Preliminary Study of Sodium Chloride Tolerance of *Rudbeckia fulgida* var. *speciosa* 'Goldsturm', *Heuchera americana* 'Dale's Variety' and *Aquilegia ×cultorum* 'Crimson Star' Grown in Greenhouse Conditions[1]

Carri Gerber[2], Laura Deeter[3], Kelsey Hylton[4] and Bryce Stilwill[5]
The Ohio State University, Agricultural Technical Institute
1328 Dover Road, Wooster, OH 44691

Abstract

Heuchera americana 'Dale's Variety', *Aquilegia ×cultorum* 'Crimson Star' and *Rudbeckia fulgida* var. *speciosa* 'Goldsturm' were grown in a soilless medium and watered weekly with either 0.00 (control), 0.05, 0.15 or 0.25 M NaCl solutions for 6 weeks. Foliage and leachate were analyzed for pH, Na^+ and Cl^-, and digital images were taken of all plants. Sodium and chloride concentrations in the leachate increased over time, while pH values remained unchanged. *Aquilegia* and *Rudbeckia* took up both Na^+ and Cl^- in increasing amounts over time. *Heuchera* took up large quantities of Cl^- initially, but lesser amounts over time, and minimal Na^+ into the foliage until the last week. At the highest treatment levels of NaCl, all plants showed significant visual damage. *Aquilegia* was the first to show visual damage at 0.05 M NaCl. *Heuchera* was intermediate in terms of symptom development. Based on the results of this study, *Rudbeckia* and *Heuchera* are listed as salt tolerant, and *Aquilegia* is listed as salt intolerant.

Index words: salinity tolerance, herbaceous perennials, columbine, coral bells, black eyed Susan.

Species used in this study: coral bells (*Heuchera americana* L. 'Dale's Variety'), black-eyed susan (*Rudbeckia fulgida* var. *speciosa* (Wender) Perdue 'Goldsturm'), columbine (*Aquilegia ×cultorum* Bergmans 'Crimson Star').

Significance to the Nursery Industry

As herbaceous plantings become increasingly common in roadside plantings, sidewalks and freeway beautification projects, it is important to have an understanding of the stresses these plants undergo and the impact of those stress factors on longevity and performance. Pollution, compaction, drought and salt stress are among the factors making plant establishment and performance in these types of landscapes difficult. Deicing chemicals not only contribute to this stress, but may also work in tandem with other stresses. In order to make appropriate plant recommendations, it is important that accurate plant stress responses be studied, and then relayed to the green industry. From this research, black eyed Susan and coral bells have potential in roadside plantings, however, columbine suffered severe necrosis from NaCl and should not be recommended in roadside plantings.

Introduction

Limited greenhouse research has been conducted with herbaceous perennials to determine NaCl tolerance, yet this chemical is utilized frequently as a deicer in winter months in the northern United States (8, 9). Plants absorb deicing chemicals through both osmosis and capillary action from NaCl present in the soil solution (6). Vehicular movement volatizes snow/salt solutions, which can land directly on plant tissues. Run-off makes salt available in the soil solution, leading to absorption by the plant (1, 5).

Sodium chloride impacts plant growth in several ways. Sodium is often preferentially absorbed over K^+, affecting water relations within the plant. Chloride, though an essential plant nutrient, can be absorbed in large enough quantities to build up to toxic levels within plant tissues (2). Plants suffering from NaCl stress exhibit the following symptoms: stunting, marginal leaf necrosis, wilting, reduced/delayed flowering, abnormal foliar growth and death (3, 11).

Deicing chemicals are utilized at varying frequencies, depending upon the severity of snow and ice accumulation on roads; thus, predictions of salt tolerance are challenging in outdoor environments. Soils in the Midwest have no natural Na^+ present (1), thus the presence of Na^+ can be attributed to deicing chemicals (1). Saline soils may increase plant susceptibility to other stresses, including temperature, drought, compaction and pollution, making it difficult to diagnose NaCl as the culprit for plant decline (4).

Plants have two basic mechanisms for coping with salinity stress: avoidance or tolerance (4). Most landscape plants are dormant during the seasons when NaCl is applied, making dormancy the most common form of avoidance for temperate, landscape plants. Tolerance can be achieved via either elimination of osmotic stress, alleviation of after-effects of osmotic stress and ion imbalance or combinations of these (6, 12).

Chloride is an essential plant microelement. It is involved in counterbalancing K^+ in regulating stomatal opening and closing, during photosynthesis at the water splitting level, helps regulate cation balance within the plant and has a possible role in disease suppression through nitrate reduction (2). Chloride is classified as a micronutrient, though in reality, plants may uptake as much Cl^- as sulfur, a secondary macroelement (2).

Sodium is not an essential plant nutrient. In fact, it is detrimental to most plant growth, with the exception of halophytes, which are adapted to highly saline areas (6); indeed, some species of halophytes require Na^+ to survive

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[2]Assistant Professor of Biotechnology and Environmental Science. gerber.140@osu.edu.

[3]Associate Professor of Horticultural Technologies. deeter.7@osu.edu.

[4]Undergraduate student. hylton.157@buckeyemail.osu.edu.

[5]Undergraduate student. stilwill.1@osu.edu.

(2). In many species of plants, Na^+ is absorbed preferentially over potassium from the soil solution. This leads to problems with water relations as stomata are opened and closed via a specialized K^+ pump within these cells (2, 12).

It is challenging, if not impossible, to separate the various stresses plants must endure in an outdoor situation (8, 10). Thus greenhouse studies are generally utilized to isolate treatment effects, in this case the effect on growth and development of three species of herbaceous perennials over a 6 week period.

Materials and Methods

Watering and general treatment. A greenhouse experiment was conducted to study the NaCl tolerance of three species of herbaceous perennials: *Rudbeckia fulgida* var. *speciosa* 'Goldsturm', *Heuchera americana* 'Dale's Variety' and *Aquilegia ×cultorum* 'Crimson Star'. One liter containers were purchased from Millcreek Gardens (Ostrander, OH) in early spring and transplanted into 6 inch azalea containers containing a soilless peat-based mixture. During the 2 week acclimation, the plants were watered as needed with tap water and fertilized once per week for two weeks with Peter's 10-10-10 solution (120 ppm nitrogen) mixed according to the label. Greenhouse climate control was accomplished via the Link4 Control Systems (Yorba Linda, CA) set to 18C (65F). Relative humidity ranged from 85–95%; no supplemental lighting was provided.

Following acclimation, plants were watered once per week with 250 ml deionized water and once per week with 250 ml of treatment solution. Additional deionized water was provided as needed based on visual determination of media conditions. Plants were arranged in a randomized complete block design. There were 3 species, 3 replications, and 4 treatments for a total of 36 plants.

Four treatment levels were selected for this experiment to represent various roadside conditions: control (0.00 M NaCl), 0.05, 0.15 and 0.25 M NaCl solutions (1, 5). The various solutions were kept in 5 liter carboys next to the plants and solutions were replenished as needed. On treatment days, 250 ml of solution was measured into a graduated cylinder and uniformly poured over the medium surface to prevent splashing off the foliage or spillage over the side of the container. Excess solutions were allowed to run through the containers; no attempt to collect leachate was made at the time of NaCl treatment.

Once per week at equal intervals, all leachate was collected from each container as they were watered with deionized water. The volume collected varied for each plant. Leachate from each individual species and treatment level were combined for an average measurement. Once per week at equal intervals, one leaf was removed from the center of each plant. The three leaves from each species at each treatment level were combined, shredded, and 0.25 g of each sample was analyzed.

Leachate and tissue samples. Leachate collected weekly from all plants was filtered through Whatman #2 filter paper to remove debris. The pH level was measured with an Orion pH meter. An Orion Cl^- specific electrode was used to measure the amount of Cl^- in the leachate. Sodium in the leachate was diluted 1:10 and ppm was measured with a Varian AA120 flame atomic absorption (slit height 5 mm; slit width 200 µm; 589 nm; acetylene/air mix). Concentration curves for both Cl^- and Na^+ were created to determine sample ion concentration.

The 0.25 g of plant sample was soaked in 5 ml of 70% nitric acid solution for 24 hrs. The solutions were slowly heated to 180C (356F) and allowed to cool to room temperature. These solutions were filtered through a 0.1 µm Anotop filter to remove undigested lignin and brought to 25 mL with deionized water. Chloride concentration was determined with the Orion Cl^- ion specific electrode and Na^+ concentrations were measured using using flame atomic absorption.

Digital images of foliage, taken each week with a Nikon D200 camera to record visual damage, were used to correlate with leaf and leachate analysis.

Descriptive statistics and tables were completed using Microsoft Excel 2007 and a three-way ANOVA used to compare means was completed using PASW Statistics version 18 (2010).

Results and Discussion

Visual analysis. Heuchera americana 'Dale's Variety' displayed very little damage at the 0.05 M NaCl level throughout the entire 6 weeks. The plants during the sixth week showed leaves with marginal purpling, 1 to 2 new leaves with contorted growth and plants visually appeared to have fewer leaves than control counterparts though this was not empirically measured.

At the 0.15 M NaCl level, plants displayed contorted foliage starting in the fourth week. Symptoms progressed over the next 2 weeks to marginal purpling, reducing flowering, and fewer leaves (visual comparison). Most new leaves emerging in the sixth week were completely purple and the plants had many necrotic leaves throughout.

At the highest sodium chloride level (0.25 M), symptoms started appearing during the second week of treatment and progressively increased through the sixth week. Images taken during the sixth week show a preponderance of dead foliage, with the remaining foliage highly contorted. All leaves displayed damage, ranging from purpling through complete necrosis. Plants were noticeably thinner with fewer leaves; however, new growth continued through the experiment.

Aquilegia ×cultorum 'Crimson Star' showed visual damage at 0.05 M NaCl even during the early weeks of the experiment, developing marginal necrosis in week 2. Starting in week 3, these plants were noticeably chlorotic and the foliage was distinctly smaller than control counterparts. Starting in week 4, marginal purpling became obvious. All plants were still alive at this NaCl level at the end of the 6 weeks; however, visual stress was readily apparent in two plants with approximately half their foliage completely necrotic.

At the 0.15 M NaCl level, older foliage of all plants was completely necrotic while newer growth displayed marginal purpling. In week 3, plants were noticeably stunted, thinner and chlorotic in addition to the above symptoms. Significant foliar distortion was apparent starting in week 4. Although none of the plants died during the 6 weeks, by the end of the experiment all plants appeared stressed with stunted growth, twisted foliage, and extreme chlorosis, and more than half the growth was necrotic.

At the 0.25 M NaCl level, *Aquilegia* 'Crimson Star' displayed the same symptoms mentioned previously, with stunting, contorted foliage, marginal purpling and chlorosis appearing in the second week. By the fifth week, all but one of the plants were dead. This lone specimen displayed very

Table 1. Summary of visual symptoms of medium applied 0.0 (control), 0.05, 0.15 and 0.25 M NaCl observed in three species of herbaceous perennials over a 6 week experiment.

Species	M NaCl	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Aquilegia	0.00[z]						
	0.05[y]		marginal necrosis	chlorosis,[x] stunted foliage	stunting of entire plant		half foliage necrotic
	0.15		older foliage necrotic	stunting, thinning plants	significant foliar stunting		half foliage necrotic
	0.25		stunting, marginal purpling, chlorosis			death	
Heuchera	0.00						
	0.05	marginal purpling, contorted growth on 1or 2 leaves, visually fewer leaves				stunting, contortion on more than 2 leaves	
	0.15				contortion	contortion, marginal purpling, reduced flowering, visually fewer leaves	entire leaves purple, many necrotic leaves
	0.25		contorted growth, visually fewer leaves, marginal purpling, necrotic leaves				death
Rudbeckia	0.00						
	0.05					marginal necrosis	chlorosis
	0.15			marginal necrosis on older growth		leaf necrosis, plant death	plant death
	0.25				extreme necrosis both older and newer growth	no new growth forming, necrosis	foliar and crown death

[z]Blank cells indicate no noticeable symptoms.
[y]Blank cells after symptoms indicate no change in symptoms from previous cells.
[x]Once the symptoms were observed, they continued throughout the experiment. Data presented in the table above assume this continuity and additional symptoms are added as they were observed.

few symptoms of NaCl damage, although when compared with control plants was stunted and chlorotic.

Rudbeckia fulgida var. *speciosa* 'Goldsturm' displayed no visual symptoms at the 0.05 M NaCl level until week 5. At this time, all plants displayed minor marginal necrosis on the older foliage. During week 6, foliage with marginal necrosis developed chlorosis as well. New foliage growth appeared undamaged.

At the 0.15 M NaCl level, marginal necrosis appeared during the week 3; however, this damage was confined to the older leaves and overall plant health appeared unaffected. During week 4, necrotic areas increased in size and began appearing on the newer growth. During weeks 5 and 6, entire leaves were necrotic on some plants, while other specimens were completely dead.

At the 0.25 M NaCl level, all plants displayed very few symptoms until week 4 of treatments. At this point all plants became extremely necrotic over both new and older growth. During week 5, no new growth was observed and plants rapidly went from marginal necrosis to foliar death to crown death. During week 6, all plants displayed far more dead foliage than green foliage. What little green foliage existed was necrotic and appeared unhealthy.

Sodium and chloride levels in leachate. As each plant was watered with NaCl weekly, it was expected the amount of Na^+ and Cl^- in the leachate would increase as both Na^+ and Cl^- levels increased in the media. Sodium and chloride levels in leachate increased over the first 3 weeks of the experiment. Chloride leveled off at 0.006 ppm at week 4. Sodium levels followed a similar trend, leveling off at approximately 40 ppm. Levels of Na^+ absorbed by the plants were very low compared to levels available in the medium (data not shown).

Sodium and chloride levels in plant tissues. As both Na^+ and Cl^- levels increased in plant tissues, the visual appearance of damage increased. When levels of these chemicals were low, visual symptoms were also minimal, generally limited to marginal necrosis and perhaps stunting. As levels increased, additional symptoms occurred, including, chlorosis, marginal purpling, foliar necrosis and finally plant death (Table 1).

However, *Heuchera* displayed some interesting phenomena in relation to these two ions. After the first treatment, Cl^- levels in *Heuchera* increased dramatically (Fig. 1). During weeks 2 and 3, the levels remained high. However, in subsequent weeks, the levels dropped. This is perhaps indicative of the plant reaching a toxic threshold for Cl^- within the tissue and having a mechanism for eliminating some of the excess.

Intriguingly, Na^+ levels in *Heuchera* remained relatively stable until the sixth week. During the sixth week of the experiment, the level of Na^+ increased from 0.25 ppm to 1.25 ppm. This might indicate an active Na^+ pumping mechanism within this species to enable the plant to continue relatively normal water relations for a period of time. However, after 6 weeks, this mechanism was no longer effective and the plant absorbed very large amount of Na^+ (Fig. 2).

Similar to *Heuchera*, *Rudbeckia* was another species that took up Na^+ in relatively large quantities and yet seemed to display few symptoms until the end of the experiment (Fig.

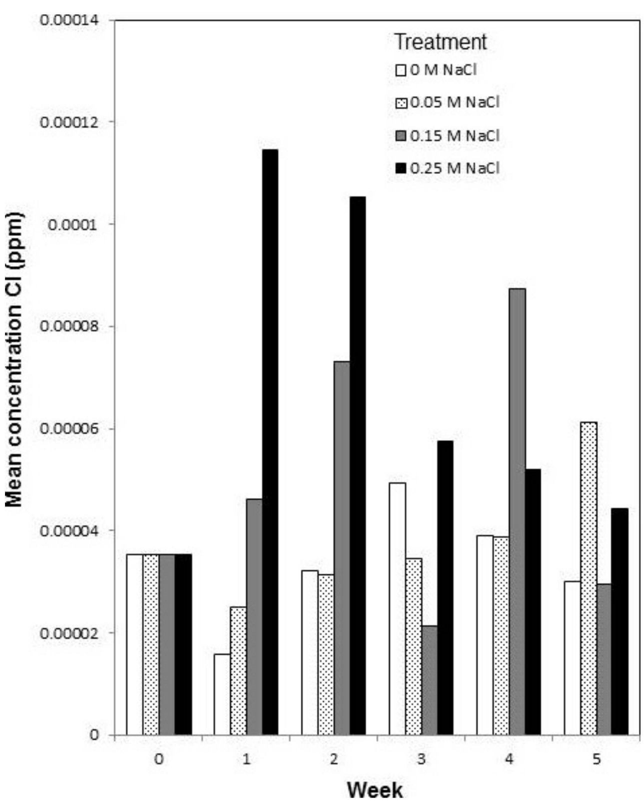

Fig. 1. **Chloride levels recorded in foliage of *Heuchera americana* 'Dale's Variety' treated with 0, 0.5, 0.15 and 1.25 M of NaCl solution to the media over 6 weeks.**

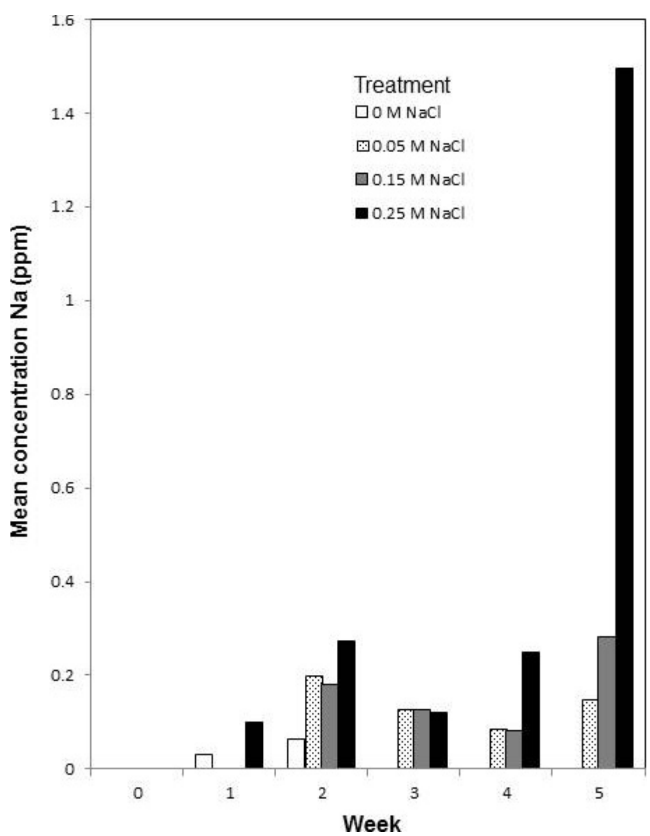

Fig. 2. **Sodium levels recorded in foliage of *Heuchera americana* 'Dale's Variety' treated with various concentrations of NaCl solution to the media over 6 weeks.**

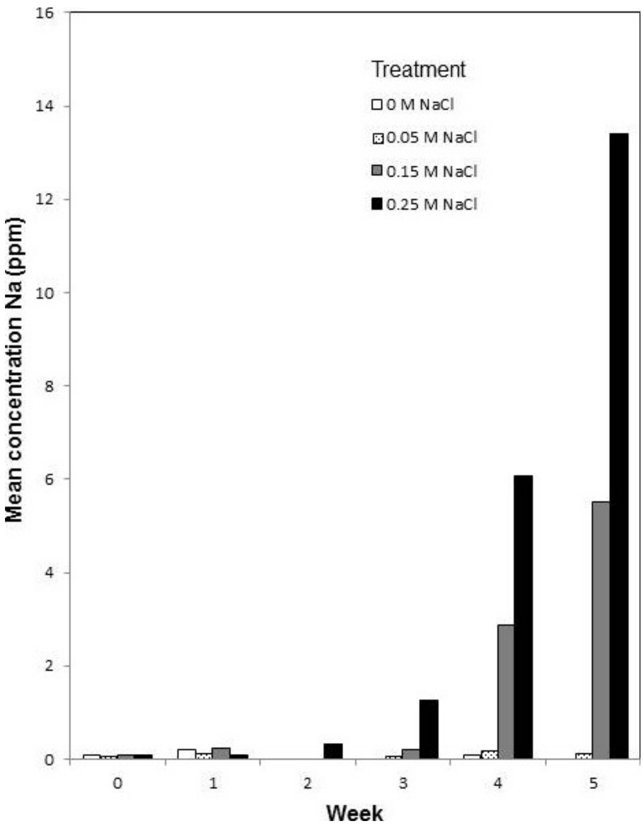

Fig. 3. Sodium levels recorded in foliage of *Rudbeckia fulgida* var. *speciosa* 'Goldsturm' treated with various concentrations of NaCl solution to the media over 6 weeks.

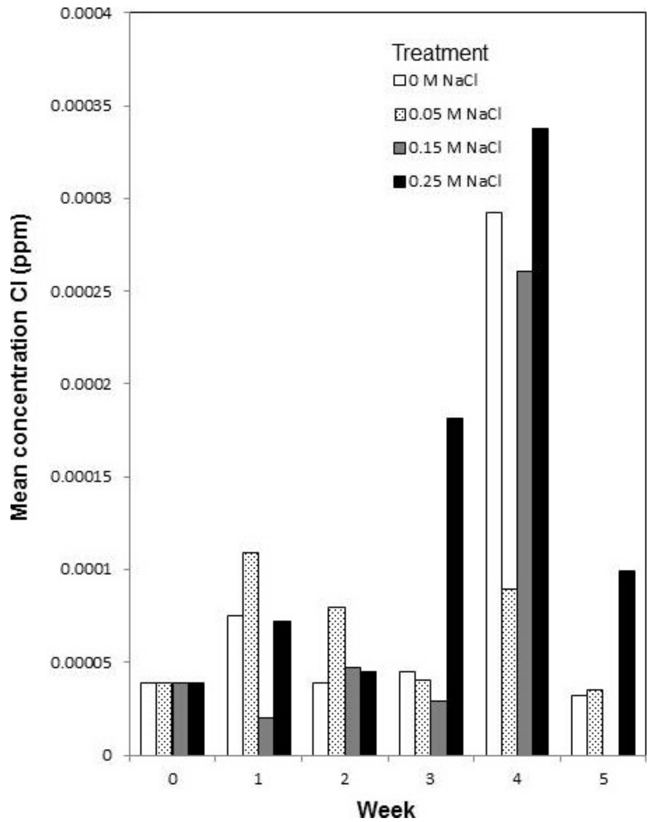

Fig. 4. Chloride levels recorded in foliage *Rudbeckia fulgida* var. *speciosa* 'Goldsturm' treated with 0, 0.5, 0.15 and 1.25 M of NaCl solution to the media over 6 weeks.

3). Visually, the plants showed minimal necrosis until week 5, and plants were essentially dead at week 6. Although Na^+ levels were increasing in the plant, damage wasn't displayed until the levels in the plant tissue reached 0.25 ppm. It is possible that *Rudbeckia* possesses a mechanism for managing Na^+ within its tissues, either through active pumps or sequestration within the cell vacuole. Further testing needs to be done to determine the exact mechanism.

Chloride levels in *Rudbeckia* remained relatively constant until week 4, when they spiked and then dropped off in week 5 (Fig. 4). During week 5 the plant displayed significant visual damage. It is unknown if Cl^- or Na^+ was the cause of the majority of the damage. Future studies could shed light on this issue.

Three-way ANOVA showed the means, the paired means and the three-way interaction are all significant ($p < 0.01$). This suggests all three variables (species, NaCl level, and time) interact to influence plant growth and development. Each species reacted differently to NaCl treatments; each week was significantly different from the previous weeks; and, each treatment was significantly different from other treatments. This confirms visual inspection of the plants, that each species showed symptoms at different times and to different degrees, and these symptoms changed with NaCl levels and also changed over the duration of the experiment.

Correlating this information to the digital images shows that during week 6, most of the *Rudbeckia* died or had so little pigmentation as to be dead. *Heuchera* continued to look damaged, yet produced new growth through the end of the experiment. Although plants were visually symptomatic, they were able to maintain some level of metabolic activity. However, until this point, the plants appeared to have few symptoms, especially when compared with the other two species, where symptoms developed within 2 to 3 weeks of the start of treatment. This indicates that both *Heuchera* and *Rudbeckia* have some mechanism for metabolizing excess Na^+ and Cl^-, at least for the short term.

Future studies with *Rudbeckia* and *Heuchera* could elucidate these mechanisms and perhaps the genetics behind the mechanism. In addition, understanding which of the two ions was more damaging would be helpful in understanding plant salt tolerance. Additional studies could include other deicing chemicals such as CaCl and MgCl. These two chemicals are often substituted for NaCl and indeed are often labeled as more environmentally friendly than NaCl.

This 6-week greenhouse study with three species would indicate that *Rudbeckia* displays good salt tolerance to 0.25 M NaCl in the short term; *Heuchera* also displays good salt tolerance, though shows increased symptoms; and *Aquilegia* is not at all salt tolerant. It is possible that dormant plants would have responded differently, however, as plants are still metabolically active during dormancy and nutrients are still taken into the plant, the results can be extrapolated to dormant conditions. *Rudbeckia* and *Heuchera* would be expected to survive longer under saline conditions, then *Aquilegia*. Indeed the statement could be made that *Aquilegia* should not be planted in areas where NaCl is utilized as a deicing chemical.

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