The effects of used motor oil on *Myzus persicae* and *Arabidopsis thaliana*

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SUMMARY

Used motor oil (UMO) is a highly toxic substance that enters the environment as run-off from roadways and other urbanized impermeable surfaces. The effects of various runoff contaminants on the environment have been studied extensively, with research focusing on efficient and effective methods of clean-up. This study serves to examine the effects of varying volumes of UMO on plants and the subsequent impacts on herbi-vores. *Arabidopsis thaliana* was used as the model plant organism due to its rapid life cycle and *Myzus persicae* was used as the model herbivore organism due to its high proliferation rate. The results showed a statistically significant effect ($F_{oil(3,64)}$=3.3853, P=0.023) of UMO treatment on plant performance. There was also a statistically sig-nificant effect ($F_{21, 256}$=2.4661, P=0.00052) due to the interaction of oil and day on the herbivore of the plant. The results indicate that contaminants can affect multiple tro-phic levels, which should be considered when looking at possible issues regarding the contamination of natural environments.

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INTRODUCTION

Bioaccumulation of contaminants within an ecological community is a threat to urban biological systems (Tchounwou et al., 2012). Being sessile, plants are among the most vulnerable to these environmental effects. It is found concentrated primarily on roadways and other urbanized impermeable surfaces and enters the environment as runoff, potentially causing harmful effects on vegetation and biological diversity (Environment and Climate Change Canada, 2010). Used motor oil (UMO) is a highly toxic substance that contains heavy metals, polycyclic aromatic hydrocarbons, and dioxins, which are some of the most common ecosystem contaminants (Australian Government, 2010). When introduced to biological organisms, heavy metals can negatively impact cellular organelles (Tchounwou et al., 2012). The exposure of *Arabidopsis thaliana* to dioxins has been shown to result in a reduction in fresh weight, a drastic decrease in chlorophyll content, and a decline in seed germination (Hanano, Almousally and Shaban, 2014). Polycyclic aromatic hydrocarbons have been shown to cause root growth reduction and trichome deformation in *A. thaliana* (Alkio et al., 2005). Therefore, UMO contamination can be used as a model to study the bioaccumulation of toxins within ecosystems. While previous studies show that UMO exposure leads to the overall reduction of plant growth in *A. thaliana*, no specific studies have looked at the resultant effects of this reduction on the subsequent trophic level. By monitoring the population of *Myzus persicae* on *A. thaliana* plants treated with varying amounts of UMO, this study creates a model for contamination effects on trophic levels. The experiment aims to answer (1) whether UMO contamination affects plant growth and (2) whether the oil contamination affects the herbivore of the plant.

STUDY SPECIES: PLANT

A. thaliana (Col) has become a staple model organism for scientific research due to the ease of its cultivation

and success at plant transformation (Meinke et al., 1998). It is a small plant of the mustard family and is native to a broad distribution of regions consisting of Europe, Asia, and North America (Meinke et al., 1998). In 1987, the modern era of *A. thaliana* research surged into the frontlines (Meinke et al., 1998). This simple angiosperm has become a pivotal factor to the scientific advances in understanding plant development (Meinke et al., 1998).

STUDY SPECIES: HERBIVORE

M. persicae is a polyphagous generalist aphid that feeds on over 40 families of plants (Blackman R.L. & Eastop V.F., 2000). The species is heteroecious holocyclic, but anholocyclic in tropical areas (Schoonhoven et al., 2005). A colony is mostly made up of females for the majority of the year, which results in rapid aphid reproduction (Blackman R.L. and Eastop V.F., 2000). The high proliferation rate validates the choice of *M. persicae* for this short 11-day study. Wingless aphids were specifically chosen because winged aphids have a lower individual fecundity (Mutti et al., 2008).

M. persicae and *A. thaliana* have been heavily involved in an evolutionary arms race leading to several different plant defenses and subsequent herbivore adaptations (Schoonhoven et al., 2005). Since *A. thaliana* is considered to be a generic plant and aphids are generalist insect herbivores, the host-attacker relationship can be used as a model in studying plant-animal interactions (Blackman R.L. & Eastop V.F., 2000). There is a plethora of applications to study from this model because of the large majority of plants and animals categorized under generalist species.

USED MOTOR OIL

The UMO was obtained from Active Green + Ross Tire Automotive Centre located at 1289 Main St. W., Hamilton. The brand and the type of car the oil was extracted from is unknown.

METHODS

EXPERIMENTAL DESIGN

Each sampling unit comprised of *A. thaliana* in the rosette life stage. The plants were placed in 5.5cm x 5.5cm x 10cm rectangular plastic pots filled with moist soil. 40 sampling units were split into two repeated tests, Block A and Block B. Both blocks consisted of a four by five arrangement of the sampling units. Four UMO treatment levels were used: 0mL, 1mL, 3mL, and 5mL. Each treatment level consisted of five plants, labelled 1-5, 6-10, 11-15, and 16-20 respectively.

Table 1: Assigned locations of plants for Block A based on a random stratified model created using Microsoft Excel software.

8	6	12	5
11	7	1	9
20	19	2	13
17	10	16	18
14	15	4	3

Table 2: Assigned locations of plants for Block B based on a random stratified model created using Microsoft Excel software.

12	15	20	18
9	1	8	17
14	4	5	11
7	3	2	6
13	10	16	19

The plant locations were determined using the Microsoft Excel software to produce a random stratified model, as shown in Table 1 and Table 2 (Microsoft, 2017). The RAND() and RANK() Excel functions were used to create a set of unique random numbers from 1 to 20 with each number corresponding to a location from the top left corner to the bottom right corner in the 4 by 5 grid, set up from left to right in each row. This process was repeated to create a new set of random numbers for Block B. This design minimized the effect of biases, causal claims, and accounted for external factors such as disproportionately distributed sunlight. The plants were placed so the plastic walls of the pots were touching, ensuring rosette leaves were not damaged.

The UMO was administered with 10 mL pipette pumps on the soil near the base of the plant. UMO contact with *A. thaliana* was carefully avoided. Each sampling unit was then inoculated with two adult female *M. persicae*. The aphids were placed at the lower base of the stem, avoiding the trichomes which could have affected the aphids. The sampling units were then placed on plastic trays with a transparent dome-shaped lid. This enclosed the plants with approximately 30cm of space between the top of the plant and the top of the lid. There was a mesh screen of about 100cm^2 that allowed for air exchange but prevented aphids from escaping.

The individual plants were not removed from the design during data collection to ensure minimal disturbance to other sampling units. At the halfway point (day 5), 3mL of water was added near the corners of the sampling units with an eye dropper. Aphid population

count was recorded on days 1, 4, 5, 6, 7, 8, and 11 to examine the effect of oil contamination on the herbivore of the plant. Rosette diameter was measured on days 0 and 11 to examine the effect of UMO on plant performance.

STATISTICAL ANALYSIS

Statistical analysis was completed using R programming language, on version number 3.4.2. Analysis of variance (ANOVA) was used to investigate the independent and correlating effects of several variables (The R Foundation for Statistical Computing, 2016). These include: UMO treatment levels of 0 mL, 1mL, 3mL and 5mL, test days from Day 0 to Day 11 (with inconsistent days in between), blocks A or B. The dependent variables that were observed are mean aphid population per sampling unit and mean rosette diameter. Mean aphid population per day was quantified by taking the total number of aphids on sampling units within a treatment level per day and dividing it by the number of sampling units. Mean rosette diameter was defined as the sum of all rosette diameters divided by the number of sampling units. A Tukey Honestly Significant Difference post-hoc analysis was used to determine which UMO treatment levels and test days had a statistically significant effect on mean aphid population and mean rosette diameter (The R Foundation for Statistical Computing, 2016).

RESULTS

UMO EFFECT ON PLANT GROWTH

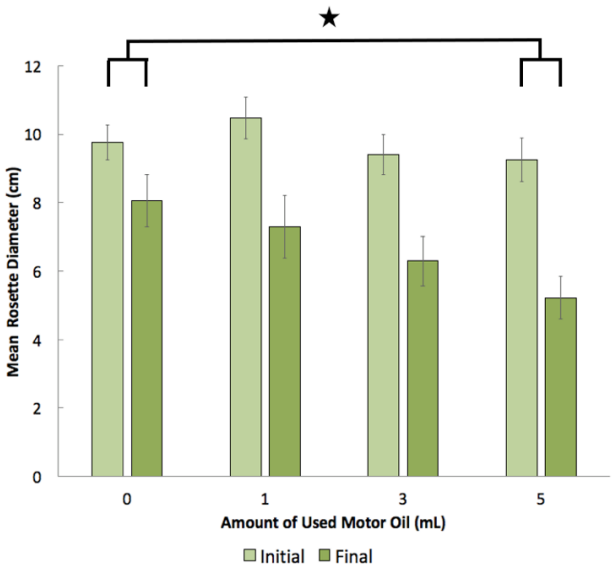

Figure 1: Initial and final mean rosette diameter measured from the farthest tips of each rosette per treatment group for both Block A and Block B. Values are means ±SD. The star denotes a difference between the control and 5mL treatment groups.

Initial and final mean rosette diameters were compared with the different treatment groups in order to investigate the effect of UMO on plant growth. It was determined that the UMO treatment levels had a statistically significant effect on the mean rosette diameter per treatment group (ANOVA, $F_{3, 64}$=3.385, P=0.023). A statistically significant difference was determined between the rosette diameters of the control and 5mL treatment level (Tukey, P= 0.048), as denoted with the star in Figure 1. It is observed in Figure 1 that there is a suggested difference which supports these statistical findings.

UMO EFFECT ON HERBIVORE

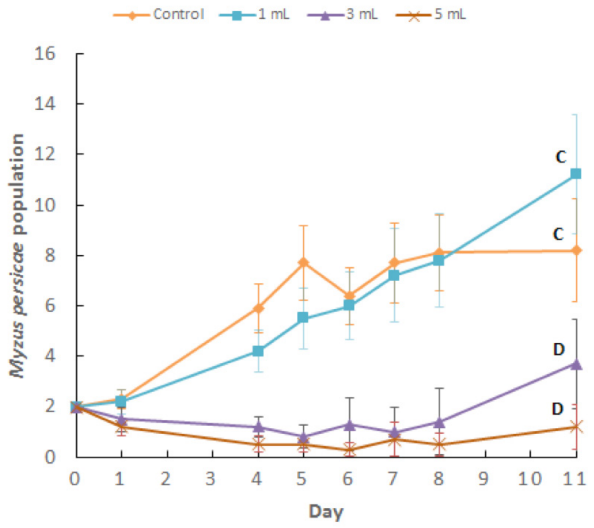

Figure 2: Daily mean aphid population per sampling unit for both Block A and Block B. Values are means ±SD. It is suggested through the illustration that there is a significant effect between treatments of 0-1mL and 3-5mL denoted as treatments C and D respectively.

Mean aphid population was plotted over the data collection period in order to investigate the effect of oil on the herbivore of the plant. The interaction between UMO treatment levels and day had a statistically significant effect on the mean aphid population per sampling unit of both Block A and Block B (ANOVA, $F_{21, 256}$=2.4661, P=0.00052). The interaction between UMO treatment and day demonstrated that the treatment levels had no significant difference within aphid count between the control and 1mL levels as well as the 3mL and 5mL treatment levels (Tukey, P=0.50; P=0.98). These treatment levels can therefore be grouped, denoted by C and D in Figure 2 and the shown graph supports this statistical significant difference.

DIFFERENCE IN TREATMENT BLOCKS

In the analysis of the differences between Block A and Block B, the same statistical conclusions were found

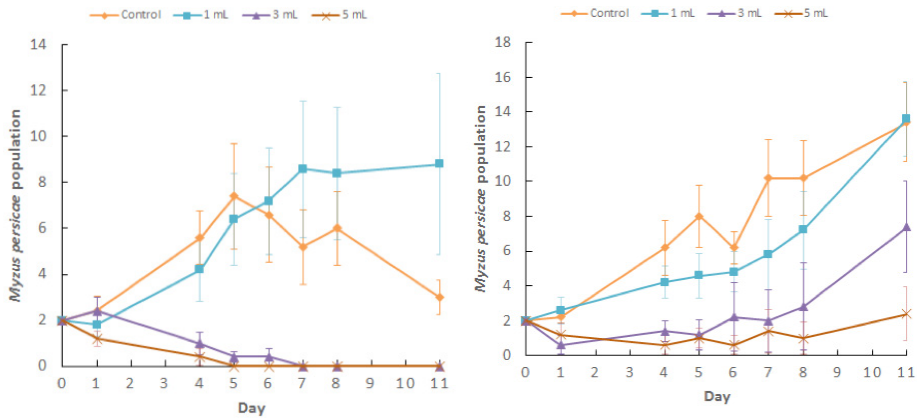

Figure 3: Mean *Myzus persicae* population per sampling unit over 11 days after being treated with various amounts of UMO for Block A (left) and B (right). Values are means ±SD. These population means are calculated through the average aphid count at a varying time on each day, roughly from the times 10am to 4pm.

as if the data of the two blocks had been run together (ANOVA, Block A: $F_{21, 288}$ = 2.286, P= 0.0013; Block B: $F_{21, 288}$ = 2.286, P= 0.0013). On each both block A and B, there was no statistically significant difference between the control and 1mL treatment levels on aphid count (Tukey, P=0.50). Additionally, there was no statistically significant effect on count when comparing the 3mL and 5mL treatment levels (Tukey, P=0.98) (Figure 3). There was a statistically significant difference between the two sub-groups, previously denoted as C and D in Figure 2. Qualitative observations, taken of Block A and Block B at the beginning and end of the experiment, shown in Figure 4, supported this data.

DISCUSSION

Figure 4: Block A (top left) and Block B (bottom left) on day 0 compared to Block A (top right) and Block B (bottom right) on day 11.

UMO EFFECT ON PLANT GROWTH

The study investigated whether or not UMO was a significant stress factor in influencing plant performance. There was a statistically significant effect (ANOVA, $F_{3, 64}$=3.3853, P=0.023, Tukey, P= 0.048) of oil on rosette diameter for plants treated with 5mL of UMO, as shown in Figure 1. On day 5, the halfway point, 3mL of water was added to each plant. A small quantity of an odourless liquid, assumed to be water, seeped out onto the paper towel underneath. This may suggest that the roots of the plants became coated with oil which prevented root uptake of water. A study conducted by Abioye, Agamuthu and Abdul Aziz (2012) found that hydrocarbons could coat root surfaces, restricting gas and water exchange. Without water, plant growth and performance are stunted and the photosynthesis process is left without a vital resource (McElrone et al., 2013). Photosynthesis plays a large role in the growth of *A. thaliana*, and with the limited presence of water, the plant must actively reprogram its metabolism and growth (Claeys and Inzé, D., 2013). This means *A. thaliana* has shown to be flexible toward water limitations. A supporting example of this claim is shown through a study by Skirycz and Inzé (2010), which showed sharp declines in leaf elongation, which was then followed by recovery to a steady growth rate known as acclimation. This could indicate that if given the opportunity to continue the experiment over time, rosette diameter could restabilize if allowed proper resources. In our plant environment with both biotic (aphids) and abiotic (oil) stress factors, it would be likely that plants focus their resources on surviving. Overall, this suggests that greater concentrated oil treatments damage plant growth in terms of rosette diameter which could be explained by *A. thaliana* allocating its resources towards survival rather than growth.

UMO EFFECT ON APHID POPULATION

The ANOVA and Tukey post-hoc results indicate that the 3mL and 5mL treatment levels of UMO had a statistically significant effect on *M. persicae* population (Figure 2). The final aphid populations on the 3mL and 5mL

treated plants were much lower than the final aphid populations in the control and 1mL plants. The 1mL treatment had a greater positive effect on aphid population than all of the treatment levels including the control group. This supports the plant stress hypothesis, which states that environmental stress on plants decreases plant resistance (Joern and Mole, 2005). It claims that herbivores favour feeding on plants under biotic and abiotic stressors more than unstressed plants (Joern and Mole, 2005). The increased performance of *M. persicae* on the 1mL treated plants could then potentially be the result of the decrease in *A. thaliana* defense mechanisms. Plant defense could have been hindered in several ways: damaged morphological features, reduction in toxic chemicals present, weakly expressed volatiles, and change in foliar chemistry (War et al., 2012; Joern and Mole, 2005). Trichome deformation eliminates physical barriers to aphid movement and decreases secretion of poisonous secondary metabolites (War et al., 2012). Plant volatiles are weakly expressed resulting in a reduction of harmful signals (Gatehouse, 2002). With change in foliar chemistry, herbivores prefer to feed on stressed plants with greater nitrogen concentrations since it has a central role in all metabolic processes (Joern and Mole, 2005; William J. Mattson, 1980).

BLOCK EFFECT

Initial ANOVA tests determined that there was a statistically significant effect on aphid count due to a possible block effect ($F_{block(1,308)}$= 7.7828, P= 0.0056). This idea may be supported by how qualitatively different Block A was compared to Block B after the testing period, as seen in Figure 4. This raised concerns about the conclusions made in response to the second research question since statistics were run on the combined Block A and B data. As such, individual ANOVA and Tukey post-hoc tests of Block A and Block B were taken; however, these tests resulted in the same conclusions and significant differences between UMO treatment levels that were previously indicated by the tests using the data from both blocks. It is possible that the block effect resulted from unequal averages of beginning rosette diameters between the two blocks. It is suggested that future experiments address this issue by having the two random stratified blocks begin with equal average rosette diameters. Another possible explanation is that the block effect resulted from the random clustering of the higher UMO treatment levels within Block A but not in Block B.

LIMITATIONS AND NEXT STEPS

The study consisted of four oil treatment groups with only 10 individual plants in each group. This is quite a small sample size, resulting in an increased influence of outliers on the data trends. A larger sample size of *A. thaliana* was not possible due to resource restrictions, but is necessary in order to further support any prevalent patterns identified. Improvement of the experimental design could potentially include increasing the number of plants to a sample size of 400. The increase by a factor of 10 allows for more specific results and increases the accuracy of any trends observed. The experiment suggested a point between 1mL and 3mL where significant negative effects occurred on the aphid count. Therefore increasing the number of treatment levels to 10 and using more specific dosage increments would allow this threshold to be further pinpointed. Resource limitations also resulted in a sample plant population with varying initial rosette diameters, meaning there was inherent error in rosette diameter change. An improved experiment would have a controlled initial rosette diameter for all plants used so the change in rosette diameter can be increasingly correlated to the treatment level.

Additionally, the experiment was carried out over a period of 11 days. Compared to the overall lifespan of *A. thaliana* of approximately six to eight weeks (Koornneef and Scheres, 2001), the experiment time frame was considerably shorter, potentially resulting in less significant observed changes in rosette diameter, leaf number and size, and stem length. An improved experiment would involve measuring results over a larger time period - potentially the entire lifespan of *A. thaliana*. Furthermore, contamination effects were studied on *A. thaliana* only in the rosette stage. In order to achieve more accurate results, contamination effects would need to be observed during several different plant life stages, accounting for factors such as variation in plant biomass, defense, and nutrient uptake.

The effects of UMO on plants and herbivores should be further examined in greater detail. Possible areas of future research include studying the effects of UMO contamination on higher trophic levels in an environment and the results of biomagnification in affected ecosystems, studying UMO quantity limits before negative impacts on fauna are distinguishable, and studying how plants in different life stages respond to UMO contamination.

CONCLUSION

The exposure of *A. thaliana* to 5mL of UMO had a statistically significant negative effect on plant rosette diameter compared to the control, indicating that oil contamination negatively affects plant growth. The 3mL and 5mL treatments had a statistically significant negative effect on aphid population while the 1mL treatment was statistically equivalent to the control,

indicating that oil contamination negatively affects the herbivore of the plant once a critical point of contamination is reached. This study indicates that pollutants can influence multiple trophic levels, which should be taken into account when looking at future contamination of natural environments.

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AUTHOR CONTRIBUTIONS

C.C., B.C., Y.G., D.J., and S.M., conceived and carried out the experiments. S.M. took the lead in running statistics, with Y.G. collaborating with C.C. to integrate the necessary statistics for figures. B.C. and D.J. had a primary focus experimental design and analysis of results. All authors contributed to the final writing and editing process of the manuscript.

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