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Effects of Salinity and Freezing on *Acer platanoides*, *Tilia cordata*, and *Viburnum lantana*[1]

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Abstract

The purpose of this study was to evaluate the effects of NaCl and freezing temperatures on dormant lateral buds of *Acer platanoides* L. (Norway maple), *Tilia cordata* Mill. (littleleaf linden), and *Viburnum lantana* L. (wayfaringtree viburnum). The role of bud morphology was also examined. Buds were exposed to three NaCl concentrations [0, 2000, or 16,000 mg/liter (0, 2000, 16,000 ppm)] and eleven freezing temperatures [4, –4, –8, –12, –16, –20, –24, –28, –32, –36, and –40C (39, 25, 18, 10, 3, –4, –11, –18, –26, –33, –40F)] in November 2001 and January and March 2002. Electrolyte leakage and visual ratings of outer and inner bud tissue browning were used to assess injury. Bud injury generally increased as NaCl concentrations increased and temperatures decreased. Buds exposed to NaCl and freezing temperatures had greater electrolyte leakage than buds exposed to freezing temperatures alone. Norway maple buds had the highest electrolyte leakage, followed by wayfaringtree viburnum, and littleleaf linden in response to freezing temperatures and NaCl. The naked buds of viburnum had significantly more inner tissue browning than the scaled buds of maple and linden in response to freezing temperatures and NaCl in January 2002. Wayfaringtree viburnum exhibited increased tissue injury in response to NaCl and low temperature treatments in March 2002.

Index words: bud morphology, cold hardiness, dormancy, electrolyte leakage, NaCl, salt tolerance.

Species used in study: *Acer platanoides* L. (Norway maple); *Tilia cordata* Mill. (littleleaf linden); *Viburnum lantana* L. (wayfaringtree viburnum).

Significance to the Nursery Industry

Each winter deicing salts and freezing temperatures cause injury and death to landscape plants, resulting in replacement costs and aesthetic losses. Consequently, plants selected for winter hardiness should tolerate exposure to both low temperatures and salinity. By midwinter, naked buds of wayfaringtree viburnum were less resistant to freezing temperatures and NaCl than scaled buds of Norway maple and littleleaf linden. All species became susceptible to freezing and NaCl treatment injury in March. Landscape managers and horticulturists should select species with adequate cold hardiness, and use caution when planting species possessing naked bud morphology in areas exposed to salt spray. Salt applications near vegetation should be reduced or avoided in late winter and spring due to increased bud susceptibility to salt injury.

Introduction

Deicing salts are used to melt ice on roadways, walkways, and parking structures for public safety purposes. Sodium chloride (NaCl), or rocksalt, is commonly used because of its effectiveness, availability, and comparatively low cost. Deposition of salt spray on plants from traffic inhibits budbreak and causes stem dieback and plant death in severe cases (7, 16). Injurious effects of salt spray deposition have been noted on roadside plants up to 378 m (1,240 ft), and increased sodium levels have been found in plant tissue as far as 1,081 m (3,340 ft) (10). As urbanization and roadway expanses continue, an increasing number of plants will be exposed to salt spray.

Landscape plants located in northern climates suffer repeated exposure to freezing temperatures. The formation of ice crystals causes injury and death in plant tissues (31). A plant's capacity to either avoid or tolerate ice formation governs its ability to survive (21). Woody ornamentals vary in their resistance to freezing injury. Numerous studies recognize cold hardiness as an important selection criterion for survival (5, 14, 18), but only a few studies have incorporated salinity into cold hardiness testing.

With regard to the interaction of salt and freezing temperatures, Sucoff and Hong (26) and Sucoff et al. (27) noted injury of cold hardy woody species adjacent to roadways and questioned whether NaCl altered cold hardiness. The experimental evidence suggested that salt spray decreased cold hardiness in twigs of *Syringa vulgaris* L. (common lilac) and *Fraxinus pennsylvanica* Marsh. 'Marshall's Seedless' (Marshall's seedless green ash). Conversely, Percival and Fraser (20) used chlorophyll fluorescence to test the foliage of six genotypes of *Crataegus* L. (hawthorn). Although combined effects of salt and freezing treatments were greater than salt treatments alone, interactions between freezing temperatures and salinity were not significant. Further interpretations regarding the interactions of salinity and freezing temperatures are needed to determine if integrating salinity into cold hardiness screenings is justified.

Salt tolerance and cold hardiness of woody ornamentals can change as the winter season progresses. Hofstra and Lumis (9) reported that sodium and chloride ion concentra-

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tions did not start accumulating in dormant twigs of roadside trees until mid-February. Similarly, Headly and Bassuk (7) found that soil salt uptake was generally low in mid-winter months, but higher in October, March, and April. Landscape plants exhibit a comparable response to freezing temperatures. Shortened daylengths and decreasing temperatures in fall promote the onset of cold acclimation in many trees and shrubs (6). Numerous genera exhibit some degree of cold acclimation by late September (19). Cold hardy species commonly show maximum resistance to freeze injury between mid December and early January (14, 18, 19, 22). Dehardening occurs as spring approaches (19, 22). Prior to budbreak, the ability to resist freezing injury is often lost (1).

Dormant buds are good indicators of freezing and salt tolerance because buds are present at the time of exposure to winter stresses, and are often more susceptible to freezing and salt injury than other plant tissues (2, 4, 22). Morphological factors such as bud size and the nature of scales often affect salt uptake (8). Plants with resinous buds or buds submerged in the twig tend to accumulate salt slowly (25), whereas naked buds (lacking scales) are susceptible to salt spray (16). Bud characteristics also impact a plant's resistance to freezing temperatures. Bud scales protect against primordial freezing in some species (11, 12, 13). Knowledge of the relationship between a bud's characteristics and its mechanism of cold hardiness and salt tolerance could be important information for appropriate plant selection.

Therefore, the objectives of this study were to investigate the effects of NaCl and freezing temperatures on dormant buds. Norway maple, littleleaf linden, and wayfaringtree viburnum are common urban landscape plants in the northern United States, therefore information regarding the cold hardiness and salt tolerance of these species is useful. Norway maple [mature height of 12.2–18.3 m (40–60 ft)] and littleleaf linden [mature height of 18.3–24.4 m (60–80 ft)] are shade trees frequently used in street settings, parks, and residential and commercial landscapes. Wayfaringtree viburnum [mature height of 2.4–4.6 m (10–15 ft)] is a flowering ornamental shrub used for hedges, screens, and shrub borders. Species were selected for their similar range of cold hardiness and differing bud characteristics. Norway maple and wayfaringtree viburnum are USDA cold hardiness zone 4 species [–28 to –34C (–20 to –30F)], and littleleaf linden is a zone 3b species [–34 to –37C (–30 to –35F)] (3). Norway maple buds are small, 3–6 mm (0.125–0.25 in) long, and have numerous, tightly-arranged bud scales. Littleleaf linden buds are 6–9 mm (0.25–0.33 in) long, and have two to three bud scales. Wayfaringtree viburnum buds are large 13–25 mm (0.5–1.0 in) long, and have a naked bud morphology that entirely lack scales. The effects of NaCl and freezing temperatures, along with the role of bud morphology were examined for each species from winter to early spring.

Materials and Methods

Plant materials. Nodal samples containing lateral vegetative buds were collected from Norway maple, littleleaf linden, and wayfaringtree viburnum. Samples were collected at McKay Nursery in Waterloo, WI (42° 57'N). One lateral branch node was taken per plant. A total of 198 plants were sampled for electrolyte leakage testing and a total of 108 plants were sampled for visual observation testing at each collection. In addition, eight branch samples from each species were placed in vases with tap water for three weeks at

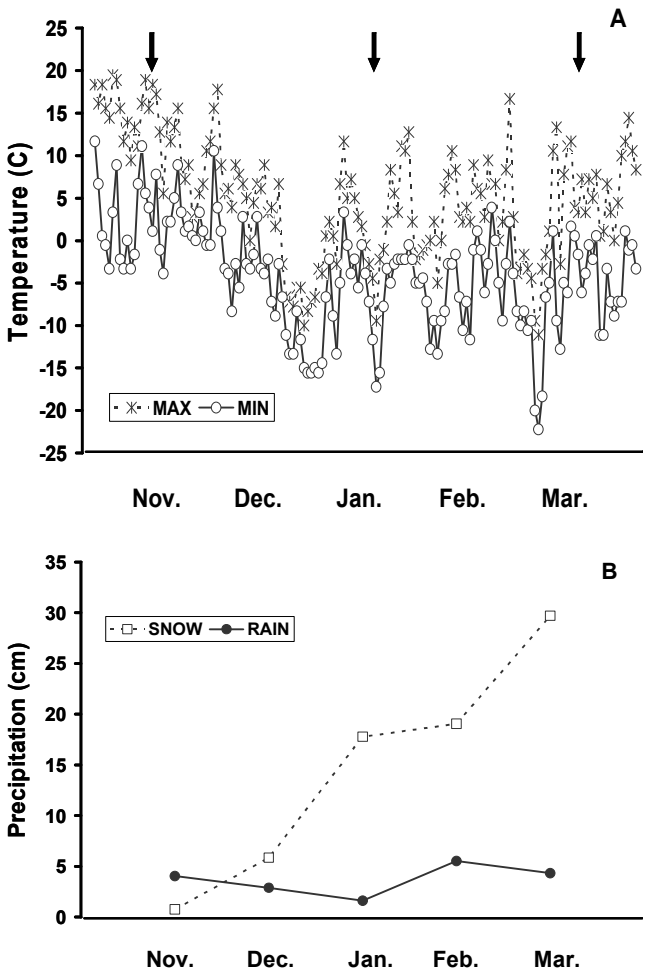

Fig. 1. Daily maximum and minimum air temperatures (A) and total monthly snowfall and rainfall (B) from November 2001 to March 2002 in Madison, WI (43°7' N latitude, 89°20' W longitude). Arrows indicate collection dates.

ambient temperature [21C (70F)] under a fluorescent light for 16 h/d to evaluate if budbreak occurred.

Sodium chloride was used in this study, as it is a standard deicing chemical used on roads. Preliminary research was conducted and the lowest concentrations of NaCl that induced noticeable bud injury were chosen for the study. The experimental design within each sampling date was a completely randomized design with a factorial arrangement of treatments and subsampling (individual buds) repeated three times throughout the 2001–2002 winter season on the following dates: November 13, January 11, and March 22 (Figs. 1A–B). Treatments consisted of buds from three species (Norway maple, littleleaf linden, and wayfaringtree viburnum), three salt concentrations [0, 2000, 16,000 mg/liter (0, 2000, 16,000 ppm) A.C.S. certified crystalline NaCl], and eleven temperatures [4, –4, –8, –12, –16, –20, –24, –28, –32, –36, and –40C (39, 25, 18, 10, 3, –4, –11, –18, –26, –33, and –40F)].

Freezing procedure. Nodal samples were rinsed in deionized water for 30 s, placed into 60 ml (2.0 fl oz) vials containing one of three salinity solutions: 0, 2000, 16,000 mg/

liter (0, 2000, 16,000 ppm) NaCl, and shaken on an Innova® 2100 platform shaker (New Brunswick Scientific Co., Inc., Edison, NJ) at 140 rpm for 20 hr at 4C (39F). Samples were removed from solutions and rinsed in deionized water for 1 min to remove exterior salinity. Six bud samples from each species/NaCl treatment were placed on 15 × 15 cm (6 × 6 in) aluminum foil squares lined with white cheesecloth, and nucleated with several milliliters of tap water and ice chips. Controls were placed in a refrigerator at 4C (39F) with the remaining packets placed in a programmable, air-circulating deep freezer (ScienTemp 51–12, Adrian, MI), and maintained at –2C (28F) overnight. The following day, the temperature was decreased at a rate of 4C (7F) per hour down to –40C (–40F). Temperature reductions were performed by the programmed computer within the freezer. Treatment samples were removed after each designated temperature treatment. After freezing, bud injury was determined using electrolyte leakage and by visual observation using methods similar to those reported by Shirazi and Fuchigami (24).

Electrolyte leakage. Nine packets (1 per species/NaCl treatment) were removed at 4C (7F) intervals for the eleven test temperatures. Packets used in electrolyte leakage tests were placed in a refrigerator overnight at 4C (39F). One bud was cut from each node and placed into a 25 ml (0.84 fl oz) vial containing 8 ml (0.27 fl oz) of deionized water. Vials were shaken for 20 hr at ambient temperature to facilitate electrolyte leakage from injured tissues. Initial electrical conductivity measurements were recorded for each vial using an Acromet AR20 electrical conductivity meter (Fisher Scientific, Chicago, IL). Vials were then immersed in a hot water bath (Fisher Isotemp, Indiana, PA) at 80C (176F) for 1 hr to induce cell rupture. The vials were again placed on the Innova 2100 platform shaker for 20 hr at ambient temperature, and final conductivity was measured for each vial. Percent electrolyte leakage for each sample was calculated as: (initial conductivity / final conductivity) × 100.

Visual browning. Nine packets (1 per species/NaCl treatment) were removed at 8C (14F) intervals [4, –8, –16, –24, –32, –40C (39, 18, 3, –11, –26, –40F)] for a total of six test temperatures. Six temperatures instead of eleven were used due to time constraints and limited plant material. Buds were then placed in 15 cm (5.9 in) Petri dishes containing filter paper, moistened with deionized water, sealed with parafilm, and incubated in the dark in coolers at 21C (70F) for 10–12 d. One bud per node was then removed, cut longitudinally, and examined under a dissecting microscope for tissue browning (6 buds per treatment). Buds were rated on outer tissue browning, inner (primordial) tissue browning, and fungal growth. Outer tissue of scaled buds was defined as the outer, lignified budscales and immediately interior non-lignified tissue. Outer tissue for naked buds included the tomentose surface covering of primordial leaves and the outer edge of leaf primordia. Inner tissue was defined as any bud tissue located interior to outer bud tissue. Browning was rated on a scale of 1–5: 1 = green/yellow tissue (living tissue), 2 = detection of light colored brown tissue (< 10%), 3 = light colored brown tissue (>10%), 4 = medium colored brown tissue (100%), 5 = very dark brown/black tissue (100% necrotic tissue). Fungal growth was rated on a scale of 1–3: 1 = no fungal growth, 2 = detection of fungal growth (5–10%), 3 = severe fungal growth (>10%).

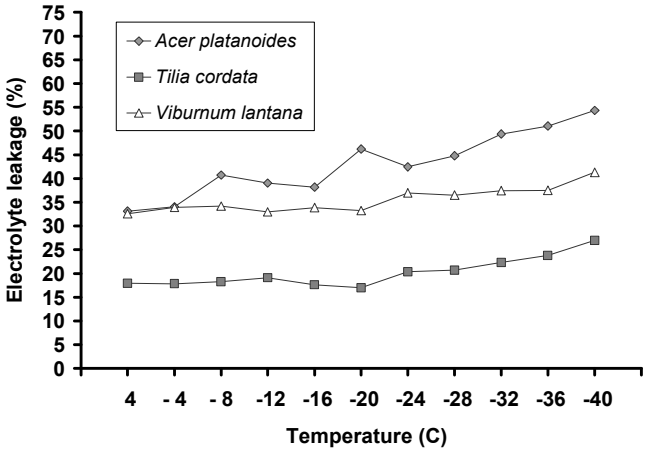

Fig. 2. Effect of temperature and species on percent electrolyte leakage of November 2001 collected buds combined over three NaCl concentrations. Standard error of the treatment mean difference = 2.20. Each point represents the mean of 18 buds. Quadratic equations for the species by temperature response: *Acer platanoides,* $Y = 0.003x^2 + 0.37x + 33.93$; *Tilia cordata,* $Y = 0.008x^2 + 0.26x + 33.48$; *Viburnum lantana,* $Y = 0.01x^2 + 0.17x + 19.24$; where Y = electrolyte leakage, and X = temperature.

An arcsin transformation of percent electrolyte leakage data was conducted, and results were similar to the untransformed data, therefore the untransformed data are presented. The experimental units were the foil packets containing six bud subsamples. By virtue of design, it was not possible to separate the three-factor interaction (species × salt × temperature) from packet to packet variability, therefore, the interaction was treated as the error term for a conservative analysis. Preliminary statistical analysis revealed that the three-factor interaction was insignificant. Data were then subjected to analysis of variance procedures and regression analysis. All mean separations were performed pairwise t-test comparisons at $P \leq 0.05$. Analysis was conducted using PROC MIXED, SAS Software Version 8 (SAS Institute Inc., Cary, NC).

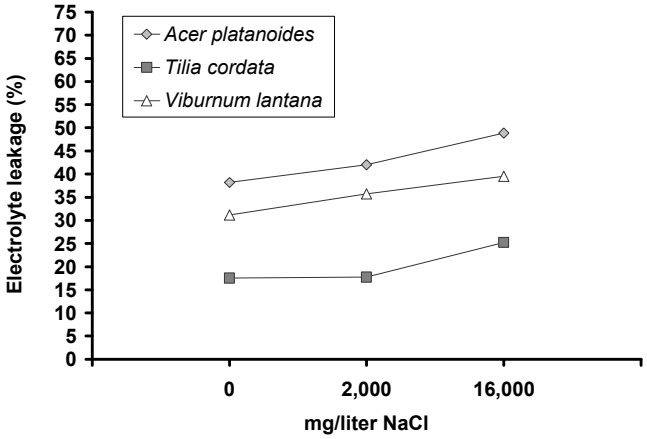

Fig. 3. Effect of NaCl and species on percent electrolyte leakage of November 2001 collected buds combined over eleven temperatures. Standard error of the treatment mean difference = 1.15.

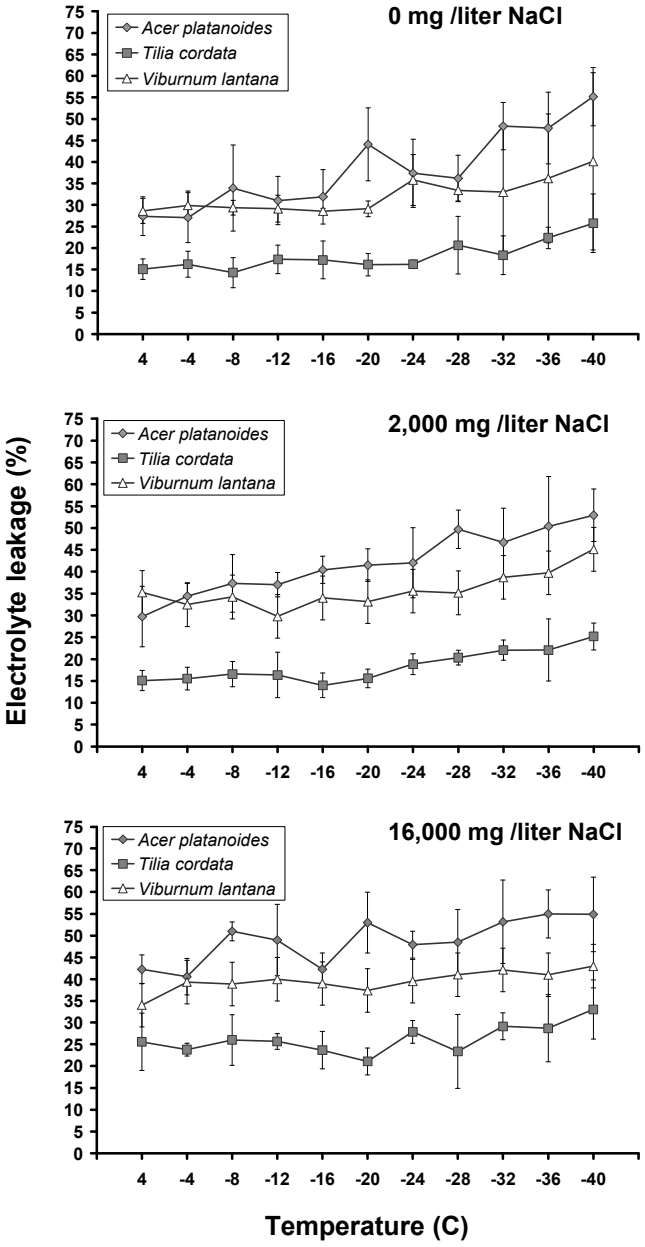

Fig. 4. Effect of temperature and species on percent electrolyte leakage of November 2001 collected buds for the three NaCl concentrations. Vertical bars represent ± SD of subsampling error. Each point represents the mean of 6 buds. Quadratic equations for the species by temperature response based on data pooled across NaCl treatments: *Acer platanoides,* $Y = 0.003x^2 + 0.37x + 33.93$; *Tilia cordata,* $Y = 0.008x^2 + 0.26x + 33.48$; *Viburnum lantana,* $Y = 0.01x^2 + 0.17x + 19.24$; where Y = electrolyte leakage, and X = temperature.

Results and Discussion

Electrolyte leakage. Patterns for electrolyte leakage were similar for November, January, and March (2001–2002), therefore, only November 13, 2001, data are presented (Figs. 2–4). Electrolyte leakage from buds increased as NaCl concentration increased and temperatures decreased (Figs. 2–4). Mechanical injury from ice formation and Ca^{2+} ion displacement from excess Na^{+} ions disrupt plasma membranes, possibly accounting for the increased electrolyte leakage measurements (28).

Electrolyte leakage results showed significant differences in injury among species in response to freezing temperatures ($P \leq 0.001$) and NaCl ($P \leq 0.05$) (Figs. 2, 4). Buds of littleleaf linden ranked lowest in electrolyte leakage, followed by wayfaringtree viburnum, then Norway maple to freezing temperatures and NaCl respectively (Figs. 2, 3). Buds of Norway maple, littleleaf linden, and wayfaringtree viburnum had a significant quadratic response to temperature ($P \leq 0.05$), showing an increased rate of injury, especially at –20 to –24C (–4 to –11F). Bud scales of Norway maple did not protect against electrolyte leakage, as Norway maple buds experienced higher electrolyte leakage than the other species, especially at temperatures below –24C (–11F). Low to moderate electrolyte leakage in control buds 4C (39F) was likely a result of environmental stresses (i.e., low temperatures and wind desiccation) prior to bud collection (Fig. 1A), and stress from the collection itself.

Interactions between NaCl and temperature were not significant for any of the three collection periods (data not shown). Buds subjected to both NaCl and freezing treatments had greater electrolyte leakage than buds subjected to freezing temperatures alone, however the increased electrolyte leakage from NaCl remained constant across all temperatures (data not shown). The occurrence of increased electrolyte leakage at warmer temperatures in NaCl treated buds would have indicated the existence of a salt-induced reduction of cold hardiness. However, the lack of interaction between NaCl and freezing temperatures suggested that each factor contributed to injury independently. Sodium chloride did not influence the effects of freezing temperatures and vice versa. Thus, results from this study do not support pre-

Table 1. Effect of temperature on outer and inner tissue browning of November 2001 and January 2002 collected buds combined over three NaCl concentrations and three species.

Temp. (C)	Tissue browning rating	
	Outer	Inner
	November 13, 2001	
4	2.0[z,y]a[x]	1.7a
–8	2.1a	1.9ab
–16	2.7b	2.4b
–24	3.4c	3.2c
–32	3.7cd	3.6cd
–40	3.9d	3.9d
	January 11, 2002	
4	2.0a	1.7a
–8	2.8b	2.5b
–16	3.2bc	2.8b
–24	3.3c	3.0b
–32	4.2d	3.7c
–40	4.7e	4.2d

[z]Represents the mean of 54 buds for each temperature.

[y]Rating scale: 1 = green/yellow tissue, 2 = detection of light colored brown tissue (<10%), 3 = light colored brown tissue (>10%), 4 = medium colored brown tissue (100%), 5 = very dark brown to black tissue (100%).

[x]Mean separation within columns by pairwise t-tests, $P \leq 0.05$.

Table 2. Effect of NaCl and species on outer tissue browning of January 2002 collected buds combined over six temperatures.

Species	NaCl (mg/liter)		
	0	2000	16,000
Acer platanoides	3.1[zy]ab[x]	3.5a	3.1a
Tilia cordata	2.7a	3.4a	3.0a
Viburnum lantana	3.4b	3.4a	4.1b

[z]Represents the mean of 36 buds for each species.

[y]Rating scale: 1 = green/yellow tissue, 2 = detection of light colored brown tissue (<10%), 3 = light colored brown tissue (>10%), 4 = medium colored brown tissue (100%), 5 = very dark brown to black tissue (100%).

[x]Mean separation within columns by pairwise t-tests, $P \leq 0.05$.

vious reports that NaCl *caused* decreased cold hardiness in highway-grown plants (27).

It is feasible that salt spray could increase cold tolerance by lowering the freezing point of water remaining in dormant buds, or decrease cold hardiness by weakening cell membranes that function as barriers to intracellular ice propagation. Future work using infrared video thermography to monitor ice nucleation temperatures and propagation patterns could provide further insight regarding interactions between salt and low temperatures (32). Regardless of whether significant interactions between NaCl and freezing temperatures exist, deleterious effects of NaCl and freezing temperatures were additive. Consequently, there is a demonstrated need for winter hardiness screenings of potential urban landscape or roadside species to include both salinity and freezing temperature treatments.

Visual observations. Visual observations were conducted to determine whether leakage was occurring from the outer tissue or inner tissue. Tissue discoloration, fungal growth, and a water-soaked appearance were indicators of freezing temperature and NaCl injury. Partitioning visual observation ratings into outer and inner tissue browning was useful in determining the location of injury within the bud. Injured bud tissue turned brown to dark brown after the 10–12 day incubation period. Patterns of visual observation data varied over time. Therefore, November, January, and March data are presented (Tables 1–5). Fungal growth provided little information, but increased with the occurrence of outer and inner browning (data not shown).

In the week prior to the November 2001 collection, minimum daily air temperatures ranged from –3 to 9C (26 to 48F), and maximum daily air temperatures ranged from 11 to 19C (52 to 67F) (Fig. 1A). Outer and inner tissue interactions between species and NaCl, species and temperature, and NaCl and temperature were not significant in November (data not shown). Differences in outer and inner tissue browning were both significant among temperature treatments ($P \leq 0.0001$) for November and January collected buds (Table 1). Tissue browning increased as temperatures decreased. Outer tissue browning was typically more severe than inner tissue browning. Buds appeared acclimated as tissue browning was low to moderate in all species at temperatures at or above –24C (–11F). Shortened daylengths (30) and low minimum night temperatures (6) were adequate for the onset of cold acclimation, despite the occurrence of unseasonably warm daytime temperatures.

Injury from freezing temperatures is characteristically the result of ice crystal formation within cells (31); hence the absence of severe tissue browning in November collected buds suggested that little or no intracellular ice formation occurred. Ice formation may have been localized to intercellular spaces, or was possibly avoided by means of a supercooling mechanism, which maintained water in a liquid state during exposure to below-freezing temperatures (28). Higher ratings for tissue browning were expected around –32C (–26F) as Norway maple and wayfaringtree viburnum are cold hardy only to –28 to –34C (–20 to –30F), and littleleaf linden to –34 to –37C (–30 to –35F). Ratings for tissue browning were high at –40C (–40F) due to spontaneous ice nucleation that occurs at a temperature around –40C (–40F) (1, 28).

Interactions between NaCl and temperature remained insignificant in January (data not shown). Species differences in outer tissue browning in response to NaCl were evident ($P \leq 0.05$) (Table 2). Large, naked buds of wayfaringtree viburnum exhibited greater outer tissue browning than smaller, scaled buds of Norway maple and littleleaf linden at the 16,000 mg/liter (ppm) NaCl treatment. Lumis et al. (17) similarly reported that naked buds of *Rhamnus frangula* L. (glossy buckthorn) were more injured by salt spray than *Rhamnus catharticus* L. (common buckthorn), a scaled species in the same genus. Salt injury has been correlated with the accumulation of Na+ and Cl– ions in plant tissues (9, 27). Morphological factors such as bud size and the nature of scales can affect salt uptake (27). Wayfaringtree viburnum buds were susceptible to NaCl accumulation, partially due to their lack

Table 3. Effect of species on inner tissue browning combined over three NaCl concentrations and eleven temperatures of January 2002 collected buds.

Species	Browning rating
Acer platanoides	2.5[zy]a[x]
Tilia cordata	3.0b
Viburnum lantana	3.4c

[z]Represents the mean of 36 buds for each species.

[y]Rating scale: 1 = green/yellow tissue, 2 = detection of light colored brown tissue (<10%), 3 = light colored brown tissue (>10%), 4 = medium colored brown tissue (100%), 5 = very dark brown to black tissue (100%).

[x]Mean separation within columns by pairwise t-tests, $P \leq 0.05$.

Table 4. Effect of species on outer tissue browning of March 2002 collected buds combined over three NaCl concentrations and eleven temperatures.

Species	Browning rating
Acer platanoides	4.9[zy]a[x]
Tilia cordata	4.7b
Viburnum lantana	5.0a

[z]Represents the mean of 108 buds for each species.

[y]Rating scale: 1 = green/yellow tissue, 2 = detection of light colored brown tissue (<10%), 3 = light colored brown tissue (>10%), 4 = medium colored brown tissue (100%), 5 = very dark brown to black tissue (100%).

[x]Mean separation within column by pairwise t-tests, $P \leq 0.05$.

Table 5. Effect of temperature and species on inner tissue browning on March 22, 2002 collected buds combined over three NaCl concentrations.

	Temp. (C)					
Species	**4**	**–8**	**–16**	**–24**	**–32**	**–40**
Acer platanoides	3.6[z,y]a[x]	4.1a	4.1a	4.6a	4.5a	4.8a
Tilia cordata	4.8b	4.2a	4.3a	4.9a	5.0a	4.8a
Viburnum lantana	5.0b	4.8b	5.0b	5.0a	4.9a	5.0a

[z]Represents the mean of 18 buds for each species.

[y]Rating scale: 1 = green/yellow tissue, 2 = detection of light colored brown tissue (<10%), 3 = light colored brown tissue (>10%), 4 = medium colored brown tissue (100%), 5 = very dark brown to black tissue (100%).

[x]Mean separation within columns by pairwise t-tests, $P \leq 0.05$.

of scales and large, bud surface area. Bud scales and cuticular wax on scale surfaces may have inhibited NaCl penetration, accounting for the comparatively lower surface injury in Norway maple and littleleaf linden buds (8, 17).

Species differences in inner tissue browning were significant across all temperature and NaCl treatments in January ($P \leq 0.0001$) (Table 3). Norway maple had the lowest ratings for inner tissue browning, followed by littleleaf linden, and wayfaringtree viburnum. Scales on buds of Norway maple and littleleaf linden may have delayed freezing temperature and NaCl injury to inner, primordial tissues.

According to the National Weather Service, temperatures preceding the January 11, 2002 bud collection were warmer than average. Daily minimum temperatures ranged from –13 to –1C (8 to 31F) and daily maximum temperatures ranged from 1 to 12C (33 to 53F) (Fig. 1A). The occurrence of these above-average temperatures may have affected the cold hardiness of buds. Fluctuations in temperatures are capable of causing corresponding fluctuations in the cold hardiness (5); however, acclimation and deacclimation are often controlled by endogenous seasonal rhythms (6, 31), thereby maintaining bud cold tolerance to mid-winter temperature fluctuations. January collected buds appeared to maintain cold tolerance as ratings for tissue browning were similar to November data. Outer and inner tissue browning were highly correlated ($R^2 = 0.91$), however, Norway maple buds exhibited consistently lower inner tissue browning compared to outer tissue browning. Higher injury in bud scales and outer tissue suggested that buds of Norway maple withstood freezing temperatures via a mechanism termed extra-organ freezing. This type of freezing results in water translocation from inner, primordial tissues into the bud scales, which enhances primordial supercooling by increasing the cell solute concentration (13, 28). Freezing readily occurs in the bud scales due to the high water content. The ability for the primordia to supercool and the containment of ice crystals within bud scales is critical for bud survival. Kang et al. (14) found that budbreak did not occur when the primordia of persimmon (*Diospyros kaki* L.) buds were injured. Patterns of tissue browning in littleleaf linden and wayfaringtree viburnum buds were less pronounced, and the mechanism(s) used to resist freezing injury were not identified by visual observations alone.

Severe injury occurred in all treatments of the March 2002 collected buds (Tables 4, 5). In addition to severe tissue browning, many buds exhibited a water-soaked appearance. Minimum daily temperatures ranged from –11 to –1C (12 to 31F) and maximum daily temperatures ranged from 1 to 7C (33 to 45F) in the week prior to the March 22 collection (Fig. 1A). Budbreak occurred in the branch samples of all species within two weeks of the collection (data not shown), indicating a late stage of dormancy termed ecodormancy (15). Differences in species ratings for outer tissue browning across freezing temperatures and NaCl, and species inner tissue browning in response to freezing temperatures were significant among species ($P \leq 0.05$ for both) (Tables 4, 5), however, visually observed injury was severe in all treatments. Buds from all three species exhibited severe outer tissue browning in response to NaCl and freezing temperatures combined ($P \leq 0.05$) (Table 4). With the exception of Norway maple buds at 4C (39F), buds from all three species also exhibited severe inner tissue browning in response to temperature (Table 5). Numerous studies have noted decreased cold hardiness of woody ornamentals in spring (14, 22, 30). As spring and warmer temperatures approach, the capacity of buds to supercool is lost, and the presence of freezable water increases as vascular systems resume water uptake (1, 5). Rinne et al. (23) found that the water content increased in buds of downy birch (*Betula pubescens* Ehrh.) prior to bud burst in spring. Inner tissue browning of scaled species in this study probably occurred as a result of freezing injury from water translocation back into primordial tissue from bud scales (5). State of dormancy, exposure to warm temperatures, and morphological changes during bud development also may have affected cold hardiness (1, 29, 30). In addition, warmer temperatures and increased physiological activity of plants in March may have increased Na and Cl ion intake, resulting in lethal injury (7). Regarding outer tissue browning, the interaction between NaCl and temperature was insignificant (data not shown), however, the interaction between these two variables for inner tissue browning was significant ($P \leq 0.05$). However, with the exception of moderate injury of buds at the –8C (18F) by 2,000 mg/liter (2,000 ppm) NaCl treatment, buds from all treatments exhibited severe inner tissue browning (data not shown).

Results herein indicate that salt spray contributes to winter injury, however, it does not affect cold hardiness of dormant buds of woody ornamentals. Structural features and physiological processes occurring in buds appear to work together as an interconnected system that determines tissue sensitivity to NaCl and freezing temperatures. Species possessing buds with naked morphology appear more susceptible to freezing temperatures and NaCl than species with scaled buds. Bud tissue injury from freezing temperatures and NaCl applications is greatest in spring, as buds are coming out of dormancy. Combining electrolyte leakage tests and

visual observation tests provided a more detailed representation of bud hardiness and salt tolerance than measuring one variable alone. Norway maple experienced high electrolyte leakage; however, visual observations showed that the majority of injury occurred in the outer bud tissues, thereby preserving the sensitive, inner primordial tissues. Results should be interpreted cautiously. Bud response to salt can be influenced by factors including: genetic differences, type of exposure (soil salt or salt spray), exposure intensity, biotic or abiotic, and climatological/seasonal factors. Bud salt tolerance and cold hardiness may or may not reflect whole plant tolerance of a given species.

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