The Effects of Beech Bark Disease on Photosynthetic Rates of American Beech Trees in Northern Michigan

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Abstract

The health of American Beech trees (*Fagus grandifolia*) is declining due to Beech Bark Disease (BBD). BBD is an infection of an insect and fungus that girdles beech trees, leading to their death. The goal of this study is to examine whether or not the infection and girdling caused by BBD negatively affect the photosynthetic ability of the leaves of more diseased beech trees. We took leaves from beech trees in Colonial Point that were healthy or only slightly affected by BBD and from severely infected trees to compare their photosynthesis performance. Using a PhotosynQ MultispeQ, we took samples of relative chlorophyll, Phi2, or the ratio of incoming light that the leaves use for photosynthesis, non-photochemical quenching (PhiNPQ), and light that was lost to non-regulated processes (PhiNO). One-sided, two-sample t-tests revealed that healthier trees had more relative chlorophyll and higher Phi2 and PhiNO ratios than more infected trees, but had a lower PhiNPQ ratio. The relative chlorophyll and Phi2 supported our hypothesis, but PhiNPQ and PhiNO did not. These results have implications for the way BBD puts stress on the plants, and reveals a knowledge gap about how the trees respond to the disease.

Introduction

The health of American Beech trees (*Fagus grandifolia*) is declining due to Beech Bark Disease (BBD). Earliest records of BBD suggest that it originated around 1890 in Eastern Canada and may have spread into Michigan as early as 1985 (Hewitt, Deichsel). Since its arrival in Michigan, BBD has killed about 7% of beech trees in the state (State of Michigan, n.d.).

BBD is caused by a sap-feeding scale insect (*Cryptococcus fagisuga*) and a phytopathogenic fungus of the *Neonectria* species (Deichsel, 2009). Infection begins when scale insects feed on the bark, creating small fissures that make room for the fungus to invade the tree (Koch, 2010). The fungus kills bark tissue and begins to girdle the tree, usually leading to death (Koch, 2010). Removal of bark from controlled girdling can decrease the leaf water potential of trees and cause unusual nutrient accumulation in the leaves, depending on the tree species and severity of girdling (Sellin, et al., 2013). We also know that trees can behave differently in response to pathogenic infections (Ennos, 2015). These responses can affect productivity and rates of photosynthesis (Lovett et al., 2006). We want to investigate if pathogenic stress from BBD can affect photosynthetic performance in beech leaves by measuring various aspects of photosynthesis.

Photosynthesis begins when pigments such as chlorophyll capture photons from sunlight and pigment molecules are excited to higher energy states, becoming exitrons (Singhal et al., 2013). An exitron can either continue through photosynthesis, or be emitted as heat or fluorescence. (Singhal et al., 2013). Leaves have different amounts of chlorophyll, which affects how many photons they can absorb, so relative chlorophyll content is a measure of differing photosynthetic ability (Li, et al., 2018).

When the exitron goes to photosynthesis, a chlorophyll–protein complex called photosystem II (PSII) uses its energy to transfer electrons from water molecules to electron carriers (Johnson, 2016). The percentage of incoming light that goes into PSII is the quantum yield of PSII (Phi2) and is a measure of its operating efficiency (Körner, et al., 2015). The electron transfer reactions of PSII create a proton gradient that ultimately powers the production of ATP for the plant (Johnson, 2016).

Plants can be damaged when photosynthesis is saturated and there is still incoming light energy, so they have evolved an alternative pathway for excitons called non-photochemical quenching (NPQ) to avoid harmful radiation (Muller, 2001). NPQ is an energy consuming process in which plants dissipate light energy into heat rather than use it for photosynthesis (Ruban, 2016). The ratio of incoming light that goes towards NPQ is called PhiNPQ (Kuhlgert, et al., 2016). Some light energy does not go into photosynthesis nor NPQ, and is lost to non-regulated processes, which is measured in a ratio called PhiNO (Kuhlgert, et al., 2016).

We want to investigate if beech photosynthetic ability is affected by the severity of BBD. We expect more severely diseased trees to have lower ability to do photosynthesis than the healthier beech trees in the same forest.

Methods

We collected leaves from beech trees (*Fagus grandifolia*) with BBD at Cheboiganing Neyaashi, or Colonial Point, an area of protected forest at the University of Michigan Biological Station (45.4761224°N, -84.6786522°W) where the BBD infestation is particularly prevalent.

We created a rating system for BBD severity adapted from Hancock et al. and Seeburger, and catalogued trees by this system (Table 1) (2008 and 2018). While most severe signs of BBD are present after the beech tree's death, we only looked at living trees so we could measure photosynthesis. We took samples from healthy specimens (level 1) and severely infected but living trees (level 3). We measured the height of each tree using a clinometer, and diameter at breast height (DBH).

We cut one branch from each sampled tree at a height of approximately 4.6 m. We placed the branch in water immediately after pruning, and used a PhotosynQ MultispeQ to measure relative chlorophyll, light to photosystem II output (Phi2), non-photochemical quenching (PhiNPQ), and light lost to non-regulated processes (PhiNO) for each leaf. We held each leaf parallel to the ground while measuring it and measured ten leaves from each tree.

Using R-studio, we calculated the mean of each MultispeQ variable, grouping them by disease severity. We performed a two sample, one-sided t-test to assess differences in mean for each variable. We graphed regressions for all variables to tree height and DBH.

Results

Using a one-sided two sample t-test, we found a statistically significant difference between the leaves of level 1 and level 3 trees for photosystem II output, relative chlorophyll, non-photochemical quenching, and non-regulated processes. Mean output of photosystem II ($p = 3.4 \times 10^{-4}$), relative chlorophyll content ($p = 2.3 \times 10^{-3}$) and PhiNPQ ($p = 3.61 \times 10^{-6}$) were greater in level 1 trees (Figure 1, 2, and 3). Level 3 leaves had a lower mean for PhiNO ($p = 3.7 \times 10^{-4}$) (Figure 4) .

We also calculated the mean DBH and tree height for each of the severity levels and found a statistically significant difference for each using a one-sided two sample t-test. Trees of level 3 had greater DBH ($p=2.2 \times 10^{-6}$) and height ($p = 1.2 \times 10^{-10}$) (Figure 5 and 6). To assess whether these differences affected the results of our four variables, we ran a correlation of DBH and tree height versus each variable. The maximum correlation between DBH and tree height versus any of these variables was 22% (Figure 7).

Discussion

Output from MultispeQ

Our data somewhat supports our hypothesis that photosynthetic ability is lower in more diseased trees. We expected level 1 beech trees to have higher Phi2 levels, as Phi2 is essentially a measure of photosynthesis efficiency. We found evidence of this, meaning that level 1 beech trees had better ability to perform photosynthesis. This also means that healthier trees directed a larger percentage of incoming light into the photosystem II pathway. We also expected relative chlorophyll to be higher in healthier trees, since studies have found that stressed plants have lower chlorophyll levels (Andrade et al. 2015). Our data supports this, as relative chlorophyll is higher in level 1 trees, indicating that healthier trees have more chlorophyll to absorb more photons to power photosynthesis.

We expected more diseased trees to have lower PhiNPQ, as they would have less energy to devote towards actively resisting light damage. We found the opposite—level 3 trees had a higher mean for PhiNPQ. This did not support our hypothesis, but still might represent evidence of BBD negatively affecting tree health, as putting energy towards resisting light damage could be an ecological tradeoff of the tree trying to resist infection. We cannot make a definitive conclusion without further investigation. We also expected the percentage of light going to non-regulated processes (PhiNO) to be higher in damaged plants, since stressed plants can be more at risk for photo-oxidative damage (Urban, et al., 2017). We found that PhiNO was lower for level 3 trees than level 1. A possible explanation for this could be that the BBD is causing harm to the trees but is not stressing them to the point of losing significant control of regulated processes. BBD is girdling the trees, but perhaps enough nutrients are still getting up to the leaves to support major use of regulated processes. Again, we cannot conclude the cause for this result without further investigation into how BBD stresses the leaves of beech trees.

Confounding Variables

Older beech trees tend to have more severe BBD infections than younger trees, a pattern that is consistent with our data (Stephanson et al., 2017). On average, our level 3 trees were taller and had larger DBH than level 1 trees. We compared DBH and tree height to each MultispeQ variable and found no significant correlation. Therefore, the differences found in photosynthetic performance between level 1 and level 3 trees is attributable to BBD infection rather than DBH or height.

We were not able to control for the number of trees growing around our samples. Tree branches in denser areas may have received less light or had to compete with more trees for nutrients. We also did not control for the concentration of diseased versus healthy trees that surrounded the ones we took samples from, which could have affected the way our trees responded to BBD infection.

Limitations

There are also factors that could have affected the amount of light reaching the leaves. We were limited in selecting branches to those we could reach, so the orientation of the branches on the tree and the cardinal direction they faced varied.

Ecological Implications

Understanding how BBD affects the health of beech forests is worthwhile because beech trees play a key role in forest ecosystems in Northern Michigan (Lovett et al., 2006). Beech leaves take longer to decompose than those of other species. (Lovett et al., 2006). This impacts the litter quality of the forest floor and nutrient cycling, changing the composition of tree species in beech-dominated forests. (Lovett et al., 2006). BBD tends to affect mature beech trees more than younger trees, clearing older tree canopies and allowing competitive species to dominate. (Lovett et al., 2006). Chipmunks, deer, bears, foxes, and many more animals feed on beech nuts as an important component in their diet (Kirchner, 2006). Since beech trees play an integral role in forest ecosystems, widespread infection such as BBD threatens not only the survival of the trees themselves but many beech-dependant or coexisting organisms where they are found. If we understand how BBD affects beech trees, we can improve efforts to stop or slow its spread.

Future studies

While we found that photosynthesis efficiency is less in trees more heavily infected with BBD, exactly why this is happening is still unknown. BBD kills beech trees through girdling, but future studies could examine whether it is girdling specifically that reduces photosynthesis efficiency of leaves, or if it is the presence of an infection at all. To further investigate the unexpected results for PhiNO and PhiNPQ, we would recommend doing a study on other similar tree infections to see if these trends are common for infection stress in other trees, or if they are specific to BBD. An example of a similar pathogenic tree disease is the cytospora canker found in spruce trees, which is also caused by a fungus and results in girdling (Worrall et al., 2010).

Maps and Figures

Severity Level	**Description**
1	Healthy tree. Scale insect completely absent or hardly apparent on tree bark.
2	Mild BBD infection. Scale insect uniformly distributed across bark.
3	Moderate to severe BBD infection. Scale insect uniformly distributed across or covering bark. Heavy vertical cracking apparent on bark.

Table 1: Classification of BBD severity adapted from Hancock et al. and Seeburger (2008 and 2018).

Variable	**Level 1 Trees**	**Level 3 Trees**	**P-value**
Phi2	0.49	0.45	3.40×10^{-4}
PhiNPQ	0.27	0.34	3.61×10^{-6}
PhiNO	0.24	0.22	3.70×10^{-4}
Relative Chlorophyll	23.59	22.10	2.30×10^{-3}

Table 2: A table representing the mean value of each of the variables grouped by severity level. P-value was obtained with a two-sample, one-sided t-test for significant difference.

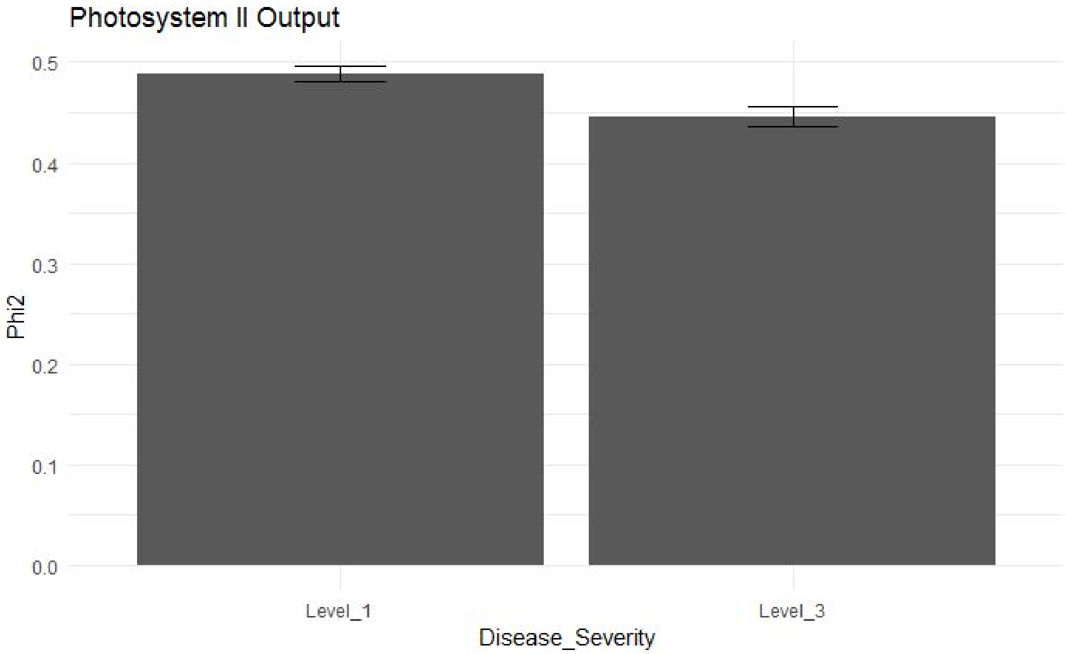

Figure 1: Mean Phi2 (percent of electrons that go into photosystem II) as a function of disease severity ($p < 3.4 \times 10^{-4}$).

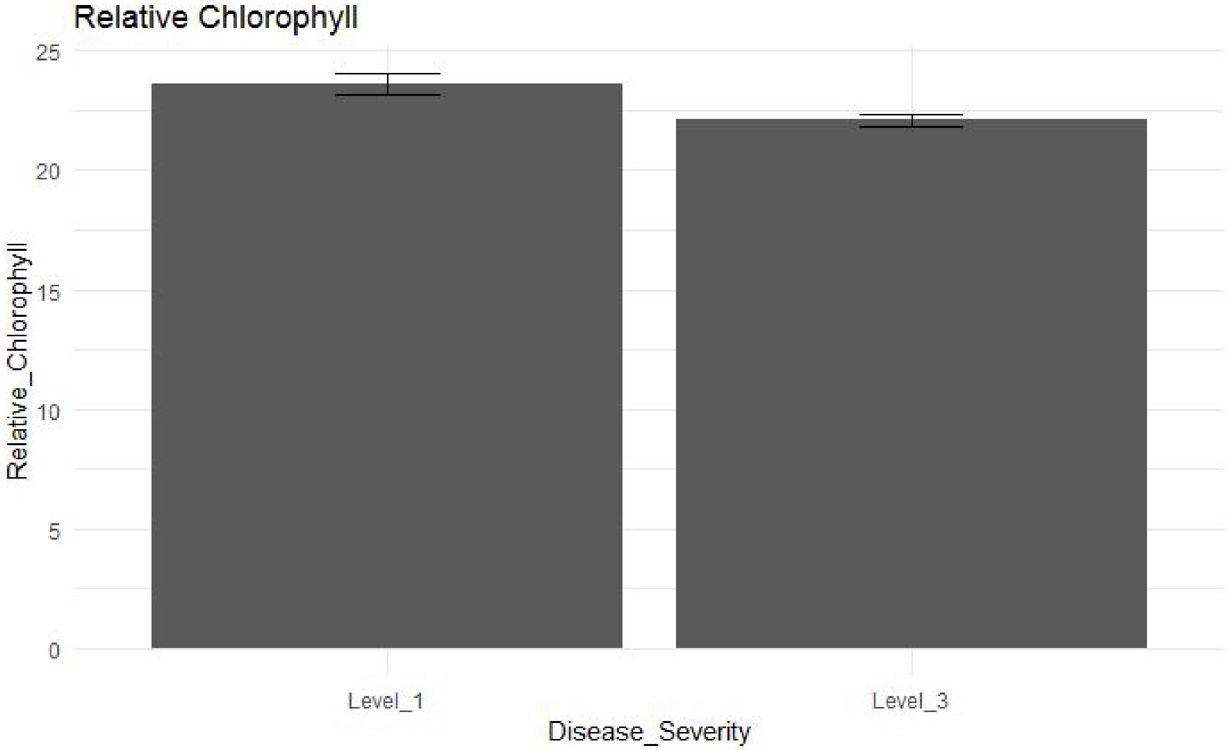

Figure 2: Mean relative chlorophyll content measured in Special Products Analysis Division (SPAD) units as a function of disease severity ($p < 2.3 \times 10^{-3}$).

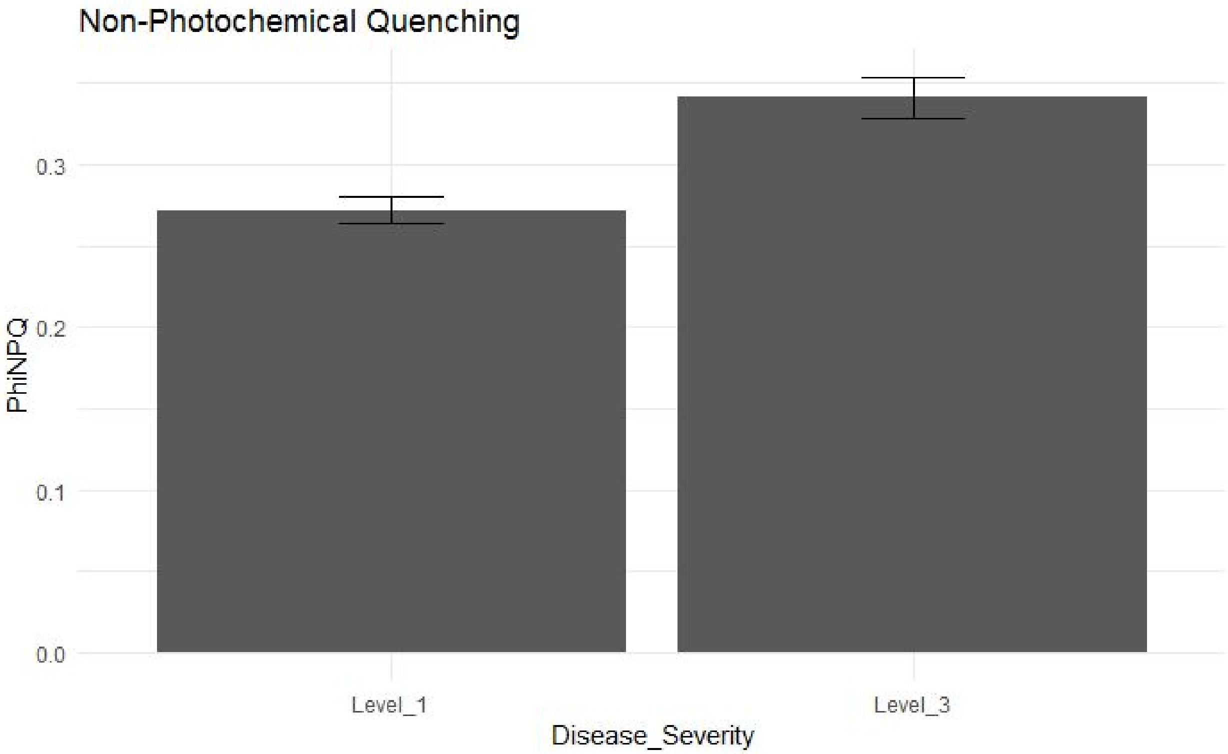

Figure 3: Mean measurement of Non-photochemical Quenching (PhiNPQ) as a function of disease severity ($p < 3.61 \times 10^{-6}$).

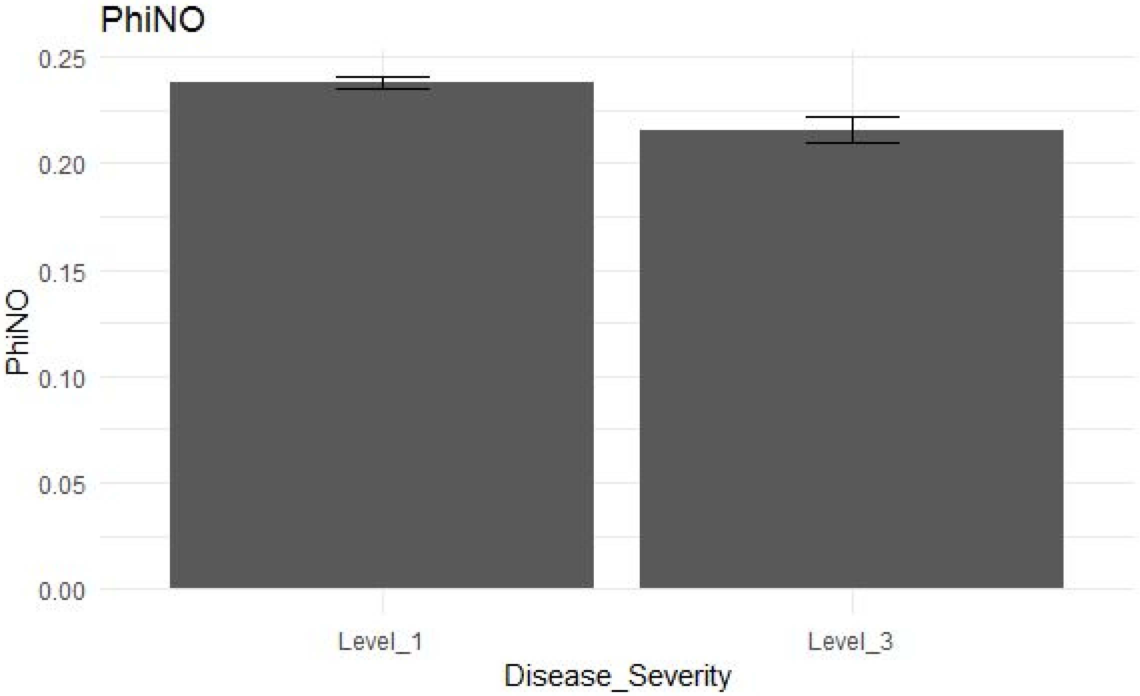

Figure 4: Mean measurement of non-regulated processes (PhiNO) as a function of disease severity ($p < 3.7 \times 10^{-4}$).

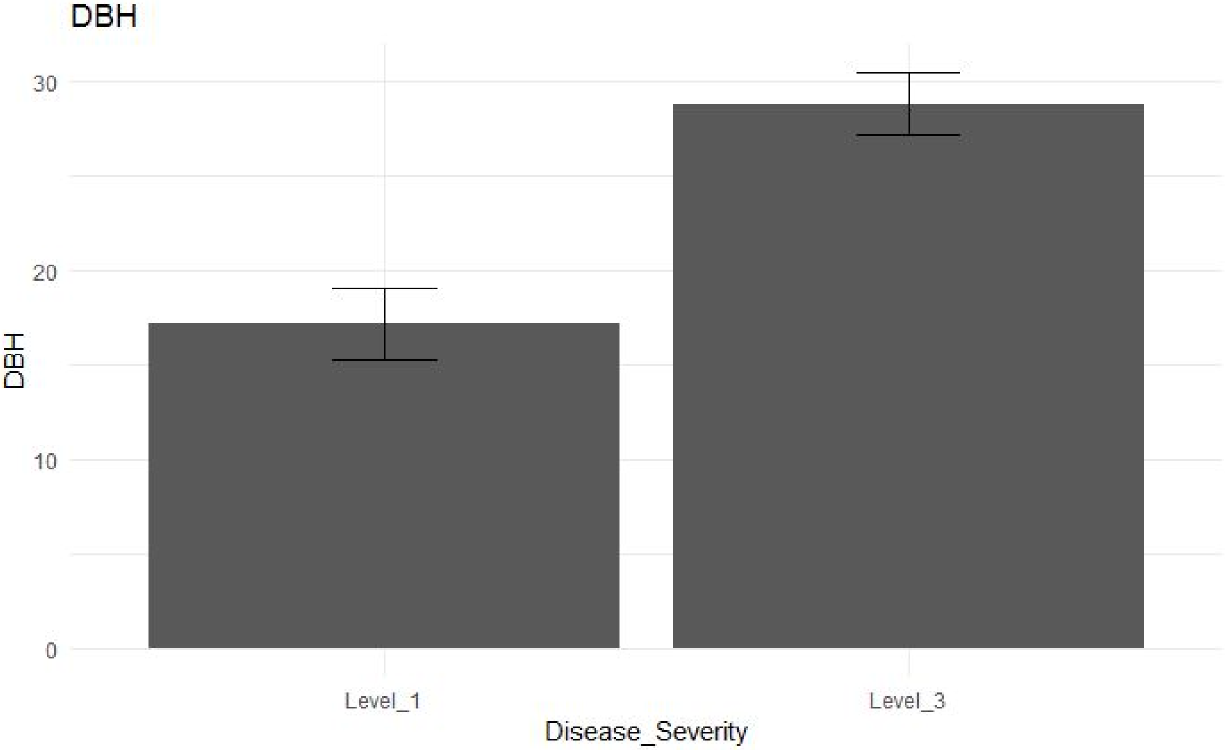

Figure 5: Mean DBH of in centimeters as a function of disease severity ($p < 2.2 \times 10^{-6}$).

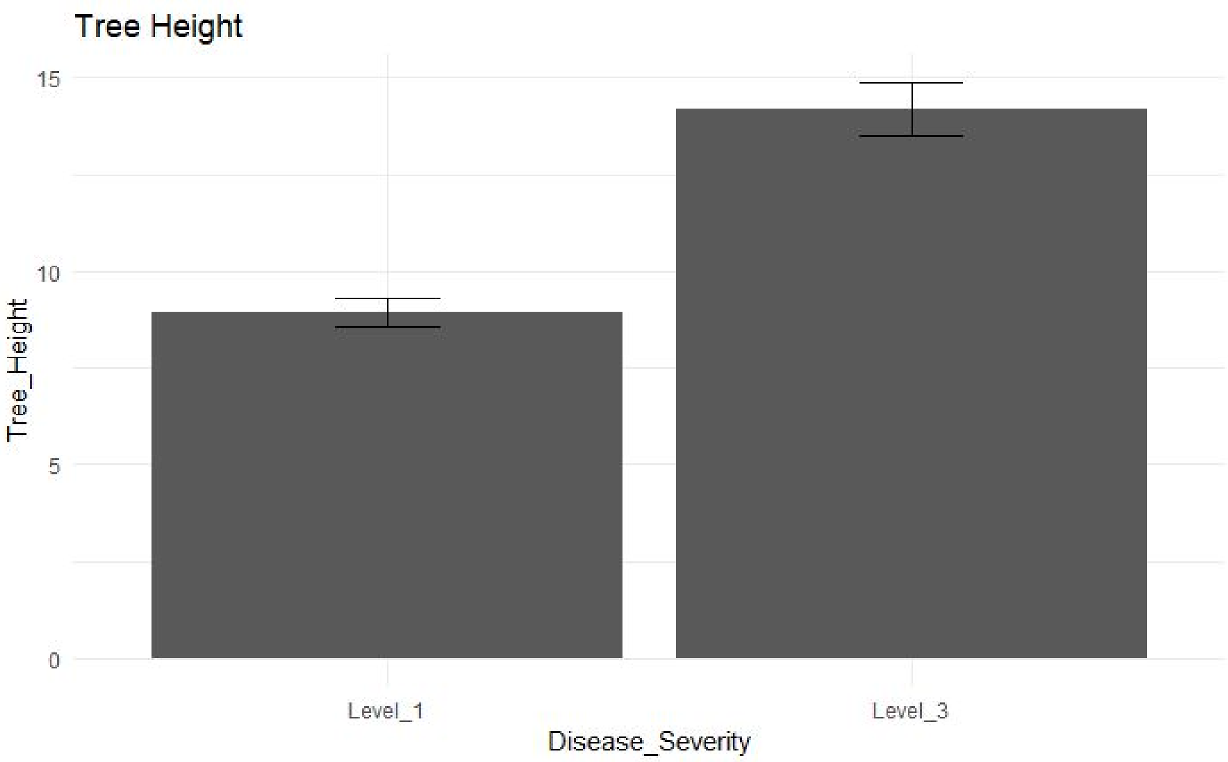

Figure 6: Mean DBH of in centimeters as a function of disease severity ($p < 1.2 \times 10^{-10}$).

MultispeQ Variables vs. DBH and Tree Height

DBH

Tree Height

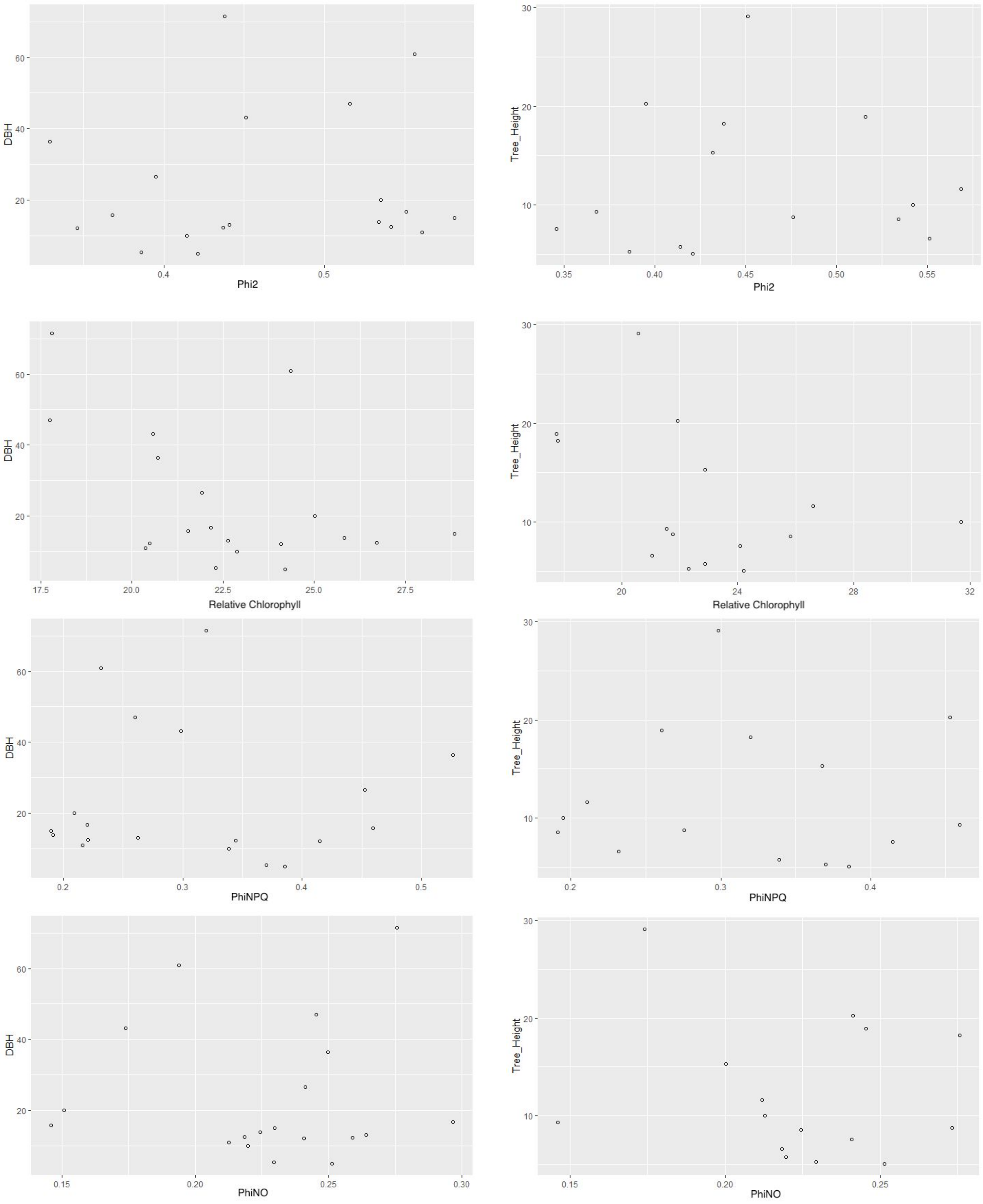

Figure 7: Shows scatter plots of correlations of DBH and Tree Height. No significant correlations (highest r^2 0.22).

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