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The Effect of Hydrocarbons on the Growth and Germination Patterns of the Plant *Brassica rapa*

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ABSTRACT

This experiment focuses on the effect of soil hydrocarbons have on Rapid Cycling *Brassica rapa* (RCBr). RCBr was chosen because of its ability to reach full maturity in thirty days. Eight soil samples were taken adjacent to oil wells in a salt marsh found thirty miles west of Hutchinson, Kansas, at the Quivira National Wildlife Refuge (QNWR). Sixteen plants were grown in these samples to determine the effect hydrocarbons have on the growth and germination patterns. The soil porewater salinity was determined by refractometry. The eight salinity levels ranged from 2ppt to 37ppt. Soil organic carbons, determined by ashing at 600°F, ranged from 1.25% to 4.25% by weight. Percent oil in the soil, determined gravimetrically following carbon tetrachloride extraction, ranged from 0.3% to 1.8% by weight. A multiple linear regression of percent oil by weight and salinity in ppt.on percent germination yielded the following equation: %Germination = 57.9-(1.73*%oil)-(1.65*Salinity), r^2 = 0.624. An analysis of a variance shows that salinity explains most of the effect on germination. Variables and effects still under investigation include soil texture, soil moisture, seed production, root and stem growth, and biomass accumulation.

INTRODUCTION

Hydrocarbons affect all plants by altering either their growth, their reproduction, or their structure. Hydrocarbons in fact create toxic soils. These soils either kill the plants or produce a defect in the plants' structure. Recent studies on hydrocarbons prove that they affect plant production. Some studies have identified toxic levels of hydrocarbons. Current studies on hydrocarbons are determining what elements of hydrocarbons hinder plant development and structure.

Salinity levels also modify plants' growth and reproduction. Because salinity reduces the activity of major nutrient ions and produces large ion ratios in soil solutions (Gratton and Grieve, 1991), the plant will experience nutritional disorders. Mass and Hoffman (1990) identify three different ways salinity interacts with a plant's nutritional values: (1) no effect on salt-tolerance, (2) increased salt-tolerance, (3) decreased salt-tolerance. The soils analyzed in this study have all three types of these interactions. Watching the plants grow in these types of soils, I determined which interaction most influenced the plants. While other researchers have studied the effects of either salinity or hydrocarbons, I was unable to find studies assessing the combined effects of salinity and hydrocarbons. I intend to determine the amount of hydrocarbons and salinity levels in the soil and how they affect the plant *Brassica rapa.*

A major difficulty in understanding plant nutrition as it is affected by soil salinity is reconciling results obtained in experiments conducted in the field with those conducted in sand or solution cultures (Gratton and Grieve, 1991). A plant's nutritional value contains the amount of minerals it needs to survive in a fertile, non-saline, non-hydrocarbon environment. Changing the nutritional value will cause the plant to change in a form different then it is adapted to. The addition of the new materials will cause they plants structures to undergo a new environmental change other then what usually takes place. The reduction of nutrient ions and the increase of the already high concentrations of major ions in the soil solution, will have an influence on the plant. (Gratton and Grieve, 1991).

Salinity and nutrient concentrations also vary spatially and temporally. Salt marshes are inundated with high salinity water. With the adding of tidal regime, freshwater, soil texture, and marsh elevation, spatial and temporal variations in the salt and oxygen concentrations of the interstitial, water in salt marsh soils will result. (Wijte and Gallagher, 1996).

Hydrocarbons have always been present in the world. They are seen in everyday activities. For example, they are found in the cars we drive. Hydrocarbons can also be found under ground. By means of oil rigs, oil is pumped from the earth's floor to holding tanks designated for oil. This process is done by using a series of pipes. The oil is traveled through pipes. However, occasional breaks or leaks in the pipes cause the oil to leak out into the soil and damage the soil for future use. Sometimes the oil leak is so bad it can be seen on the surface and dead plants are rotting away in the middle of the oil spill. This is why I focused my research in this area. I wanted to determine how much effect hydrocarbons have on plants.

In studying hydrocarbons, scientists have determined several things' whether a plant will live or not. One thing would be the amount of hydrocarbons that influence the plant. Another would be if the plant has some sort of defense system that will help fight off the harmful hydrocarbons.

In my research I intend to take samples of soils from around oil well sites in a salt marsh, and determine the toxicity of hydrocarbons that are present in the soil.

I will be doing this by using rapid-cycling *Brassica Rapa* as the subject for my research. Because of its small size, short life cycle (30d), easy to study, and having the ability not to have to tend to it every day (Daugherty and Musgrave, 1994) I can get a more accurate conclusion. "The plants are well suited for

laboratory investigations of many ecological and genetic questions due to their short generation time, small stature, lack of seed dormancy, and ease of breeding, and considerable genetic variability" (Gurevitch, et all, 1996). The typical Brassica rapa plant grows between 20cm and 25cm. The plant is a relative of the mustard plant and grows much like it does. IT grows tall will one base stem and several branches coming from the stem.

MATERIALS AND METHODS

I gathered soil samples from the Quivira National Wildlife Refuge. Located in south central Kansas, Quivira National Wildlife Refuge (NWR) lies in the transition zone of eastern and western prairies. The length of the marsh is roughly eleven miles long and at the wider part of the marsh is about six miles. In total the refuge's acres reach about 21,820. I focused my attention in the northern end of the marsh, where oil wells are present.

I excavated eight soil samples. These included three from near oil wells currently in operation, one from near a water-take-off tank, one from near an oil well that is not in use, one from an area where four holding tanks are present, and two controls (one control bare ground with no plants growing there, and one control that has plants growing). At each site I measured out the length and width of each area and measured three meters from the center of each tank. I then drew a diameter around each site, three meters away and randomly took ten samples of soil in that diameter. I obtained my samples with a soil core remover. The core remover had a diameter of 40mm. I then took the samples in a range between 200mm and 250mm deep. Then I placed the soil in a bucket and mixed the soil together. Next I placed the soil in zip-lock bags, to keep them from drying out, and place them in the fridge when I got back to the lab.

The same procedure took place at each site. I used the same procedure with the controls. The only difference was that instead of measuring out three meters for my controls I picked an area that was one meter by one meter from which to take my samples.

In the lab, I first took each bag and distributed the soils into smaller bags. I made sure I had at least three bags (that weighed 1500g) for each soil and placed them in small zip-lock bags. With the first bag I used the soil weighing 1500g to plant my seeds. Another bag was put in the oven at 100 degrees Celsius to determine the moisture content of the soil. The last bag was used to determine how much oil was present in the soil by running a test.

With the bag I designated to be placed in the oven, I took the soil out and placed the soil in paper bags. I left the bags in the oven at 105C for 48 hours and then weighed the soil again. This procedure could tell me how much moisture was in the soil. Then I could use the dried soil to run two more tests. In the first test I learned how much ash free content I had in the soil. To do this I weighed out 2.00g of the dried soil and placed it in the muffle furnace at 600C for three hours. This burned off all the organic material, leaving only the mineral content, or ash free dry weight minerals in the soil. I then weighed the material and recorded the difference.

The second test I did was to measure the sand/silt/clay percentages of each soil using the procedure of Bower (1991).

To plant my seeds I used the first bag of each soil (The procedure I used to plant my seeds can be found in the handbook for the Wisconsin Fast Plants Growing Instructions, published by the Carolina Biological Supply Company (1989)). After I planted my seeds, I placed them into a growth chamber.

My plant cycle was divided into days. Day one I planted my seeds. On day 2-3 I watered my plants and on days 4-5 I thinned my plants until there were only one seed in each cell. On days 6-11 I checked the water and watched for growth and development. On days 12-18 I cross-pollinated my plants. On days 17-35 I watched for seed pod development and embryos maturing to occur. On day 36 I removed the plants and day 30 and day 60 I harvested the seeds. I then weighed the plant while it was wet and then again after it dried for 48 hours at 105C and determined the percent dry weight and the percent moisture in the plant (using the same procedure as the soil). Next I measured out 2.00g of the oven dried plant material and placed it into the muffle furnace at 600C and determined the amount of organic material in the plant.

With the third bag of soil I ran a test on the soil to determine the amount of hydrocarbons that were present in the soil. To do this I measured out 150g of soil in a beaker and added 200g of carbon tetrachloride to the soil. I then filtered out the combined mixture until I had a small amount of liquid. Then I weighed this material and heated it until the carbon tetrachloride evaporated. The hydrocarbon material that remained was weighed to determine the percent of hydrocarbons for each soil.

RESULTS

Figure 1 shows a comparison of four things. The relationship between the amount of seeds grown, that amount of plants that grew to full maturity, the percent of oil in the soil, and the percent of salinity each soil had. This graph shows that salinity had a more direct effect then the hydrocarbon did. Where there were high levels of salinity there were low to none amounts of plants grown to maturity. Soil number 8 grew the best according to the graph and as the graph shows it should have. This soil has the lowest salt and hydrocarbon mixture among all of the soils combined.

The eight different soils can be broken down into one of ten categories. These ten categories include clay, sandy clay, silt clay, clay loam, silty clay loam, sandy clay loam, loam, silt loam, silt, sandy loam, loamy sand, and sand. The eight samples of soils fell

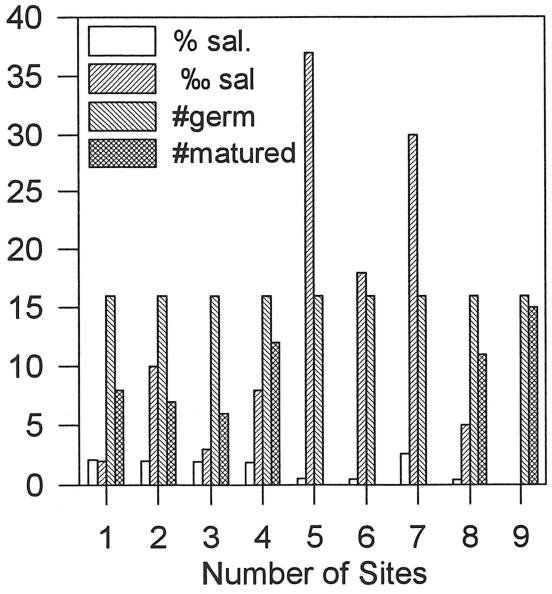

Figure 1. Soil test parameters (% oil, ‰ sal., #germ, and #matured) for samples from the Quivira N.W.R.

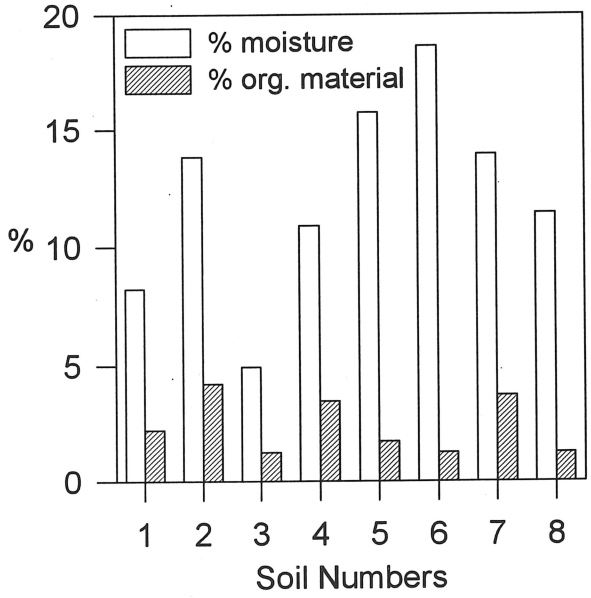

Figure 2. Soil test parameters (%moisture, %organic matter) for samples from the Quivira N.W.R.

into one of these groups. Soil 1 was in sandy loam, Soil 2 was silt loam, Soil 3 was loamy sand, Soil 4 was loamy sand, Soil 5 was sand, Soil 6 was sand, Soil 7 was loamy sand, and Soil 8 was also loamy sand. Using the U.S. Department of Agriculture (USDA) System I could obtain the correct particle diameter for each category.

Figure 2 shows a comparison between % moisture and % organic material. This graph shows the different levels of each for each of the eight soils.

The plants with the greatest amount of oil in their soil showed a difference to the control plants. Soil 7 had the greatest amount of oil and no plants were grown. Soil 4 also had a great amount of oil present and these plants grew in all shapes and sizes. Some plants grew with a very small height with many branches, much like a bush. The plants would contain no central stem but would have 20 to 30 different branches and the base of the plant. These plants produced grew no seeds. Most of the plants leaves in soil 4 grew 2 to even 3 times bigger then the control plants. Some leaves were so heavy they touched the ground. The plants with the big leaves produced a large amount of seeds. One plant produced 18 seeds. Another 58 seeds, another 38 seeds, and the last plant produced no seeds.

The total seed count of all 9 soils was 731 seeds. Soil 1 had a total of 8 plants germinate and 154 seeds produced. Soil 2 had a total of 7 plants germinate and 85 seeds were produced. Soil 3 had only 4 plants grow to a mature level and 28 seeds were produced. Soil 4 had a total of 10 plants grow to a mature level and 120 seeds were produced. Soil 5,6, and 7 had no plants produce and no seeds produce. Soil 6 did have a plant live for 7 days but it then perished. Soil 8 had 11 plants and 217 seeds produced. Soil 9 (which was the control group) had 15 plants grow to a mature level and produced 127 seeds.

DISCUSSION

In this experiment, the salinity level of the soil out weighted the level of hydrocarbons in plant production. With the data I obtained, the salinity level of the plants had a much greater impact on plant production versus the hydrocarbon levels. Figure 1 helps explain this. The graph shows a high amount of salinity in soils 5-7 and the outcome was no plants being produced. Then if you look at soils' 1-4, you can see high levels of oil present in the soils and the outcome was produced plants. Soil 7 had the most oil present in the soil also but compared with the salinity level, this is a greater amount of salinity added to the soil then oil.

In a corresponding study by (Ware, 1997) she took *Brassica rapa* sees and put them in a petri dish. In the dish she added different levels of salinity that ranged from 0ppt to 37ppt. At 0ppt 30 seeds out of 30 seeds germinated. At 2ppt 29 seeds germinated. 5ppt had 28 seeds produced and at 8ppt 30 seeds germinated. At 10 ppt 29 seeds germinated and at 18ppt only 8 seeds germinated. Then at 25ppt 2 seeds germinated and at 30 and 37ppt 0 seeds germinated.

In all 9 soils 16 plants were germinated and each soil produced a different number of plants. Soil 9 (which was the control plant) produced the greatest plants. This soil had no added materials in the soil and was only used as a control group. Soil 4 (which came from an area around the water run off tank) had the second highest number of plants produced. This soil the fourth lowest amount of salinity and about the same amount of oil as 1-3 did. Yet, it produced more plants then 1-3. Soil 8 (which came from an area near the holding tanks) had the third best amount of plants produced. Besides the control group this group had the lowest amount of

soil present in the soil. It also had the second lowest salinity level. From the data I collected the higher the salinity level the lower the plants produced and the higher the amount of oil did not have as much as an impact on the plant production.

The hydrocarbon level did have a greater impact on the plant's genetics then did the levels of salinity. Soil 4 had the greatest amount of oil present with plants that survived. These plants looked different then the control groups. Of the 16 plants germinated 12 were grown to a mature plant. From these 12 plants 10 of them had some defect to them. Of the 12 plants that germinated 4 of them grew like small bushes. When looking at the plant no stems were visible. Instead there were about 10-12 branches on each plant that grew from the ground. The plant still produced an adequate amount of flowers but it did not look like the control plants. This was due to the amount of oil present in the soil. The oil must have affected the genetic process of the plant to inhibit plant growth in the way the plant was use to growing.

Of the 10 plants that had some defect to them 6 of them had bigger leaves, stems, roots, and branches then the controls. This caused the plants branches to become heavier and broader then it previously did in the control group. These plants did not look healthy. The leaves were so big they sometimes touched the ground and would turn colors. The plants also had a weltering look to them. The leaves and branches reminded me of a weeping willow tree dying.

How much sand, silt, and clay also had an influence on the amount of plants grown. The plants that grew the best tended to have less sand and more silt and clay. The soils with higher amounts of sand did not do as well as the soils with lower amounts of sand. The plants that had the higher amounts of sand also tended to have high amounts of salinity. I would have liked to have another control group with no salt added and just have different levels of sand in the soil. Then I can decide how much sand the *Brassica rapa* can handle in its soil and still produce plants.

If I had done this expiration again, I would have added more plants. I would have split 36 plants into two groups. In one group I would grow plants in potting soil and have increasing amounts of oil present in the soil. This way I can decide how much oil it would take to determine how much oil it took to make the plant toxic to oil. With the other group I would put plants in different amounts of salinity levels and do the same experiment. In a corresponding study (Ware 97) this experiment was accomplished. As her results show the higher, the salinity level produced a lower amount of plants produced. The salinity levels had an immense impact between 10ppt and 18ppt. This helps in my conclusion in stating that the salinity does affect seed germination the higher you increase the ppt.

From the data I have collected, I would conclude that the oil wells that are found at Quivira National Refuge are currently influencing the soil in a negative way. All eight samples that I took from QNR had amounts of hydrocarbons in them. One soil I took was from an old run down oil well. This oil well had the largest amount of oil present in the soil. No plants were seen growing in a 100ft square area of the oil well. This soil (number 7) also produced no plants. Soil 4 had the second largest amount of oil present in the soil. The plants produced there were all affected in some form. The plants produced did not look like the control plants grown and the seed count was also lower.

Oil present in soil has no-good result that I have found. It looks bad, smells bad, and hinders the plant's ability to produce the best possible plant. I feel it would be in the best interest of the National Parks if the government would stop placing oil wells in the parks and start focusing in on the wildlife and plants that occupy them.

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