | | |
|---|---|---|---|
| Salinity treatment | 41 d of treatment | 75 d of treatment | Difference[z] |
| Control | 26 a[y] | 37 a | 22** |
| NaCl (moderate) | 12 b | 4 b | –8* |
| $CaCl_2$ (moderate) | 14 b | 7 b | –7* |

[z]Difference in means within row are significantly at $*P \leq 0.05$ and **0.01.

[y]Means (n = 5) followed by a different letter within a column are significantly different at $P \leq 0.05$. Plants were grown in nutrient solution containing no additional salt (control) or low and moderate levels of NaCl or $CaCl_2$. Plants in the control and moderate salt concentration treatments were also inoculated or not with the, *Rhizophagus irregularis*. There was no evidence of mycorrhizal colonization in the noninoculated plants (data not shown).

plants was allocated to roots, whereas Ca and Cl were allocated primarily to leaves and stems (Fig. 4). However, there were a few exceptions. For example, plants treated with NaCl allocated a considerable portion of Na to stems by 75 d, particularly with AMF, where the inoculated plants allocated only 45% of the total Na to roots and allocated 50% to stems (Fig. 4A). Plants treated with NaCl also allocated more Cl to leaves and less to stems than the control treatments ($P \leq 0.01$), whereas those treated with $CaCl_2$ allocated more Cl to leaves and less to stems than the NaCl treatments ($P \leq 0.01$; Fig. 4C).

By 75 d, plants treated with low and moderate levels of NaCl had Na concentrations of 18.8–24.4 $mg \cdot g^{-1}$ in the roots, 2.5–6.1 $mg \cdot g^{-1}$ in the stems, and only 0.1–1.0 $mg \cdot g^{-1}$ in the leaves and flowers. The NaCl treatment had the largest influence on Na concentrations in roots and stems at 75 d when root and stem Na concentrations in NaCl treated plants were 10 to 40 times greater than in controls. NaCl had little influence on Na concentrations in flowers at 75 d when Na concentrations in flowers were less than four times greater than controls (data not shown). Plants treated with low and moderate levels of $CaCl_2$ had Ca concentrations of 46–50 $mg \cdot g^{-1}$ in the leaves, 29–31 $mg \cdot g^{-1}$ in the flowers, and 8–17 $mg \cdot g^{-1}$ in the roots and stems at 75 d. In contrast to Na, $CaCl_2$ treatment affected Ca concentrations

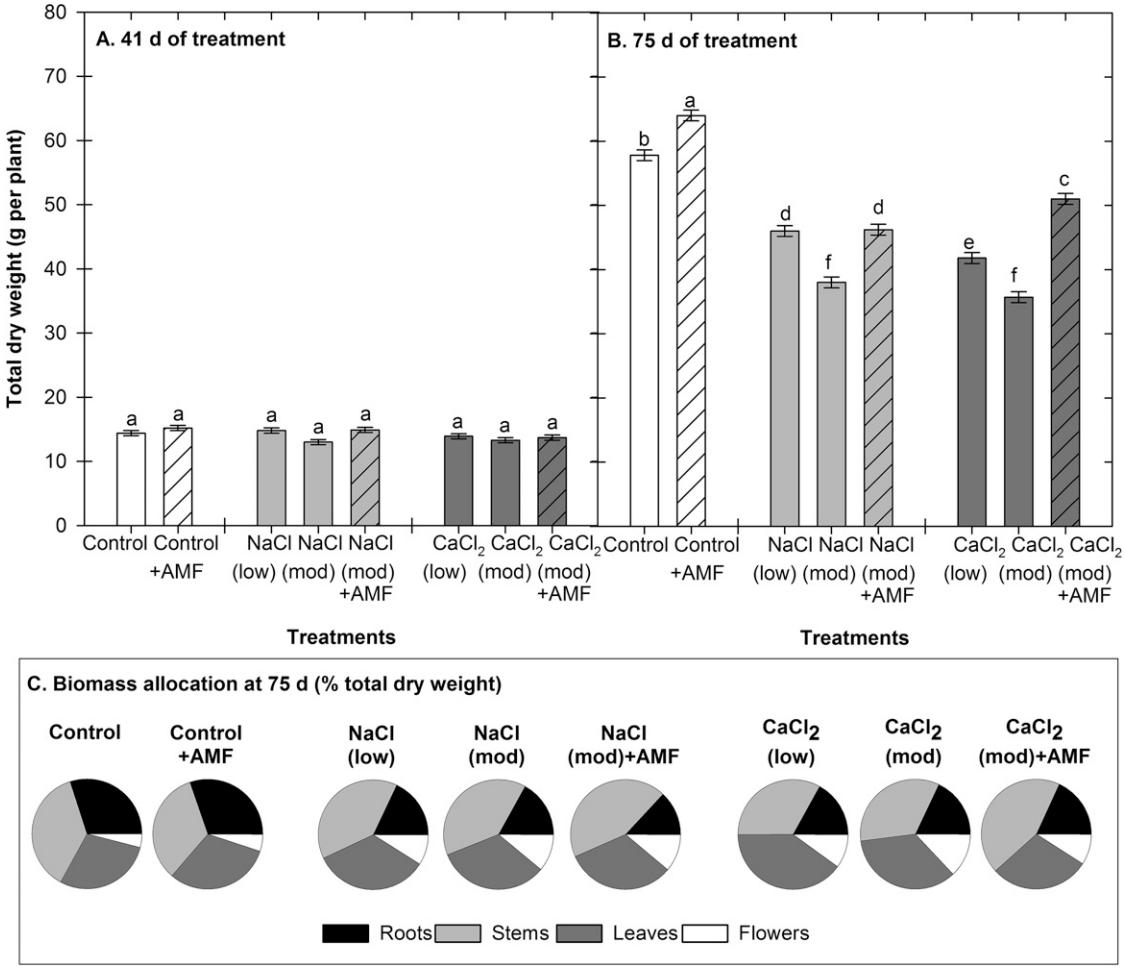


Fig. 2. Effects of NaCl and $CaCl_2$ salinity and arbuscular mycorrhizal fungi (AMF) on total dry weight of 'Siam Queen' basil at (**A**) 41 and (**B**) 75 d after start of salt treatment, and on (**C**) allocation of biomass at 75 d. Plants were grown in nutrient solution containing no additional salt (control) or low and moderate (mod) levels of NaCl or $CaCl_2$. Plants in the control and moderate salt concentration treatments were also inoculated or not with the arbuscular mycorrhizal fungus (+AMF), *Rhizophagus irregularis*. Flowers are a total of flowering stems, flowers, and seeds (if present). (**A**, **B**) Columns and error bars are, respectively, means and standard errors (n = 5). Means denoted by different lower case letters are significantly different at $P \leq 0.05$.

Table 3. Effects of NaCl and $CaCl_2$ salinity and arbuscular mycorrhizal fungi (AMF) on leaf stomatal conductance ($g_S$) in 'Siam Queen' basil.

| | $g_S$[z] (mmol·m$^{-2}$·s$^{-1}$)[z] | | |
|---|---|---|---|
| Salinity treatment | No AMF | AMF | Difference[y] |
| Control | 195 a[x] | 185 a | 10[NS] |
| NaCl (low) | 168 b | – | |
| NaCl (moderate) | 159 b | 175 ab | 16* |
| $CaCl_2$ (low) | 165 b | – | |
| $CaCl_2$ (moderate) | 141 c | 172 b | 31** |

[z]Values are an average of five weekly measurements taken between 27 and 62 d of treatment. Plants were grown in nutrient solution containing no additional salt (control) or low and moderate levels of NaCl or $CaCl_2$. Plants in the control and moderate salt concentration treatments were also inoculated or not with the *Rhizophagus irregularis*.
[y]Difference in means within row are significant at $P \leq 0.05$ (*) and 0.01 (**) or nonsignificant (NS).
[x]Means (n = 25) followed by a different letter within a column are significantly different at $P \leq 0.05$.

of all plant parts at 75 d when $CaCl_2$ treated plants had ≈4 times greater Ca concentrations than controls (data not shown).

Concentrations of Cl in NaCl treated plants at 75 d were 25–38 mg·g$^{-1}$ in the leaves, 18–28 mg·g$^{-1}$ in the roots, 13–18 mg·g$^{-1}$ in the stems, and 6–10 mg·g$^{-1}$ in the flowers. Plants treated with low and moderate levels of $CaCl_2$ had slightly higher Cl concentrations in the leaves (47–68 mg·g$^{-1}$) compared with NaCl treated plants, but similar Cl concentrations in other plant parts (20–27 mg·g$^{-1}$ in the roots, and 15–19 mg·g$^{-1}$ in the stems and flowers). Both NaCl and $CaCl_2$ salinity had the largest influence on Cl concentrations in leaves at 75d when leaf Cl concentrations in NaCl plants were 25 to 35 times greater than controls and leaf Cl concentrations in $CaCl_2$ plants were 47% to 68% greater than controls (data not shown). In contrast, Cl concentrations in other plant parts were less than eight times greater than controls.

*Other nutrients.* The effects of salinity on other essential nutrients was similar at 41 and 75 d but was more apparent on the latter date (41 d data not shown). On average, salinity from both salts reduced relative uptake of S and Fe (Fig. 5A). Additionally, $CaCl_2$ salinity reduced the relative uptake of Mg and Mn, whereas NaCl salinity reduced relative uptake of B. Salinity also increased relative uptake of specific nutrients. For example, both salts increased relative uptake of N, Cu, and Zn and NaCl treatment increased relative uptake of Mn. Inoculation with AMF only increased relative uptake relative uptake of N, K, Fe, and Cu in the no salt treatment (Fig. 5B). In most cases, AMF had no or a negative effect on relative uptake of nutrients in NaCl and $CaCl_2$ treated plants.

## Discussion

### Response of basil to NaCl and $CaCl_2$ salinity

*Tolerance to salinity.* 'Siam Queen' basil was moderately tolerant to NaCl and $CaCl_2$ salinity in the present study. Salinity levels reached as high as 8 dS·m$^{-1}$ in the leachate but had no effect on plant growth within the first 41 d of treatment. However, growth was reduced by long-term exposure to salinity. By 75 d of treatment, plants exposed to low and moderate levels of NaCl and $CaCl_2$ had 20% to 38% less DW than those fertigated with a standard Hoagland's solution. The EC values in the present study were within or above the range considered detrimental to many vegetable and herb crops (3–4 dS·m$^{-1}$;

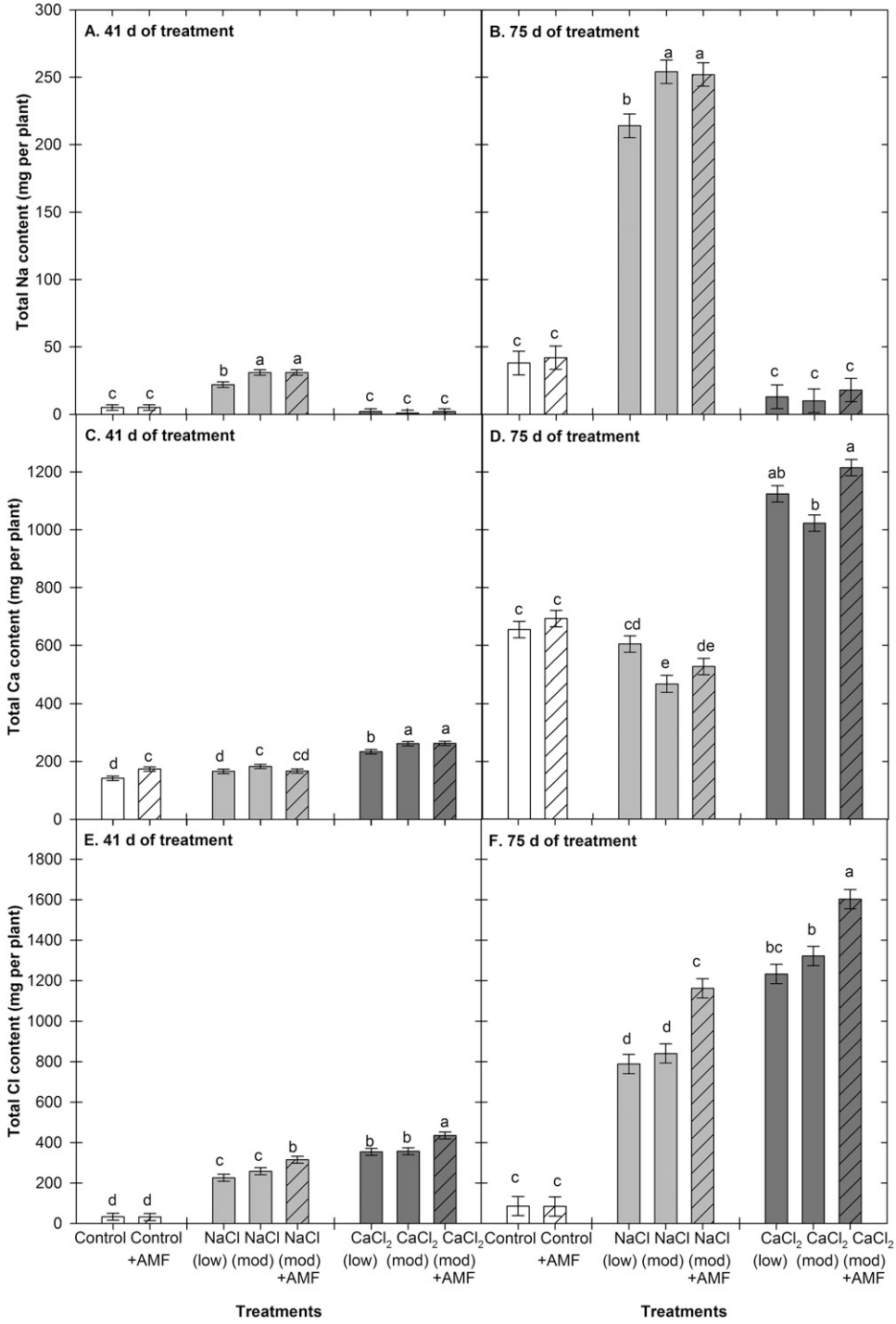


Fig. 3. Effects of NaCl and $CaCl_2$ salinity and arbuscular mycorrhizal fungi (AMF) on total content of (**A**, **B**) Na, (**C**, **D**) Ca, and (**E**, **F**) Cl at (**A**, **C**, **E**) 41 and (**B**, **D**, **F**) 75 d in 'Siam Queen' basil. Plants were grown in nutrient solution containing no additional salt (control) or low and moderate (mod) levels of NaCl or $CaCl_2$. Plants in the control and moderate salt concentration treatments were also inoculated or not with the arbuscular mycorrhizal fungus (+AMF), *Rhizophagus irregularis*. Columns and error bars are, respectively, means and standard errors (n = 5). Means denoted by different lower case letters are significantly different at $P \leq 0.05$.

Shannon and Grieve, 1999) but was within the range thought to be acceptable for basil (4.3–9.1 $dS \cdot m^{-1}$; The Herb Society of America, 2003). It should be noted, however, that salinity tolerance can vary among basil species and cultivars (Barbieri et al., 2012; Prasad et al., 2007; Ramin, 2006; Said-Al Ahl et al., 2010). For example, Heidari (2012) found that growth of *Ocimum minimum* was susceptible to a NaCl salinity level of 3 $dS \cdot m^{-1}$, while *O. basilicum* was susceptible at 6 $dS \cdot m^{-1}$, whereas Bernstein et al. (2010) found that growth of 'Perrie' basil (*O. basilicum*) was reduced by a NaCl salinity level of only 1 $dS \cdot m^{-1}$ within 20 d of treatment. In this latter case, the plants were grown hydroponically. Plants are often more susceptible to salinity in hydroponic systems than in soil or soilless substrates because there is no buffering capacity in such systems and the roots are exposed to salts continuously (Bazihizina et al., 2012; Tavakkoli et al., 2010).

Timing of salt exposure in relationship to plant age can also influence plant response to salinity (Zeng et al., 2001). In our study, salt treatments were imposed after plants had produced four full sets of leaves and may have been at less sensitive developmental stage than plants in Bernstein et al. (2010). Even though salt treatments in our study caused no detectable differences in plant DW at 41 d, salinity treatments could still alter physiology (e.g., nutrient uptake and

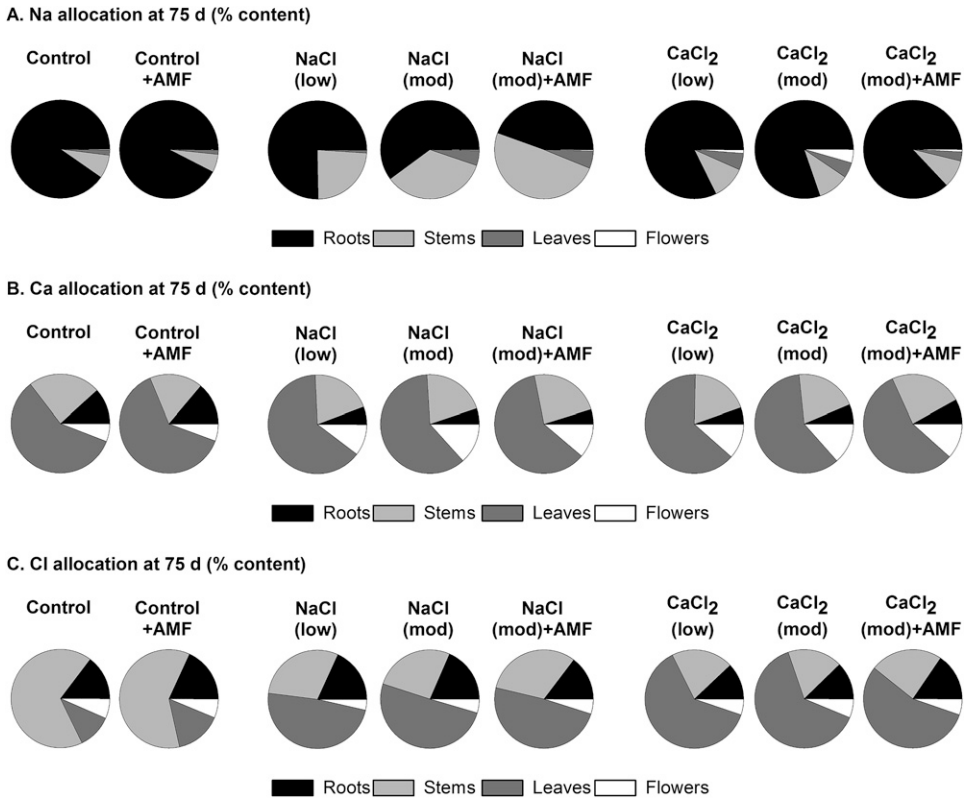


Fig. 4. Effects of NaCl and $CaCl_2$ salinity and arbuscular mycorrhizal fungi (AMF) on allocation of (**A**) Na, (**B**) Ca, and (**C**) Cl at 75 d in 'Siam Queen' basil. Plants were grown in nutrient solution containing no additional salt (control) or low and moderate (mod) levels of NaCl or $CaCl_2$. Plants in the control and moderate salt concentration treatments were also inoculated or not with the arbuscular mycorrhizal fungus (+AMF) *Rhizophagus irregularis*. Flowers are a total of flowering stems, flowers, and seeds (if present).

$g_S$). Similarly, Sabra et al. (2012) treated *Echinacea* with 0, 50, 75, and 100 mM NaCl in a hydroponic system and even though salt had no influence on DW, greater salt concentrations decreased photosynthetic rate.

*Differences between NaCl and $CaCl_2$ salinity.* Basil appeared to be equally sensitive to NaCl and $CaCl_2$ salinity. Reductions in plant growth were similar when the plants were exposed to the same concentration of each salt. However, the reductions were not similar between the salts when the values were expressed based on EC or Cl concentrations. For example, while plant DW was similar at the moderate salinity levels at 75 d, plants treated $CaCl_2$ were exposed to solutions with nearly twice the EC and twice as many Cl ions. Tarchoune et al. (2010) reported that 'Genovese' basil plants had a greater sensitivity to $Na_2SO_4$ than NaCl after 30 d of growing in hydroponic solutions with the same Na equivalents (25 mM $Na_2SO_4$ and 50 mM NaCl). Since the EC of NaCl is greater than a similar concentration of $Na_2SO_4$, this suggests that EC thresholds for basil will vary among salt source or different mixes of salts. Sensitivities to different salts has also been demonstrated for other crops, including cucumber, where plants were considered more susceptible to NaCl than to equal EC values of $CaCl_2$ (Trajkova et al., 2006).

*Salinity altered allocation of biomass.* Salinity increased biomass allocation to leaves and flowers in basil at the expense of the roots. Clearly, root growth was much more sensitive to salinity than shoot growth in the present study and resulted in much lower root-to-shoot DW ratios at low and moderate levels of NaCl and $CaCl_2$ (0.15–0.22 in each salinity treatment vs. 0.43 in both control treatments). Most studies on glycophytes report the opposite and find that shoot growth, particularly of the leaves, is more sensitive to salinity than root growth (Lauchli and Epstein, 1990). However, the response of root growth can vary within many crop species, differing among cultivars, growing medias, and ionic composition of the salts applied (Cramer et al., 1988; Snapp and Shannon, 1992).

Salinity also hastened anthesis by 2–3 d in basil, and nearly doubled the DW of flowers on the plants at 75 d. Exposure to salinity often hastens reproduction in salt sensitive plants (Parida and Das, 2005). Increased flowering under salt stress has been reported for several crop species and is thought to be mediated by phenylalanine ammonia lyase activity (Wada and Takeno, 2010).

*Salinity reduced $g_S$.* Both NaCl and $CaCl_2$ reduced $g_S$ in basil, which presumably resulted in lower photosynthetic rates in the plants (Sabra et al., 2012). Reductions in conductance occurred within 25 d of treatment and were detectable well before any differences in plant growth occurred. Barbieri et al. (2012) likewise found that NaCl salinity reduced $g_S$ quickly in 'Napoletano' and 'Genovese' basil that were grown hydroponically. In both cases, $g_S$ was similar when plants were exposed to low and moderate levels of NaCl (i.e., 100 and 200 mM NaCl). However, this was not the case for $CaCl_2$. In the present study, $g_S$ was reduced by the increased level of $CaCl_2$. Leaf Ca concentrations were high when plants were treated with $CaCl_2$ and reached 5% at the moderate salinity level. High Ca concentrations can inhibit stomatal regulation in certain species, such as *Aster tripolium* and *Gerbera jamesonii* (Albin-Garduño et al., 2007; Perera et al., 1995).

*Salinity effects on nutrient uptake.* With the exception of Na, Ca, and Cl, the effects of salinity on nutrient uptake were minimal in the plants during the first 41 d of treatment. However, by 75 d, salinity from either salt substantially increased uptake (expressed on a plant DW basis) of N, Cu, Zn, and Cl, and reduced uptake of S and Fe. Furthermore, NaCl salinity increased uptake of Na, as expected, and reduced uptake of Ca, whereas $CaCl_2$ salinity increased uptake of Ca and reduced uptake of Mg and Mn. Salinity often affects nutrient uptake by influencing the availability of soil nutrients and changing the mobility and utilization of certain nutrients within the plant (Shannon and Grieve, 1999). For example, high concentrations of $Ca^{2+}$ often inhibit P uptake by forming insoluble Ca–P complexes in certain soils and soilless substrates (Bazihizina et al., 2012). High $Ca^{2+}$ may also compete with other divalent cations, such as $Mg^{2+}$ and $Mn^{2+}$, for nutrient uptake at exchange sites within the root cell walls (Parida and Das, 2005). High concentrations of $Na^+$, on the other hand, often reduces uptake of $K^+$ and $Mg^{2+}$ (e.g.,

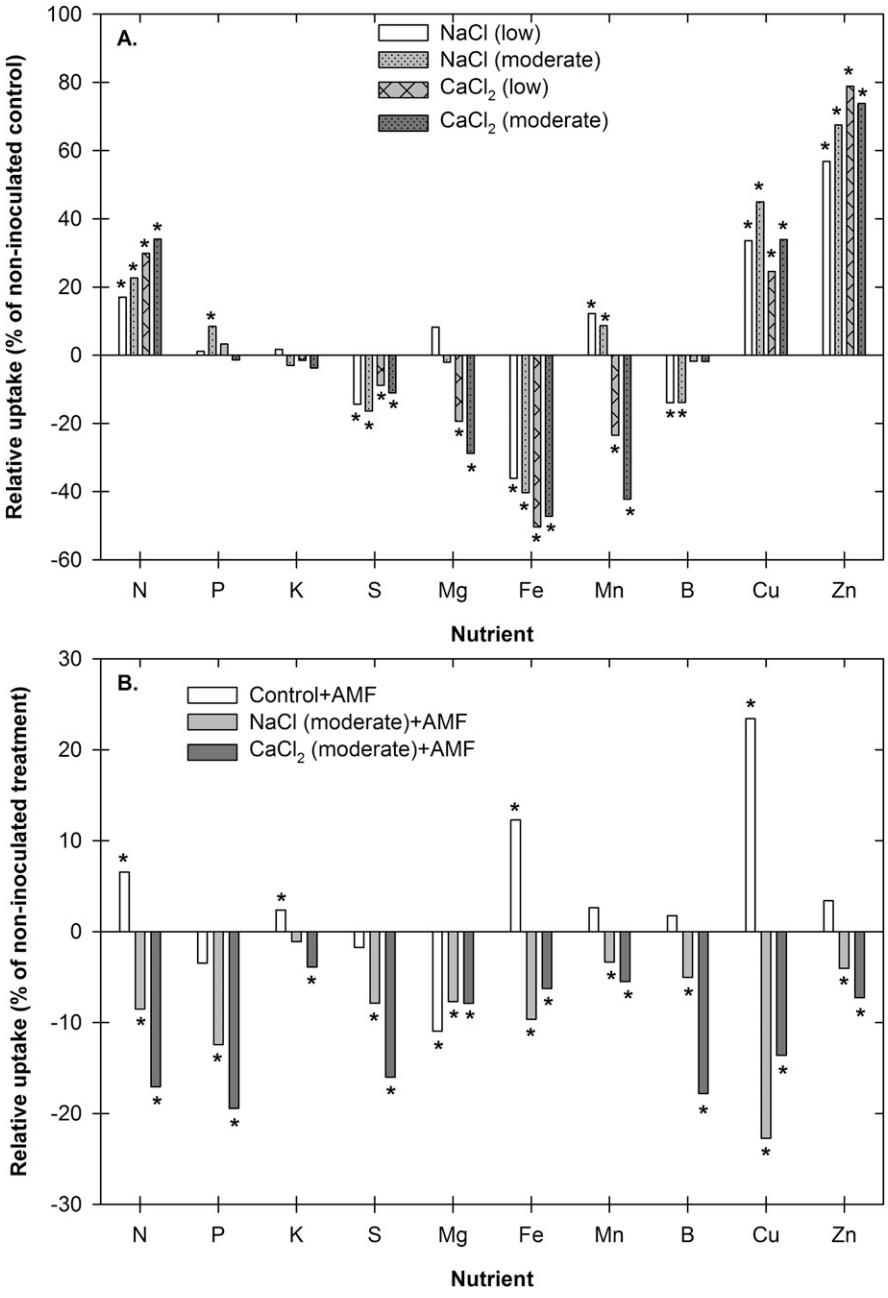


Fig. 5. Effects of NaCl and $CaCl_2$ salinity and arbuscular mycorrhizal fungi (AMF) on the relative uptake of macro- (N, P, K, Mg, and S) and micronutrients (B, Cu, Fe, Mn, and Zn) at 75 d in 'Siam Queen' basil. Plants were grown in nutrient solution containing no additional salt (control) or low and moderate levels of NaCl or $CaCl_2$. Plants in the control and moderate salt concentration treatments were also inoculated or not with the arbuscular mycorrhizal fungus (+AMF), *Rhizophagus irregularis*. An asterisk indicates the nutrient concentration that was significantly increased or decreased by (**A**) the salt treatment compared with control and (**B**) inoculation compared with noninoculated plants in the same salt treatment ($P \leq 0.05$).

Neocleous et al., 2014). Surprisingly, NaCl salinity had no effect on relative uptake of K or Mg in the basil plants. It is well known that $Ca^{2+}$ can mitigate NaCl salinity and enhance net uptake of $K^+$ (counter transport) at the expense of $Na^+$ (Marschner, 2002). Perhaps, $Ca^{2+}$ in the Hoagland's solution was sufficient enough in the present study to counteract the effects of high $Na^+$ levels on uptake of $K^+$ (and $Mg^{2+}$) in the NaCl treatments.

Currently, there is little information on the effects of salinity on micronutrients (Hu and Schmidhalter, 2005). Interestingly, Zn applications can improve tolerance of *Salvia officinalis* L. to salinity (Hendawy and Khalid, 2005), suggesting that increased Zn uptake may help mitigate the negative effects of NaCl and $CaCl_2$ salinity in basil.

### Mechanisms of salt tolerance in basil

Exclusion of Na from the leaves has been reported to increase tolerance to NaCl in certain plants (Hu and Schmidhalter, 2005). Basil appears to be one of those plants. By 75 d, the plants treated with low and moderate levels of NaCl had no more than 1.0 $mg \cdot g^{-1}$ of Na in the leaves and had <0.25 $mg \cdot g^{-1}$ in the flowers. Most of the Na was concentrated in the roots. As a result, the plants had very little salt damage in the leaves by the end of the study. Typically, salt damage occurs when leaf Na concentrations are >2.5 $mg \cdot g^{-1}$ (Sabra et al., 2012). Salt exclusion is the predominant strategy in most crop species, and it usually involves reduced transport of salts from the roots to the leaves in general and to expanding leaves and the terminal buds and flowering structures in particular (Greenway and Munns, 1980).

In general, Cl toxicity occurs at Cl concentrations of 4–7 for Cl-sensitive and 15–50 $mg \cdot g^{-1}$ DW for Cl-tolerant plant species (White and Broadley, 2001). Based on these ranges, 'Siam Queen' basil would be considered a salt tolerant plant since Cl concentrations in all salt treatments were greater than 15 $mg \cdot g^{-1}$ DW. Others have reported that basil is tolerant to saline conditions from NaCl (Omer et al., 2008; Zahedi et al., 2011). To our knowledge this is the first report of basil tolerance to salinity from $CaCl_2$. Although a reduction in vegetative growth was observed in 'Native mass' basil obtained under growing conditions with higher EC from $CaCl_2$ (Zahedi et al., 2011).

Some plant species also increase salinity tolerance by restricting transport of $Cl^-$ to the shoots (Storey and Walker, 1999). However, there was no evidence of this in basil. Plants treated with NaCl or $CaCl_2$ had higher concentrations of Cl in the leaves than in the roots. The flowers, however, had much lower concentrations, suggesting that basil may preferentially block accumulation of $Cl^-$ in the flowers. Others have reported that floral tissues generally have lower Cl concentrations that other structures in many glycophytes and halophytes (Xu et al., 2000).

### Effects of AMF on plant growth and nutrient uptake under moderate salinity conditions

*Salinity reduced colonization by AMF.* Moderate levels of NaCl and $CaCl_2$ salinity reduced root colonization by AMF in the basil plants. Salinity is well known to negatively affect AMF and hamper colonization, spore germination, and hyphal growth of the fungus (Evelin et al., 2009). To our knowledge, there are no reports on the effects of $CaCl_2$ salinity on AMF colonization. However, others have reported that 75–150 mM NaCl reduced colonization by different AMF species in basil, including *G. intraradices and Glomus mosseae* (Shekoofeh et al., 2012; Zuccarini and Okurowska, 2008). Percent root colonization was relatively low in the present study, averaging 37% without salinity after 75 d of growth, and only 7% with NaCl or $CaCl_2$ salinity. Previously, AMF colonization ranged from 59% to 72% after 112 d in four cultivars of basil, including Cinnamon, Red Rubin, Sweet Dani, and Siam Queen (Scagel and Lee, 2012). In that study, the plants were grown in a peat-based substrate,

which was perhaps more conducive to root colonization by AMF than the calcined clay used in the present study.

*AMF increased salinity tolerance in basil.* Inoculation with AMF increased growth of basil exposed to moderate levels of NaCl and $CaCl_2$ salinity. Others have reported that AMF can increase plant growth under saline conditions. A previous study on basil found that growth was better with than without AMF when the plants were exposed to 50 mM of NaCl for 56 d (Zuccarini and Okurowska, 2008). Inoculation with *Funneliformis mosseae*, *G. intraradices*, *and Claroideoglomus etunicatum* also increased growth of the medicinal herb, *Sesbania sesban* (L.) Merr., when plants were grown in a saline soil (7 $dS \cdot m^{-1}$) with 0, 75, and 150 mM NaCl for 60 d (Abd Allah et al., 2015). Plant growth was likewise enhanced by AMF under saline conditions in tomato, lettuce, onion, and *Sesbania* (Al-Karaki, 2000; Al-Karaki et al., 2001; Cantrell and Linderman, 2001; Giri and Mukerji, 2004).

Inoculation with AMF also altered allocation of biomass in the basil plants exposed to $CaCl_2$ salinity but not in those exposed to NaCl salinity. When the plants were exposed to a moderate level of $CaCl_2$, AMF increased biomass allocation to stems and reduced allocation to flowers and leaves. However, AMF had no effect on allocation of biomass to roots in any of the treatments. Kaya et al. (2009), in contrast, reported in pepper (*Capsicum annuum* L.) that AMF increased biomass allocation to roots when the plants were exposed to 50 mM NaCl and decreased biomass allocation to roots when the plants were exposed to 100 mM NaCl.

Mycorrhizal fungi may have improved growth of basil under salinity conditions by altering gas exchange and water relations of the plants. Inoculation improved $g_S$ in the presence of salts, particularly when the plants were exposed to $CaCl_2$. Others have reported similar results in lettuce (*Lactuca sativa* L.) and corn (*Zea mays* L. ssp. *mays*) and found that greater $g_S$ with AMF increased photosynthesis under low and moderate levels of NaCl salinity (Ruiz-Lozano et al., 1996; Sheng et al., 2008).

A number of studies have shown positive effects of AMF on nutrient uptake under salinity (Evelin et al., 2009). However, AMF did not appear to enhance nutrient uptake under NaCl and $CaCl_2$ salinity in the present study. Increased Cl uptake by plants with AMF grown under saline conditions has been reported previously for basil (Zuccarini and Okurowska, 2008). Although high concentrations of Cl are toxic in many crops and often result in leaf chlorosis and leaf scorch, such damage was minimal in the present study. Apparently, basil is somehow able to mitigate the toxic effects of high Cl concentrations in the plant tissues, possibly by restricting Cl import into younger leaves and inflorescences (Marschner, 2002).

Interestingly, AMF gradually increased leachate pH under nonsaline conditions in the present study. Similar effects of AMF on leachate pH have been reported by others and is likely due to differences in nutrient uptake between mycorrhizal and nonmycorrhizal plants (Carpio et al., 2005; Corkidi et al., 2011; Zinati et al., 2011; Zuccarini and Okurowska, 2008). Greater pH may reduce the availability of macronutrients, such as P, K, S, and Mg (Orozco-Patiño and Medina-Sierra, 2013), and may partially account for the negative effects of AMF on relative uptake of these nutrients in the present study.

In conclusion, our results indicate that 'Siam Queen' basil is moderately tolerant to salinity, due at least in part to exclusion of Na from the shoots, and AMF can increase plant tolerance to both NaCl and $CaCl_2$ salinity. Tolerance of 'Siam Queen' basil to salinity varies with duration of exposure, salinity level in the root environment, and salt source. Short-term exposure to salinity with an EC of 4 to 8 $dS \cdot m^{-1}$ has little influence on DW and nutrient uptake of plants, whereas EC $\geq$ 8 $dS \cdot m^{-1}$ has a negative impact on nutrient and biomass accumulation. Plants were more sensitive to NaCl than $CaCl_2$ indicating salt sensitivity is not solely a function of EC and that plants may have different mechanisms for dealing with salinity depending on salt source. This highlights the importance of understanding the source of salinity in irrigation waters and soil for crop response.

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