The Use of Indicator Species to Determine the Effects of Pesticide Spray on Soil Toxicity

Taryn Smith

Introduction

Anthropogenic effects imposed on the environment are a growing concern as scientists continue to determine the consequences resulting from human habits. Advancements in farming, such as pesticides, have led agricultural factories to increase production of food for worldwide distribution. One such way to increase production is to increase land use, as of 2016, 37% of global land is used for agriculture (Food and Agriculture Organization 2019). The increase land use as well as increasing technologies have led to a more efficient industry, for example as of 2019 world cereal production is up by 117% since 2009 (Food and Agriculture Organization 2019). One such technology, pesticides, while intended to be beneficial for crop protection, are harmful to the environment that surrounds the fields (Sanchez-Bayo et al., 2002). Chemicals are sprayed over large areas and in great volumes which allows for large amount of pesticide runoff (van der Werf, 1996). Following initial spray 5-10% of pesticide runoff goes into the soil surrounding the field potentially causing harmful effects on the organisms that inhabit it (van der Werf, 1996).

Soil invertebrates in the presence of pesticides are found to have both physiological damage as well as behavioral changes. Soil invertebrates are vital to soil processes such as the breakdown of detritus, maintaining the food web, and filling the role of ecological engineers (Ritchie et al., 2019). However, these important soil-dwelling organisms are inadvertently being targeted by pesticides (Ritchie et al., 2019). While there is some species-specific variation to susceptibility of pesticide damage, earthworms, as well as many other soil invertebrates, experience reduced adult survival as well as a reduction in offspring production at sublethal levels (Ritchie et al., 2019). Additionally, in the presence of pesticides, earthworms have a reduction in body weight and a decrease in burrowing behavior (Capowiez & Bérard, 2006). Burrowing behavior is important for soil health, as it allows for increased water infiltration, stimulation of microbial activity, improved soil water-holding capacity, and production of channels for root growth (Rusek, 1998). Due to the importance of these soil invertebrates to the health of the subterrain ecosystem, they are a great study organism to determine how the pesticides affect them on a physiological basis.

Study organisms, or indicator species, are a valuable tool for determining the toxicity of their environment. Indicator species demonstrate the effects of pollutants on both biotic and abiotic factors of an environment (Hodkinson & Jackson, 2005). These indicator species demonstrate the environmental effects through their behavior and changes to physiological and reproductive success. This study looks at how the toxicity of pesticides in the environment affects the growth rate of the indicator species. The hypothesis of this study is that soil toxicity will be highest closest to the edge of the field. The amount of pesticides will be highest the closer to the field due to initial deposition of chemicals during runoff. The higher pesticide concentration will cause an increased soil toxicity resulting in a depressed growth rate of the indicator species along the transect. This study looked at the growth rate of redworms to determine the soil toxicity along a transect moving away from the field.

Methods

Indicator Species

Redworms (*Eisenia foetida)* were used as an indicator species to assess soil toxicity. Redworms have a fast rate of sexual maturation, 40-60 days, and it is during this time frame that the growth rate will be the fastest and the most susceptible to environmental effects (Venter & Reinecke, 1988). The use of redworms is advantageous because they are subject to accumulating high levels of environmental toxins as a result of their detritivore diet (Cortet et al., 1999). In previous studies, high levels of pesticides have been shown to have fatal effects on redworms (Das Gupta et al., 2011). The growth rate of the indicator species in soil samples collected along transects from agricultural fields was analyzed to determine the toxicity of the soil.

Study Site

Kentland Farm is the study site for this experiment. Kentland is a 3000 acre plot of land with 400 acres devoted to farming. The farm is adjacent to the New River and therefore there is a high potential of runoff into the body of water. Four fields across Kentland Farms were chosen (Figure 1) and from each field a transect was drawn and soil samples were collected. A variable that has a big effect on the runoff potential is the topography of the area(Schriever et al., 2007). Using a topographic map, the fields with the most obvious drainage route to a nearby river were determined.

Soil Sampling

The drainage route from each field was determined and along this, 100 meter transect was drawn. At 20 meter increments along the transect a soil sample was collected. There were five sites of collection along the transect. At each site of soil collection, several 3 centimeters diameter holes were dug using a soil corer with the depth not surpassing 23 centimeters. The soil that was collected was a combination of top layer soil as well as any vegetation or detritus that was on the surface.

Growth Rate

The soil samples collected were used as the medium in which the redworms lived for the span of the experiment. The soil that was collected was homogenized to make for a more even distribution of layer of soil between all the cups filled. Next, the soil cups were filled with an equal amount of soil between each collection site. One pre-weighed juvenile redworm was placed in each soil cup and was weighed twice a week for the duration of the experiment to determine growth rate. The cups were stored in an incubation chamber set to 24 degrees celsius.

At the two week mark, water was added to the soil after deaths due to dehydration were observed. The growth rate of the worms were determined, grouped according to distance from the field, and compared.

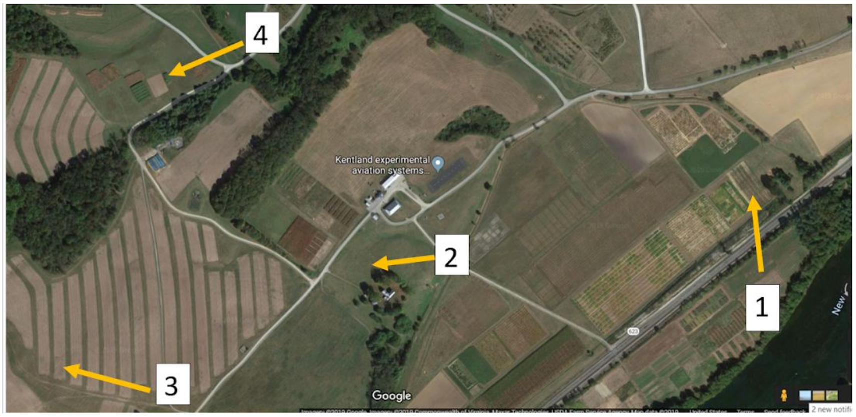

Figure 1: Site map of Kentland Farms. The four fields are designated by the yellow arrows and are numbered according to the order in which the soil samples were collected. Image Credit: Google Maps

Data analysis

All statistics were conducted using R-Studio and graphs produced using the packages *ggplot2* and *RColorBrewer*. Relative growth rate of each worm was calculated in Microsoft Excel using the equation, RGR= $\frac{(final\ mass - initial\ mass)}{initial\ mass}$. To determine the effect of distance from the field on growth rate, we used a linear mixed model with growth rate as the response, distance from the field as a fixed effect, and the field identity as a random effect. Prior to analysis, an outlier was removed from the data set (value= 6.17, IQR=0.655), a value of 1 was added to all data points, due to some values being negative, and the log was taken to approximate a normal distribution. To test whether distance was a significant predictor of growth, we used a likelihood ratio test comparing the full model to a simplified version with field identity only as a random effect.

Results

The distance from the field had a positive effect on the relative growth rate of *E. foetida* (X^2 = 10.124, p-value=0.001; Figure2). In Figure 2, a line of best fit was drawn that had a positive slope, indicating an increase in the mean growth rate as distance from the field increased.

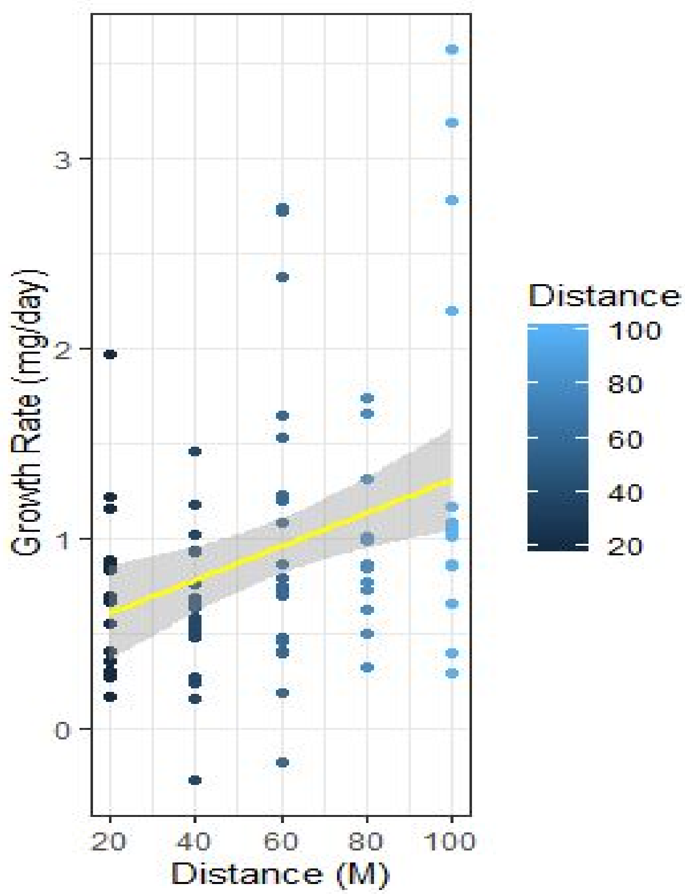

Figure 2: The effect of distance from the field on relative growth rate of *E. foetida*. The linear regression line is in yellow (b=0.430, m=0.009). Distance from transect is demonstrated by a blue color gradient.

Discussion

The goal of this study was to examine the soil toxicity of a particular farm, and test how the distance from the field affects the growth rate of an indicator species, *E. foetida.* Pesticides sprayed onto crops leech into the environment, resulting in an increased soil toxicity(van der Werf, 1996). Many studies have looked at the impact of soil toxicity on growth rates of indicator species (Aebischer, 1990, Cortet et al., 1999, Hodkinson & Jackson, 2005, Capowiez & Bérard, 2006, Jänsch et al., 2006, Das Gupta et al., 2011, and Ritchie et al., 2019). The methodology and information gained through these studies and reviews, were applied when looking at fields that were sprayed with the herbicide Glyphosate. This study found that the growth rates of redworms were higher at farther distances from the field.

In this study growth rate was used to demonstrate the presence of soil toxins (Ritchie et al., 2019). Therefore, the worms that had a slower growth rate were in soil that had a higher concentration of soil toxins. The higher soil toxicity would be a result of pesticide runoff. Past studies have shown that soil invertebrates exposed to pesticides have negative effects on their physiology(Correia & Moreira, 2010). The negative effects on their physiology would likely result in reduced growth rate in increased concentrations. These results support the hypothesis, the positive growth rate indicates a decrease in soil toxicity as the distance from the field increased. However, the results are correlational and should be cautiously interpreted. The actual concentrations of pesticides in the soil were unknown and the results were interpreted using the methodology of previous studies. There are additional factors that could have contributed to the difference in growth rates, such as, nutrient concentration, sediment composition, gradient of transect, and or soil moisture content.

The low R^2 value could be explained by a few errors during the duration of the experiment. During the weighing, many of the worms were coated in soil. While a good bit of soil was removed from the worms before they were weighed, there was some variability in the success of the soil removal. This variability of soil removal could have resulted in skewed numbers during the weekly weighings. An additional error that could have contributed to a low R^2 value was the amount of water in the soil. The soils all started off fairly damp however through the duration of the experiment the temperature of the incubator caused the water to evaporate. It was during the second to last weigh in that a decrease in mass in most of the worms as well as worms dying was noted. Water was added to prevent future deaths or growth rate effects, however this could have been a factor in the worms ability to grow properly.

Using the information gained from this study, there are many future directions that can be taken. One would be to do a generational study to determine if the herbicides cause enough damage to see generational effects. Multiple studies have shown that there are detrimental effects of both pesticides and herbicides on the reproduction of *E. foetida* (Ritchie et al., 2019, & Rusek, 1998). However, these studies haven't looked at to what extent abnormalities caused by the chemicals potentially impact future generations. Another direction to take this study would be to expose the worms to a known concentration of herbicides and compare the growth rate differences between known and unknown pesticide concentrations. A review by Jänsch and his team emphasized the importance of backing up field toxicology tests with the use of a laboratory standard (Jänsch et al., 2006). The use of known concentrations when looking at soil toxicity would strengthen the results found. Lastly, another study that could be conducted is to demonstrate the effect of multiple types of pesticides in conjunction with others in a direct to determine how the use of many types of chemicals impact the organisms. It is not uncommon that private consumers or larger commercial farms would use multiple types of pesticides. The combination of these chemicals in the environment could pose considerably more damage than a single type of pesticide.

Pesticides runoff results in increased soil toxicity. It is this increased toxicity that causes physiological and behavioral damage to the organisms in immediate field edges, and potentially other organisms in the environments surrounding the fields. More studies need to be conducted looking at all effects of pesticides not only on immediate soil ecology, but also to look at the larger ecological consequences of introducing pesticides into the environment. The pesticides are introduced to lower levels of the food chain and have the potential to introduce bottom up issues into the ecosystem. Therefore it is vital to emphasize the effects of pesticides on a greater ecological picture to businesses and the public consumers.

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