Deicing Salt Spray Injury in Selected \textit{Pinus} spp.\textsuperscript{1}

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Abstract. Needle surface characteristics and NaCl penetration rates were compared and related to deicing salt spray injury for resistant Austrian pine, \textit{Pinus nigra} Arnold, and susceptible Eastern white pine, \textit{Pinus strobus} L. Stomata in longitudinal rows separated by parallel ridges characterized needle surfaces of both species; surface fine structure was free of trichomes or other recognizable structures. \textit{Pinus nigra} in comparison to \textit{P. strobus} had greater surface area (3.64 cm\textsuperscript{2}/needle vs. 1.87 cm\textsuperscript{2}/needle) and larger quantities of epicuticular wax 183 \mu g/cm\textsuperscript{2} vs. 75 \mu g/cm\textsuperscript{2}). Thin-layer chromatography indicated no distinct differences in epicuticular wax chemistry. Surface wettability, measured by contact angle, was similar. Retention of an aqueous solution was similar when needles were attached to fascicles. Penetration of \textsuperscript{36}Cl was significantly greater in needles of \textit{P. nigra} on a surface area basis (\textit{P. nigra} = 9,839 dpm/cm\textsuperscript{2}, \textit{P. strobus} = 3,503 dpm/cm\textsuperscript{2}). No differences in penetration occurred when expressed on a fresh weight basis. Electron microprobe analysis substantiated a greater penetration of Na\textsuperscript{+} and Cl\textsuperscript{−} in needles of \textit{P. nigra}. Levels of Na\textsuperscript{+} were higher than Cl\textsuperscript{−} in both species. Triphenyl tetrazolium chloride studies indicated greater sensitivity to increasing concentrations of NaCl in needles of \textit{P. strobus} and \textit{P. nigra}. Differences in species sensitivity appears to be related to protoplasmic sensitivity rather than to differences in penetration of Na\textsuperscript{+} and Cl\textsuperscript{−} ions.

Foliar injury in Eastern white pine from deicing salt spray injury is well documented (2, 11, 13, 16, 24, 36); equally numerous are observations that Austrian pine is resistant to salt spray (11, 16, 24, 36). Injured needles exhibit chlorosis at the tips, followed by bronzing and ultimate browning.

Foliar penetration of applied compounds has been extensively investigated (18, 31, 38, 39). Although not specifically related to deicing salt spray injury, evidence exists for penetration of foliar applied Na\textsuperscript{+} (4, 20, 26) and Cl\textsuperscript{−} (4, 39, 40). According to Bukovac (5), pathways for absorption of water soluble or ionic compounds are unclear, yet lipid soluble compounds are thought to be absorbed and diffused through lipidic components of the cuticle.

To induce a given physiological response, a foliar applied substance must come in contact with and be retained by the plant surface, penetrate the cuticle, and be translocated to an active site for response induction (3). Deicing salt spray injury in coniferous species has been attributed to high levels of internal Na\textsuperscript{+} and Cl\textsuperscript{−} (2, 13, 16, 19, 33), while resistance has been associated with thick waxy cuticles excluding toxic ions (16, 24). Bowers and Hesterburg (2) postulated that salt coatings on needles act as non-selective contact herbicides, creating osmotic stress, subsequent water loss, and injury.

To understand the dynamics of penetration, it is important to characterize aspects of surface morphology. The plant cuticle, the prime barrier to penetration of foliar applied substances, is composed of a cutin matrix and is separated from the underlying epidermal cell walls by a layer of pectinaceous material. Deposited on the outer surface of the cutin matrix is a layer of epicuticular wax (28).

Scanning electron microscopy (14, 21, 22) has been used to observe conifer surface fine structure. Reports on epicuticular wax quantities are limited, yet excellent survey information has been presented by Herbin and Sharma (15) on cuticular wax chemistry, specifically \omega-hydroxy acids present in estolide (short chain hydroxy-fatty acids) fractions. Surface wettability has been determined by contact angle measurements and has been reported in relation to water loss (21, 22).

This research characterizes needle surfaces of \textit{P. nigra} and \textit{P. strobus} and their relation to salt spray resistance or injury. Coupled with these investigations were studies on penetration and protoplasmic viability to further assist in determining differences in sensitivity.

Materials and Methods

\textit{General needle characteristics.} One-year old needles were randomly selected from trees growing under field conditions at the Horticulture Research Center at Michigan State University. Needle characteristics of length (cm), Surface area (cm\textsuperscript{2}), volume (cm\textsuperscript{3}) and fresh weight/unit surface area (\mu g/cm\textsuperscript{2})
were determined. Surface area (SA) was calculated using formulas of geometric shapes modeling needle morphology. Formulas used were that of a half cylinder for *P. nigra* L. (SA = \( \pi r^2 + 2rl \)), where \( r = \) radius and \( l = \) length) and a prism having equilateral sides for *P. strobus* (SA = 3sl, where \( s = \) side measurement and \( l = \) length). Basal and terminal portions of needles were deleted from calculations, since these areas did not significantly add to the total surface area (<0.02 \( \text{cm}^2 \)) or absorptive sites. Measured values of length were used in surface area determinations; however, radius and side measurement values were calculated from volume displacement studies.

**Surface morphology.** Sections of needle tissue (20 mm) were freeze-dried, attached to stubs with double-stick tape, coated (about 20-30 nm) with gold-palladium alloy (Au 60%, Pd 40%), and observed with a scanning electron microscope (Advanced Metal Research Model 900) operated at 21 kv. Both adaxial and abaxial surfaces were observed at varying distances from the fascicle end at magnifications of 150, 400, and 1,650X.

**Epicuticular wax quantity and chemistry.** Epicuticular wax extracted by dipping needles into 800 ml of redistilled chloroform (4 successive 10 sec dips, 200 ml/dip). Washings were combined and filtered; chloroform was removed on a rotary evaporator at a temperature not exceeding 40°C. Epicuticular wax was redissolved in chloroform and transferred (3 successive 1 ml washings) to tared tubes. Tubes were dried at 40°C and epicuticular wax weight was determined. Data were expressed in \( \mu \)g of epicuticular wax/cm² of needle surface area. Seven determinations were made for each species (needles/determination: *P. nigra* = 250, *P. strobus* = 500).

Qualitative differences in epicuticular wax chemistry were determined by thin-layer chromatography. Epicuticular wax was redissolved in 1 chloroform:1 ethyl acetate (by volume) at a concentration of 200 mg/ml and spotted (2 \( \mu \)l) on precoated silica gel G thin-layer plates (250\( \mu \), Uniplate, Analtech Inc., Newark, Del.). Thin-layer plates were pre-washed in distilled benzene and dried at 110°C for 30 minutes. Wax constituents were isolated on plates using distilled benzene. Spotted plates were run (10 cm), as a mobile solvent, and identified by spraying with concentrated H\( _2 \)SO\( _4 \), and charring at 200°C. *Pinus* samples were co-chromatographed with samples of *Brassica oleracea*. Epicuticular wax chemistry was identified by comparison with published results for *B. oleracea* (1, 12, 30).

**Surface wettability.** Surface wettability was assessed by measuring contact angles of 1 \( \mu \)l drops of deionized water on adaxial surfaces of both species according to the procedure outlined by Mark (25).

**Surface retention of aqueous solutions.** Retention of aqueous solutions by needle surfaces was determined using \( ^{14} \text{C} \) labelled solution (\( ^{14} \text{C}-\text{naphthaleneacetic acid, 16 } \mu \text{c/M, 0.1 } \mu \text{c/ml} \)). Needles of both species were dipped to a consistent length (80 mm for *P. nigra*, 70 mm for *P. strobus*). Immediately following, needles were rinsed twice in 30 ml of 95% ethanol to remove the retained aqueous (\( ^{14} \text{C} \) labelled) solutions. Radioactivity was determined by liquid scintillation (2 ml aliquots of combined EtOH rinses in 15 ml of scintillation fluid) using a Beckman LS-100 Liquid Scintillation Counter. Scintillation cocktail consisted of 5 g of 2,5-diphenyloxazole dissolved in 1 liter of p-dioxane. Corrections were made for quenching and efficiency. Three determinations were made for each species with and without fascicle ends attached (needles/determination: *P. nigra* = 20; *P. strobus* = 50). Retention data were expressed as \( \mu \)l of solution retained/cm² of needle surface area.

**Measurement of penetration.** To determine penetration, needles were placed into culture tubes (20 \( \times \) 150 mm) containing 3 ml of 1.0 NaCl solution (\( ^{26} \text{Cl} \) – sodium, 1.1 \( \mu \)c/ml, 2.7 \( \mu \)c/\( \mu \)l) and rotated to insure uniform coverage. Fascicle ends were sealed with parafilm to prevent infiltration through detached surfaces. After 24 hr, needles were removed from the treating solution and thoroughly rinsed with distilled H\( _2 \)O. Needles were homogenized with 2 ml of 80% ethanol followed by 2 additional rinses of the homogenization tube. The combined 6 ml extraction was brought to a 10 ml volume, and 1 ml aliquots (3 replications/sample) were pipetted into planchets. Aqueous solutions were evaporated from planchets at 30°C. Activity was determined using a Beckman Low Beta II proportional gas flow counter.

Data were expressed in dpm/cm² surface area and dpm/mg fresh weight and represent the mean of 5 determinations (1 fascicle unit/determination). Corrections were made for background, self absorption, and efficiency.

**Distribution patterns of Na⁺ and Cl⁻ penetration.** Distribution patterns of Na⁺ and Cl⁻ following penetration were determined using an electron microprobe X-ray analyzer (Model EMX-SM, Applied Research Laboratories) operating at an accelerating voltage of 15 kv and 0.025 \( \mu \)A sample current. Needle pretreatment was identical to that used in isotope studies. Needles were placed into culture tubes (20 \( \times \) 150 mm) containing 3 ml of 1.0 NaCl for 24 hr; needles placed in deionized water served as controls. Prior to examination, needles were thoroughly rinsed with distilled H\( _2 \)O, placed into sample holders, frozen in liquid N\( _2 \), and cross-sectioned while frozen to prevent redistribution of Na⁺ and Cl⁻ ions during sectioning. The sample holder was designed with a liquid N\( _2 \) reservoir to insure that samples remained frozen during observation. Distribution patterns of Na⁺ and Cl⁻ were observed by a line scan through needle cross sections.

**Protoplasmic viability.** Species sensitivity to NaCl was determined using a modified procedure of Stepokus and Lanphear (35). Sections (2 mm) of one-year-old needles were placed into culture tubes (20 \( \times \) 150 mm) containing 5 ml of varying concentrations of NaCl (0.00, 0.01, 0.10, 1.00, and 6.10 n) for 30 hr at 18°C. Sodium chloride solutions were vacuum infiltrated into needle tissues to insure uniform penetration.

After NaCl treatment, sections were rinsed with distilled H\( _2 \)O and solutions (5 ml/culture tube) of 1% (weight/volume) triphenyl tetrazolium chloride (TTC) were vacuum infiltrated into needle sections. For comparative purposes, non-viable needle sections (frozen in liquid N\( _2 \)) were similarly treated. After 15 hr, needle sections were rinsed with distilled H\( _2 \)O and placed in 95% ethanol (10 ml/culture tube). The reduced formazan derivative was extracted in boiling ethanol for 5 min; tubes were cooled and diluted to 10 ml with 95% ethanol. Transmittance was measured at 530 nm. Viability was expressed as a percentage of non-viable tissue (100% transmittance). Data were the mean of 5 determinations (sections/determination: *P. nigra* = 50, *P. strobus* = 100).

**Results**

**General needle characteristics.** Needles of *P. nigra* were two-sided and closely resembled a half cylinder, and the morphology of *P. strobus* closely resembled a prism having equilateral sides. Needles of *P. nigra* had significantly greater length, more surface area, and greater fresh weight/cm² than *P. strobus* (Table 1).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>P. nigra</em></th>
<th><em>P. strobus</em></th>
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</thead>
<tbody>
<tr>
<td>Needle length (cm)</td>
<td>1.02a</td>
<td>0.84b</td>
</tr>
<tr>
<td>Surface area (cm²/needle)</td>
<td>3.64a</td>
<td>1.87b</td>
</tr>
<tr>
<td>Volume (cm³/needle)</td>
<td>0.020a</td>
<td>0.007b</td>
</tr>
<tr>
<td>Fresh wt/unit surface area (µg/cm²)</td>
<td>283a</td>
<td>101b</td>
</tr>
<tr>
<td>Epicuticular wax (µg/needle)</td>
<td>657a</td>
<td>141b</td>
</tr>
<tr>
<td>Epicuticular wax (µg/cm²)</td>
<td>1,650a</td>
<td>75b</td>
</tr>
<tr>
<td>Contact angle (º)</td>
<td>67a</td>
<td>73b</td>
</tr>
<tr>
<td>Retention (without fascicle, µl/cm²)</td>
<td>2.48a</td>
<td>2.61a</td>
</tr>
<tr>
<td>Retention (without fascicle, µl/cm²)</td>
<td>2.25a</td>
<td>1.41b</td>
</tr>
</tbody>
</table>

*Mean separation within rows by Tukey’s \( \omega \) test, 5% level.*
Surface morphology. Surface morphology was uniform on both adaxial and abaxial surfaces of both Pinus spp. regardless of location in regard to the fascicle end (Fig. 1). Stomata occurring in longitudinal rows separated by parallel ridges characterized medial abaxial needle surface morphology for both species (Fig. 1, A,D). Surface fine structure in both species was free of trichomes or other recognizable structures. Scanning electron micrographs indicated P. strobus had more

Fig. 1. Scanning electron photomicrographs of medial adaxial needle surfaces of Pinus nigra (A, B, C) and P. strobus (D, E, F) at varying magnifications: A, D-100μm; B, E-25μm; C, F-10μm.
pronounced epicuticular wax fine structure than \textit{P. nigra} (Fig. 1, E,B). At 1,650× (Fig. 1, F,C) stomatal occlusion by wax wax evident in \textit{P. strobus} but not in \textit{P. nigra}. These observations support the findings of Hanover and Reicosky (12).

\textbf{Epicuticular wax quantity and chemistry.} Needles of \textit{P. nigra} had more wax/unit surface area than \textit{P. strobus} (Table 2). No qualitative differences in wax chemistry were observed between \textit{Pinus} sp. by thin-layer chromatography (Fig. 2). Major constituents co-chromatographed with fatty acids, primary and secondary alcohols of \textit{Brassica oleracea} epicuticular wax (1, 12, 30).

\textbf{Surface wettability.} There were no significant differences in needle surface wettability (contact angle) between \textit{P. nigra} and \textit{P. strobus} (Table 1). Contact angles ranged from 67° for \textit{P. nigra} to 73° for \textit{P. strobus}.

\textbf{Surface retention of aqueous solutions.} Individual \textit{P. nigra} needles without fascicles attached retained more water than \textit{P. strobus} needles (Table 1). There was no significant difference in retention between species with fascicles attached.

\textbf{36Cl Penetration.} Penetration of 36Cl was significantly greater in needles of \textit{P. nigra} (9,839 dpm/cm²) than \textit{P. strobus} on a surface area basis (3,503 dpm/cm²). There was no difference in uptake when expressed on a fresh wt basis (Table 2).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Species & 36Cl Penetration \textsuperscript{y} & 36Cl Penetration \textsuperscript{z} & 36Cl Penetration \textsuperscript{z} \\
& (dpm/cm²) & (dpm/cm³) & (dpm/mg fresh wt) \\
\hline
\textit{P. nigra} & 9,839\textsuperscript{a} & 443,996\textsuperscript{a} & 34,684\textsuperscript{a} \\
\textit{P. strobus} & 3,503\textsuperscript{b} & 32,650\textsuperscript{b} & 34,762\textsuperscript{a} \\
\hline
\end{tabular}
\caption{Cuticular permeability in \textit{Pinus nigra} and \textit{P. strobus} as indexed by penetration of 36Cl – sodium chloride.}
\end{table}

\textbf{Protoplasmic viability.} There was a significant difference in % transmittance (viability) between species at all concentrations NaCl. At 1.0 N, a clear difference in viability was observed between species; 100% (0% viability) for \textit{P. strobus}, while \textit{P. nigra} was 50% (50% viability). Saturated (6.1N) NaCl solutions were lethal to both species (Table 3).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Species & 0.00 N & 0.01 N & 0.10 N & 1.0 N & 6.10 N \\
\hline
\textit{P. nigra} & 44\textsuperscript{a} & 42a & 42a & 50b & 94c \\
& d & d & d & d & d \\
\textit{P. strobus} & 60a & 63a & 64a & 100b & 100b \\
& e & e & e & e & e \\
\hline
\end{tabular}
\caption{Effect of increasing concentrations of NaCl on viability of \textit{Pinus nigra} and \textit{P. strobus} as indexed by the triphenyl tetrazolium chloride method.\textsuperscript{y}}
\end{table}

\textbf{Discussion}

General needle morphology does not explain the differences
Fig. 4. Relative intensity as measured by microprobe analysis of Na⁺ and Cl⁻ in sodium chloride (1.0 N) treated and non-treated needle cross sections of Pinus strobus. Full scale equals 300 counts/sec; background levels were 10 counts/sec.

in sensitivity of P. strobus and P. nigra to deicing salt spray. Needles of P. nigra are longer with greater surface area for potential interception of saline spray. This difference is significantly reduced when comparing retention of solutes on a fascicle basis. Injury in P. strobus may be explained in part to the significantly lower fresh weight/unit surface area. If equal penetration occurred in both species, salt ions could be potentially more concentrated in P. strobus.

Surface morphology and fine structure provides little insight into resistance of injury. The major difference observed in surface fine structure was occlusion of stomates in P. strobus. Recent evidence (32) indicates that even with surfactants, stomatal infiltration of solutions is virtually impossible. There-fore, wax occlusion in P. strobus is likely of little consequence in limiting stomatal penetrations of salt solutions, unless the articular ledges of guard cells and/or guard cells themselves are acting as preferential uptake sites for polar compounds, as has been shown for bean (3).

Surface wettability, measured by contact angle was not significantly different, yet individual needles of P. nigra retained significantly more solution/cm² than P. strobus. The absence of a difference in wettability is supported by the similarity in surface morphology, surface fine structure, and epicuticular wax chemistry. Epicuticular wax, by nature of its fine structure and chemistry, determines to a large extent the wetting properties, and thus retention by the plant surface (3, 7, 8, 9, 14, 34). Increased retention by needles of P. nigra is most likely a result of the geometric shape of needles. A potential explanation for sensitivity of P. strobus may be related to aqueous solution retention expressed on a fascicle basis. When wetted, needles coalesce resulting in trapping of solutions. Potentially, solutions at this interface are less subject to the forces of weathering in comparison to those retained by needles of P. nigra which remain separated resulting in increased penetration. Luckwill and Lloyd-Jones (23) have suggested that reduction in penetration of solutions over time is associated with rate of droplet evaporation.

Resistance to salt spray in P. nigra could not be explained on the basis of greater epicuticular wax quantity limiting penetration of Na⁺ and Cl⁻. Results of isotope and electron microprobe studies demonstrated greater penetration of salt ions into resistant P. nigra. Investigations by Surcoff (37) have indicated that penetration of 2,4-D was more influenced by wax chemistry rather than cuticle thickness. Further, penetration of polar compounds applied in aqueous solutions is impeded and influenced not only by quantity, but by quality and orientation of waxes present within and on the outer surface of the cuticular membrane (3, 10, 18, 29).

Viability studies indicated that the threshold level for injury due to penetration of Na⁺ and Cl⁻ ions was less in P. strobus than in P. nigra. These data support Surcoff (37) who found injury levels of Na⁺ and Cl⁻ to be considerably lower in P. strobus than in P. nigra. Also, Buschbom (6) has stated that after penetration of damaging ions has occurred, the protoplasmic tolerance to penetrating salt ions determines susceptibility.

We conclude that the resistance of needles of P. nigra to deicing salt injury cannot be attributed to the exclusion of Na⁺ and Cl⁻ ions, but rather to a higher level of protoplasmic tolerance to the penetrated ions. Sensitivity of P. strobus needles is due to a lower level of protoplasmic sensitivity to the penetrated ions.

References


Translocation and Metabolism of Carbohydrate Fraction of 14C-photosynthates in 'French' Prune, Prunus domestica L.1

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Additional index words. source-sink relationship, sorbitol, sucrose, starch

Abstract. When 14CO2 was administered to leaves on girdled 'French' prune spurs, the label was incorporated into sorbitol, sucrose, glucose, fructose, starch, and amylose-insoluble assimilates. The rates of export of soluble sugars, and sorbitol, and mobilisation of starch from leaves were proportional to the rate of fruit growth. The deposition of amylose-inert assimilates in leaves exceeded that of starch, which may account for the gradual increase in specific leaf weight in prunes. The proportion of sorbitol to total sugars in leaf blades and petioles, stem, and peduncle was nearly constant during the 22-day experimental period but changed abruptly in the fruit. 14C-sorbitol fed to fruits via their peduncles was metabolized to 14C-sucrose, but the reverse reaction was barely detectable.

Sorbitol, a sugar alcohol, is a common carbohydrate constituent in members of the Rosaceae family. The fruit of the 'French' prune is relatively rich in this compound, especially when ripe (14). Reid and Bielecki (13) identified it in apricots, Prunus armeniaca L., and reported that the ripening fruit relied on carbohydrate reserves, rather than on current photosynthates, as carbon sources. They based their interpretation on the inability of the apricot fruit to metabolize sorbitol, a major carbohydrate in the translocation stream, to sucrose. In 'French' prune, a light to moderate crop caused a slight diminution of starch in the stem (14) while a heavy crop in 'Sugar' prune exhausted it (4). This study was undertaken to elucidate the transport and metabolism of soluble carbohydrates and starch produced by 'French' prune leaves after administration of 14CO2. Source-sink relationship is particularly important in this cultivar because overcapping often leads to potassium deficiency symptoms under some California conditions (10).

Materials and Methods

14CO2 administration and sampling of treated spurs. On July 5 and 25, 8 spurs having 20 to 70 leaves and bearing 2 to 7 fruits were selected on a 6-year-old 'French' prune tree growing in the University Orchard at Davis, CA. The spurs were girdled at their bases to prevent radioactive contamination of the tree. Some of the leaves, but no fruit, were