The effect of habitat on constitutive levels and inducibility of leaf phenolics in American beech (*Fagus grandifolia)*

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Abstract

Plants use chemical defenses to ward off herbivory. Phenolics, a carbon-based chemical defense, are present in all woody plants. There are many biotic and abiotic factors that can affect the levels of phenolics present in plant tissue. Two factors that can affect phenolic levels are carbon and nitrogen availability. Often these factors vary with habitats. Glacial moraines often have mesic, nitrogen rich soil. Glacial outwash plains often have xeric soil with less nitrogen. To see the effects of carbon and nitrogen availability on constitutive levels and inducibility of phenolics, we tested constitutive levels and inducibility of phenolics in American Beech (*Fagus grandifolia*) in both habitats. Trees from the outwash plain had higher constitutive levels of phenolics. Though both sites induced phenolics in response to leaf damage, neither site produced a significantly greater amount.

Keywords: Phenolics – Herbivory – *Fagus grandifolia* – American Beech – Defense – nitrogen/carbon availability

Introduction

Carbon and nitrogen are crucial to plant growth: carbon, acquired during photosynthesis from CO_2, is made and stored in leaves, and nitrogen is taken up from the soil and is necessary for plants in order to synthesize DNA and RNA which provides the basis for all plant chemical processes. Nitrogen is the main component for plant development, and is usually the limiting factor for growth. Carbon is not directly limiting for growth, however, carbon is essential to build all organic materials. When nitrogen quantities are low, fewer organic molecules can be synthesized, regardless of the amount of carbon available.

Plants have evolved chemical defenses against herbivores, which can also provide resistance against fungal, bacterial, and viral infections (Harborne, 1980 in Tomova, 2005). Phenolics are one of the major groups of carbon-based plant chemical defenses, and include tannins, which are known to exist in many common foods, such as tea and wine. Phenolic compounds help protect trees against infection by microorganisms and injury by herbivores (Coder, 1999).

In trees, phenolics can occur in both bark and leaves. Structurally, phenolics are compounds that contain an aromatic phenol group. Carbon is required to synthesize the phenol structure, while nitrogen is required for RNA synthesis and enzyme production, which is necessary to build the phenolic compound. Levels of phenolic compounds within leaves are not constant, and it has been observed that levels of phenolics increase in response to herbivore damage. (Karban and Baldwin 1997)

Tree leaves obtain nitrogen primarily from the soil, and carbon primarily from sunlight. Because distinct habitats provide different soil nutrient (nitrogen), light (carbon), and moisture levels, and thus differ in growth patterns of similar species, habitat may limit the plant's ability to both produce constitutive levels of phenolics and induce phenolics when damaged. Since phenolics are carbon-based, it is possible that levels of carbon in the leaf will limit its ability to produce both constitutive phenolic levels, as well as post-damage phenolic levels. However, general nutrient availability, more specifically nitrogen availability, may also affect the plant's ability to induce phenolics in response to damage. Therefore, nitrogen availability may also be a limiting factor in phenolic production, since it is required to build phenolic compounds. Decreases in water and nitrogen levels have been shown to correspond to increases in tannin and phenolic glycosides in Quaking aspen (*Populus tremuloides)* leaves (Roth et al., 1998)

The two habitats in which phenolic levels were observed are products of glaciations: a terminal moraine and an outwash plain. A terminal moraine marks the furthest extent of the glacier; here, the glacier deposits unsorted sediment, or till. Till is nutrient rich mesic soil. An outwash plain is usually a flat area downstream from a terminal moraine. Here, glacial run-off deposited larger particles such as sand and gravel that were carried from the moraine. Outwash plains have generally drier xeric soil and are lower in nutrients, although they also receive more sunlight than moraine environments (Karowe, 2007).

American beech is found generally in the eastern and southern United States. It grows best in deep, rich, well-drained mesic soils of various textures (Yale, 2001), but also grows successfully in xeric soils (Burns and Honkala, 1990). Little is known about the chemical defenses of *Fagus grandifolia*, or American beech.

Flowers bloom in late April or early May when the leaves are about one third of their full size. Growth of American beech occurs primarily in the spring and summer and can only start after the leaves have fully expanded, which is usually in the middle of spring (Burns and Honkala, 1990). Defoliation of *F. grandifolia* is caused by a few different insect species. *Heterocampa guttivitta* (saddled prominent) is the most damaging herbivorous species. *Malacosoma disstria* (forest tent caterpillar), *Lymantria dispar* (gypsy moth), as well as *philapometaria* (fall cankerworm) and *Operophtera bruceata* (Bruce spanworm) feed upon the American beech (Burns and Honkala, 1990).

Beech trees in northern Michigan are found in abundance in both moraines and outwash plains habitat types; thus they are an ideal species to use in order to study the effect of habitat on phenolic activity.

In this study, we will compare constitutive levels and inducibility of phenolics in *F. grandifolia* in two habitats of northern Michigan, a glacial outwash plain and a terminal moraine. In this study, we will attempt to answer the following questions:

1. Does habitat affect constitutive levels of phenolics in *F. grandifolia*?
    a. Does constitutive leaf carbon to nitrogen ratio affect constitutive phenolic levels?
    b. Does diameter at breast height (DBH) affect constitutive levels?
    c. Does leaf surface area affect constitutive levels?
2. Does induction occur in *F. grandifolia*?

3. Does habitat affect inducibility in *F. grandifolia*?
    a. Does habitat affect absolute change in phenolics levels?
    b. Does habitat affect percent change in phenolics levels?

Materials and Methods

Effect of Habitat on Constitutive Levels of Phenolics

To determine whether habitat has an effect on constitutive levels of phenolics in *F. grandifolia*, we sampled 50 beech trees in two habitats in northern Michigan: 25 in the outwash plain on the north side of Riggsville Road across from the gorge, and 25 in a terminal moraine on the south side of Riggsville Road west of Bryant Road. Both sites are located on University of Michigan Biological Station property near Pellston, MI. Each tree sampled was taller than 6 feet and at least 5 feet from any other beech tree in the area. We sampled leaves in position 2 on the branch (the second leaf from the terminal leaf), unless that leaf was damaged. Additionally, we measured DBH of each tree, test leaf length and width, and air temperature directly above and below the test leaf, and height from ground of the test leaf.

The first samples (undamaged leaves) were collected on May 26, 2007 between noon and 4:00pm. Half of the test leaf was removed using standard scissors, leaving the midrib intact, and immediately placed in liquid nitrogen to halt chemical processes. The remaining half of the test leaf and the three surrounding leaves (leaves in positions 1, 3, and 4) were damaged using a garlic press in order to simulate herbivore damage. The midribs were not damaged. After 24 hours (May 27, 2007) we returned to the sites and collected the damaged halves of the leaves, again without the midribs. These leaves were also immediately placed in liquid nitrogen. All leaves were stored in a -80°C freezer upon return to lab.

After drying the frozen leaves in a lyophilizer for 48 hours, leaf samples were ground to a fine powder using a ball grinder. The Folin-Denis phenolic analysis method was used to determine total phenolic concentrations in the leaf samples by spectrophotometry. In order to perform this analysis, leaf material was extracted from 10 mg samples of dry leaf powder using a solution of 70:30 acetone:water. Once extracted, the leaf material was diluted 1:50 using deionized water and combined with the Folin-Denis reagent and 2N Na_2CO_3 and then absorbance was read at 700nm. Standards of tannic acid were made in concentrations of 2, 4, 8, 16, 32, and 64 ppm, and diluted and processed in the same way as the leaf extracts. To calculate phenolic content as percent dry leaf weight, the following formula was used:

[(PPM)(50)(0.0015)(100)]/sample weight in mg= % dry weight phenolics

where PPM=concentration based on standard curve
50=dilution factor
0.0015=total extraction volume
100=conversion to percent

Statistical analysis

Before means were compared, all data was tested for normality using descriptive statistics. In order to determine whether beech trees have constitutive levels of phenolics, we performed independent t-tests to compare mean constitutive levels of phenolics between the two habitats. In order to determine whether constitutive leaf carbon to nitrogen ratios affect constitutive levels of phenolics, a linear regression was performed comparing constitutive carbon:nitrogen to constitutive phenolics levels. In order to determine whether tree size affects constitutive levels of phenolics, a linear regression was performed comparing DBH to constitutive levels. In order to determine whether leaf age affects constitutive levels, a linear regression was performed comparing leaf surface area to constitutive levels. Leaf surface area was, for simplicity, calculated to be a rhombus ($1/2*d_1d_2=1/2*$length*width).

To determine whether induction of phenolics occurs in *F. grandifolia*, we performed paired t-tests to compare mean constitutive levels and mean post-damage levels for each of the two habitats.

To determine whether habitat affects inducibility of phenolics, we compared mean absolute change and mean percent change in phenolics using the Mann-Whitney U test because both variables were not normal.

Results

For trees in both habitats, the ratios of both kurtosis and skewness to their respective standard errors was less than two for constitutive levels and therefore their distributions were considered normal. Once normality was verified, independent t-tests were performed assuming unequal variances. The mean value (+/- standard error) for constitutive levels of phenolics within the outwash plain (8.4 +/- .44 % dry leaf weight) was significantly greater than the mean value for constitutive levels of phenolics within the moraine (5.2 +/- .29% dry leaf weight) ($t=-6.131$, $df=42.018$, $p<0.0005$) (Figure 1).

In the moraine, there was a positive correlation between the carbon to nitrogen ratios and the constitutive phenolic levels ($R^2=0.76$, $df=5$, $p=0.023$). However, in the outwash, C: N did not have an effect on constitutive phenolic levels, according to our data ($R^2=.40$, $df=5$, $p=.179$).

Tree size, measured as DBH, did not affect constitutive levels in either the outwash plain or the moraine ($R^2=0.048$, $df=49$, $p=0.128$).

Similarly, leaf surface area did not affect constitutive levels in either the outwash plain or the moraine ($R^2=0.024$, $df=49$, $p=0.279$).

In both habitats, the mean post-damage levels were greater than mean constitutive levels of phenolics, indicating that induction occurs (outwash plain: $t=-4.706$, $df=24$, $p<0.0005$; moraine: $t=-10.788$, $df=24$, $p<0.0005$). In the outwash plain, the mean difference between post-damage and constitutive levels was 4.3 +/- .91% dry leaf weight. In the moraine, the mean difference

between post-damage and constitutive levels was 3.7 +/- .35% dry leaf weight (Figures 2,3 and 4).

The effect of habitat on absolute change in phenolics (inducibility) is nearly statistically significant ($p=0.079$, $Z=-1.756$). The difference in mean absolute change in phenolics between habitats is 0.5% dry leaf weight. The mean percent change in phenolics does not indicate that habitat has an effect on inducibility ($p=0.265$, $Z=-1.116$) (Figure 5).

Discussion

After our data analysis, we are confident in saying that habitat affects the constitutive levels of phenolics in *F. grandifolia*. The constitutive levels of phenolics were significantly higher in samples from the outwash plain. This may be attributed to the higher levels of sunlight to which the sample trees are exposed. Sunlight drives photosynthesis, which produces the carbon for phenolic production.

Nitrogen is essential for nucleic acid synthesis, and thus essential to the production of enzymes to stimulate phenolic assembly. Because the mesic soil of the moraine has higher nitrogen levels than the xeric soil of the outwash, our analysis suggests that nitrogen availability is not a direct limiting factor in phenolic production. This may be because high nitrogen availability will be allocated to growth instead of defense, as the plant will use the available carbon to growth and direct the remaining nitrogen to phenolic production. In the outwash plain, nitrogen is limited, causing only a limited amount of corresponding carbon to be used for growth. This leads to the tree dedicating more of its resources to defense. The excess carbon will be used for phenolic compound synthesis, which requires less nitrogen.

F. grandifolia is able to induce phenolics following damage in both habitats; however, neither habitat has significantly higher inducibility than the other. Because the results were nearly significant, it may be necessary to test this further to make definitive conclusions on magnitude of induction between the two habitats. Further experimentation may show that inducibility is higher in the outwash plain, again because of the higher carbon availability, which is used for greater phenolic production.

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Appendix: Figures

Figure 1

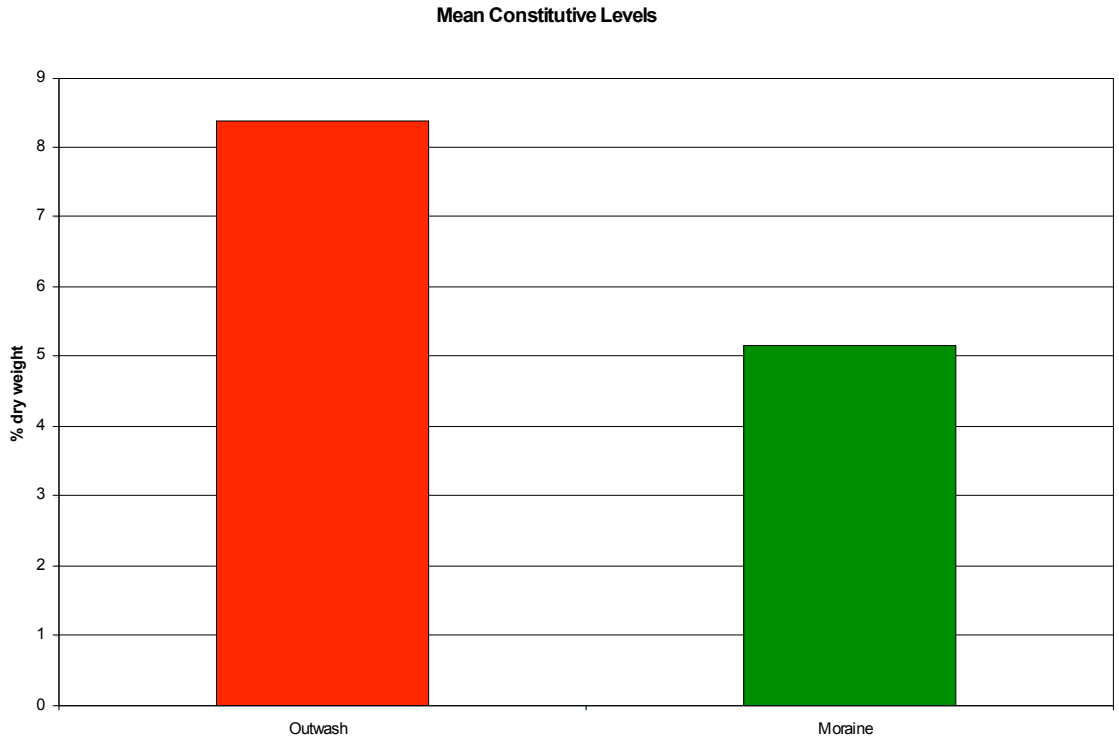


Figure 2

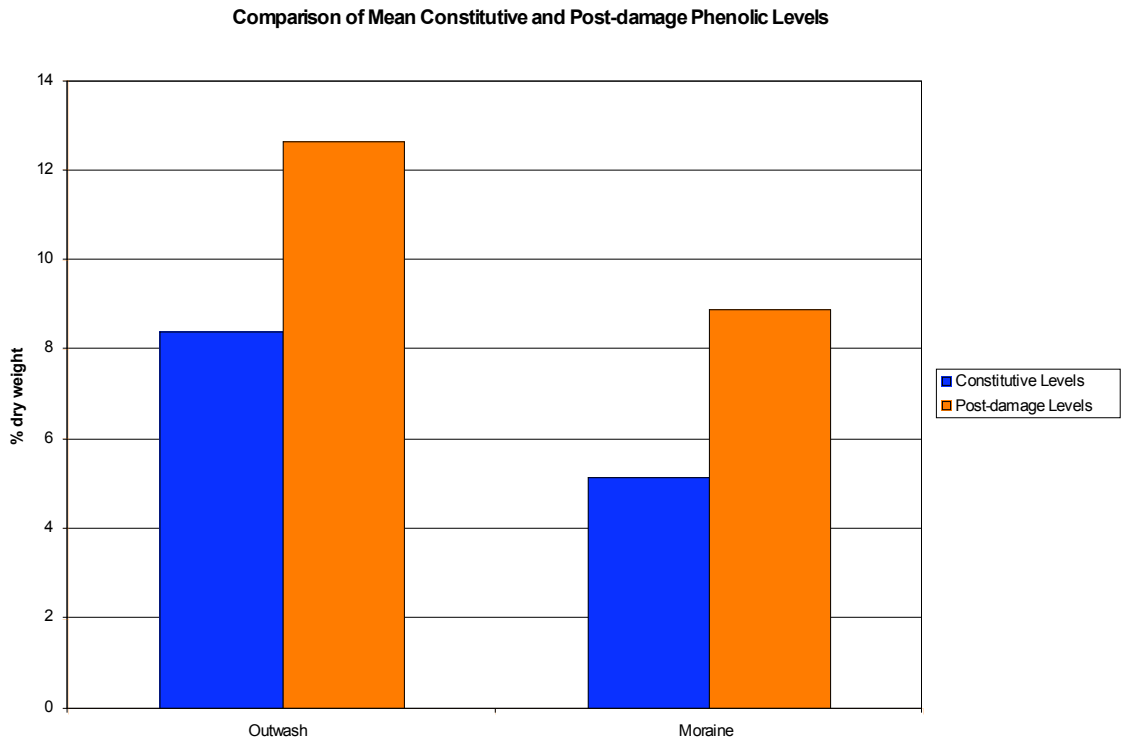


Figure 3

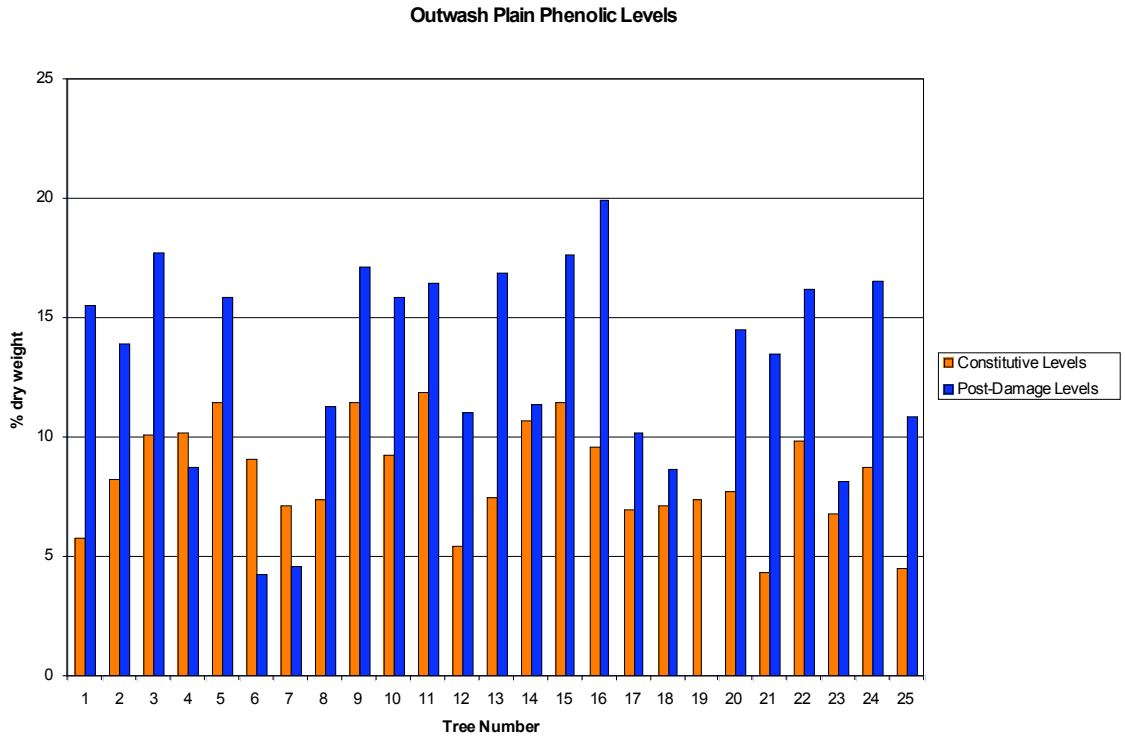


Figure 4

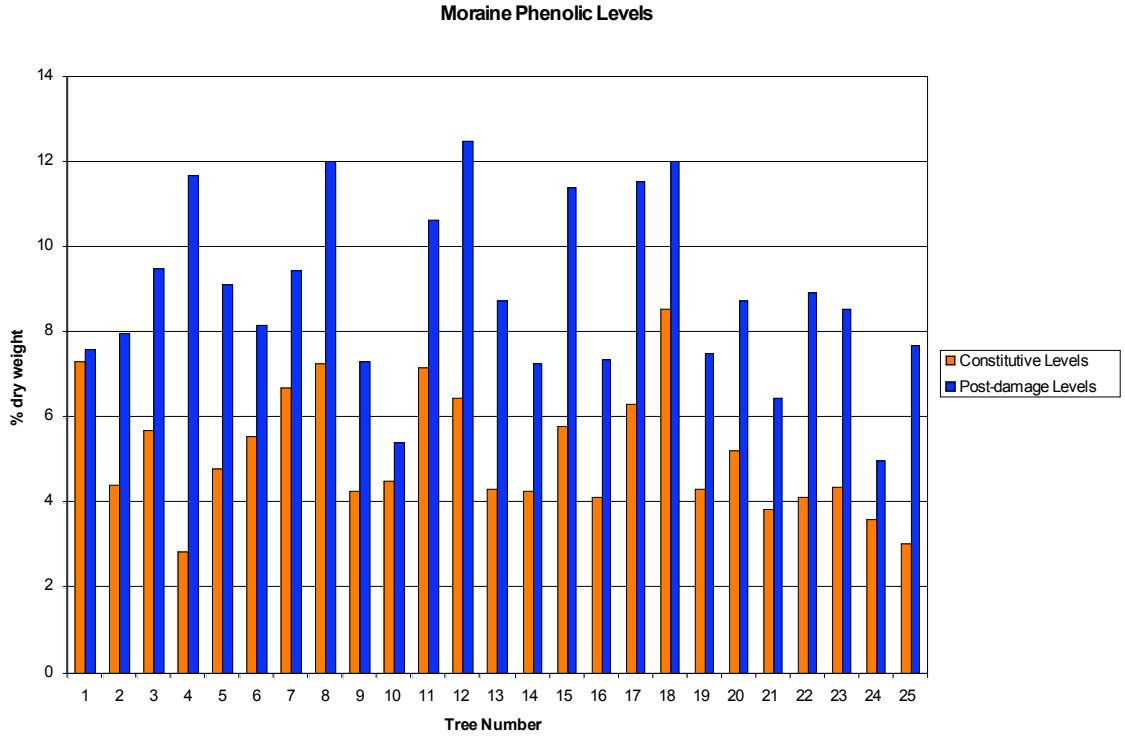
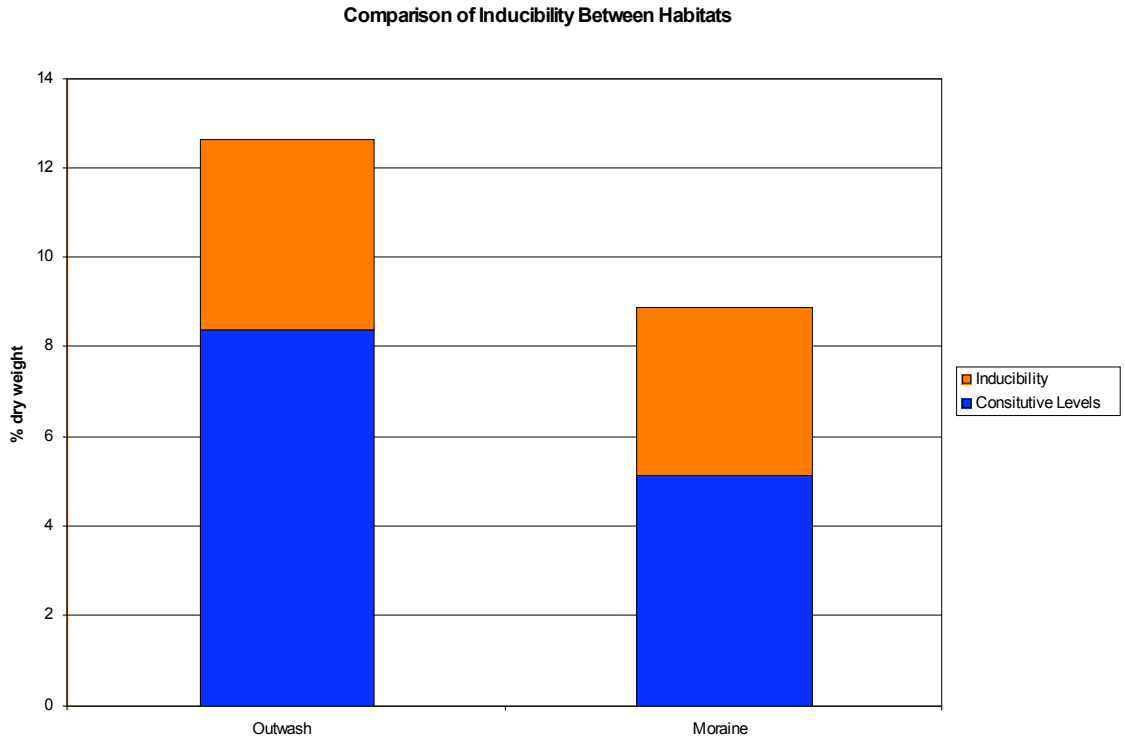


Figure 5


Comparison of Inducibility Between Habitats
% dry weight
14
12
10
8
6
4
2
0
Outwash
Moraine
Inducibility
Consitutive Levels

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