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NORTHERN WHITE CEDAR

RESULTS OF 2018–2019 ASSESSMENT OF *THUJA OCCIDENTALIS* POPULATIONS AT THE FOX RIVER FEN

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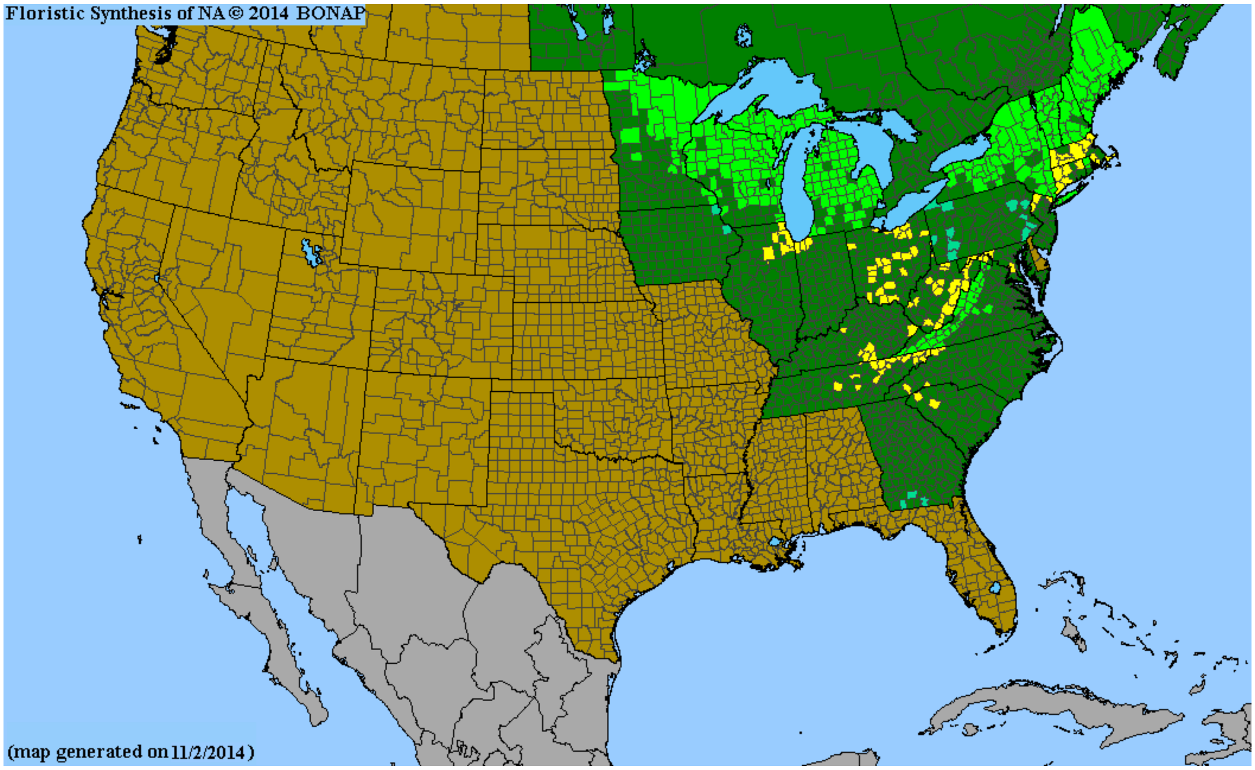
EXECUTIVE SUMMARY

- ▹ Collected seed from mature individuals of *Thuja occidentalis* at Trout Park and Chicago Junior School, as well as 3 control sites for comparison
- ▹ Measured seed set, seed weight, and seed morphology to determine site variability
- ▹ Conducted germination trials in growth chambers under incremental salt concentrations to determine the effect of salt on seed germination
- ▹ Conducted periodic soil samples over the span of 1 year to assess salt levels at Trout Park and Chicago Junior School
- ▹ Found differences between Trout Park and Chicago Junior School for reproductive metrics and soil salt levels, but not for germination

INTRODUCTION

Northern White Cedar (*Thuja occidentalis* L.; Cupressaceae) is an evergreen tree native to northeastern North America (Plate 1). The southwest range of this species extends into the northeastern portion of Illinois (Map 1) where it is generally rare and uncommon outside of planted populations. In Illinois, *Thuja occidentalis* was formerly listed as a state-threatened species but was delisted in 2004, based on stable population numbers in the state (Mankowski, 2012). This species is slow-growing and prefers to live in areas with saturated, nutrient-rich soil but often occurs in well-drained, dry sites as well. Many Northern White Cedar individuals are found on cliffs or rocky limestone outcroppings near lake and river shores (Boulfroy et al., 2012). Other populations in the state are known along the bluffs of Lake Michigan and major rivers such as the Fox and Illinois (Carroll-Cunningham et al., 2018).

The Illinois State Tollway I-90 in Elgin, IL (Kane County) bisects 1 of the most extensive remaining populations of *Thuja occidentalis* in the state. This area constitutes the Fox River Forested Fen, a wetland complex encompassing our study sites, Trout Park and Chicago Junior School (Map 2). The Chicago Junior School was acquired in 2013 by the Kane County Forest Preserve District and was recently dedicated as a nature preserve, completing the protection of a contiguous natural community with neighboring Trout Park, designated as an Illinois Nature Preserve in 1972 (INPC, 2017). This site represents one of the only remaining fen and seep communities of the Fox River Valley in Illinois. This nature preserve is now named the Fox River Fen, however, for consistency with previous studies at this site, we will continue referring to this site as the Chicago Junior School. The diversity in plant and animal life is attributed to constant flowing groundwater that upwells from calcareous bedrock and periodic flooding by the Fox River during the growing



Map 1. Biota of North America (BONAP) map of *Thuja occidentalis* range in North America (2014).
KEY: Dark green= species present in state and native, light green= species present and not rare, yellow= species present and rare, brown= species not present in state, teal= species native, but adventive in state

Plate 1. Site photo of large upland grove surrounding seep at Chicago Junior School. Photo taken October 2019.

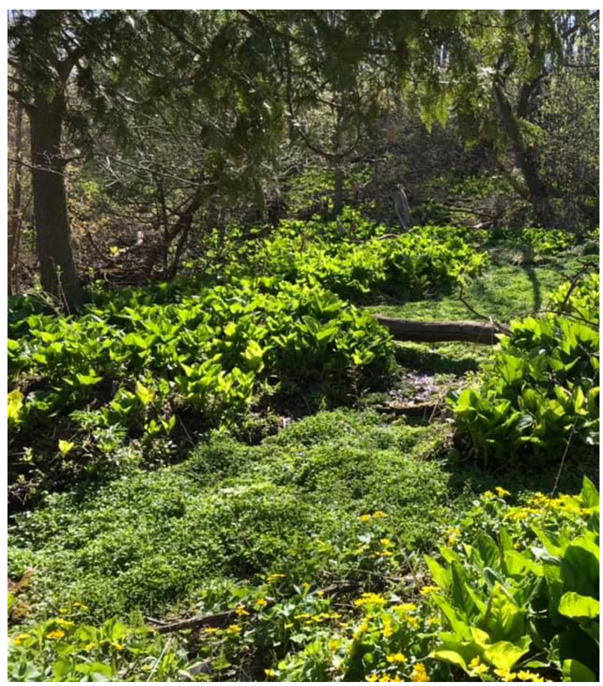


Plate 2. Forested seep ground layer dominated by Skunk Cabbage (*Symplocarpos foetidus*) in Trout Park, Kane County. Photo taken April 2019. *Thuja occidentalis* overhead in the foreground.

season (INPC, 2017). Both sites have a rich herbaceous plant community hosting many rare and unique species. However, they are subject to many threats including invasive species, the impact of waste and runoff, and consistent human trampling and disturbance (Carroll-Cunningham et al., 2018).

In 2017, a survey by Illinois Natural History Survey (INHS) botanists found 568 and 299 trees (82 of them dead) at Chicago Junior School and Trout Park, respectively (Carroll-Cunningham et al., 2018; Plate 3). Site visits described large and healthy groves of mature multi-stemmed individuals at Chicago Junior School. Individuals at Trout Park, however, were in poor condition with many living trees growing from stem sprouts of fallen trees. Seedlings were seen during site visits, particularly at Chicago Junior school in 2019 (Plates 4 and 5) and at Trout Park in 2017. Around 20 young trees and seedlings were documented to be growing on nurse logs at Trout Park in 2017 (Carroll-Cunningham et al., 2018) but very few seedlings were found at Trout Park in 2019. Nurse logs with several seedlings were seen at Chicago Junior School in 2019 as well as other seedlings scattered across the site grounds. In addition, few intermediate stage saplings were found on site and seedlings seen earlier in the year were often not able to be relocated at later visits. Carroll-Cunningham et al. (2018) suggested that causes of decline in health and recruitment of these populations, particularly at Trout Park, could be multi-faceted including deer browsing of seedlings, which may limit seedling survivorship, as well as salt spray and run-off from the Illinois Tollway into the southern population at Trout Park.

The Illinois Tollway uses an average of 47,000 tons of salt per year to maintain safe driving conditions on toll roads during Chicago winters. Commercial deicers frequently used are calcium chloride, sodium chloride, and roadway abrasives (Illinois Tollway, 2018). Road salt is known to be damaging to roadside plant populations by infiltrating the water table (Kelly et al., 2012), restricting nutrient uptake and mimicking drought conditions at the root level (Delahaut and Hasselkus, 1999), and causing foliar injury, particularly in conifers (Trahan and Peterson, 2007). Systems that are dependent upon a delicate balance of hydrology and nutrient load may be even more sensitive to salt levels in the groundwater. Increased salt levels not only have damaging effects on the health of mature plants, but have been shown to limit recruitment and long-term seedling survival (Cochrane,

Plate 3. *Thuja occidentalis* individuals at Chicago Junior School (left) and Trout Park (right). Photos taken during site visits in 2017 (Carroll-Cunningham et al., 2018).

Plate 4. *Thuja occidentalis* seedling on forest floor at Chicago Junior School. Photo taken April 2018

Plate 5. *Thuja occidentalis* seedlings on forest floor at Trout Park. Photo taken October 2018.

2018). Studies on seeds under varying salt conditions show a reduction or inhibition of germination with multiple studies showing a stark decline in germination with concentrations as low as 250 ppm or mM (Bojović et al., 2010; Daddario et al., 2017; Özdemir et al., 2016).

In the case of Chicago Junior School and Trout Park, salinity levels in the groundwater have varied over time from around 200 ppm (mM), about double normal levels at the site, to some areas spiking far above 800 ppm (mM), especially in drainage areas close to the tollway. Before a reconfiguration of the I-90 and IL Route 25 drainage network in 2014, the Illinois State Geological Survey (ISGS) monitoring wells documented a decrease in groundwater levels and elevated pollutants from roadway runoff. After reconstruction, elevated concentrations of both sodium and chloride were observed in 2 of 3 ISGS monitoring wells at Trout Park, likely due to an influx of residual road salts as local groundwater levels increased after the removal of a storm sewer. About 5,454 tons of salt were applied to this section of tollway (Tollway Maintenance Unit M-6) in the winter of 2018–2019, the highest amount since pre-construction in the winter of 2013–2014. (Plankell et al., 2020).

To determine the drivers behind differences in adult health and seedling success between populations, we conducted a series of studies to assess the impact of varying salt concentrations on germination and examined differences in salt concentrations in the soil at each site. In addition, we assessed several aspects of the reproductive ecology of the species at both sites, including cone biomass, seed biomass, seed set, and seed morphometrics as additional components of population health.

MATERIALS AND METHODS

Germination Trials

Cones were collected on September 14, 2018 at Trout Park (TP) and Chicago Junior School (CJS) from 5 and 15 mature adult trees, respectively. For the purpose of comparison, cones were collected from 3 trees on September 19, 2018 at Chicago Botanic Garden (CBG) and from 15 trees at Morton Arboretum (MA). Trees were not selected at random as the number of trees with cones were limited at sites. Cones were collected from each tree using a pole pruner to snip out-of-reach branches and/or pruners (Plate 6). Cones were dried in a lab oven for 24 hours at a low temperature of 110°F (43°C) to facilitate cone dehiscence and to ensure seeds were dry before cold storage. Seeds were cold, dry stratified for more than 60 days at 40°F (4°C) (Schopmeyer, 1974). In addition to these seed collections, seeds were acquired from Pakulak Seed and Nursery Co. (PAK) in Michigan to conduct preliminary germination studies and to be used as a control for comparison.

Preliminary germination trials were conducted in January 2019 and March 2019 to establish a baseline rate of germination using seeds from PAK. These trials were also used to determine appropriate salt concentrations to be used during the germination study. Salt concentrations were measured in millimolars (mM) or parts per million (ppm) for easy comparison to soil and water quality results. Preliminary trial solutions utilized varying increments ranging from 0 mM to 1000 mM NaCl to test the lower and upper ranges of possible salt concentrations. Nine solutions ranging from 0 mM to 1000 mM (0, 25, 50, 100, 200, 400, 600, 800, 1000) were utilized in January 2019, while 13 solutions ranging from 0 mM to 1000 mM (0, 5. 10, 15, 20, 25, 50, 100, 200, 400, 600, 800, 1000) were utilized in the March 2019 trial. Very low germination occurred for *Thuja occidentalis* seeds in solutions over 400 mM. Thus, this was set as our maximum level for our germination trial. The final germination trial was run in May 2019 using a series of 10 salt solutions ranging from 0 to 400 mM (0, 5, 10, 15, 20, 25, 50, 100, 200, 400). Seeds from all sources were used (CJS, TP, CBG, MA, and PAK) and 5 replicates were created for each seed source and salt solution combination with 20 seeds used per replicate.

Seeds were placed in petri dishes (100 mm by 15 mm) lined with 90-mm diameter, grade 1 filter paper before adding 2 mL of solution to each. Captan (an antifungal agent) was sprinkled over the filter paper and seeds to inhibit mold growth. Petri dish edges were sealed with Parafilm to prevent evaporation and placed into a growth chamber set to 8 hours light and 16 hours dark period with day temperature set to 80°F (27°C) and a night temperature of 68°F (20°C). Chambers were checked for germination after 7 days and continually monitored daily until the end of the 30-day experiment. After the initial 7 days, an additional 2 mL of solution was added to each sample and was added on a case-by-case basis until the end of the study. Germination was considered at the emergence of the radicle from the seed coat (Plate 7). Germinated seeds were removed, and petri dishes were resealed with new parafilm.

Germination data were analyzed using a generalized linear model with a binomial distribution in R, version 3.6.0 (R Core Team, 2019). The main treatment effects were site, salt concentration, and their interaction. Salt concentration was treated as a continuous variable. A Tukey post-hoc Test was used to identify differences between sites. Estimated marginal means are presented in the results.

Cone Biomass, Seed Set and Seed Morphometrics

Cones were collected at each site, with 10 cones collected from up to 15 trees (max of 150 cones per site). At Trout Park and Chicago Botanic Garden, cones were collected from only 5 and 3 individuals, respectively. Cones (with seeds) were weighed to determine cone biomass. Seeds were removed from individual cones and seed set (developed seeds / total number of ovules [i.e., 8]) was determined for each cone. Seeds were combined and collectively weighed at the site level to determine seed biomass (i.e., total number of seeds / total seed weight). Seeds were considered fully developed if the embryo was swollen. Ovules were counted when present. Each cone contains up to 8 ovules (Schopmeyer, 1974).

Plate 6. Researchers using a pole pruner to trim cones off of *Thuja occidentalis* at Chicago Junior School in September 2018.

Plate 7. Photo of radicle emerging from *Thuja occidentalis* seed coat.

Several morphological characteristics were measured on fully developed seeds, including overall length and width, embryo length and width, and right-wing length and width following Briand et al. (1992). Measurements were taken on seeds oriented with the micropylar facing up and concave surface facing toward the viewer (Plate 8). A photograph was taken of a random selection of 25 seeds from each individual and morphometric measurements were taken using IMGJ software (Schneider et al., 2012; Image J 1.x).

Morphometric data along with seed set and cone weight were analyzed using a linear mixed effects model with tree as a random effect in R, version 3.6.0 (R Core Team, 2019). Seed weight was pooled at the tree level and a linear model was used to analyze differences between sites. A Tukey post-hoc Test was used to identify differences between sites. Estimated marginal means are presented in the results.

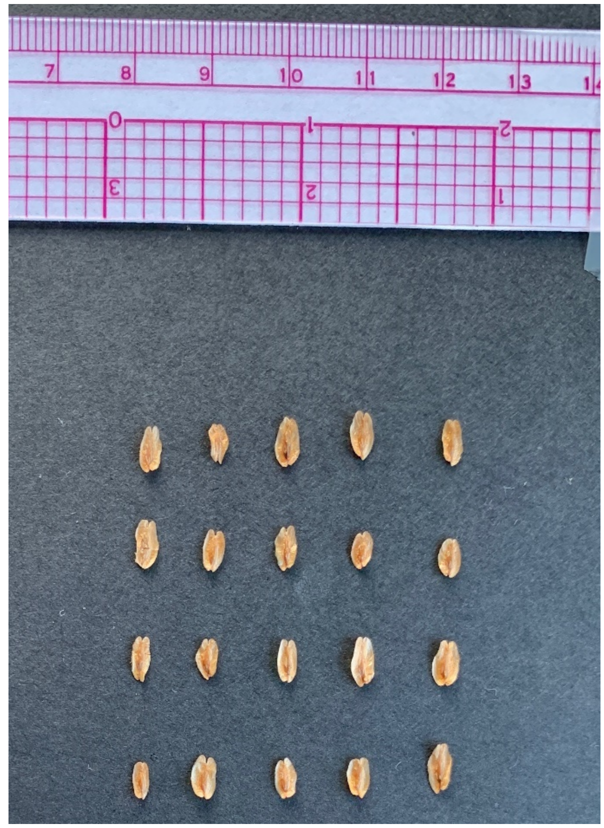


Plate 8. Photo of seeds prepared for measurement in IMG J.

Soil Collection

Soil cores were collected at Trout Park and Chicago Junior School over the span of one year (Plate 9); in October 2018 and April, August, and October 2019. Samples were used to assess soil characteristics with a focus on salt content variation at each site. Potassium (K), Magnesium (Mg), Sodium (Na), and Chloride (Cl) were measured as indicators of salt (Table 1). Initially, sampling was random and evenly distributed throughout *Thuja occidentalis* populations. Sampling locations were recorded for resampling (Map 2).

Plate 9. Researchers collecting soil cores in April 2019.

During each visit, a total of 40 soil cores were taken with 20 samples at each site. At both sites, 2 samples were taken at 5 upland and 5 lowland points. For each point, a soil core was taken near the base of a *Thuja occidentalis* tree and around 1m away from the tree. Samples were taken at a depth up to 10 inches and were about 1 inch in diameter. Samples were sent to Waypoint Analytical (https://www.waypointanalytical.com/) for analysis.

Soil analyses tested for Chloride (Cl), Soil pH, Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sulfur (S), Boron (B), Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn), Sodium (Na), soluble salts (includes additional salts such as sulfate, bicarbonate, ammonium, nitrate, and carbonate), organic matter (humus), and calculated cation exchange capacity (capacity of soil to hold exchangeable cations such as Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}). Focus was given to constituents commonly found in roadside salts during analysis such as Potassium (K), Magnesium (Mg), Sodium

(Na), and Chloride (Cl) (Large, 2017). Chloride levels were undetectable under 10ppm for all samples except for August 2019 where levels were undetectable under 20ppm. Samples under the detection limit were given a value of half the detection limit (i.e., 5 and 10, respectively) (Niles et al., 1995).

For the purpose of analysis, collection scheme was not considered as part of any analysis. Soil data were analyzed using a Wilcoxen Test in R version 3.6.0 (R Core Team, 2019). A Wilcoxen Test was used because the data were not normally distributed and many of the chloride samples were below a detection limit. Differences in Cl, Na, Mg, Ca, K, and soluble salts levels were evaluated between Chicago Junior School and Trout Park for each sampling period.

RESULTS

Germination Data

Significant differences were found among sites (ANOVA, $p < 0.001$) and treatments (ANOVA, $p < 0.001$) for germination, but not for the interaction of site and treatment (ANOVA, $p = 0.35$). Overall, Pakulak seeds had the highest probability of germination at around 35% germination followed by Morton Arboretum and Chicago Botanic Garden at less than 10% germination. Both Chicago Junior School and Trout Park had the lowest seed germination with less than 1% total germination (Fig. 1).

Also, as salt concentration increased, overall germination decreased (Fig. 2). This trend in salt treatment effect on germination was significant (ANOVA, $p < 0.001$). Seeds continued to germinate in salt (NaCl) concentrations up to 50 mM, however, average germination dropped drastically at levels of 100 mM and higher (Fig. 2). There was no germination seen in concentrations over 100 mM for Chicago Junior School, and over 200 mM for Chicago Botanic Garden and Trout Park (Fig. 3).

Cone Biomass, Seed Set and Seed Morphometrics

Significant differences (ANOVA, $p < 0.005$) were seen in seed set and cone weight among sites (Figs. 4 and 5), but no significant difference (ANOVA, $p = 0.591$) was found in seed weight among sites (Fig. 6). On average, cones collected at Trout Park weighed the least, 0.035 ± 0.013, and produced the fewest seeds of any site in the study at an average of 3.99 ± 0.41 seeds per cone (Figs. 4 and 5). Also, significant differences among sites (ANOVA, $p < 0.005$) were documented in all morphometric measurements. Throughout all morphometric measurements, Trout Park seeds were smaller than Chicago Junior School seeds except for embryo width (Fig. 7).

Soil Data

Average cations in the soil varied over the study period and significant differences were found in both chloride (Wilcoxen Test, $p < 0.005$) and sodium (Wilcoxen Test, $p < 0.001$) at Chicago Junior School and Trout Park for all sampling periods. Significant differences (Wilcoxen Test, $p < 0.05$) also were seen in soluble salts throughout

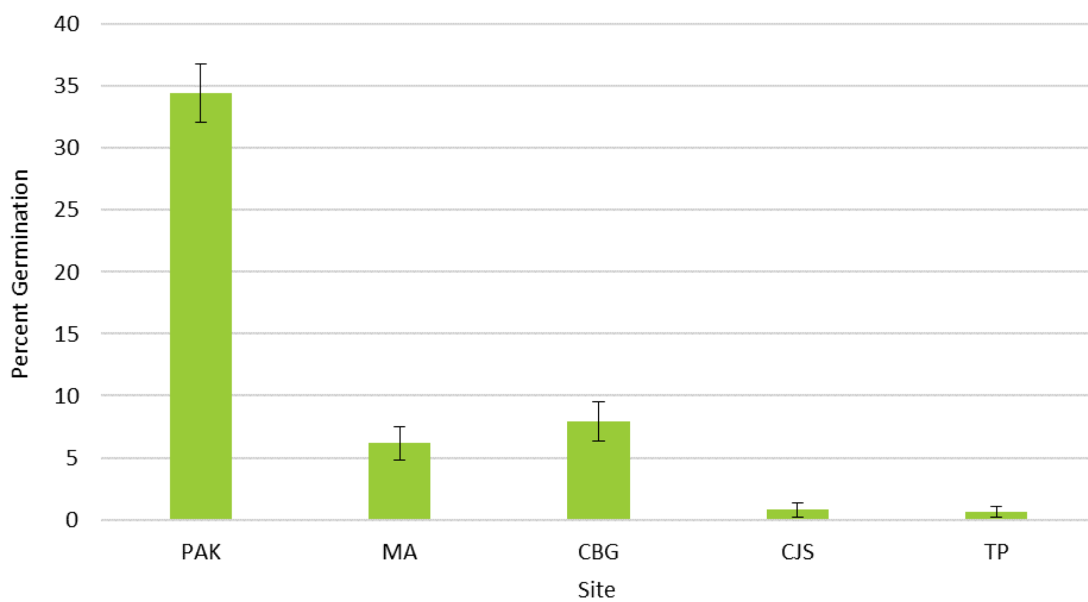


Figure 1. Estimated marginal means from a generalized linear model for germination percentage by site. Significant differences as described by a Tukey's Post Hoc Test. Error bars indicate 95% confidence intervals.

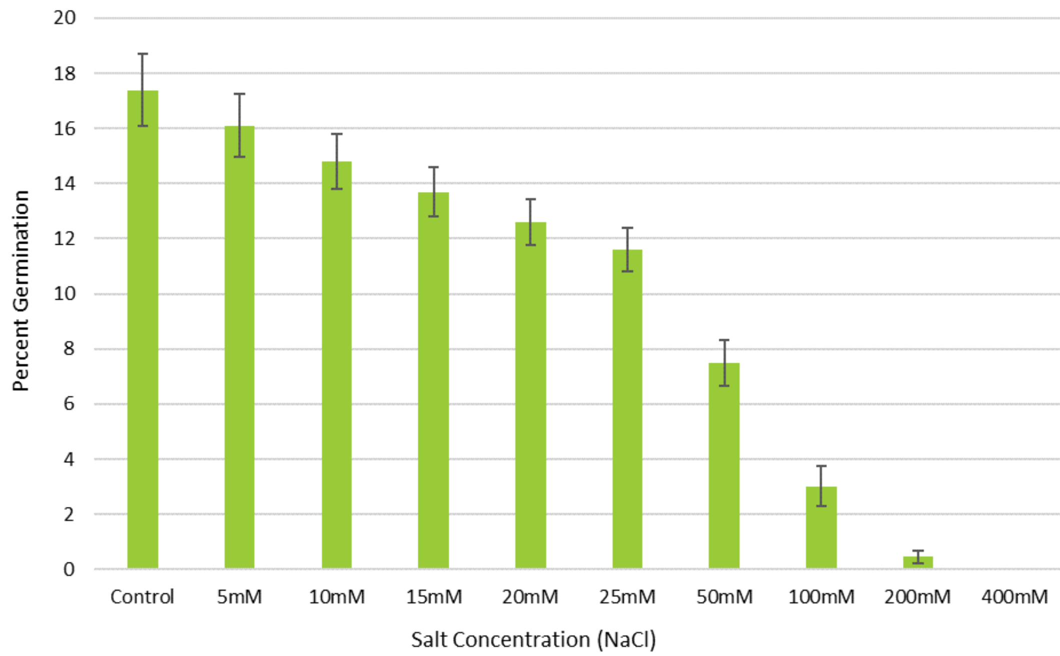


Figure 2. Estimated marginal means from a generalized linear model for germination percentage by salt concentration. Error bars indicate 95% confidence intervals.

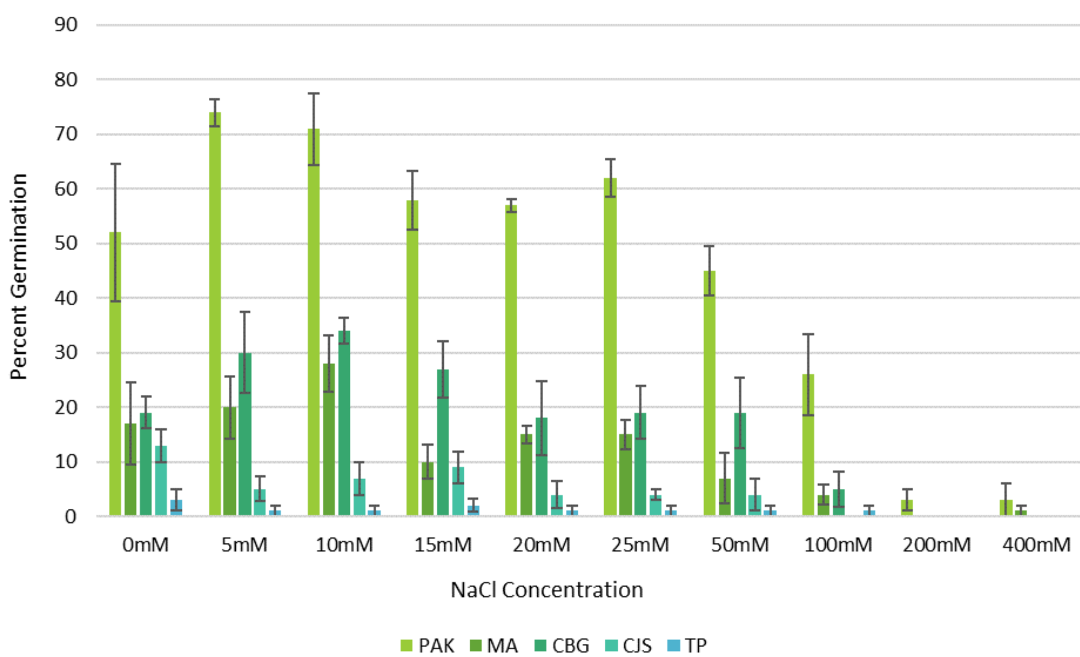


Figure 3. Seed germinated (represented as a percent) at each site as salt concentration increased, based on raw means. Error bars indicate 95% confidence intervals.

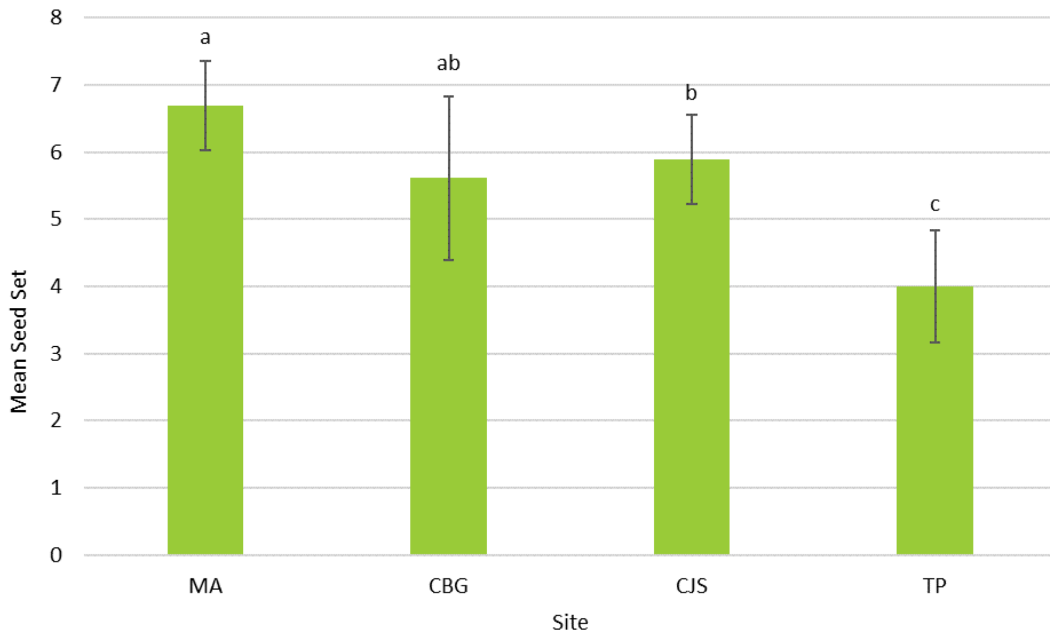


Figure 4. Estimated marginal means from a linear mixed model with tree as a random effect for seed set per cone by site. PAK seeds excluded as seeds were received loose without cones. Letters used to indicate significant differences ($p < 0.005$) in seed set between sites as described by a Tukey's Post Hoc Test. Error bars indicate 95% confidence intervals.

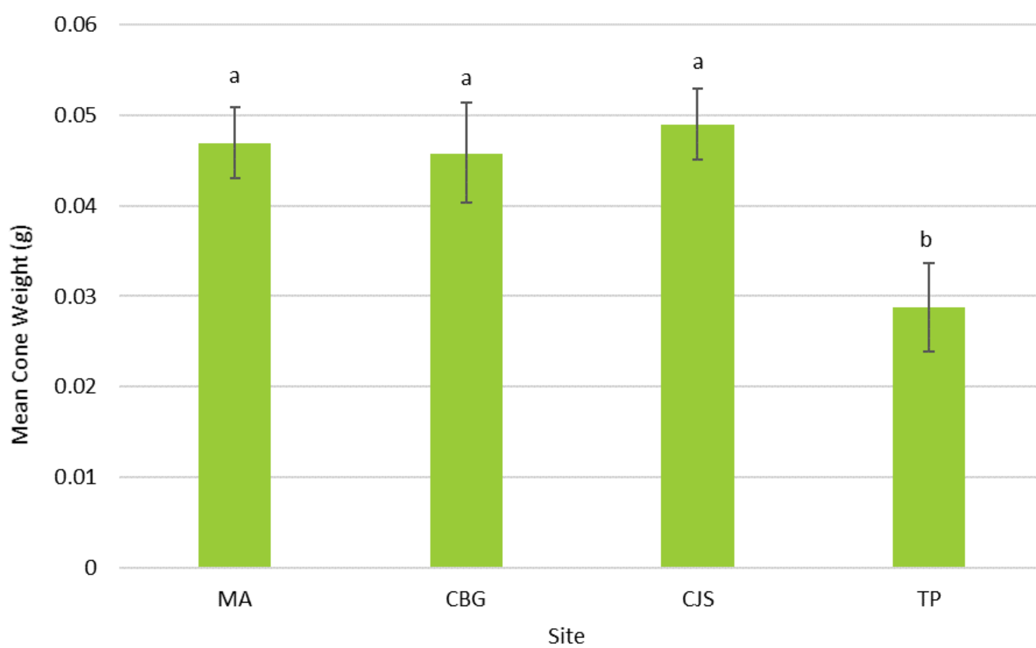


Figure 5. Estimated marginal means from a linear mixed model with tree as a random effect for cone weight by site. PAK seeds excluded as seeds were received loose without cones. Letters used to indicate significant differences ($p < 0.005$) between sites as described by a Tukey's Post Hoc Test. Error bars indicate 95% confidence intervals.

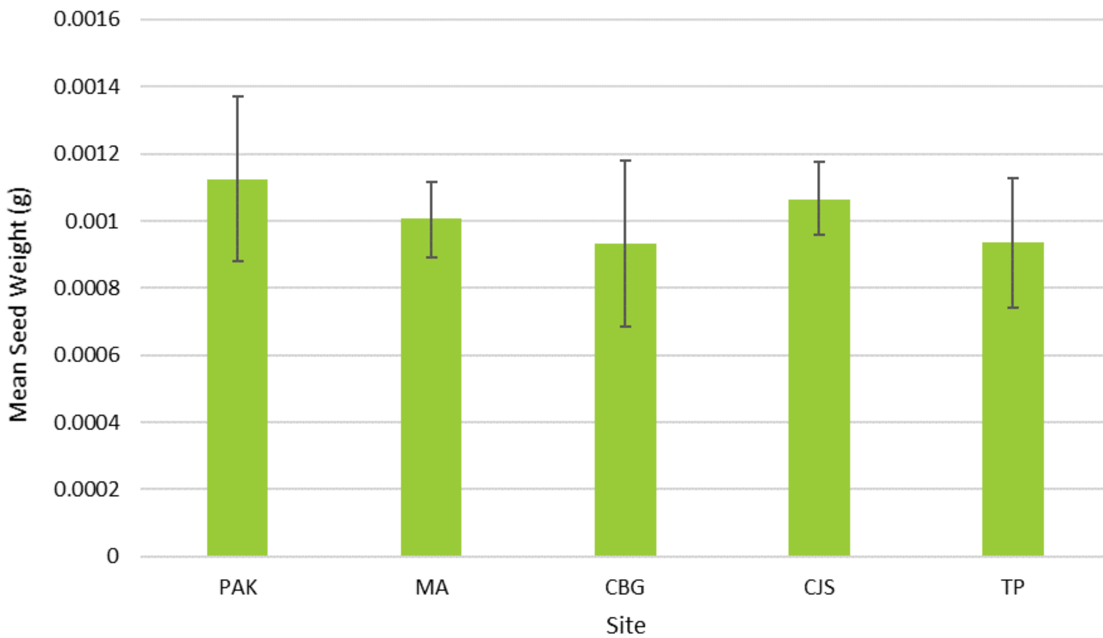


Figure 6. Estimated marginal means from a linear mixed model with trees as a random effect for seed weight by site. No significant difference in seed weight by site (p-value = .591, Linear model). Error bars indicate 95% confidence intervals.

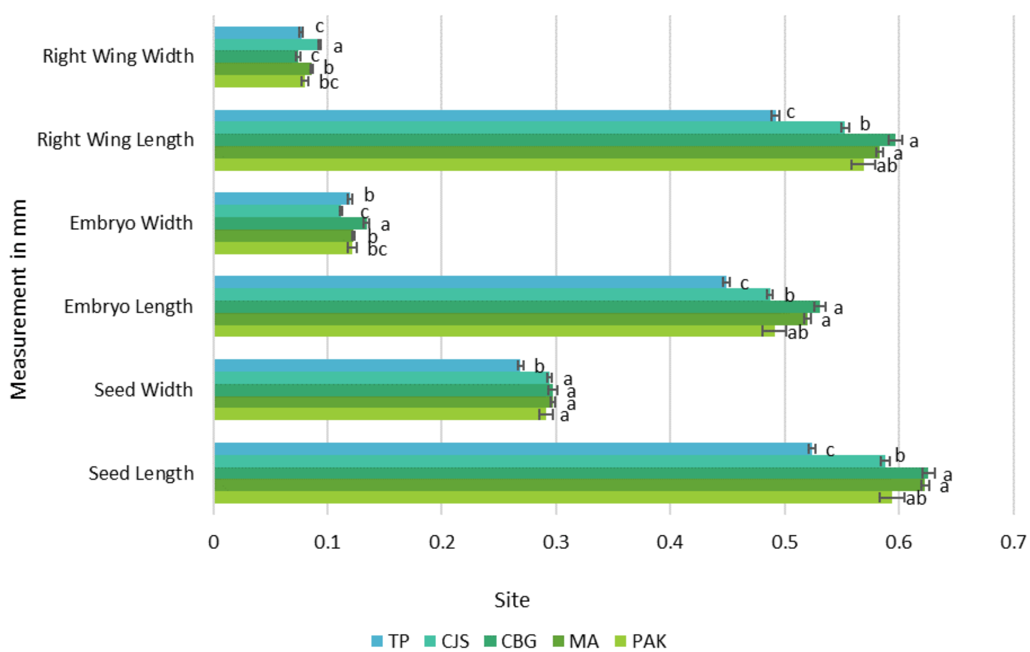


Figure 7. Summary of mean morphometric measurements (in mm) by site. Significant differences among a, b, c (p<.005, Linear mixed effects model, Tree as random effect). Error bars indicate 95% confidence intervals.

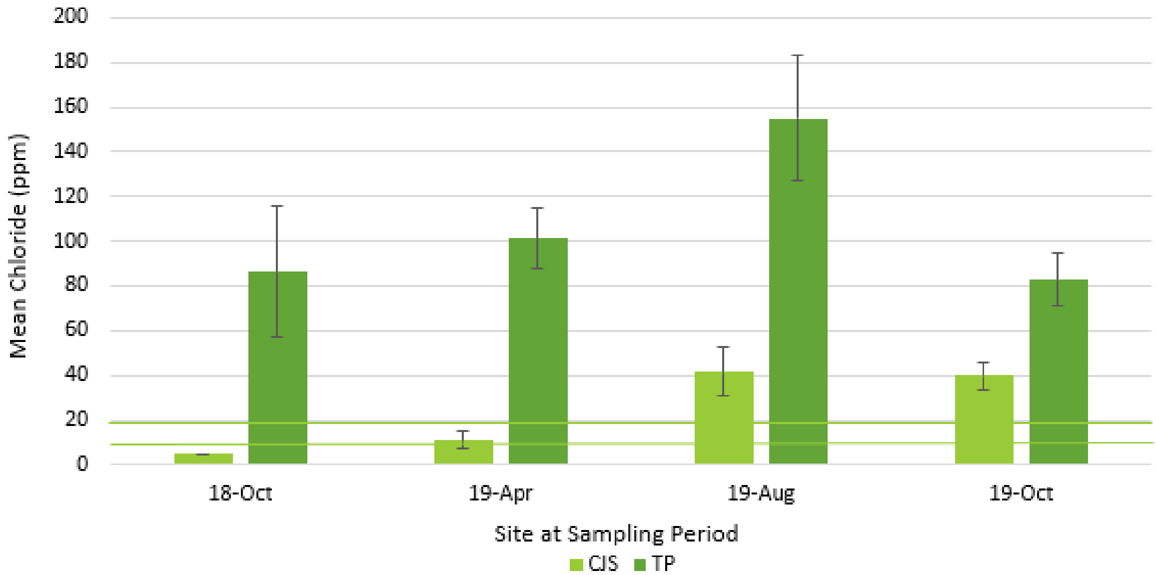


Figure 8. Sample means of chloride levels (in ppm) by site over time. Significant differences in Cl levels between Chicago Junior School and Trout Park across study period ($p < 0.005$, Wilcoxen Test; Table 1). Error bars indicate 95% confidence intervals. Green bars at 10 ppm and 20 ppm showing detection limits.

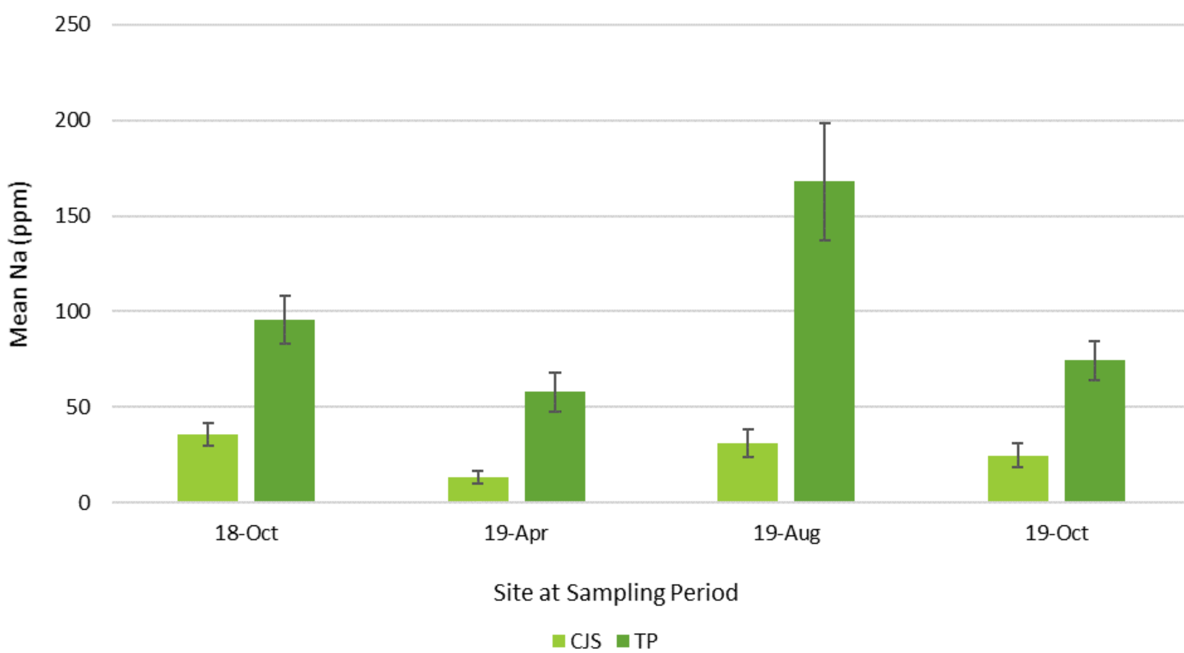


Figure 9. Sample means of sodium levels (in ppm) by site over time. Significant differences in Na levels between Chicago Junior School and Trout Park across study period ($p < 0.005$, Wilcoxen Test; Table 1). Error bars indicate 95% confidence intervals.

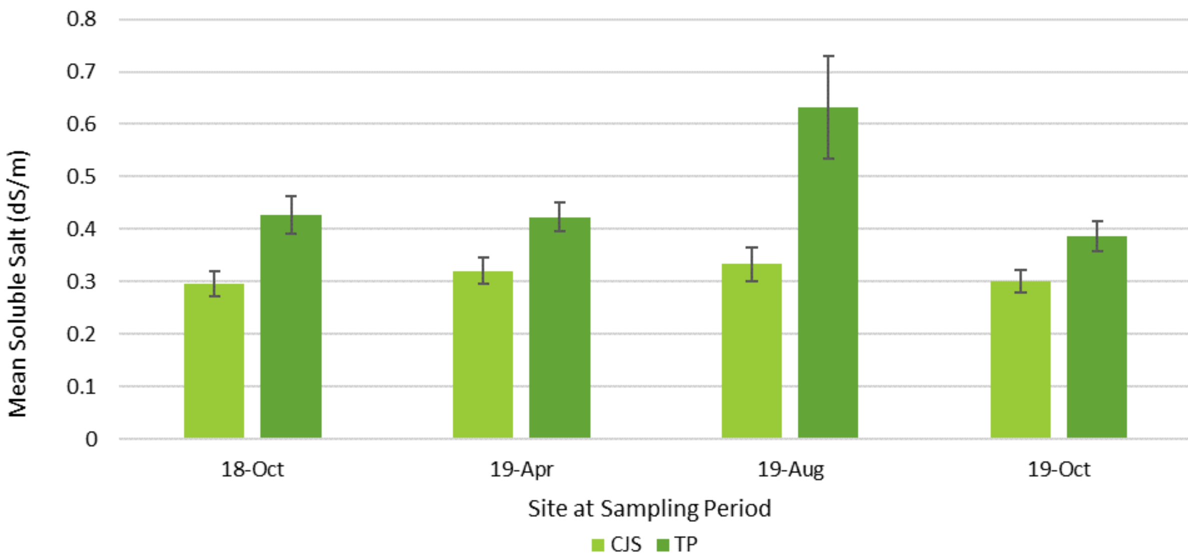


Figure 10. Mean soluble salt levels (in dS/m) by site over time. Significant differences in Soluble Salt levels between Chicago Junior School and Trout Park across study period ($p < 0.02$, Wilcoxen Test; Table 4). Error bars indicate 95% confidence intervals.

the study period and for calcium in October of 2018 (Fig. 9). Both Cl and Na were consistently higher at Trout Park than Chicago Junior School (Figs. 8 and 9; Table 1) with the highest average Cl concentrations documented at both Chicago Junior School (41.75 ± 3.52 ppm) and Trout Park (155.2 ± 28.05 ppm) in August 2019 (Fig. 8). The highest Na concentration documented at Trout Park (168.05 ± 30.65 ppm) was also recorded in August 2019 (Fig. 9). No significant differences (Wilcoxen Test, $p > 0.05$) were documented in the concentration of cations Magnesium (Mg) or Potassium (K) throughout the study period (Table 1).

DISCUSSION

This study was conducted to identify the reasons behind differences among populations of *Thuja occidentalis* at Trout Park and Chicago Junior School. In particular, the impact of road salt on seed germination, reproductive differences between sites, and soil salt concentrations. Overall, we found significant differences in seed set, cone weight, and seed morphology between sites, with fewer seeds, smaller cones, and smaller seeds seen in Trout Park individuals. Based on soil samples, there are significant differences in salt concentrations between sites with higher concentrations seen in the soil at Trout Park.

Schopmeyer (1974) reported that seed germination under control conditions for *Thuja occidentalis* is up to 37%. In our study, except for the commercial seeds, our germination results were lower (Figs. 1 and 3). Pakulak commercial seeds, outperformed all others in germination trials across treatment levels with raw average germination at 52% under control conditions. However, our 2 focal study sites had less than 5%. Despite differences among sites, all sites showed a steady decline in germination as salt concentrations increased (Fig. 2). This result is similar to other studies assessing the germination of other plant species under varying salt concentrations (Bojović et al., 2010; Daddario et al., 2017; Özdemir et al., 2016). Germination declined in all sites at salt concentrations 100 mM and over. In addition, at 2 sites, no germination occurred in concentrations over 200 mM. This concentration of NaCl, 200 mM, is less than half (555 mM; Table 1) of the most elevated salt levels documented in soil samples taken at Trout Park during the study,

In addition to germination, we examined several reproductive metrics (i.e., cone biomass, seed set, and seed morphology) to evaluate the health of *Thuja occidentalis* populations at Trout Park and Chicago Junior School. Trout Park individuals produced fewer seeds and had smaller cones than any other site in the study. Additionally, morphometric measurements were consistently smaller in Trout Park individuals, except for embryo width, than in Chicago Junior School individuals. Overall, our reproductive metrics are similar to those reported by Briand et al. (1992), but in the 2 study sites, seed set was particularly low. In addition, Briand et al. (1992) noted

Table 1. Results of salt components in soil sample analyses over sampling period of October 2018 to October 2019. Results of a Wilcoxen Test with significant differences (p-values <.05) between Chicago Junior School and Trout Park, labeled with an asterisk (*).

Soil Component	Sampling Time	Wilcoxon p-value =	Chicago Junior School Mean	Min	Max	SE	Trout Park Mean	Min	Max	SE
Cl (ppm)	October-18	*0.002	5	5	5	0.00	86.2	5	436	29.42
	April-19	*0.000	11.25	5	64	3.52	101.35	5	253	13.90
	August-19	*0.000	41.75	10	169	10.93	155.2	20	555	28.05
	October-19	*0.001	39.8	5	99	5.95	82.9	30	248	11.73
Na (ppm)	October-18	*0.000	35.55	13	100	5.95	95.95	25	211	12.55
	April-19	*0.000	13	1	55	3.26	57.8	1	201	10.19
	August-19	*0.000	30.95	12	128	7.09	168.05	10	553	30.65
	October-19	*0.000	24.7	9	117	6.16	74.25	20	244	10.49
Ca (ppm)	October-18	*0.040	12687.9	4069	14665	803.57	12737	4393	14688	733.88
	April-19	0.508	11836.2	4339	16901	1182.86	10717.6	92	16887	1237.19
	August-19	0.570	14307.55	3295	18313	1318.18	14033.45	3727	18315	1031.48
	October-19	0.457	12711.1	4982	14614	842.14	13251.15	4105	14615	594.16
Mg (ppm)	October-18	0.465	473.7	287	776	26.40	505.5	237	774	32.43
	April-19	1.000	372.7	112	792	45.63	350.25	1	703	44.29
	August-19	0.490	556.15	401	802	27.36	576.7	167	876	40.11
	October-19	0.525	513.15	246	872	36.45	485.95	233	805	37.30
K (ppm)	October-18	0.223	43.05	14	102	5.01	33.3	13	57	2.92
	April-19	0.130	43.45	1	153	10.12	28.1	1	199	9.46
	August-19	0.499	50.7	20	125	5.84	42.8	25	75	3.41
	October-19	0.176	36.6	8	104	6.28	22.7	3	46	2.92
Soluble Salts (dS/m)	October-18	*0.002	0.296	0.18	0.57	0.02	0.4265	0.22	0.85	0.04
	April-19	*0.007	0.321	0.19	0.67	0.03	0.4235	0.23	0.67	0.03
	August-19	*0.000	0.3335	0.17	0.72	0.03	0.632	0.25	2.31	0.10
	October-19	*0.017	0.301	0.2	0.47	0.02	0.386	0.26	0.85	0.03

that these reproductive and morphological differences could be the result of local environmental variation rather than genetic differences.

One environmental factor that could explain differences between Trout Park and Chicago School is salt levels. Increased and continued runoff may influence fluctuations in measured salt levels in the groundwater, which is also detectable in soil samples at both sites. An ISGS (Plankell et al., 2020) report found higher Chloride (Cl) levels at Trout Park, up to 800 ppm compared to a maximum of 555 ppm in this study. Both sites are groundwater-fed ecosystems and elevated salt concentrations could mimic drought conditions and create nutrient imbalance at the root level. *Thuja occidentalis* is sensitive to changes in hydrology, especially dry conditions, and fluctuations in water table depth, in addition to salt stress, could affect the health of adult trees. A decline in the health of mature trees could explain low seed set and seed size for these populations compared to trees in more control conditions like those grown for seed production and populations at Chicago Botanic Garden and Morton Arboretum.

There are many potential factors that could be affecting the health and recruitment of *Thuja occidentalis* in this region. In addition to previously outlined pressures on these populations, this species is surviving in an extant population at the southernmost extent of its range. While it does not explain site differences for individuals in proximity, higher regional temperature in the southern part of the species range as well as fluctuations in water table depth may explain some of the causes of decline in adult trees in the region (Housset et al., 2015). Fluctuations in soil moisture during germination and early development of seedlings could be detrimental to survival (Larouche et al., 2011). Both sites have complex legacies including a history of grazing at Chicago Junior School and a large blowdown in 1920 at Trout Park which have potentially affected the overall health of the site. Continued use for recreation at both sites may enable further trampling, disturbance, and pollution.

CONCLUSIONS

Elevated salt concentrations documented in soil samples at Trout Park indicate road salt runoff and pollution in the groundwater from adjacent roads. In addition, elevated salt concentrations in the water table and soil may affect overall health and survival of adult trees and may potentially inhibit germination of *Thuja occidentalis* seeds. Salt runoff into this unique fen may introduce additional pressure to a stressed and declining population of *Thuja occidentalis*, particularly at Trout Park. Additional studies are needed to further explore the health differences between Trout Park and Chicago Junior School. Further work using isotope analysis of adult tree tissue could provide information about differences in the health of mature trees at each site. Also, exploring microbial and fungal communities could provide additional information about the soil quality and composition at Trout Park and Chicago Junior School.

In addition, the low seedling recruitment observed at Trout Park needs further exploration as both abiotic (e.g., salt) and/or biotic factors (e.g., deer) could be playing a role. While we did not measure seedling survivorship under varying salt concentrations, studies (e.g., Bojovic et al., 2010) with other species show that seeds are capable of imbibition under elevated salt levels but at a slower pace. Additionally, recovery for non-halophytic species is low with survival in saline conditions resulting in slow growth and eventual mortality (Cochrane, 2018). While seeds may continue to germinate under elevated salt concentrations, eventual survival may be affected by chronic inundation. A more in-depth study should be considered to access the impact of deer herbivory. For example, the addition of deer enclosures around persisting seedlings could enable a long-term study of seedling survival without herbivore pressure. Lastly, genetic testing of both populations could provide insight into the genetic diversity of this split population. This could eliminate other potential factors impacting survivorship at each site and determine more causal relationships of differences between Trout Park and Chicago Junior School.

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