

# **The Spread of Earthworm Cocoons by Vehicular Traffic on Unpaved Roads**

By  
Lindsay M. Scott

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Approved: \_\_\_\_\_  
Dr. Erin Cameron  
Supervisor

Approved: \_\_\_\_\_  
Dr. Sean Haughian  
Thesis Reader

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## **Abstract**

The introduction and spread of non-native species have been heavily influenced by human activities, resulting in the distribution and accelerated spread of species worldwide. Earthworms are largely non-native in Canada and their presence, particularly in forested ecosystems, can cause substantial consequences as they are able to substantially alter the soil conditions. Earthworms and earthworm cocoons are easily distributed unknowingly along with soil, and earthworm populations have been found to be more concentrated in areas of frequent human use. Not much is known about how exactly human movements are able to distribute earthworms and earthworm cocoons, so more knowledge in this area would be useful. This study aimed to find out how earthworm cocoons are spread by vehicle traffic on unpaved roads, as well as some of the factors which influence this spread. I looked at how differences in cocoon size, represented by different sizes of plastic beads, wet and dry weather conditions, and two different vehicle types, a car and an all-terrain vehicle, affected the distance the beads were able to spread. Five replicate trials were completed for each unique combination, resulting in eighty trials in total. It was hypothesized that smaller beads and wet weather conditions would cause the beads to travel farthest, and that distance travelled would be unaffected by vehicle type. The results did not support my hypothesis and showed that larger beads were more likely to be moved and moved farther than the smallest bead size and this was most likely to happen under dry conditions. Vehicle type as predicted did not have a significant effect. These results suggest that earthworm cocoons can easily travel along roads as vehicles drive on them, which is important in predicting their rates of spread, although more research would be valuable in this area to obtain more accurate results.

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## **1. Introduction**

### *1.1 Invasive species*

Non-native species which have established outside of their native geographic range and threaten ecosystems, habitats, and/or other species are commonly known as invasive species (Pejchar & Mooney, 2009). Human activities introduce non-native species and accelerate their spread, both intentionally and unintentionally (Crowl et al., 2002). While greater global connectivity has increased human travel, it has also increased transport of invasive species (With, 2002). Invasive species on a global scale have been associated with a significant decrease of local native biodiversity (Kopf et al., 2017; Doherty et al., 2016) as well as causing significant, sometimes irreversible damage to the environment (Sakai et al., 2001).

### *1.2 The spread of invasive species*

After a species has been introduced into a new ecosystem, it has to reproduce and establish a population. It must then be able to successfully disperse and spread, before it can become invasive (Theoharides & Dukes, 2007; Sakai et al., 2001). Invasive species can move on their own, but in many cases their spread is facilitated by other vectors. The spread of an invasive species is affected by the species' traits, how many individuals have been introduced and established, the location and environmental conditions in which they are spreading, and the method(s) by which the species is spreading (Hastings et al., 2005; Neubert & Parker, 2004).

Invasive species may become attached to clothing, boots, or vehicles such as airplanes, boats, cars, and trains, allowing them to be transported long distances and to

areas that otherwise may have been inaccessible (Hulme, 2009). The construction of infrastructure is also an important vector for the spread of invasive species, through the movement of soil and rocks, and by disturbing and altering the landscape. This can also create relatively straight and open corridors such as canals, railways, and roads, for species to spread (Hulme, 2009; Christen & Matlack, 2009). Roads specifically have shown to be ideal corridors for the spread of invasive species (Cameron & Bayne, 2009; Cameron, Bayne, & Coltman, 2008; Hulme, 2009; Hale, 2008). For example, it has been found that roadways were significant in facilitating the spread of invasive plant species, not only during the construction of the road but also from road maintenance, age, and usage (Mortensen et al., 2009; Cameron and Bayne 2009).

The best strategy from a control and management perspective is to prevent the introduction of non-native species, which is often difficult or impossible to achieve, but trying to control the spread is the next best approach (Clout & Williams, 2009). That being said, the ability to control, manage, or even eradicate an invasive species is difficult due to the complexity of ecological systems (Buckley, 2008). It is challenging to implement effective control strategies without creating a trophic cascade effect that causes disruptions elsewhere in the ecosystem (Kopf et al., 2017). One of the main questions that must be answered to effectively manage invasions is the rate at which the species is spreading. In addition, information is needed on how to slow or stop further spread. Many studies have examined and projected rates of spread for different organisms, but simple linear or statistical methods may not be adequate for accurate predictions (Hastings et al. 2005). While much of the literature available can be useful

for reference, the large number of variables that could alter the rate of spread in varying situations remains an issue.

### *1.3 Invasive species of earthworms*

About 120 earthworm species (out of approximately 6000 globally; Csudzi, 2012) have spread on a global scale, primarily through human activities (Hendrix et al., 2008). Earthworm introductions often happen in disturbed habitats, but whether earthworms become invasive depends on climatic, edaphic, land use factors, and the biology of the species (Hendrix et al. 2008). Non-native and native earthworms can coexist in some instances, particularly if they occupy slightly different niches; however, non-native earthworms typically displace native earthworms if they are in competition for similar resources (Hendrix et al., 2006; Hendrix et al., 2008).

Earthworms have a significant effect on ecosystems, both below and above-ground, as they alter the soil in many ways. Invasive earthworms have particularly large effects in northern forests where they consume plant litter, accelerate decomposition rates, mix soil horizons, and create burrows underground (Bohlen et al., 2004; Frelich et al., 2006). Earthworms are able to shift the distribution of fine root systems, reduce germination rates, change the mycorrhizal community, and change the productivity of the ecosystem by influencing the plant communities, all of which have significant effects on the ecosystem (Frelich et al., 2006; Bohlen et al., 2004; Hale et al., 2005).

### *1.4 The spread of earthworms*

Earthworms have a limited ability to disperse on their own, travelling only 2-4 meters per year on average (Marinissen & van den Bosch, 1992). However, as their

cocoons are small, ranging in length from 1.87 mm to 6.24 mm depending on the species (Edwards & Lofty, 1977), it is easy for them to be carried in water, soil, or boot/tire treads, allowing them to travel much farther. Earthworms of all life stages can be transported in the soil on tractor tires (Marinissen & van den Bosch, 1992), and other studies have found that boots and bike tires picked up and transported beads of a similar size to small earthworm cocoons (Hardiman et al., 2017). It has been suggested that the spread of earthworm cocoons through movement of soil both in and on vehicles contributes to an accelerated rate of spread (Hale, 2008).

Human activities have increased both the distance and rate of earthworm spread, through discarding fishing bait, constructing roadways and paths, or even by simply accessing different areas, whether that is on foot, bicycling, driving, or off-roading (Hale, 2008). High earthworm populations are concentrated around urban areas, as well as around boat launches, common fishing areas, roads, trails, and campsites, making it easier and more likely for them to be picked up and spread unintentionally (Hale, 2008; Cameron, Bayne, & Coltman, 2008).

Roadways in particular are important in facilitating the spread of non-native species and understanding the extent to which vehicular traffic on roadways may aid in their dispersal is vital information to have for effective management (Barbosa et al., 2010). While some studies have examined the spread of earthworms in general, more information on the various introduction mechanisms and rate of spread of earthworms and earthworm cocoons is needed.

### *1.5 Earthworms in Canada*

In Canada, non-native earthworms have been found in every province and territory (Addison, 2009). They occur in the coastal and Columbian forests, as well as boreal forests, montane forests and around the Great Lakes and St. Lawrence forests (Addison, 2009). Currently there are eight known native species, and nineteen known non-native species of earthworms living in Canada (Addison, 2009).

Almost all of the currently known non-native earthworms in Canada are European in origin, although most research has been conducted in the last several decades and less is known about their distributions prior to that (Addison, 2009). It is highly probable that additional exotic earthworm species from other parts of the world are also present in Canada but there is a deficiency of information in current literature on this topic, possibly in part due to lack of taxonomic expertise in the country (Addison, 2009). Studies have found that invasive earthworms are spreading in the boreal forests of Canada, and human facilitation is increasing this rate of spread (Cameron, Bayne, & Clapperton, 2007).

The current literature agrees that further distribution and invasions of non-native earthworms into Canadian forests could cause substantial changes to these ecosystems, but there is a lack of research available on what specific impacts they have had since their introduction and on what scale. As earthworms are difficult to remove once they have become established (Cameron, Bayne, & Clapperton, 2007), it is of importance for both the control and management of non-native earthworms to better understand their current distribution and spread as well as their impacts on Canadian forests.

### *1.6 Objectives*



The objectives of this research were to determine the potential distance that earthworm cocoons spread due to vehicular traffic, as well as assess the factors that influence this spread. I tested how cocoon size, weather conditions (wet vs. dry), and vehicle type affected the likelihood that cocoons will be picked up by vehicles, and the distance they travel, as this should reflect their ability to spread and invade new areas.

I hypothesized that the smaller cocoons would more easily be picked up with soil or flung by the tire tread and travel farther than the larger cocoons, regardless of vehicle type due to their smaller diameter and lighter weight. I also hypothesized that wet conditions would make it easier for them to be picked up as the extra moisture might make it more likely they would be combined with mud or soil and adhere to either the vehicle or the tire due to the adhesive properties of water. I did not expect much difference between the vehicle types because it seems likely that the movement of beads is relatively unaffected by which vehicle passes over them.

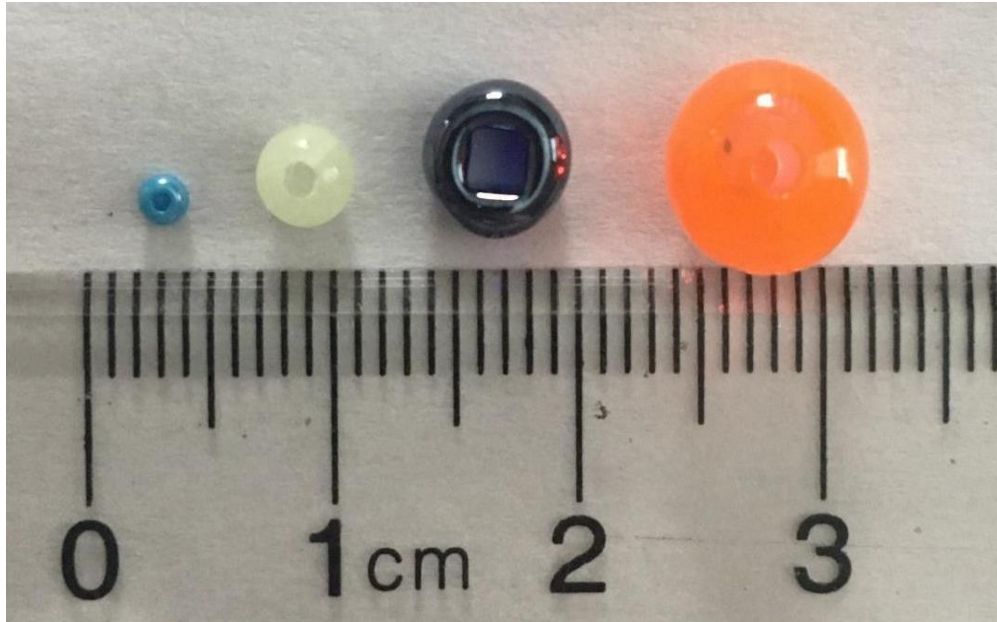
## **2. Methods**

### *2.1 Data Collection*

In order to determine the distance that earthworm cocoons can travel due to vehicle movement, plastic beads were used to represent earthworm cocoons. I examined the effects of bead size, weather condition, and vehicle type on distance travelled by the plastic beads. The trials took place on November 10<sup>th</sup>, November 29<sup>th</sup>, and December 16<sup>th</sup> of 2020, on a narrow dirt road located in Colchester County, Nova Scotia, Canada at approximately 45°11'40.1" N and 63°15'49.0" W.

Four bead sizes were used (2, 4, 6, 8 mm; Figure 1). Bead sizes were chosen to closely resemble the various sizes of earthworm cocoons typically found in Canada. For example, the species *Eiseniella tetraedra* has a small cocoon which is approximately 1.87 mm long and the species *Lumbricus terrestris* has a larger cocoon approximately 7.3 mm in length (Edwards & Lofty, 1977; Sims & Gerard, 1985). Twenty beads of each size were spread relatively evenly on the ground within a 10 cm<sup>2</sup> area. The beads were then driven over by a vehicle travelling 50 km/h. Two vehicle types, a car and an all-terrain vehicle (ATV), were tested under both wet and dry weather conditions. The weather condition was determined to be wet if the road had visible water on it, no dust was seen in the air when driving on the road, and there was a recent rain event and/or it was raining during the trial. The weather condition was determined to be dry if the road was deemed not wet either visibly or to touch, there had been no recent precipitation events, and driving along the road caused dust clouds behind the vehicle. For each combination of bead size, vehicle type, and weather condition, five replicate trials were performed. Thus, a total of 80 trials were conducted.

The car used was a Hyundai Elantra which had Joy Road studded winter tires (size 205/55/R16), and the ATV was a Honda Foreman with Maxxis mud tires (size 25 x 8-12). The beads used were as follows: “11/0 Czech seed beads” for the 2 mm diameter size, plastic 4 mm round beads, “2/0 Czech seed beads” for 6 mm diameter, and plastic 8 mm round beads (purchased from [ibeadcanada.com](http://ibeadcanada.com); Figure 1).



**Figure 1.** Plastic beads used for the study in order of size, 2 mm, 4 mm, 6 mm, and 8 mm, each representing different sizes of earthworm cocoons, with ruler for scale.

After driving over the beads, I measured the distance the beads travelled from the original area with a tape measure and collected them for subsequent trials. I counted the number of beads to ensure all beads were located, and if not I proceeded to inspect the area both along and beside the road as well as in the tire tread to try and find them, until I either found them all or was confident I was unable to locate them. I then recorded any instances where beads remained in the tire tread or could not be found. This process was repeated for each bead size and vehicle type under both weather conditions until all trials were completed.

## *2.2 Data Analysis*

To examine how bead size, weather condition, and vehicle type affected the distance the beads travelled, I calculated the mean distance the beads travelled for each

trial ( $n = 80$ ). After initial data exploration, this data on mean distance travelled was found to be not normally distributed so I log-transformed the data in order to run a general linear model.

I also examined how bead size, weather, and vehicle type affected the number of beads which moved per trial. This count data was over-dispersed ( $\text{Theta} = 19.0$ ) indicating that the variance was greater than the mean for the data set (Ver Hoef, & Boveng, 2007) and consequently a negative binomial generalized linear model was used.

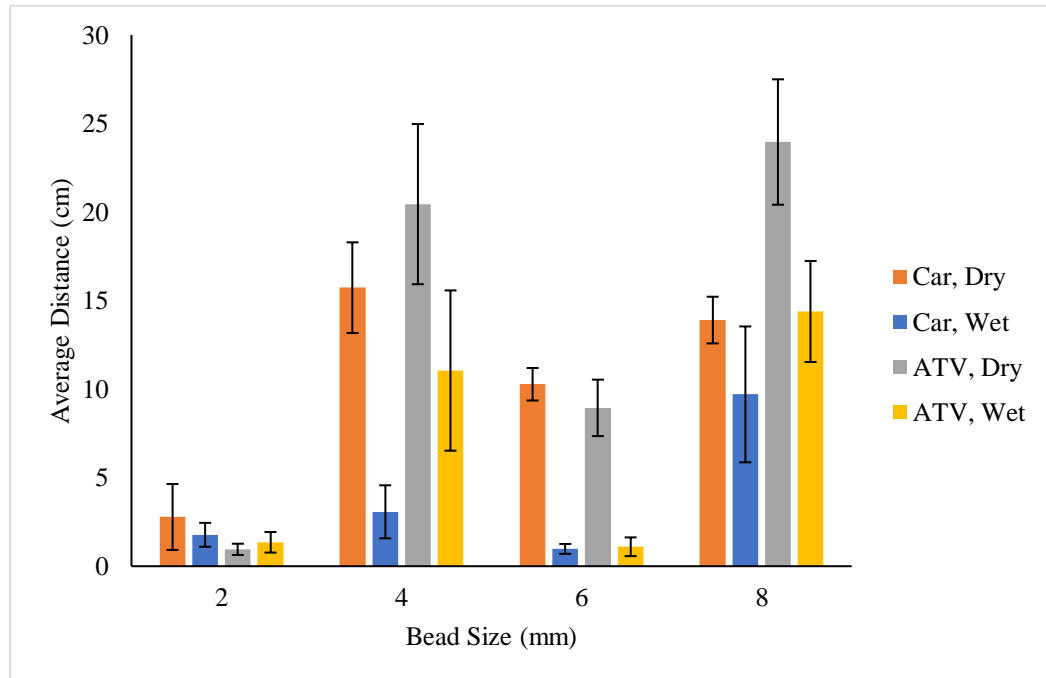
The statistical analysis was completed through R 4.0.2 within the R Studio environment (R Core Team, 2021; RStudio Team, 2021). The packages *rstatix* (Kassambara, 2020) and *MASS* (Venables & Ripley, 2002) were used for the analysis. For the results of the analysis, a  $P$ -value less than 0.05 was considered to be statistically significant.

### **3. Results**

#### *3.1 Overview*

Overall, the 4 mm and 8 mm sized beads moved farther on average than both the 2 mm and 6 mm sized beads, and typically beads moved farther under dry weather conditions than wet (Figure 2). The larger beads moved significantly farther compared to the smallest 2 mm bead (Table 1) in both wet and dry conditions, and the beads that travelled the farthest on average were the 8 mm beads under dry conditions with the ATV (Figure 2). In addition, the car moved the 2 mm and 6 mm beads farther than the ATV during dry weather, and the ATV moved 4 mm and 8 mm beads farther compared to the

car (Figure 2). The results from each trial individually show similar patterns (Appendix 1).



**Figure 2.** The mean distance travelled in centimeters for each bead size according to weather condition and vehicle type, averaged over all trials. The error bars show standard error.

Bead size had a significant effect on the distance beads travelled (Table 1), where larger bead sizes moved farther than smaller beads, with  $P$ -values  $<0.001$  for 4 mm (coefficient = 1.019) and 8 mm beads (coefficient = 1.157) and a  $P$ -value of 0.002 for 6 mm beads (coefficient = 0.637). Additionally, the 6 mm bead size in wet weather on average did not travel as far as the 2 mm bead size in dry weather, which was also found to be statistically significant (coefficient = -0.611,  $P = 0.033$ ; Table 1).

**Table 1.** Results of linear regression model with significant *P*-values (<0.05) indicated by an asterisk.

<b>Coefficient</b>	<b>Estimate</b>	<b>Standard Error</b>	<b>T value</b>	<b>P value</b>
Intercept	0.219	0.140	1.566	0.122
Car	0.213	0.198	1.077	0.286
4 mm bead	1.019	0.198	5.153	<0.001*
6 mm bead	0.637	0.198	3.222	0.002*
8 mm bead	1.157	0.198	5.848	<0.001*
Wet weather	0.030	0.198	0.151	0.881
Car x 4 mm bead	-0.326	0.280	-1.166	0.248
Car x 6 mm bead	-0.043	0.280	-0.155	0.877
Car x 8 mm bead	-0.422	0.280	-1.508	0.136
Car x Wet weather	-0.155	0.280	-0.555	0.581
4 mm bead x Wet weather	-0.305	0.280	-1.091	0.279
6 mm bead x Wet weather	-0.611	0.280	-2.185	0.033*
8 mm bead x Wet weather	-0.284	0.280	-1.016	0.313
Car x 4 mm bead x Wet weather	-0.318	0.396	-0.803	0.425
Car x 6 mm bead x Wet weather	-0.085	0.396	-0.214	0.831
Car x 8 mm bead x Wet weather	0.129	0.396	0.326	0.746

Bead size also had significant effects on the frequency of movement, with larger beads moving more frequently than smaller beads. The 4 mm (coefficient = 1.504, *P*-value < 0.001), 6 mm (coefficient = 1.204, *P*-value < 0.001), and 8 mm (coefficient = 1.705, *P*-value < 0.001) bead sizes were moved significantly more times on average than the 2 mm bead size, and the 6 mm bead size specifically in wet weather (coefficient = -1.322, *P*-value = 0.034) moved significantly less often (Table 2). No other variables were statistically significant in this model.

**Table 2.** Results of the negative binomial regression model with significant *P*-values (<0.05) indicated with an asterisk.

<b>Coefficient</b>	<b>Estimate</b>	<b>Standard Error</b>	<b>Z value</b>	<b>P value</b>
Intercept	0.875	0.306	2.858	0.004*
Car	0.154	0.419	0.368	0.713

4 mm bead	1.504	0.351	4.291	<0.001*
6 mm bead	1.204	0.360	3.347	<0.001*
8 mm bead	1.705	0.346	4.931	<0.001*
Wet weather	-0.288	0.464	-0.620	0.535
Car x 4 mm bead	0.091	0.479	0.190	0.850
Car x 6 mm bead	0.474	0.485	0.978	0.328
Car x 8 mm bead	-0.013	0.474	-0.028	0.978
Car x Wet weather	0.288	0.616	0.467	0.640
4 mm bead x Wet weather	-0.706	0.552	-1.278	0.201
6 mm bead x Wet weather	-1.322	0.622	-2.126	0.034*
8 mm bead x Wet weather	-0.031	0.522	-0.059	0.953
Car x 4 mm bead x Wet weather	-0.695	0.751	-0.926	0.354
Car x 6 mm bead x Wet weather	-0.799	0.834	-0.957	0.338
Car x 8 mm bead x Wet weather	-0.539	0.703	-0.767	0.443

The maximum distance a bead travelled and was found, was just over 418 cm.

This was a 4 mm size bead on a wet day using the ATV (Table 3). The other larger distances include over 327 cm for a 4 mm bead on a dry day with the ATV and 340 cm with an 8 mm bead on a wet day with the car. The combinations that resulted in the shortest maximum distances involved the 2 mm and 6 mm beads (Table 3).

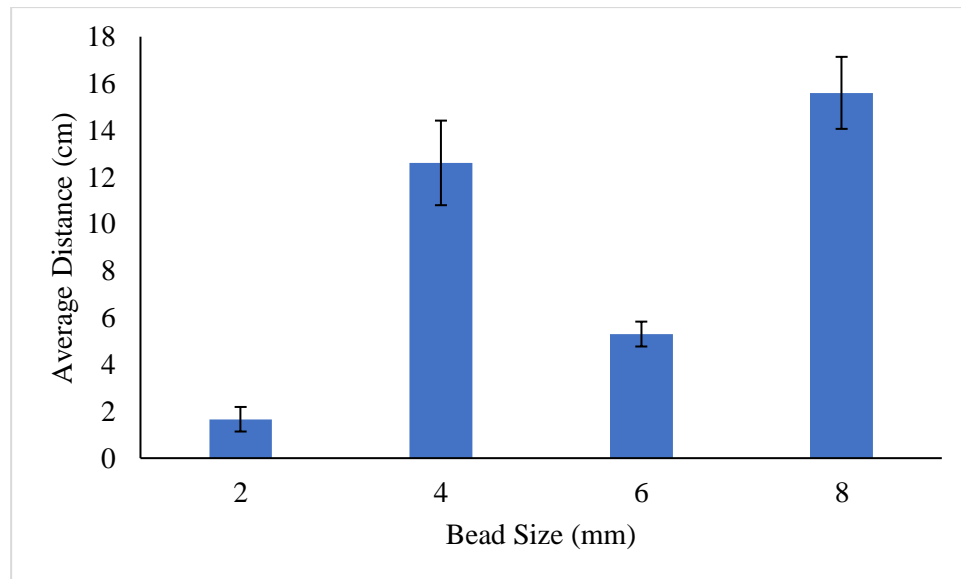
**Table 3.** The maximum distance that beads travelled in centimeters, based on bead size, weather condition and vehicle type.

Bead Size	Weather Condition	Vehicle Type	Maximum Distance (cm)
2	Dry	ATV	17.8
2	Dry	Car	185.4
4	Dry	ATV	327.7
4	Dry	Car	120.7
6	Dry	ATV	66.0
6	Dry	Car	44.5
8	Dry	ATV	260.4
8	Dry	Car	53.3
2	Wet	ATV	39.4
2	Wet	Car	40.6

4	Wet	ATV	418.5
4	Wet	Car	136.5
6	Wet	ATV	43.2
6	Wet	Car	15.2
8	Wet	ATV	144.8
8	Wet	Car	341.0

### 3.2 Effects of Each Variable

Beads tended to move more often and farther distances with increasing bead size (Figure 2; Tables 1&2). If we look only at the average distance each bead size travelled across both weather conditions and vehicle types, it is clear that the 2 mm beads moved the shortest distance, while 8 mm beads and 4 mm beads moved farthest (Figure 3).

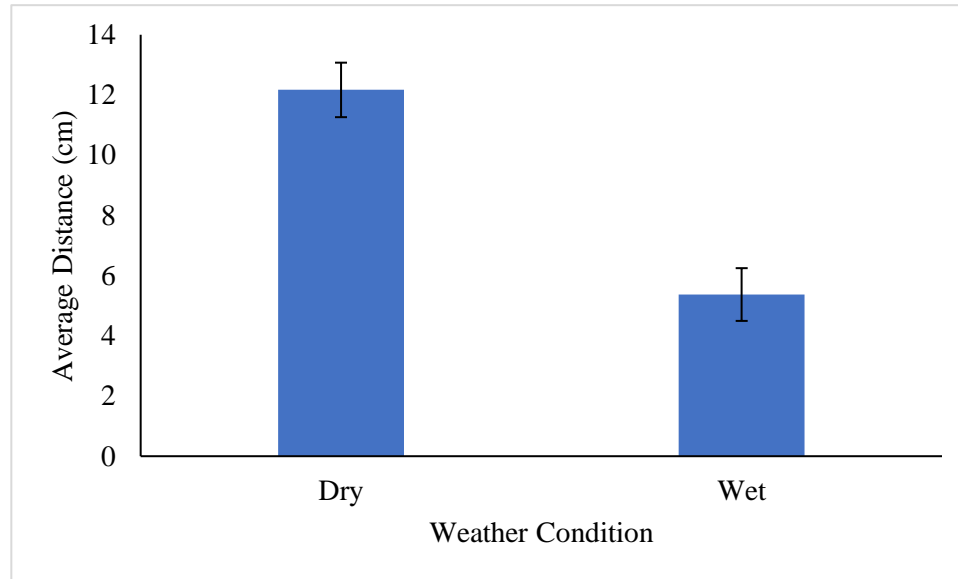


**Figure 3.** The average distance in centimeters travelled by each bead size, averaged over all trials, weather conditions, and vehicle types. The error bars show standard error.

On average, the beads travelled much farther on dry days compared to wet days (Figure 2; Figure 4). Under wet conditions, the vehicles on average moved beads just over 5 cm whereas beads moved just over 12 cm on average under dry conditions (Figure 4).

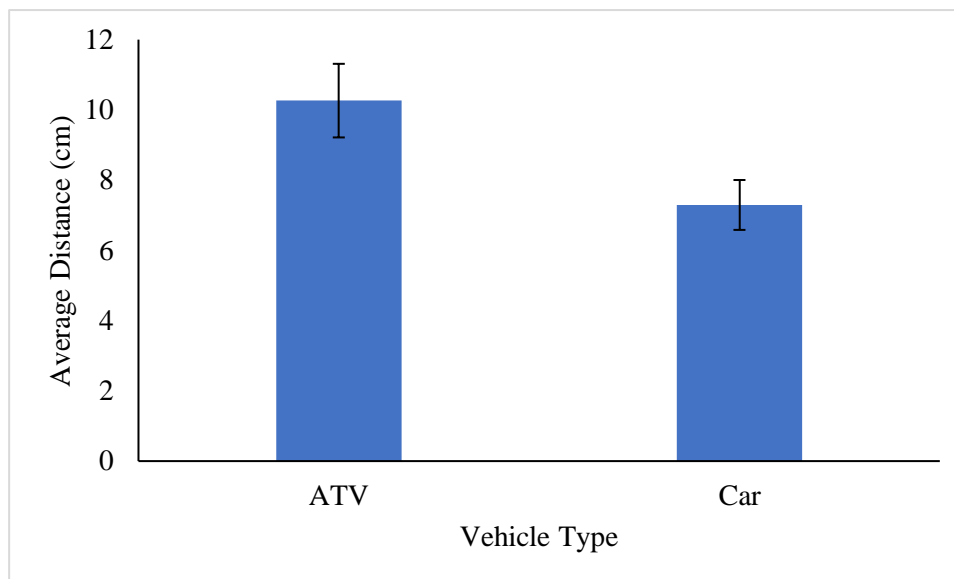


Considering the maximum distance travelled (Table 3) wet weather can still allow for beads to travel far distances, but dry weather is more likely to move the beads farther distances compared to wet weather.



**Figure 4.** The average distance the beads travelled in centimeters based on weather condition, averaged over all trials, bead sizes, and vehicle types. The error bars show standard error.

Vehicle type was found to have less effect on the distance the beads travelled compared to the other variables bead size and weather condition. On average, the ATV did tend to move beads farther than the car, but only by about 3 cm (Figure 5). The summary of maximum distance also shows that beads can travel far distances with both vehicle types (Table 3) but there were no statistically significant differences found in the distance beads moved or the number that moved between the two vehicle types (Table 1; Table 2).



**Figure 5.** The average distance in centimeters beads travelled based on vehicle type, averaged over all trials, bead sizes, and weather conditions. The error bars show standard error.

### 3.3 Lost Beads

Across all trials ( $n = 80$ ), 35 beads were lost, accounting for 2.2% of all the beads which were used. Of these lost beads, the majority were 4 mm beads (49%) and 2 mm beads (32%), while 14% of the lost beads were the 8 mm size, and 6% were the 6 mm size.

Beads were lost more often under wet conditions compared to dry, as 80% of the beads were lost on wet days. Additionally, beads were lost more when the car was used compared to the ATV, as 74% of the lost beads happened with the car vehicle type. Thus, the combination which was most likely to result in beads which could not be located after a trial was 4 mm beads on wet days with the car vehicle type. Beads that were lost were assumed to have moved and thus were included in the counts of the number of beads

moved per trial. However, as the distance that they moved was unknown, they were excluded from the calculations of mean distance moved.

## **4. Discussion**

### *4.1 Influence of Bead Size*

The size of the bead affects the likelihood that it will be moved by a vehicle, and contradicting my hypothesis, this study found that larger beads were more likely to be moved and, furthermore, to be moved farther as compared to the smallest bead size. This is also in partial contrast with other literature which has found that smaller sized seeds are more likely to be found on vehicles, and moved by the airstream produced by vehicles, which has been attributed to their light weight and greater abundance (Rew et al., 2018; von der Lippe & Kowarik, 2012). These differences could be due to the way the data was collected in this experiment, as the movement or attachment of seeds to a vehicle is not directly comparable to beads being driven directly over.

Some studies have found that seeds travel farthest by vehicle when they become physically attached to the vehicle in some way, either through being picked up with mud or soil, and/or being stuck in the undercarriage of the vehicle (Rew et al., 2018). Other studies indicate that once attachment has occurred, release will occur either relatively quickly or else they will be stuck for a long period of time and be more likely to travel farther before becoming dislodged (Wichmann et al., 2009). Based on what I have found, cocoons are more likely to be moved and potentially picked up by a vehicle passing over them if they are larger than 2 mm in size. Whether the beads became attached or not is

unknown from this study, as only a small proportion of beads were never located, and none were found on the vehicles or in the tire tread. This information could be useful in part for determining the rate of spread, as well as being applicable to the spread of other objects or species of similar sizes.

Since beads larger than 2 mm in size were found to be more likely moved by travelling vehicles, this indicates that earthworm species which lay cocoons larger than 2 mm in size may be more likely to disperse through vehicle facilitation compared to earthworm species which lay smaller cocoons. There are many earthworm species which lay cocoons larger than 2 mm (Edwards & Lofty, 1977), so depending on the species of interest (and the associated cocoon size) for a particular area they may be more or less likely to be moved on roadways by vehicles. For example, the earthworm species *Lumbricus terrestris* has a relatively large body size as well as large cocoon size (Edwards & Lofty, 1977) and is an invasive species present in most provinces and territories of Canada (except for Nunavut and the Northwest Territories; Addison, 2009). The species *L. terrestris* rapidly consumes leaf litter, as well as seeds, and distributes these materials into the lower mineral soils through deep burrows, reducing both the overall organic matter in the soil and the number of viable seeds, which affects the aboveground plant communities (Yavitt et al., 2015; Yatso & Lilleskov, 2016; Melampy, Mansbach, & Durkin, 2019). Due to the impacts *L. terrestris* has, if their ability to spread is potentially increased due to the ability of their large cocoons to be more easily transported by vehicles, this should be considered when predicting their rates of spread since it would also affect which areas may be at higher risk for invasion of this species.

#### *4.2 Weather Condition Effects*

Variation in the weather and road conditions would influence the distance cocoons or other materials move due to travelling motor vehicles, and I found that beads were typically moved more frequently and farther in dry weather conditions compared to wet. This is not consistent with my hypothesis, but it does support some of the current literature where dry conditions allowed for seeds to be transported by vehicles for much longer once already attached (Taylor et al., 2012). However, other studies have found that wetter conditions facilitated greater attachment to vehicles, bike tires, and boot soles (Rew et al., 2018; Hardiman et al., 2017). My study did not address attachment to vehicles and none of the beads were found attached to vehicles in either wet or dry conditions, but more beads were lost due to wet conditions and it is possible they did become attached somewhere on the vehicles.

Wet conditions have been found to make it more likely that mud, soil, and seeds will attach to vehicles, but also more likely for them to be washed off. In contrast, dry conditions allow for any material on the vehicle to be carried a longer distance, as studies have found more seeds on vehicles driven in dry conditions than in wet (Ansong & Pickering, 2013; Taylor et al., 2012; Zwaenepoel, Roovers, & Hermy, 2006). Based on how wet the conditions are, the distance of potential seed or cocoon transport is likely to vary. Considering the conditions of this experiment, the wet weather trials were done in very wet conditions, with 35 mm of total rainfall over the 5 days prior to the wet trials (Canada, 2021). If more trials were to be conducted on days which are less wet, it might allow for further travel than seen in these results.

When considering the variation in moisture levels, the road type and texture could also affect the likelihood of materials, such as beads, cocoons, or seeds, being picked up

either on their own or along with mud or soil (Clifford, 1959). Unpaved roads with higher proportions of silt and clay appear more likely to carry seeds and adhere to travelling vehicles (Clifford, 1959). The road used for this study was unpaved, but sandy in texture, and little mud was seen during the wet conditions possibly due to both the texture and over-saturation of the road during the wet trials.

#### *4.3 Vehicle Type Impacts*

There were no significant differences found between vehicle types in terms of the distance or number of beads which were moved in this study. This supports my hypothesis and agrees with another study where there was no difference in vehicle type comparing attachment of seeds to ATV and four-wheel drive vehicles on unpaved roads (Rew et al., 2018). On average in my study, the ATV did move beads farther compared to the car and considering that an ATV is more likely to be travelling off-road or on unpaved roads, there is typically a greater chance for an ATV to encounter and disperse earthworm cocoons. Any vehicle travelling more frequently on unpaved roads would be more likely to disperse and accumulate seeds, or earthworm cocoons, than vehicles travelling primarily on paved roads (Rew et al., 2018).

#### *4.4 Issues and Limitations*

While the number of lost beads was minimal compared to all the beads used, the distance they travelled is unknown. It is plausible that the beads could have become stuck on the vehicle, or simply moved far enough that I could not find them. Thus, while overall the number of beads lost was not a large proportion of the beads in the study, it is still important to consider as they spread an undetermined distance from where they

originated. As the beads used represent earthworm cocoons and can also be similar sizes to seeds or other small organisms, the implications of the lost beads indicate that they can be easily transported by vehicles, and are able to travel considerably far, potentially into new areas.

One of the issues I found in my study was with the 6 mm bead used, which had two flat sides instead of being spherical like the other beads. I believe this could account for the shorter distance travelled by the 6 mm beads compared to both the 4 mm and 8 mm beads. Unfortunately, there was no time to order different 6 mm beads prior to starting, as time to complete the trials was limited. This is unlikely to have strongly affected the results, but it may indicate that variation in shape could also affect the distance travelled by cocoons.

#### *4.5 Future Research*

There is limited research available regarding the vehicular transport of earthworm cocoons, and it would be beneficial for future research to investigate various conditions which would affect their spread and likelihood to be transported by human movements. For the purposes of this study, many factors which could affect the dispersal of cocoons were kept consistent, but several other variables would be useful to investigate in the future. Comparing road types or different road surfaces and textures, as well as looking at travel completely off-road would be interesting to compare and evaluate for any differences in road type. Comparing varying degrees of vehicle traffic and changing the abundance of cocoons would also be beneficial to discovering more about their dispersal potential as these variables may affect the results and could better represent different conditions. Looking at non-spherical shapes as well would also be interesting in order to

assess how shape affects their ease of transport. Future studies should consider using 3D printing technology to reproduce various cocoon sizes and shapes in more detail, as that would provide more accurate results as well.

There is considerable opportunity for future research into the dispersal of earthworms and earthworm cocoons, and how humans influence their spread. More research into determining the rate of species spread and the ability to calculate this rate more accurately for various scenarios would be useful and relevant for the management of invasive species.

#### *4.6 Summary*

Based on the results of this study, the size of the cocoon and the weather condition would alter the chances that a vehicle travelling on an unpaved road would move a cocoon and transport it from its original position. The chances of the cocoon being moved would be increased if the cocoon is larger than 2 mm in size, and the conditions are dry. Both findings were in contradiction to my hypotheses and did not consistently support other studies which looked at seed transport by vehicles.

The distances beads travelled had a large range, with many not moving at all, and the farthest reaching more than four metres from its starting location. Even though only some of the beads were able to travel longer distances, research has shown that rare long distance dispersal events contribute significantly to the spread of species, especially invasive species (Neubert & Caswell, 2000; With, 2002; Trakhtenbrot et al., 2005). As well, some of the beads which were never found but had moved out of their original position and therefore have travelled unknown distances. These movements indicate that



it is possible for earthworm cocoons to travel considerable distances solely from vehicles driving on roadways which is important to consider for their overall spread and dispersal.

## **5. Conclusion**

The spread of non-native species, such as earthworms, is important to understand as their current and future distributions will impact local ecosystems (Frelich et al., 2006; Bohlen et al., 2004). Humans have been facilitating the spread of non-native species and accelerating the rate at which these species have been able to invade the landscape (Hale, 2008). A better understanding of the different vectors by which humans transport materials will allow for more accurate predictions of species dispersal. Determining a relatively accurate rate of spread for various species is important for their effective management (Arim et al., 2006), particularly if they are invasive or become invasive. The potential distance vehicles can move cocoons, seeds, or other propagules is a key factor to consider in modelling spread rates for species occurring along roads.

The results of this study, which found that larger sized beads are travelling farther and moving more frequently, especially in dry weather conditions, can contribute to our overall understanding of how vehicles may be moving species around. These results were not entirely as expected, which also suggests that further research on how other factors (e.g., cocoon shape, intermediate moisture levels) affect movement of cocoons by vehicles is needed. The results of this study regarding the spread of earthworm cocoons are relevant, but the gaps which were recognized in the current literature (e.g., regarding the current distributions and invasiveness of earthworm species), as well as the

identification of areas for future research surrounding this topic, are equally important to consider.

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## Appendix – Summarized Data

This table shows a summary of the data used for the study, organized by trial number, where there are 5 replicate trials of each combination of bead size, weather condition, and vehicle type. For each trial (n = 80) the number of beads moved, the mean distance the beads moved in centimeters, as well as standard deviations, have been calculated across all of the beads which were used per trial (n = 20). Values of NA indicate the only beads moved in that trial were never located.

<b>Trial</b>	<b>Bead Size (mm)</b>	<b>Weather Condition</b>	<b>Vehicle Type</b>	<b>Beads Moved</b>	<b>Mean Distance (cm)</b>	<b>Standard Deviation</b>
1	2	Dry	Car	0	0.000	0.000
2	2	Dry	Car	2	9.398	41.435
3	2	Dry	Car	2	0.635	1.997
4	2	Dry	Car	6	2.286	3.653
5	2	Dry	Car	4	1.588	3.420
6	4	Dry	Car	16	7.049	6.851
7	4	Dry	Car	12	6.016	7.330
8	4	Dry	Car	17	22.024	20.514
9	4	Dry	Car	11	7.461	12.971
10	4	Dry	Car	13	37.497	42.752
11	6	Dry	Car	14	8.827	7.636
12	6	Dry	Car	15	6.668	6.750
13	6	Dry	Car	20	15.653	6.022
14	6	Dry	Car	7	6.096	10.922
15	6	Dry	Car	19	14.129	9.914
16	8	Dry	Car	19	13.621	8.456

17	8	Dry	Car	13	19.082	18.606
18	8	Dry	Car	12	10.382	12.568
19	8	Dry	Car	19	10.541	6.591
20	8	Dry	Car	13	16.711	14.768
21	2	Dry	ATV	8	3.397	5.651
22	2	Dry	ATV	0	0.000	0.000
23	2	Dry	ATV	3	0.699	1.722
24	2	Dry	ATV	0	0.000	0.000
25	2	Dry	ATV	1	0.667	2.982
26	4	Dry	ATV	14	9.620	9.040
27	4	Dry	ATV	4	5.937	18.833
28	4	Dry	ATV	12	21.336	25.668
29	4	Dry	ATV	14	46.355	89.326
30	4	Dry	ATV	10	18.987	23.058
31	6	Dry	ATV	13	18.288	21.309
32	6	Dry	ATV	11	15.113	18.912
33	6	Dry	ATV	3	0.762	2.345
34	6	Dry	ATV	8	7.366	15.165
35	6	Dry	ATV	5	3.175	6.201
36	8	Dry	ATV	15	14.859	12.977
37	8	Dry	ATV	13	22.384	24.119
38	8	Dry	ATV	15	39.751	56.323
39	8	Dry	ATV	14	21.114	17.521

40	8	Dry	ATV	9	21.654	43.976
41	2	Wet	Car	5	1.638	3.288
42	2	Wet	Car	0	0.000	0.000
43	2	Wet	Car	2	0.714	2.858
44	2	Wet	Car	0	0.000	0.000
45	2	Wet	Car	6	7.085	13.402
46	4	Wet	Car	5	10.093	32.001
47	4	Wet	Car	1	1.016	4.544
48	4	Wet	Car	4	1.237	3.337
49	4	Wet	Car	2	0.535	2.331
50	4	Wet	Car	1	NA	NA
51	6	Wet	Car	1	0.381	1.704
52	6	Wet	Car	0	0.000	0.000
53	6	Wet	Car	4	1.810	3.817
54	6	Wet	Car	1	NA	NA
55	6	Wet	Car	3	1.715	4.366
56	8	Wet	Car	3	1.080	2.772
57	8	Wet	Car	12	26.971	77.625
58	8	Wet	Car	6	3.683	7.028
59	8	Wet	Car	13	6.414	7.476
60	8	Wet	Car	9	12.332	34.286
61	2	Wet	ATV	1	0.508	2.272
62	2	Wet	ATV	1	NA	NA



63	2	Wet	ATV	0	0.000	0.000
64	2	Wet	ATV	2	2.921	9.075
65	2	Wet	ATV	1	1.969	8.803
66	4	Wet	ATV	4	24.479	93.196
67	4	Wet	ATV	1	1.524	6.816
68	4	Wet	ATV	5	8.541	27.565
69	4	Wet	ATV	6	15.081	25.478
70	4	Wet	ATV	4	5.620	13.761
71	6	Wet	ATV	0	0.000	0.000
72	6	Wet	ATV	3	0.984	2.407
73	6	Wet	ATV	1	0.381	1.704
74	6	Wet	ATV	2	1.136	4.953
75	6	Wet	ATV	2	3.048	10.247
76	8	Wet	ATV	10	9.843	14.149
77	8	Wet	ATV	6	18.574	43.133
78	8	Wet	ATV	4	3.778	10.413
79	8	Wet	ATV	8	13.811	32.436
80	8	Wet	ATV	20	25.908	26.767

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