Title: The Influence of Roadside Conditions on the Shoot Growth of Wisconsin Fast Plants (*Brassica rapa*)
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Abstract

Urban runoff from snowmelt and rain events is caused by impermeable surfaces such as roads and parking lots, and has the ability to carry an array of pollutants into roadside environments. Contaminants in runoff such as salt from road deicing efforts, motor oil from leaking cars, or excess water can affect surrounding soils and bodies of water, and therefore vegetation. To see how different roadside conditions and seed varieties affect the aboveground growth of Wisconsin Fast Plants (*Brassica rapa*), three seed varieties were grown under conditions in which they were watered weekly with varying concentrations of saline (230 mg/L NaCl, 4 g/L NaCl, or 35g/2L NaCl), grown in motor oil polluted soil (5% oil w/w or 10% oil w/w), or were grown in compacted soils (1.5cm or 3.0 cm compaction). Seed variety had a significant effect on aboveground growth ($p < 0.05$). Increased salinity and oil pollution in soil caused significant declines in aboveground growth ($p < 0.05$), while soil compaction prior to planting caused a marginally significant decline in plant growth ($p = 0.0547$). These results indicate that urban runoff should be monitored to ensure that roadside plants continue to grow successfully and aid in flood control and soil erosion.

Introduction

Runoff from urban roads and paved areas is a major contributor to nonpoint pollution in surface waters (Shrestha et al, 2018) and surrounding environments. In particular, federal and local roads can create large problems for roadside soil, waters, and the surrounding flora and fauna. In the United States alone, there are roughly 6.2 million kilometers of public roads which are utilized by 200 million vehicles (Forman, 1988). In order to create roads, the removal of vegetation such as trees or grasses is required, and these roads are often paved with asphalt, an impervious material. Since where roads are, water cannot infiltrate the ground, runoff becomes an issue.

Urban runoff is made by snowmelt events or from stormwater and as the water moves, it picks up a multitude of pollutants along the way (Müller et al, 2020). Pollutants from urban runoff can be absorbed by plants, deposited in soils, or can be introduced to stream ecosystems and can be diluted and carried further from the source (Forman et al, 1988). Among the pollutants transported by urban runoff are solids/floatables like bottles or plastic bags, sediment from the road like sand and sediment from construction, nutrients like phosphorus and nitrogen, pesticides, metals like lead, arsenic, and mercury, road salt, petroleum hydrocarbons, and even pathogens such as *Escherichia coli* which can cause gastroenteritis in humans (NJDEP, 2004). The pollutants picked up by urban runoff can be deposited in soils and introduced to waterways near the roads. One form of sediment, road dust, can be transferred roughly 10-20m from the road surface and can provide nutrients for plant growth, damage vegetation, or change the pH of the soil (Forman et al, 1988). When sediment is carried by runoff into waterways such as streams or lakes, it can increase turbidity in those water bodies. When nutrients like nitrogen and phosphorus are transported to water bodies, algae blooms could result, which can lower the dissolved oxygen content of that ecosystem (WSU, n.d). Furthermore, metals like nickel, lead, copper, and manganese typically contaminate the road surface due to normal use of vehicles (Müller et al, 2020). These metals are almost always deposited in the soil close to the road surface affecting local organisms. For example, lead was found in the tissues of small mammal species that were in close proximity to the road (Forman et al, 1988).

A component of urban runoff that is of concern in temperate areas is road salt. Road salt (NaCl) is used in cooler climates as a deicing agent and is typically applied either as rock salt after snow or ice events, or as a brine solution (23% NaCl) prior to snow or ice events (Ohio LTAP). Following snowmelt or a storm, runoff from roads can carry these salts far distances, affecting soils, vegetation, aquatic life, and aquatic environments. In New Jersey, the average concentration of chlorides in stormwater is 230mg/L (njstormwater.org). When this water moves into local bodies, it can increase salinity of the aquatic environments. According to Beasley, 2020, rural lakes that receive runoff from roads can have a salt concentration of 150 mg/L, ponds can have a concentration of 4 g/L, streams can have a concentration of 4.3 g/L, and urban impoundments can have a salt concentration of 5 g/L. In these water bodies, an increase of salinity can be damaging to the function of wildlife, especially amphibians (Beasley, 2020). The accumulation of salts in soil can also be damaging to roadside vegetation. Excess salt can increase water stress in plants, can affect the uptake of nutrients like potassium and phosphorus, can stunt growth and lead to premature drop of leaves (Gould, 2013). Previous research done by Crocker et al involved watering wild rice plants with saline solutions of either 0 mg/L, 100 mg/L, 500 mg/L, 1000 mg/L, 3000 mg/L, or 5000 mg/L, and found that higher salt concentrations had a negative effect on growth of these plants.

Another pollutant of concern is motor oil since it can cause harm to both plants and animals. According to the Massachusetts Department of Environmental Protection, just one quart of motor oil can pollute over 250,000 gallons of water. Furthermore, oil can be deposited in soil for a long time, subsequently affecting soil properties and therefore plant growth. Oil contamination can clog pores of soil, reducing aeration and water infiltration in soils, affecting the amount of water and oxygen available to roots, thereby affecting plant growth (Klamerus-Iwan et al, 2015). In a previous study, *Amaranthus hybridus* L. seedlings were grown in soil with 0-5% v/w of spent engine oil. Mean heights of the plants as well as leaf area were measured. In the 5% v/w treatments, the mean height was 27.0 ± 1.25cm and the leaf area was $5.63\pm0.36 cm^2$, whereas in control plants (0% v/w) the mean height was 41.4 ± 0.8cm and the leaf area was $13.44\pm0.22 cm^2$ (Odjegba et al, 2002).

In addition to chemically, urban runoff can affect nearby soils and waterways physically. Runoff can be responsible for a reduction in percolation and aquifer recharge rates due to impermeability of the pavement, alter channel morphology, increase the discharge rate of streams, and increase the rate of erosion (Forman, 1988). When the rate of infiltration of soils is exceeded by the runoff rate from roads, flooding can ensue since the water cannot be taken up quickly enough to prevent runoff. Flooding can harm vegetation by preventing the proper uptake of oxygen (Wiebold, 2009).

The conditions created in a roadside environment can also limit the richness of vegetation present. According to Forman, 1988, a roadside is defined as an intensively managed strip, often by mowing, that is dominated by herbaceous vegetation, and is adjacent to a road surface. Roadside vegetation often grows rapidly with ample light and by the moisture from road drainage (Forman, 1988). Roadsides often serve as good habitats for opportunistic invasive plant species since high traffic volume creates greater disturbance, creating conditions favorable to invasive species spread (Joly et al, 2011). While invasive species may be able to survive in the conditions created in roadside environments, native and introduced species may not have as great of survival. To see how roadside vegetation may react to the conditions of a roadside environment, Wisconsin Fast Plants (*Rapid Cycling Brassica rapa*) will be used. Wisconsin Fast Plants are plants that are selectively bred to have a short flowering time and be fully grown forty days after planting (fastplants.org). According to the fast plants website, the closest vegetable relatives to these plants are turnips. Turnips (*Brassica rapa subsp. rapa*) have shoots that are moderately tolerant to salinity, and roots that are moderately sensitive to salinity, with salinity having little effect on seed germination (Shannon et al, 1999). Since the turnip is the closest vegetable relative to Wisconsin Fast Plants, it is expected that the fast plants will react similarly to increased salinity. To gauge how different plant types may react to these conditions, standard plant, petite plant, and tall plant seed varieties will be used.

In order to mimic conditions created in roadside environments, Wisconsin Fast Plants will be subjected to either increased salinity (230 mg/L, 4 g/L, or 230 g/L NaCl), soil polluted with motor oil (5% w/w or 10% w/w), or soil that is compacted (1.5 cm or 3.0 cm) prior to growth. It is important to observe which treatment and seed type affects the growth of the shoot over the 5-week study period. It is hypothesized treatments of salinity, oil and compaction will cause a reduction in growth in those plants than when compared to the control.

Methods

Preparation of Control Groups for Salinity, Oil, and Compaction Experiments

In order to prepare the control groups, either standard, petite, or tall Wisconsin Fast Plant seed varieties were planted in 3” nursery pots with standard pre-moistened potting soil. Six pots per seed variety were used per control group, with three seeds of each variety being planted in their respective pots. After the seeds were planted, ten Osmocote fertilizer pellets were placed on top of the soil of each pot, and each pot was watered with 50ml of water. Control plants for the salinity treatments were watered with distilled water, while control plants for the oil and compaction treatments were watered with 50ml of tap water. The plants were placed under a grow light immediately after watering.

The plants in the control groups were watered three times a week with 50ml of either distilled water or tap water. Once a week, the aboveground growth at the tallest point of the plant was measured and recorded. Plants in the control group were allowed to grow for five weeks since their initial planting date.

Salinity Treatments

In order to mimic the effects of urban runoff from brine application, 230 mg NaCl/L, 4 g NaCl/L, and 230 g NaCl/L treatment groups were created. The nursery pots for the salinity treatments were prepared using the same methods used for the control pots. Likewise, each treatment had a total of eighteen nursery pots, with six pots per treatment being devoted to each of the three seed varieties (standard, petite, and tall plant). Rather than distilled water, treatments were watered with 50ml of either a 230 mg/L, 4 g/L, or 230 g/L solution of NaCl dissolved in distilled water. To prevent premature death, treatments in the experimental groups were only watered once a week with their respective saline solutions. The other two days, they were watered with 50ml of distilled water.

Shortly after the experiment began, the 230g/L solution was changed to a 35 g/L solution which was then diluted to a 35 g/2L solution. This procedural change was made in order to prevent the lack of germination and/or immediate death of the high salinity treatment. Plants in the salinity treatments were allowed to grow for a five-week period past their planting date, and the aboveground growth was measured and recorded once a week.

Oil Treatments

In order to mimic the effect of motor oil pollution from urban runoff, we planted seeds in soil contaminated with different quantities of oil (5% motor oil w/w or 10% motor oil w/w) in place of standard potting soil. In order to create the oil-polluted soil, a bigger batch of soil was created in order to maximize uniform mixing. For the 5% oil w/w soil, a total of 1520g of standard potting soil was polluted with 80g of Castrol Edge advanced full synthetic motor oil and mixed thoroughly with a glove. For the 10% oil w/w soil, 1800g of potting soil was mixed thoroughly with 200g of Castrol Edge advanced full synthetic motor oil. Following the mixing of the soil, the standard, petite, or tall plant seed varieties were planted in their respective pots using similar methods used to prepare control plants. As seen with previous treatments, each treatment had a total of eighteen nursery pots, with six pots per treatment being devoted to each of the three seed varieties. Three seeds were planted per pot, and ten pellets of Osmocote fertilizer were placed on top of each pot. Each pot was watered with 50ml of tap water. Planters were then placed under a grow light.

The plants in these treatments were watered three times per week with 50ml of tap water. Once a week, the aboveground growth of each plant was measured and recorded. Plants were allowed to grow for a five-week period past their planting date.

Compaction Treatments

In order to mimic the flooding that roadside environments experience due to excess runoff, 1.5cm and 3.0cm soil compaction treatments were created. Per treatment, a total of 18 pots were used, with six pots per treatment being devoted to either the standard, petite, or tall plant seed variety. Pots were filled to about an inch from the top with pre-moistened potting soil. The soil in the pots was then compressed either 1.5cm or 3.0cm from the rim of the pot using the bottom of another nursery pot. Following compaction, a small amount of potting soil was sprinkled on top to allow for seed germination, and then three seeds of the proper variety were planted and ten Osmocote fertilizer pellets were placed in each pot. Each pot was watered with 50ml of tap water. Planters were immediately placed under a grow light, and allowed to grow for a 5-week period.

Planters were watered three times per week with 50ml of tap water. Once a week, the aboveground growth of each plant was measured and recorded.

Data Analysis

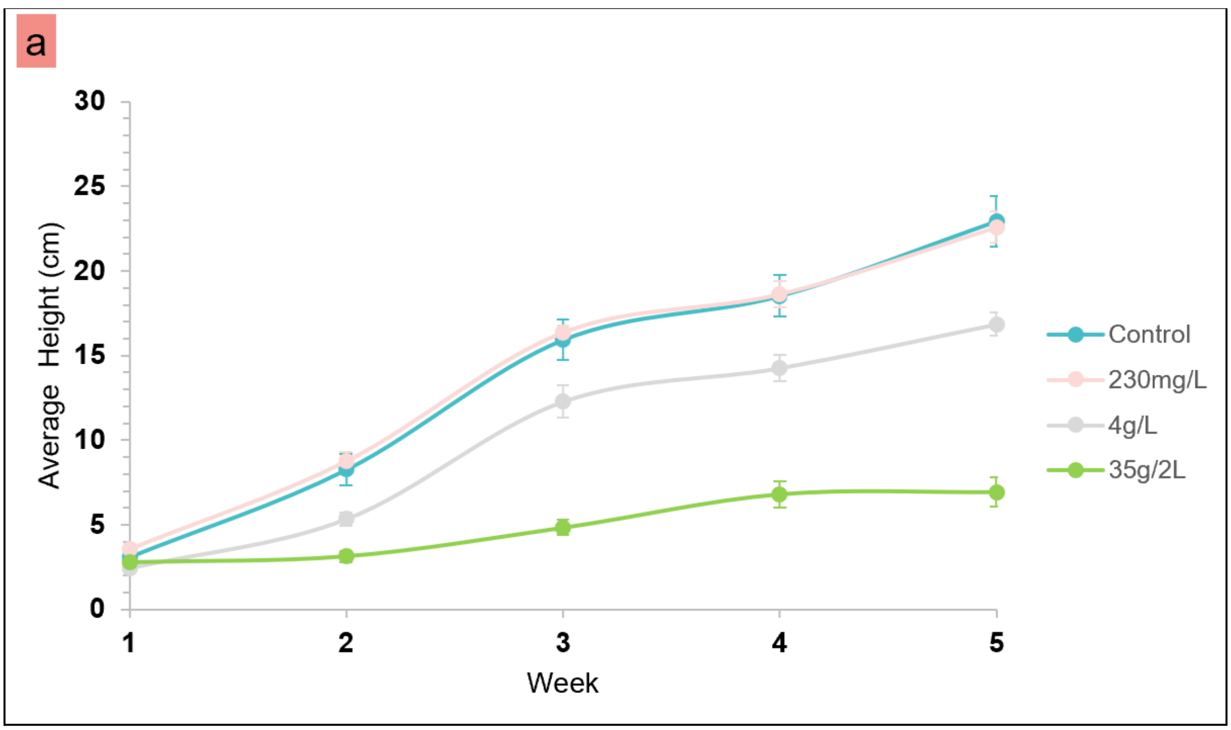
Multi-factor analyses of variance (ANOVAs) were used to quantify the relationship between plant height and treatment variables, which included different combinations of seed variety, time, salinity, oil contamination, and soil compaction. All statistical analysis were conducted in Jmp statistical analysis software (JMP 15, SAS Institute, Cary, NC).

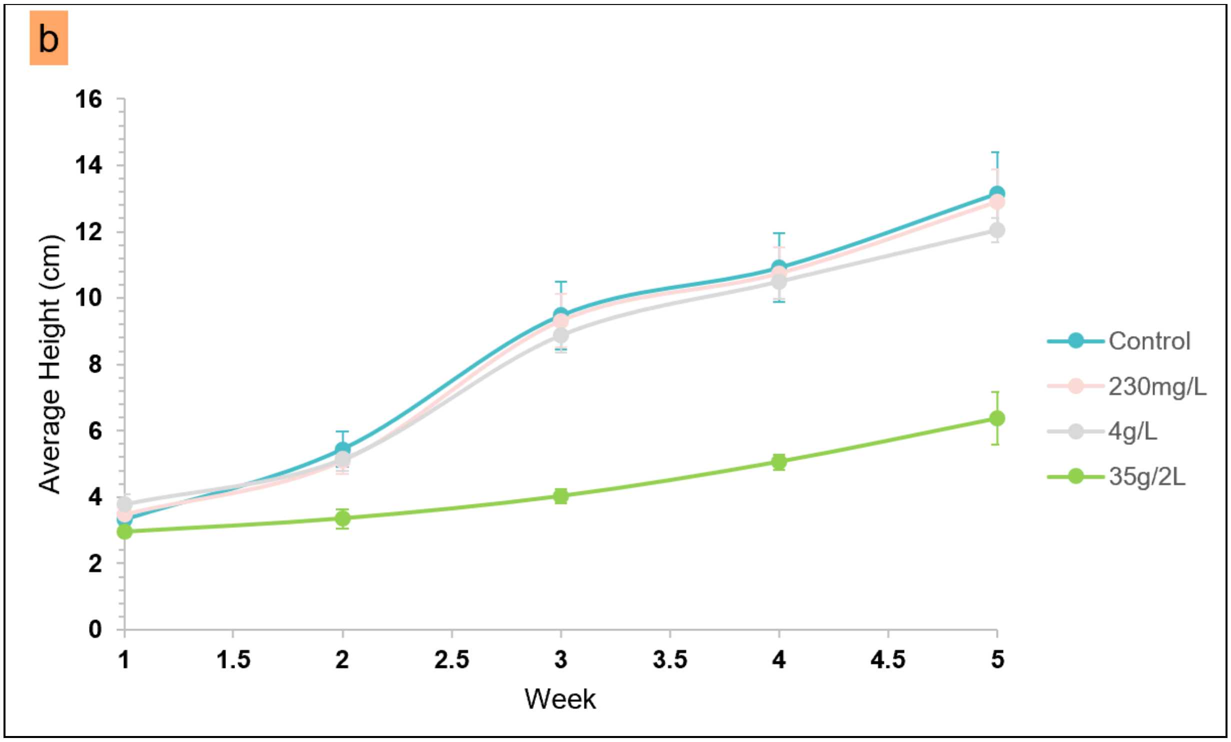
Results

Salinity Treatments

Overall, as salinity increased, average height of the plants for all seed varieties decreased (Table 1, Figure 1). Growth for the seed varieties was as expected with the tall plant variety having the greatest growth (cm) followed by the standard plant variety and the petite variety (Table 1). For the petite and tall seed varieties, plant height in the 230 mg/L and 4 g/L treatments was similar to height in the control group over the 5-week period (Figure 1b, 1c). For the standard variety, plant height was also similar between the 230 mg/L treatment and the control, but the 4 g/L treatment caused a significant decline in plant height (Figure 1a). For all seed varieties, the 35 g/2L treatment reduced average height of the plants over the five week period (Figure 1).

Similar results are seen for the week five results (Figure 2, Table 2). For the petite and tall plant seed varieties, only the 35 g/2L treatment led to significantly reduced height in the plants. For the standard seed variety, both the 4 g/L and 35 g/2L led to a significant reduction in height (Table 2).





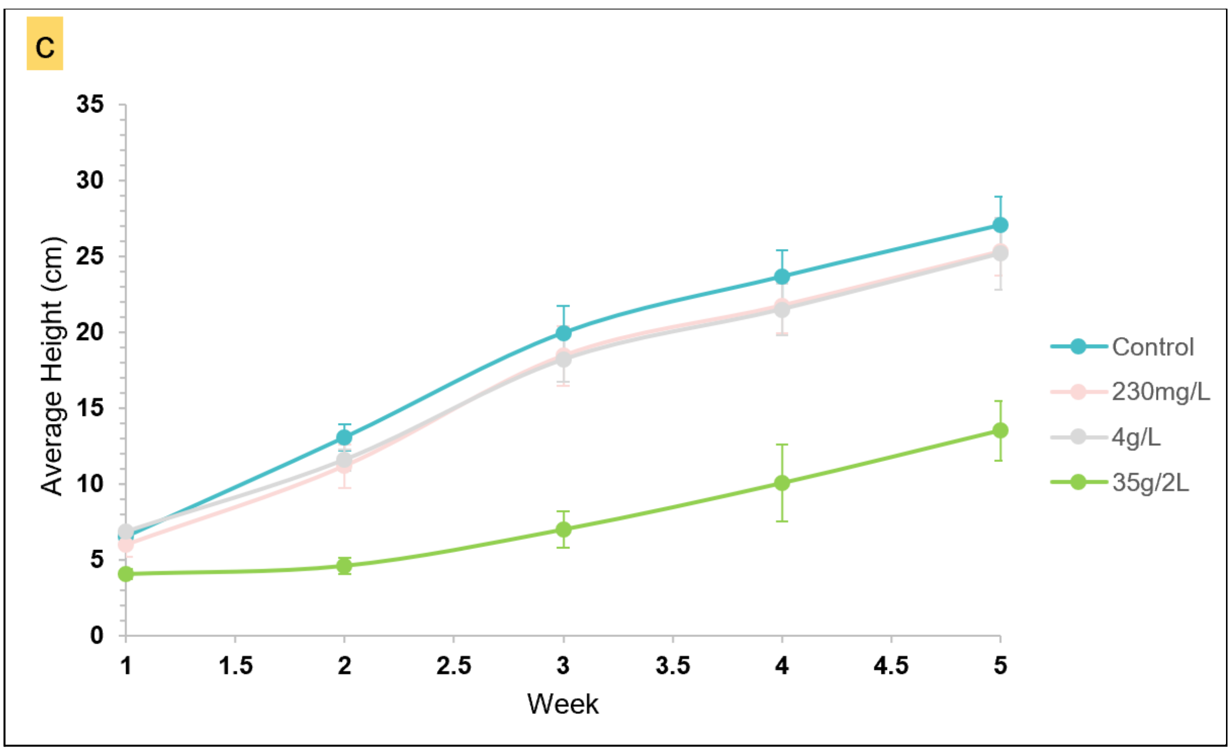


Figure 1 5-week average height measurements (cm) and standard error for the standard (a), petite (b), and tall plant seed varieties (c) of the control, 230 mg/L, 4 g/L and 35 g/2L salinity treatments. The p-values are shown in Table 1.

Effect	*F*-value	*p*-value	Full ANOVA	
Salinity	171.80	**<0.001**	*p*-value	**<0.001**
Seed variety	264.53	**<0.001**	R^2	0.91
Time	320.85	**<0.001**	*n*	352
Salinity * Time	11.99	**<0.001**		
Seed variety * Time	13.92	**<0.001**		
Salinity * Seed variety	12.86	**0.014**		

Table 1. Multi-factor ANOVA effects of salinity, seed variety, and time on plant height of Wisconsin Fast Plants (Brassica rapa) grown over a 5-week period.

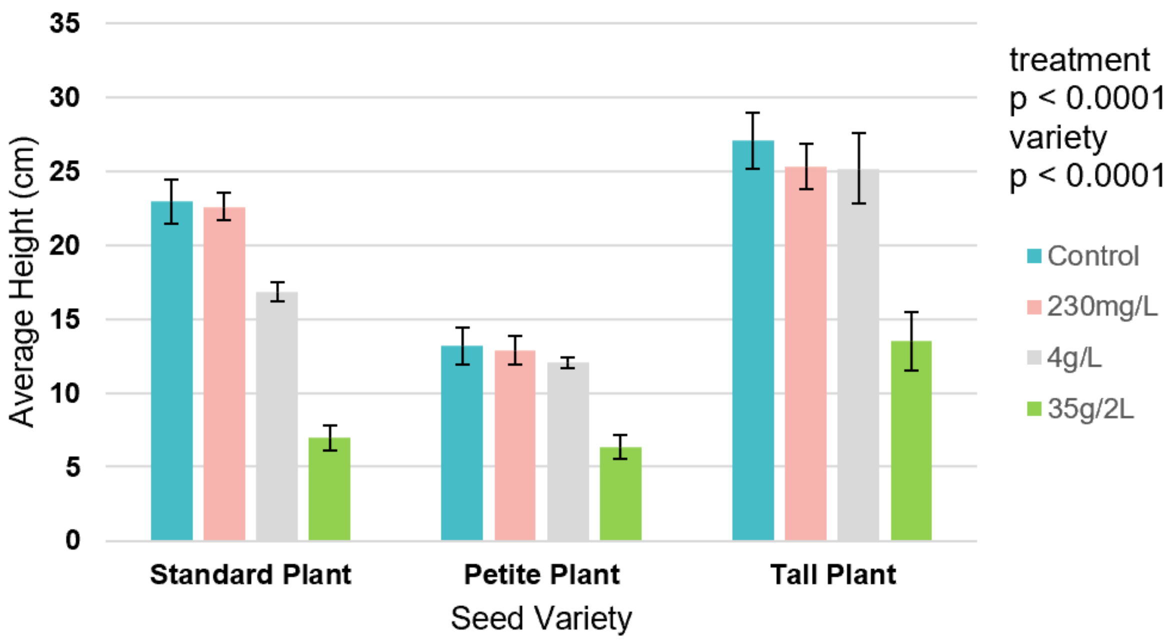


Figure 2 Mean height (cm) and standard error for each of the seed varieties of the control, 230 mg NaCl/L, 4 g NaCl/L and 35 g NaCl/2L treatments. The p-values are shown in Table 2.

Effect	*F*-value	*p*-value	Full ANOVA	
Salinity	39.72	**<0.001**	*p*-value	**<0.001**
Seed variety	67.33	**<0.001**	R^2	0.85
Salinity * Seed variety	2.96	**0.014**	*n*	67
Oil contamination	126.94	**<0.001**	*p*-value	**<0.001**
Seed variety	18.65	**<0.001**	R^2	0.88
Oil * Seed variety	2.73	**0.041**	*n*	52
Soil compaction	3.10	*0.054*	*p*-value	**<0.001**
Seed variety	26.39	**<0.001**	R^2	0.59
Compaction * Seed variety	1.59	0.193	*n*	54

Table 2. Multi-factor ANOVA effects of salinity, soil contamination, and soil compaction on plant height of Wisconsin Fast Plants (Brassica rapa).

Oil Treatments

For the oil treatments, oil contamination of the soil led to reduced height (Table 2). Growth for the seed varieties was as expected with the tall plant variety having the greatest growth (cm) followed by the standard plant variety and the petite variety (Table 2). The addition of any weight of oil to the growth medium led to a reduction in aboveground growth (Figure 3). For the standard seed variety, the 5% w/w and 10% w/w oil treatments led to a similar reduction in growth. For the petite plant and tall plant seed varieties, the 10% w/w treatments led to an even greater reduction in average height than the 5% w/w treatments (Figure 3).

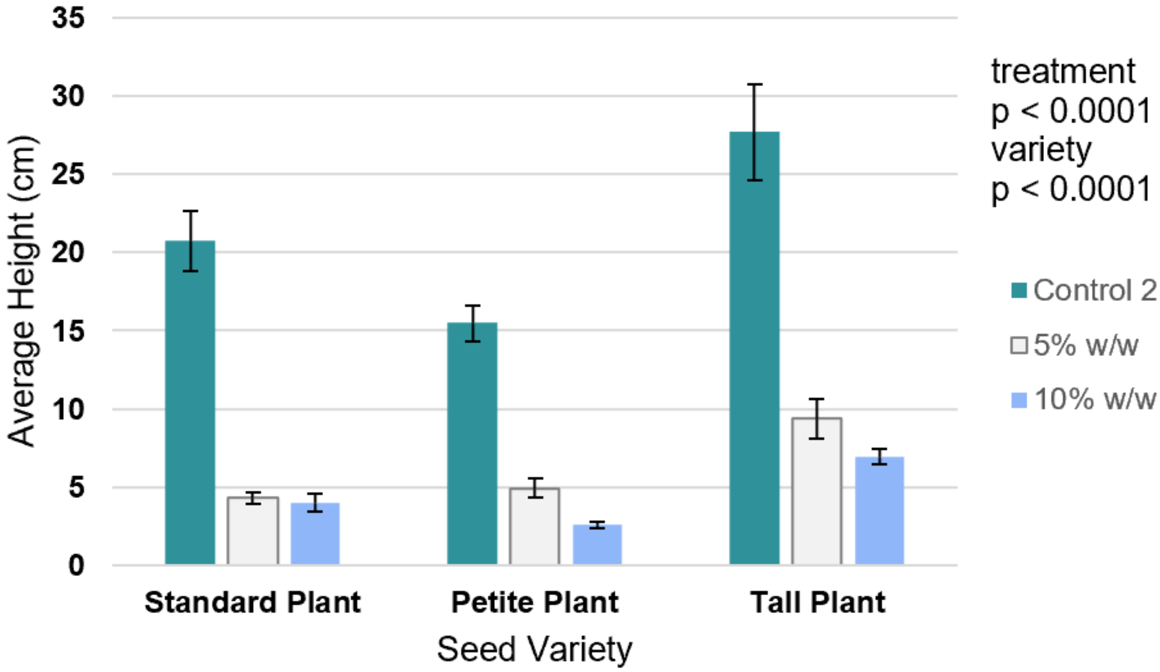


Figure 3 Mean height (cm) and standard error for each of the seed varieties of the control, 5% w/w, and 10% w/w oil treatments. The p-values are shown in Table 2.

Compaction Treatments

Growth for the seed varieties was as expected with the tall plant variety having the greatest growth (cm) followed by the standard plant variety and the petite variety (Table 2). All seed varieties were tolerant of some soil compaction with the results being marginally significant (Table 2). Compaction did not have an effect on average height for the standard plant variety or petite plant variety (Figure 4). Compaction had a minor effect on the tall plant variety with the 1.5cm and 3.0cm treatments leading to a marginally significant reduction in average height (Figure 4).

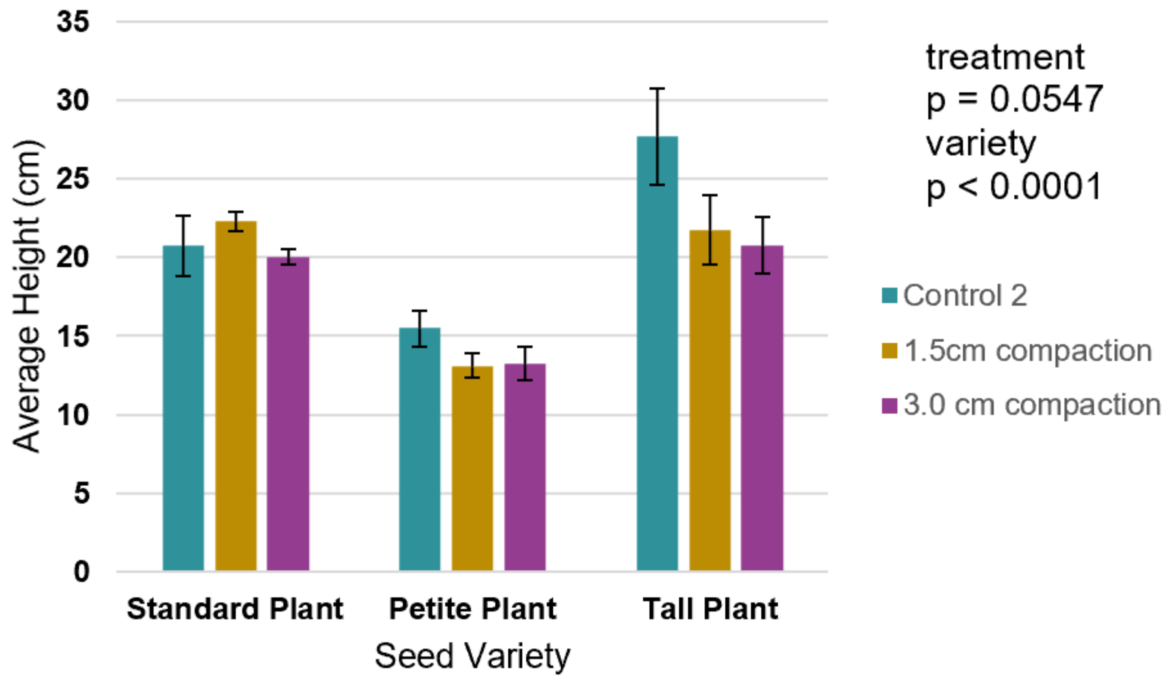


Figure 4 Mean height (cm) and standard error for each of the seed varieties of the control, 1.5cm compaction, and 3.0cm compaction treatments. The p-values are shown in Table 2.

Discussion

The results from this study mostly support our hypothesis and suggest that Wisconsin Fast Plants are sensitive to higher levels of salinity and to oil polluted soil ($p < 0.0001$). However, they are tolerant to some amount of soil compaction ($p = 0.0547$).

The results of the salinity experiment support the hypothesis that the growth of the plants in the treatment group will be negatively affected when compared to the control plants. Overall, for all three seed varieties, the 35 g/2L salinity treatment led to a reduction in growth when compared to the control. For the standard plant variety, the 4 g/L treatment also led to a reduction in average height over the duration of the experiment. According to the Encyclopedia of Life (n.d.), the standard seed variety has a medium tolerance to salinity, meaning it can tolerate 4.1-8.0 ds/m or 2.6-6.4 g/L NaCl. This may be why all seed varieties grew similarly to the control plants when faced with a low level of salinity (230 mg/L). In the tall plant seed varieties, upwards of twelve times more gibberellic acid is produced than in the standard seed variety (fastplants.org). Gibberellic acid is a plant hormone that is responsible for many plant processes including seed germination and seedling growth (Vishal et al, 2018). Previous studies have shown that while gibberellic acid levels and signaling may decrease, this hormone is also linked to stress tolerance in plants (Colebrook et al, 2014). This may help explain why although growth was reduced in the high salinity treatment, the tall plant seed variety was tolerant of the medium salinity treatment (4 g/L) while the standard plant variety was not.

It is important to note that at first, plants in the high salinity treatment were watered with 35 g/L NaCl. After noticing a lack of germination, and poor health in the seedlings that did germinate, the solution was diluted to 35 g/2L in order to prevent further harm. The initial use of the higher salinity may have led to a poorer long-term outcome for plants in this treatment group.

The findings from the oil pollution experiment are consistent with previous studies. Researchers from one study found that when soil was polluted with either 10% w/w or 20% w/w crude oil, seed germination and growth of lettuce plants was significantly reduced (Ilyas et al, 2021). The significant decline in plant growth for plants in these treatments may have been caused by a change in soil properties influenced by the oil contamination. When plants in these treatments were watered, it appeared as if the water ran through the soil without picking up soil particles along the way, which was unlike the control. The soil texture became harder than the control soil, which may have prevented the roots from penetrating the soil. Results from Klamerus-Iwan et al (2015) found that as oil pollution in soils increased, soil bulk density (g/cm^3) increased which in turn decreased the air-filled porosity of the soil. According to Phillips (2015) oil has the potential to harm vegetation by blocking oxygen absorption via the root system. The creation of an anaerobic environment soils can cause oxidative stress in the plants, which can therefore damage the plant further (Noori et al, 2012; Odukoya et al, 2019). Perhaps the change in soil texture reduced the oxygen available to plants in the treatment groups, which then led to a decline in overall aboveground growth.

Compaction of soil prior to growth led to a marginally significant decline in plant growth in the treatment groups ($p = 0.0547$). According to studies done by Unger et al (1994), water and nutrient uptake, and therefore plant growth, only declined when soil strength reached critical levels, meaning that no water drainage was possible. In these treatments, there was a loose soil level on the top of the compacted soils in order to allow for seed germination, and although there was temporary surface flooding, water was able to infiltrate the soil and drain. Furthermore, they found that weather events could sometimes enhance or diminish the effects of compacted soils on the root system, and therefore alter the associated limitations (water and nutrient uptake) to crop growth (Unger et al, 1994). Since the plants were watered three times a week, the movement of water through the soil, as well as the root growth, may have lessened the effects of the compacted soil on plant growth.

Data from these experiments support the notion that elements of roadside environments (salinity, oil pollution, and compaction) affect the growth of Wisconsin Fast Plants, and therefore may also affect other roadside plant species. Based on these results, it is clear that brine application to clear icy roads and vehicle upkeep practices can have effects on our roads and the surrounding vegetation. To prevent further harm to roadside vegetation, permeable surfaces and a non-harmful alternative to brine should be investigated. To prevent harm done by motor oil pollution, it is important to spread awareness about proper vehicle maintenance, and perhaps even create a future alternative to motor oil. It is important to keep these factors in mind as we continue to encroach on surrounding habitats and build new roads, possibly installing water catchment systems to collect runoff in particularly polluted areas rather than allowing contaminated water into roadside environments. Alternatively, we can also plant species that are capable of tolerating harsh conditions in strips around these roadside plantings, which can act as buffers and remove some of the contaminants before reaching other plant species. For example, species adapted to coastal environments such as day lilies, bayberry, and *Rosa rugosa* are generally salt-tolerant due to the sea spray and sandy soils they naturally grow in.

In a future study, it may be interesting to use spent motor oil in place of new motor oil since it is more likely that roadside vegetation will come in contact with that form of oil. Furthermore, it may be beneficial to investigate the interactive effects of multiple conditions (e.g., oil polluted soil and salinity) on plant growth. Lastly, in future studies using species commonly planted in roadside habitats such as black-eyed susans and Carolina lupine in place of Wisconsin Fast Plants would allow for more accurate predictions of the effects of roadside contamination.

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