

The effects of water salinity on *Myzus persicae* and *Arabidopsis thaliana*

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SUMMARY

Road salt is often used during the winter months as a cheap and efficient way to melt snow. However, it also poses some serious environmental issues. Runoff containing road salt can lead to soil salinization, which can impact plant health. This has detrimental effects on not only the organisms that feed on these plants, but also on the surrounding ecosystem. The purpose of this experiment was to study how salinity affects plants and the herbivores that feed on them. Wild type *Arabidopsis thaliana* plants and *Myzus persicae* (green peach aphid) were the model organisms used in the experiment. It was hypothesized that watering the plants with different concentrations of saline solutions would affect the health and growth of the plants as well as the *M. persicae* populations. Wild type *A. thaliana* plants were inoculated with *M. persicae* and watered with either regular tap water, a 20 mM saline solution, or a 60 mM saline solution. Rosette leaf surface area and aphid population were recorded for each plant over the course of the experiment and analyzed to determine the effect of salinized water on *A. thaliana* and *M. persicae*. The saline treatments did not have a significant effect on the aphid population growth, but did significantly affect the leaf surface area. There was no significant difference between the control and the high salt treatments or the control and low salt treatments. However, statistical significance was found between the low and high salt treatments, suggesting that salt did have some effect on plant fitness. The lack of effect on the aphids could be due to two main reasons. Firstly, the plant may have allocated its resources towards survival rather than defence against the aphids. Secondly, the regions of the plant preferred by the aphids may not have been affected by the saline solution. Since the aphids do not appear to be affected by salinization, plants will have to face the double burden of salt stress and herbivory.

Received: 11/04/2017

Accepted: 03/15/2018

Published: 03/15/2018

URL: <https://theiscientist.com/2018/03/15/TakahashiEffects.html>

Keywords: salinity, *Arabidopsis thaliana*, *Myzus persicae*, soil, road salt, de-icing, ecology, plant-animal interactions

INTRODUCTION

Road salt is one of the most widely-used environmental pollutants in Canada since it is a cheap and efficient way to melt snow and ice during the winter months. Road salt is usually made up of sodium chloride, and when used to de-ice roads and sidewalks, it significantly increases the concentrations of sodium in bodies of freshwater in North America (Godwin, Hafner and Buff, 2003). Moreover, studies have shown that the increase in sodium concentration has led to groundwater

contamination and plant toxicity issues (Forman and Alexander, 1998). Increasing soil salinity also has detrimental effects to plant health, affecting the plant's ability to uptake water and photosynthesize (Bryson and Barker, 2002). Consequently, it is important to study the impacts high salinity would have on not only the plants, but also on the ecosystem that the plants inhabit. While the negative effects of sodium on plant species may cause noticeable changes in plant health, any influence on the rest of the ecosystem and other species may be less apparent.

Although population size is regulated through both top-down and bottom-up interactions, the influence of either varies in each population. A discussion in the American Naturalist argues that community structure is controlled by bottom-up interactions, meaning every trophic level is “food-limited” (Hairston, Smith and Slobodkin, 1960). It is also argued that community structure is regulated by top-down interactions, whereby each trophic level limits the population size of the trophic level below it (Hillebrand, et al., 2007). In a community that operates via bottom-up interactions, an increase in soil salinity would likely negatively impact the rest of the ecosystem, which depends upon the affected plant species for energy. The influence of the salt would be seen first within the primary producers and then within herbivore populations such as insects. Any impact on the size of these insect populations would be sure to have repercussions elsewhere in the food chain. Therefore, it is important to determine whether road salt influences plant growth and the effect this has on herbivores.

To address this issue, a 13-day experiment was designed to observe the interactions between the plant species *Arabidopsis thaliana* and one of its predators, *Myzus persicae*, more commonly known as the green peach aphid. The study was conducted in order to answer the following set of questions: (1) is the growth rate of *A. thaliana* affected by an increase of soil salinity, and (2) how does the concentration of salinity affect the size and growth of the *M. persicae* populations feeding on *A. thaliana*?

Watering the *A. thaliana* plants with salinized water was predicted to cause decreased plant growth and a reduction in aphid population size for several reasons. Continual high salinity in plants results in the cessation of plant leaf surface expansion, a decrease in the productivity of photosynthesis, and plant death (Parida and Das, 2005). This causes aphid populations, which rely on the phloem sap of plants for nutrition (Giordanengo, et al., 2010), to be left without a food source. Additionally, high salinity in plants increases plant mesophyll and epidermal thickness (Boughalleb, Denden and Tiba, 2009). This may make it more difficult for the aphids to pierce the leaves for food (Giordanengo, et al., 2010).

By observing the effect of saline soil on *A. thaliana* and *M. persicae*, the present study aims to better understand the effects of road de-icing on primary producers and the organisms that feed on them. The results of the study may have important implications for the future of ecosystems both around Cootes Paradise and around the world.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

For the experiment, 24 healthy wild type *A. thaliana* rosettes were chosen out of a selection of plants grown in the McMaster University Greenhouse in Hamilton. These plants were deemed healthy based on their lack of discoloration or torn leaves. All chosen plants either had not begun the bolting stage or had bolts that had been previously removed. The study used 96 viviparous female *M. persicae* (green peach aphid) that had been reared on either *A. thaliana* or tobacco leaves. Randomization of the treatments was done using a random number table (Petrie and Sabin, 2009). To make the randomization process more efficient, a four-number range was chosen for each plant. For example, numbers one to four corresponded to plant A, numbers five to eight corresponded to plant B, etc. (Table 1). The random number table was then used to assign treatments to plants in the systematic pattern of “no salt”, “low salt”, and “high salt”, resulting in eight plants per treatment.

Table 1: The *Arabidopsis thaliana* plants and their corresponding random table number ranges and treatments. Although only plants A-G are shown in the table, the remainder of the plants were assigned a letter, a four-number range, and a treatment in the same manner.

Plant	Range of Numbers	Salt Level
A	1-4	High
B	5-8	None
C	9-12	None
D	13-16	Low
E	17-20	Low
F	21-24	None
G	25-28	High

This random number table was used again to determine the placement of the plants in the two trays. The sequence of two-digit numbers in the table dictated the order in which plants were placed in the tray. Plants were placed one at a time into Tray 1, first filling up a row of three and then going on to the next until there were four rows of three plants each. Tray 2 was filled in the same manner (Figure 1). Plants were arranged in a staggered fashion to minimize the contact between plants and to reduce the chance of aphids migrating between plants.

SALINE TREATMENT SET-UP

Micropipette tips were used to facilitate the watering of the plants. 96 tips were retrieved and cut at the second line from the bottom so that each tip was 4.5 cm long. This allowed water to travel through at a fast-

Tray 1		
G	W	K
Q	O	B
S	I	H
U	R	M

Tray 2		
A	X	D
L	E	V
T	J	C
F	N	P

Figure 1: Plant treatments (red - no salt, green - low salt, blue - high salt) and tray placement.

er rate. Four of the modified micropipette tips were placed near the edge of the container in the middle of each side. They were then pushed in at an angle approximately 65° from the horizontal into the soil until their tops aligned with the surface (Figure 2).

The plants were watered with a 20 mM salt solution, a 60 mM salt solution, or regular tap water depending on their treatment group. Two 500 mL solutions were created for each concentration so that plants could be watered with the same solution each time. Tap water was used instead of distilled water to ensure that the plants still received some of the minerals that are found in tap water.

The plants were watered one tray at a time. A micropipette was used to fill each of the four modified micropipette tips with tap water in all the plants in the “no salt” treatment group (i.e. the control plants) on the first tray. Each of the 4 micropipette tips were first filled with 1 mL of water, adding up to a total of 4 mL per plant. Immediately after, the same plants were watered with another 4 mL of tap water, resulting in a final volume of 8 mL of tap water in each control plant. The water was allowed to sink into the soil through the micropipette tips while the plants in Tray 2 were being watered in the same manner. This process was repeated one more time, so that in the end, each control plant had received a total of 16 mL of tap water. The process was repeated in its entirety using the 20 mM and the 60 mM salt solutions for the plants in the “low salt” and “high salt” treatments, respectively.

INOCULATION OF *M. PERSICAE*

Every plant was first inoculated with two aphids using a metallic probe and a magnifying glass. This number was chosen to ensure that there was a sufficient number



Figure 2: A top-view image of a plant that was taken using the quadripod. The four circles at the north, east, south, and west sides of the plant pot are the micropipette tips that were inserted at an angle of approximately 65° and used to water the plant.

of aphids for all 24 plants. Each plant was put on the lab bench after inoculation to avoid mixing up inoculated and uninoculated plants. After every plant had received two aphids, a second round of inoculation occurred, whereby each plant was populated in the same way with two more aphids. In the end, each plant had a total of four aphids.

DATA COLLECTION

Front-view and top-view photos were taken of each plant for comparison throughout the study. A quadripod was built out of a cardboard box in order to ensure that the top-view photo of every plant was taken from the exact same position, height and angle each time (Figure 3). Several holes were cut out of the box to allow the maximum amount of light to reach the plant. An outline was drawn on the top of the box to ensure that the photography device (an iPhone 6) was placed in the same location for each photo. A sheet of blank paper with an outline of the bottom of the plant pot was attached to the legs of the quadripod to aid with the placement of the plant. A small piece of cardboard was used to make a frame with a white background to give sufficient contrast for the pictures. A ruler was taped to the cardboard to provide a scale for pictures. The photos of all the plants were taken in the exact same

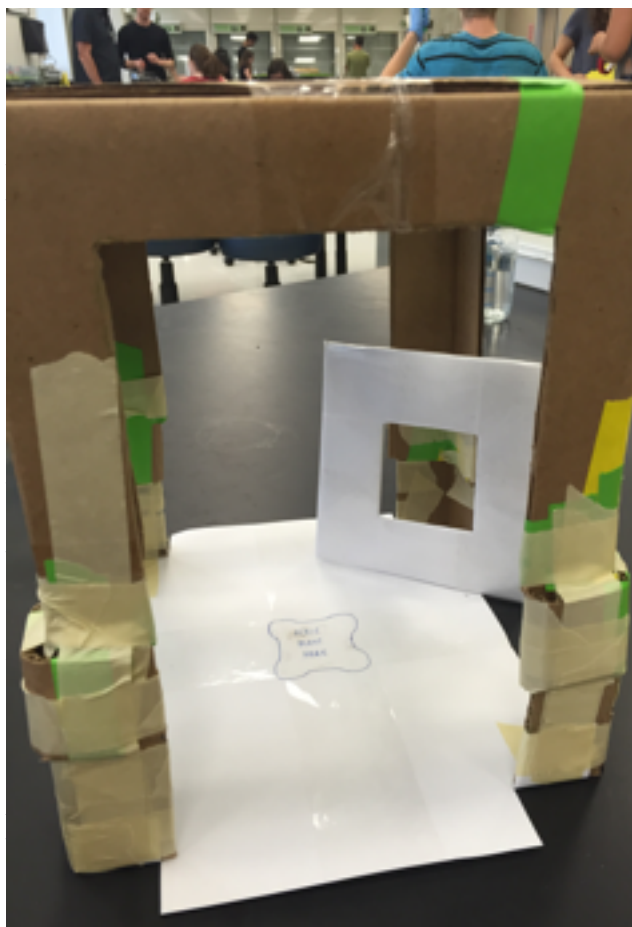


Figure 3: The cardboard quadripod for taking top-view images of the plants (the ruler is not present in the photo). The square frame was used to provide a white background to enable better visualization of the rosette leaves. The plant pot was placed in the outlined area on the sheet of paper and the iPhone 6 was positioned using the outline on the top of the quadripod.

visualization and analysis of the data as the effect of treatment was separated from date. A two-way analysis of variance (ANOVA) test was conducted to see whether treatment and date had a significant effect on aphid population growth and whether there was a significant interaction effect from the two variables. This interaction effect would indicate that variance in aphid populations between treatments depended on the number of days elapsed.

The aerial photos of the plants were analyzed using an image processing software, ImageJ. Using the software, the surface area of the exposed rosette leaves (in cm^2) of each plant was calculated for every observation day. This value was used as an estimate of total plant leaf surface area. The scale of the program was calibrated using the ruler in the plant image. A boundary was drawn by hand around the plant leaves to accurately select the area for measurement. This data was analyzed using the same statistical method (a two-way ANOVA) as the aphid population data to see if treatments had an effect

on the *A. thaliana*.

Any significant results ($p < 0.05$) from the two-way ANOVA analysis were further explored using a post-hoc Tukey's Honest Significant Difference (HSD) test to compare the means of the different treatment groups.

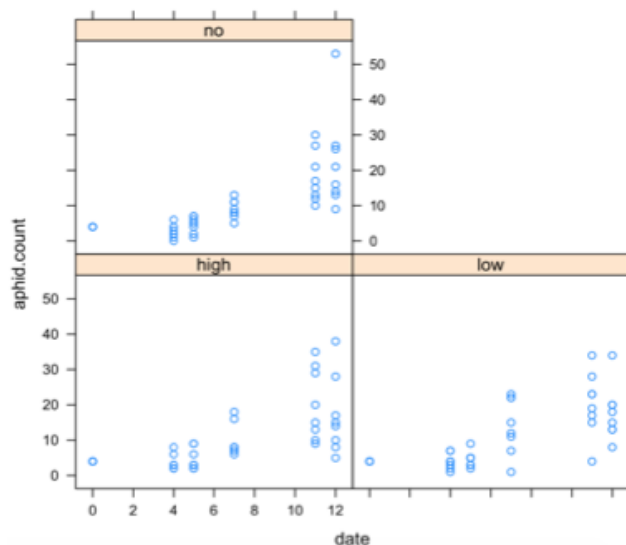


Figure 4: Aphid population change throughout time on plants watered with normal water, with a low-concentration salt solution, and with a high-concentration salt solution.

RESULTS

APHID POPULATION CHANGE

Within the different treatments, there were no noticeable trends with respect to aphid population growth. Both of the treatments and the control saw the aphid population increase with time, and there was no noticeable difference regarding the manner in which the populations increased (Figure 4). This suggests that the salt treatments did not have an impact on the aphid population growth. This is also evident when the average aphid population count is compared within each of

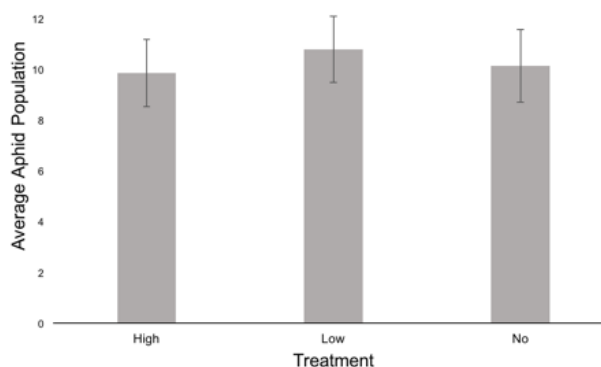
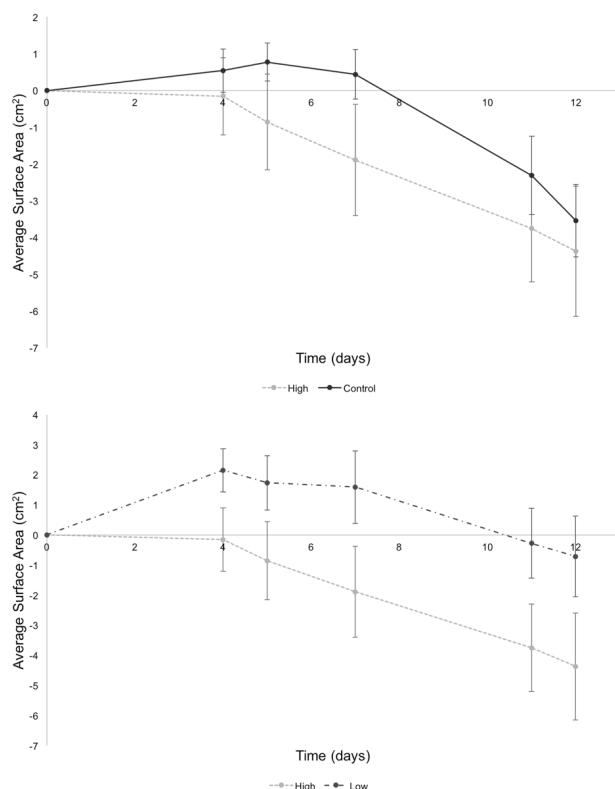


Figure 5: The average population of aphids on plants watered with tap water, a low-concentration salt solution and a high-concentration salt solution. The error bars represent $\pm \text{SE}$.



the three treatments (Figure 5).

A two-way ANOVA test was conducted on the relationship between the aphid population growth and both the treatment and the date. The relationship between aphid population growth and the treatment was deemed insignificant ($p = 0.76$). The only variable that was significant in relation to aphid population growth was the date ($p < 0.001$). In other words, the different salt solution used to water the plants did not have any impact on the growth of aphid populations.

PLANT SURFACE AREA CHANGE

Using ImageJ, the surface area of the exposed rosette leaves of each plant was measured for each of the days that they were surveyed. Each plant began with a unique and distinct surface area. In order to account for this difference, the surface area change of each plant through time was compared. Initially, it was difficult to notice any trends in the data collected. As well, each plant's growth and shrinkage pattern was unique, making visual analysis of the data impossible.

Due to the three distinct variables (high salt, low salt, and no salt), a two-way ANOVA was used. The relationship between surface area change and treatment was

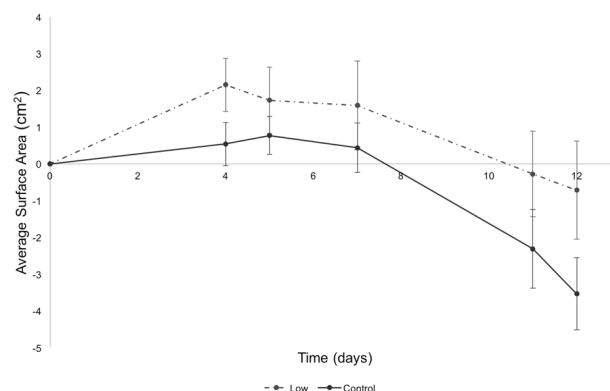


Figure 6: Average plant surface area change throughout time of plants watered with a high-concentration saline solution (60 mM), a low-concentration saline solution (20 mM), and tap water. (A) shows the relationship between the “high salt” treatment and the control; (B) shows the relationship between the “low salt” treatment and the control. (C) shows the relationship between the “high salt” treatment and the “low salt” treatment. The Tukey HSD test indicated that there was a significant difference between the high and the low treatments ($p = 5.276 \times 10^{-4}$). All the error bars were calculated by measuring the standard error of the data points.

significant ($p = 3.27 \times 10^{-4}$). It should be noted that time also had a significant effect on the plant surface area ($p = 2.45 \times 10^{-5}$). However, time and treatment affected the plants independently of each other ($p = 0.856$). Thus, the manner in which the date affected the surface area change did not impact the way in which the treatment affected the surface area change.

To determine which treatment had an effect on the plant surface area change, a Tukey HSD test was run (Table 2). The test revealed that there was a significant difference between the high and the low treatments ($p = 5.276 \times 10^{-4}$). Interestingly, it also revealed that there was no significant difference between the control and the high salt treatment or the control and the low salt treatment ($p = 0.1884$ and $p = 0.09536$, respectively).

To determine how the distinct treatments affected the plant surface area growth with respect to time, the change in surface area of all plants for each treatment was averaged for each day that data was collected. The averages of the variable interactions in Table 2 were graphed against each other (Figure 6). The plant surface area of the plants in the high salt treatment group decreased the most throughout time; however, all of the treatments had a net negative impact on plant growth

Table 2: Result of a Tukey Honest Significant Difference test with a 95% family-wise confidence level analyzing the differences between the impact of the salt treatments on *Arabidopsis thaliana* plants.

Variable Interactions	Difference	Lower bound	Upper Bound	Adjusted p-value
Low - High	2.497	0.9595	4.035	0.0005276
No - High	1.140	-0.3980	2.678	0.1884
No - Low	-1.358	-2.895	0.1804	0.09536

over time. There was no significant difference between the control plants and the low salt treatment group or between the control and the high salt treatment (Figure 6A and 6B). The significant difference between the low and high treatments is visible since the error bars of the graph do not overlap (Figure 6C). In other words, the three graphs support the results obtained using the Tukey HSD test.

DISCUSSION

These results give some insight into the impact of winter road salting on both plant and aphid populations. First, this study suggests that varying saline concentrations in the soil have an impact on *A. thaliana* growth. The results of this experiment did not entirely confirm the initial hypothesis because the control group did not differ from either treatment groups. Had the hypothesis been entirely accurate, the decrease in plant surface area would have been significantly greater in the high salinity plants than in the control, which was not the case. However, the results of this study did indicate that increasing the salt concentration from 20 mM to 60 mM negatively impacted plant leaf area. Salted soils are known to impede the growth of many plant species (Qados, 2011). Increasing salinity has been shown to decrease water uptake by the roots, alter leaf osmotic potential, modify protein content, increase sodium and chloride ions in the chloroplasts, reduce the quantity of photosynthetic pigments present in the leaves, and inhibit photosynthesis (Ueda, Tsutsumi and Fujimoto, 2016; Heidari, et al., 2011; Qados, 2011; Gama, et al., 2009). This, in turn, can have a negative effect on leaf number, plant dry mass, and leaf area (Qados, 2011). Additionally, exposure to salt stiffens the epidermal cell wall of the plant (Zörb, et al., 2015). The present study indicates that *A. thaliana* may be susceptible to salt stress and that exposure to salted soils might negatively impact its fitness.

Contrary to *A. thaliana*, however, the *M. persicae* population was in no way affected by increased salinity. This rejects the hypothesis that was initially postulated. *M. persicae* feeds by sucking the phloem of the plant (Dutton, et al., 2002). If the salinity of the soil had caused a change in phloem chemical composition or availability, it should have negatively impacted the population growth of *M. persicae*. Additionally, since salt causes plants to thicken and stiffen their epidermis (Zörb, et al., 2015), it could have been possible that the aphids were unable to pierce it with their stylets and therefore died from lack of food. However, no such effect was observed.

There are a few potential reasons why the aphid population was not affected by the increase in salinity. *A. thaliana* defends itself from aphids in multiple differ-

ent ways. Its constitutive defences include trichomes and toxic antibiotics in its phloem (Louis and Shah, 2013). The plant also makes use of inductive defences such as increasing starch and glucosinolate concentration when attacked, or inducing premature death of leaves on which *M. persicae* colonies are established (Louis and Shah, 2013). While effective, these inductive mechanisms of defence are costly to the plant. It is possible that, when under stress from increased salt concentration, the *A. thaliana* would preferentially allocate resources to survival rather than defence. If this were the case, *M. persicae* would not be impeded in its feeding and the colonies would thrive. Another possibility is that although the leaf surface area decreased in the high-salinity treatment, this did not have a population-limiting effect on the aphids. During the study, the aphids seemed to aggregate preferentially on the stems and bolts, so perhaps a slight reduction in leaf area would not have caused a measurable impact on aphid habitat.

These findings carry some heavy implications with regards to the use of salts for road de-icing. Naturally high levels of soil salinity already impede plant growth in several locations around the world, and further salt addition through road de-icing only exacerbates the problem (Qados, 2011). By some estimates, salinization of arable land will have caused a 50% loss of agricultural space by 2050 (Heidari, et al., 2011). Furthermore, studies have shown that some plants are even more vulnerable to high salt concentrations when under drought conditions (Ha, et al., 2008). Given the water shortages currently devastating several regions of the world, the safekeeping of our agricultural lands is crucial. Additionally, if herbivores such as *M. persicae* are unaffected by the increase in salinity, plants will have to face the double burden of salinization and predation. This could have dire consequences on food production as well as on fragile ecosystems such as Hamilton's Cootes Paradise.

In light of these potential consequences, it seems vital to continue research in this direction. The present study had some limitations that future research could address. In this experiment, data was collected by manually counting the live aphids, which may have allowed for some errors during the count. In future studies, a more accurate way of assessing aphid population growth should be employed. This could include the use of microscopes as well as a greater number of independent counts of each plant. As well, ImageJ, the program used to calculate rosette leaf surface area, has limitations as it can only analyze images two-dimensionally. This did not account for the fact that some leaves naturally lie more flat while others grow in an upward direction. In future studies, a more accurate technique should be em-

ployed. Furthermore, the potted plants were kept very close to each other over the course of this experiment, which may have allowed for migration of the aphids from one plant to another. It is also worth noting that the plants did not all start off at the same stage of growth. Some were already bolting, while others were still in the rosette stage. In an effort to normalize the growth stage, the bolts were cut off of the more mature plants. However, this may have further impacted their growth. Additionally, the saline solutions used to water the plants were of relatively low salt concentrations. *A. thaliana* has been known to survive at saline concentrations of 100 mM (Ueda, Tsutsumi and Fujimoto, 2016). It is possible that repeating this experiment with higher salt levels would have a greater impact on the plants, thus yielding clearer results. Finally, and perhaps most importantly, the experiment only lasted for a duration of 13 days. While this allows us to gain some information on the effect of salinization on aphid populations, the salt levels that accumulated in the soil over 13 days are not entirely comparable to a season of road salt build-up. Future studies could investigate this system over a greater time period.

CONCLUSION

This experiment demonstrated the negative effects salt has on plant growth and health. The aphid population sizes were not significantly correlated with increased salinity levels, but the leaf surface area of the *A. thaliana* decreased with increased saline treatments.

It should be noted that, although no effect on herbivores was observed, future research should be done to determine whether this is true for other herbivores and plant types. Overall, the negative impact that salt had on *A. thaliana* in this experiment provides further justification as to why an alternative method for de-icing should be researched, as protection from salinization is crucial for ecosystem preservation.

ACKNOWLEDGMENTS

The researchers would like to thank Dr. Susan Dudley (McMaster University), Sebastian Irazuzta (McMaster University), and Russ Ellis (McMaster University) for their guidance and assistance. McMaster University Greenhouse is acknowledged for providing the *A. thaliana* and *M. persicae*.

AUTHOR CONTRIBUTIONS

All authors contributed equally towards the gathering of background research, designing of the experiment, collection of data, and editing of the final manuscript. In terms of composing the manuscript, M.T. and A.T. wrote the abstract and A.F. focused on the introduction. M.G-A. and A.T. wrote the methods, while M.T. performed the statistical analyses, wrote the results, and produced the figures. P.B. focused on the discussion and A.F. wrote the conclusion.

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