DEPENDENCE OF FUNGAL PLANT PATHOGEN HOST BREADTH ON PLANT CHARACTERISTICS

Nguyen Huynh An Markus Le

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ABSTRACT

Over time, travel between continents has become easier, allowing many diseases and pests to leave their native habitats, sometimes with disastrous results. Famous examples include the chestnut blight and woolly adelgids, which have greatly harmed ecosystems in the United States. I investigated the factors that allow these invasive species to flourish in non-native environments by examining how a plant’s susceptibility to pathogens depends on its phylogeny, traits, and native geographic range. To do so, I used grasses that varied widely across these three factors in an observational field study and in vitro inoculation experiment. Collected susceptibility data was compared to traits like leaf mass per area (LMA), growth rate, and photosynthetic rate. Initial results indicate significant susceptibility differences among different grass species. The results will help illuminate what allows pathogens to infect new hosts, allowing planning for the prevention and mitigation of species invasion.

INTRODUCTION

As time goes on, the barriers separating continents have become less and less significant. Journeys that used to take weeks to complete by ship can now be completed in mere hours on an airplane, at relatively low cost. However, people are sometimes not the only passengers that cross continents. Whether intentionally or by accident, many species have been allowed to leave their native habitat, sometimes with disastrous results. This process is known as species invasion, which occurs when species from a foreign environment establish a foothold in a new geographic area where they were not originally present. While sometimes species invasions can be beneficial, like in the case of the European honey bee, they are more often harmful to the local ecology and human population. Famous examples include Kudzu and woolly adelgids, which are
causing great harm to ecosystems in the United States, as well as bay barnacles which cause great economic costs due to their effect on man-made structures along the water.

Not every species that moves to a foreign geographic location successfully takes hold there, however. Most species fail to thrive in a foreign environment and die out. For those that do successfully survive, there are five hypotheses on why they do so. The first is the Innate Biology Hypothesis, which describes the traits inherent to the organism as the crucial determining factor. For example, an invader may be very “weedy” and generalist, allowing it to thrive in a wide variety of environments, or it may be pre-adapted to the environment to which it is being introduced (Kolar and Lodge 2001). The second hypothesis is called the Enemy Release and Biotic Resistance Hypothesis, which attributes the success of a species to take hold to the lack of predators and competition, and the resistance of invading species to diseases already in the community, or lack thereof (Montserrat, Maron, and Marco 2005). The Community Invasibility hypothesis relates empty or underutilized niches in a community, or other traits that make a community prone to invasion to the success of a species invasion (Davis, Grime, and Thompson 2000). The Propagule Pressure hypothesis correlates increased exposure frequency and intensity to an environment to how likely it will be to establish itself in the community (Verling et al. 2005). Finally, the Rapid Evolution hypothesis states that often species that successfully invade an environment can and do evolve rapidly to adapt to the local environment, allowing them to survive over generations (Davidson, Jennions, and Nicotra 2011).

My research project aims to examine the factors that allow these invasive species to flourish in non-native environments and conditions, more specifically the Community Invasibility hypothesis. I will be examining this concept by researching what allows pathogens to infect plants. More specifically, I will be looking at what determines the host breadth of fungi
infecting plants by attempting to answer the question “What aspects of a plant will allow it to be infected by any given pathogen?” Several hypotheses have been posed to answer this question. For example, the phylogenetic relationship between different tropical plant species was a strong determinant of infection rates by specific pathogens in one study, meaning that closely related plants experienced similar infection rates (Gilbert and Webb 2007). Similarly, Kumar et al (2011) hypothesized that individual plant traits could be correlated to the susceptibility of a plant to pathogens, however they found no significant relationship, since it is possible that susceptibility is determined by a combination of factors rather than individual traits. My aim is to incorporate and expand on these studies by testing my hypothesis that pathogen susceptibility depends on not just phylogeny or plant traits, but rather both simultaneously, as well as a third component, the native geographic range of the plants, which could point towards the importance of coevolution between plants and pathogens. The plant pathogen that I have chosen for the project is *Fusarium graminearum*. *Fusarium graminearum* is a generalist pathogen that infects mostly wheat, oat, and barley in cooler regions of the world and is responsible for the destructive plant disease called Fusarium head blight (Cuomo et al. 2007).

The results of this research will shed more light on what allows pathogens to spread to new hosts in which they were not originally found. The most direct importance of this would be to allow for planning the prevention of species invasion, as well as the development of guidelines and protocols that would help limit the spread of pathogens, once introduced.
MATERIALS AND METHODS

Plants and Plant Traits

The plants were chosen to allow for comparison across the three variables: relatedness, plant traits and native geographic range. For example, I would compare closely related species with similar traits, but different native geographic ranges to observe the effect of the plants’ geographic ranges. Plants with similar traits and native geographic ranges, but that are not closely related could be chosen to observe the effect of phylogeny. To observe the effect of the plants’ traits, I would compare plants that are closely related and have similar native geographic ranges, but differ in traits. As I am interested in the effects of the traits themselves on susceptibility rather than differences between species, the plants used were not genetically identical, to allow for trait variation and a more continuous trait distribution among plants.

Native geographic range estimates, the tribe to which each grass species belonged, as well as the photosynthetic pathway (C3 or C4) for each grass host species were obtained from floristic guides and other literature. For the field plant traits, growth rate, leaf mass per area and photosynthetic rate were measured, while pre-existing data were obtained from a fellow lab member, Miranda Welsh. Leaf mass per area was obtained by scanning leaf segments of each plant into a computer, calculating the area of the leaf segments with computer software and using a balance to obtain the mass of that same leaf segment. Photosynthetic rate was measured using an infrared gas analyzer (IRGA), which measures the change in gas composition in a closed chamber containing a leaf being exposed to light.

Detached Leaf Assay

Sections of plant leaves were inoculated with the fungi in vitro. This allowed for quick visual assays that showed whether the fungus is capable of infecting the chosen plants. Petri
Five dishes were prepared with water agar that contained kinetin as a senescence retarder for the leaf segments, and the fungal spores were diluted in water, since the fungi spread their spores through water in nature. The spores were diluted to a specific concentration (50000 spores per mL), with Tween 20 as an emulsifier to prevent the spore suspension from rolling off the leaves in the petri dish. The fungal spores came from separate isolates that were grown separately in petri dishes, but the spores were mixed together in the spore suspension to allow the fungus to be as generalist as possible. The fungus was a novel pathogen to all of the plants, which models how a foreign pathogen is affected by plant traits in general.

Each petri dish contained two leaf segments, each of which was inoculated using one drop of the fungal spore suspension. The petri dishes incubated for about two weeks to allow for the development of symptoms. These procedures were modeled after those used by Kumar et al (2011). Every day during the incubation period, the presence of fungal lesions was recorded, along with the approximate length and width of the lesions. For the data analysis, a day was chosen during the two week period on which all leaves that were going to become infected had already become infected, but the size of the lesions that had developed had not yet become limited by the size of the leaf segment. This allowed the length and width measurement to remain a measure of susceptibility rather than the size of the leaf itself.

Field Experiment

The plants chosen for the field experiment differed from the plant species used in the in vitro experiment, to prevent any new grasses from invading the surrounding environment. Among the grass planted in the field there were both native species, and exotic species, but only exotic species that had already been introduced to the environment. Five of each species were planted in pots in the greenhouse and kept healthy. After becoming mature enough, these pots
were transferred into the field. The area in the field chosen for the experiment had uniform vegetation, which was kept as intact as possible while putting the pots into the ground, to simulate a non-disturbed environment as best as possible. This allowed wild diseases from the environment to infect the experimental plants. Since plants in the field were both native and exotic, and the diseases in the field are mostly native, this would show the difference between infection by native and novel pathogens.

The pots were left in the field for six weeks, and every two weeks the plants were visually assessed for fungal leaf damage. Damage was recorded for every individual leaf on each plant as a percentage of the leaf area damaged by fungi. The field plants were also used to measure growth rate. A single leaf on each plant was tracked throughout the six weeks, and while taking damage data, the length and width of the leaves was measured.

*Measuring susceptibility*

The aim of both the detached leaf assay and the field experiment was to examine and compare plant susceptibility to fungal pathogens, which is approximated in the project by measuring damage caused by the pathogens. While this does not directly measure susceptibility, it can serve as an indicator. However, for damage to be an effective measure of susceptibility, several conditions have to be met. Especially in the field, the movement of pathogens can vary over time; however, in order to relate damage to susceptibility and to compare damage values between different plants, the environment for all of the plants needs to be the same, including the soil, water availability, and surrounding vegetation. This allows for pathogen prevalence and transmission to be constant among all the plants, allowing damage to be the only affected factor by the plants susceptibility to the pathogen. This is easy to achieve in the lab, since everything was controlled, including the concentration of the disease and the sterility of the environment. In
the field experiment, this was achieved by choosing a spot of uniform vegetation in the field, and by leaving vegetation around the pots intact, which helped standardize the types and amounts of pathogens, as well as transmission rates between plants. The plants were also randomized in the field plots, to further prevent location from skewing data for any one species.

Unless otherwise stated, I performed all work described in this thesis.

RESULTS

Detached Leaf Assay

Before looking at the interactions between origin, phylogenetics, and plant traits, I looked at the effect of each of these individually. There is high variation in damage between individual species; however, the differences are not statistically significant (Fig. 1). However, the same is not true when looking at the grass tribes. When the individual species are grouped together into their appropriate tribes, there is a statistically significant difference (p<0.05) in damaged leaf area between the tribes (Fig. 2). Looking at the comparisons between the data sorted by categories, origin and photosynthetic pathway, showed that there was no meaningful difference between the categories, as with the comparison between species (Fig. 3, Fig. 4). Figures 5 through 7 show scatter plots comparing the damage data to the numerical trait data, photosynthesis rate, LMA, and leaf growth rate. Of these three, only the relationship between leaf growth rate and damage was significant ($r^2=0.1776$, p<0.05). Generally, as leaf growth rate increased, the amount of damage on the leaves increased.

The remainder of the figures for the detached leaf assay (Figs. 8-16) show the same data as Figures 5 through 7, but with the data divided by the categorical plant traits (tribe, origin, and photosynthetic pathway) in order to examine the interactions between the traits that might lead to
differences in susceptibility through multiple linear models, which are statistical tests that allow for the comparison of the effects of several independent variables on the dependent variable, or in this case, damage of plant leaves caused by fungi. Of the nine models, only the models for leaf growth rate by origin, and LMA, photosynthesis rate, and leaf growth rate by photosynthetic pathway (Figs. 13, 14, 15, and 16 respectively) were statistically significant (p<0.05), showing interactions between leaf growth rate and origin, \( r^2 = 0.2893 \), LMA and photosynthetic pathway \( r^2 = 0.1627 \), photosynthesis rate and photosynthetic pathway \( r^2 = 0.1924 \), and leaf growth rate and photosynthetic pathway \( r^2 = 0.2936 \) in determining the amount of damage. However, due to the low number of samples available for C4 plants (only three of the species used were C4), the significance of the interactions and the models involving photosynthetic pathway may be exaggerated.

Also noteworthy is the model for LMA by continent of origin (Fig. 12). The model is not statistically significant, but it is marginal \( r^2 = 0.1537, p = 0.06972 \), and the interaction term between LMA and origin is significant \( p<0.05 \) which may potentially point to interaction between the two traits influencing damage. For plants from the Americas, damage decreases as LMA increases, while for plants from Europe and Asia, damage increases as LMA increases.

Field Experiment

The comparisons made for the field experiment were the same as those made for the detached leaf assay. Figures 17-23 show the damage data compared against each individual plant trait, and the remainder of the plots (Figs. 24 - 32) show scatter plots of the damage against the numerical plant traits, divided up by category. None of the simple univariate models (Figs. 17-23) were statistically significant. Of the interaction models, only the model for LMA by origin model (Fig. 28) was statistically significant \( r^2 = 0.2448, p<0.05 \), and the interaction term for the
two variables was also statistically significant (p<0.05). The model follows the same pattern as it did for the detached leaf assay: For American plants, damage decreases as LMA increases, damage increases as LMA increases for Eurasian plants.

**DISCUSSION**

In agreement with the experiment conducted Gilbert and Webb (2007), relatedness between species can help predict how susceptible they are to disease, as can be seen from the damage comparison between tribes in the detached leaf assay. The results also agree with those found by and Kumar et al (2011) that single plant traits are generally unable to predict susceptibility to disease, especially in the field. Integrating the results from these experiments in more complex models yields some interesting results.

Between the detached leaf assay conducted in the laboratory and the field experiment, the most comparable models between the two experiments are the LMA by origin models, as they are the only models the two experiments have in common that are relatively statistically significant. Additionally, the fact that both display the same trends indicates that the results obtained from the lab experiment may be able to predict susceptibility for plants in the field, since the results from both experiments suggest that the LMA and the origin of the plant interact to influence how much damage the plant accumulates. This may have several explanations. For example, LMA may be indicative of how much the plant has invested in physical defenses on its leaves. It may have a thicker outer layer, which would increase its LMA, and potentially make it more difficult for fungal pathogens to infect the leaf. However, having thicker leaves may also mean that the fungus has more nourishment to consume once it does manage to infect the plant, which could lead to an increase in infection rates as LMA increases. It would make sense for
there to be a negative correlation plants for American plants, and a positive correlation for European plants, because American plants have been able to adapt and develop resistance to local pathogens, which would make a higher LMA an asset, while for Eurasian plants which are more susceptible to local diseases, a higher LMA would be a burden.

While the most of the other models are statistically insignificant, it is interesting to note that most of the models follow the same trend as the LMA by origin models: comparing the regressions for same models between the two experiments shows that most of them share the same pattern. Where there is negative correlation in the detached leaf assay, there is negative correlation in the field experiment, and where there is positive correlation in the detached leaf assay, there is positive correlation in the field experiment. Due to the statistical insignificance of these models, it is uncertain whether these patterns are due to coincidence, but the possibility exists that patterns may be present and that they just require further research, which makes for several interesting projects that could be done in the future.

Another interesting questions that arises from these results. As mentioned before, the experiments were designed with the same ideas and principles in mind. However, the controlled petri dishes and the field are two very different environments. In the detached leaf assay, there was only one known generalist pathogen that infected the plants, while field is filled with a very wide variety of fungal diseases, many of which are highly specialized to specific grass species. This alone would lead one to expect the results between the lab and the field to be very different. If further experiments were to prove all of the similarities between the two statistically significant, that would then suggest that the susceptibility of a plant is completely independent of the pathogen, and that it relies solely on the traits of the plant, which seems highly unusual.
There are other future directions this research could take to explore different aspects of the invasion process. Instead of using a generalist pathogen like *Fusarium graminearum* for example, the leaf segments could be inoculated with a specialist pathogen and compared to a generalist pathogen, to explore the innate biology hypothesis, by showing how the traits and innate biology of the invader affects whether it can establish a foothold. Using other generalist pathogens would also produce interesting data, as it would show how widely the results from this experiment could be applied to plant pathogens in general. One could also conduct a similar experiment with different types of plants (woody or herbaceous plants, rather than grasses) to represent a different type of community and further explore the Community Invasibility hypothesis.
APPENDIX

The table below (Table 1) shows a list of all abbreviations used in the figures and the full name of species that they represent.

**Table 1.** Plant species abbreviations for species names used in figures below, along with basic relevant information to the experiments.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Species name</th>
<th>Tribe</th>
<th>Origin</th>
<th>Photosynthetic Pathway</th>
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<tr>
<td>AETR</td>
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<td>Eurasia</td>
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<td>Andropogoneae</td>
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<td>Eurasia</td>
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<td>Americas</td>
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<td>Poeae</td>
<td>Eurasia</td>
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Detached Leaf Assay

**Figure 1.** Comparison of damage between species, with standard error bars. The differences in damage between the plant species are not statistically significant.

**Figure 2.** Comparison of damage between tribes, with standard error bars. The differences in damage between the plant species are statistically significant (p<0.05).
Figure 3. Comparison of damage between plants of different origins, with standard error bars. The differences in damage between the plant species of different origins is not statistically significant.

Figure 4. Comparison of damage between species with different photosynthetic pathways, with standard error bars. The differences in damage between the photosynthetic pathways is not statistically significant.
Figure 5. Comparison between damage and photosynthesis rate, with linear regression. Photosynthesis rate does not significantly contribute to the variation in damage. The equation for linear regression for this model is \( y = -2.6976x + 95.477 \), with \( R^2 = 0.0004 \).

Figure 6. Comparison between damage and photosynthesis rate, with linear regression. LMA does not significantly contribute to the variation in damage. The equation for linear regression for this model is \( y = -31.157x + 152.61 \), with \( R^2 = 0.0095 \).
Figure 7. Comparison between damage and leaf growth rate, with linear regression. Leaf growth rate significantly contributes to the variation in damage (p<0.05). The equation for linear regression for this model is $y = 47.969x - 9.5014$, with $R^2 = 0.1776$.

Detached Leaf Assay by Tribe

Figure 8. Comparison between damage and photosynthesis rate, separated by tribe, with linear regressions. The equation for the Triticeae linear regression for this model is $y = 31.546x - 174.85$, with $R^2 = 0.2579$. The equation for the Poeae linear regression for this model is $y = -24.433x + 252.64$, with $R^2 = 0.0397$. The equation for the Bromeae linear regression for this model is $y = -55.003x + 418.35$, with $R^2 = 0.0079$. Due to insufficient data points, no linear regressions were made for Andropogoneae, Paniceae and Cynodonteae.
Figure 9. Comparison between damage and LMA, separated by tribe, with linear regressions. The equation for the Triticeae linear regression for this model is $y = -470.67x + 1283.1$, with $R^2 = 0.2579$. The equation for the Poeae linear regression for this model is $y = 116.69x - 187.07$, with $R^2 = 0.0775$. The equation for the Bromeae linear regression for this model is $y = -96.537x + 307.96$, with $R^2 = 0.0079$. Due to insufficient data points, no linear regressions were made for Andropogoneae, Paniceae and Cynodonteae.

Figure 10. Comparison between damage and leaf growth rate, separated by tribe, with linear regressions. The equation for the Triticeae linear regression for this model is $y = 19.041x - 6.9255$, with $R^2 = 0.2579$. The equation for the Poeae linear regression for this model is $y = 53.45x - 21.753$, with $R^2 = 0.2037$. The equation for the Bromeae linear regression for this model is $y = -6.8647x + 98.815$, with $R^2 = 0.0079$. Due to insufficient data points, no linear regressions were made for Andropogoneae, Paniceae and Cynodonteae.
Detached Leaf Assay by Origin

Figure 11. Comparison between damage and photosynthesis rate, separated by origin, with linear regressions. The equation for the Americas linear regression for this model is $y = 50.114x - 272.97$, with $R^2 = 0.0836$. The equation for the Eurasia linear regression for this model is $y = -22.887x + 225.77$, with $R^2 = 0.0582$. Due to insufficient data points, no linear regression was made for cultivated species.

Figure 12. Comparison between damage and leaf mass per area, separated by origin, with linear regressions. The equation for the Americas linear regression for this model is $y = -161.52x + 501.85$, with $R^2 = 0.2873$. The equation for the Eurasia linear regression for this model is $y = 121.26x - 207.13$, with $R^2 = 0.0941$. Due to insufficient data points, no linear regression was made for cultivated species. This model is marginally statistically significant (Multiple $R^2 = 0.2873$, $p = 0.0697$).
Figure 13. Comparison between damage and leaf growth rate, separated by origin, with linear regressions. The equation for the Americas linear regression for this model is $y = 108.63x - 53.301$, with $R^2 = 0.4423$. The equation for the Eurasia linear regression for this model is $y = 32.863x - 5.8817$, with $R^2 = 0.1149$. Due to insufficient data points, no linear regression was made for cultivated species. This model is statistically significant (Multiple $R^2 = 0.2893$, $p = 0.001382$).

Detached Leaf Assay by Photosynthetic Pathway

Figure 14. Comparison between damage and photosynthesis rate, separated by photosynthetic pathway, with linear regressions. The equation for the C3 linear regression for this model is $y = -14.044x + 166.84$, with $R^2 = 0.0157$. The equation for the C4 linear regression for this model is $y = 492.03x - 3598.1$, with $R^2 = 0.5853$. This model is statistically significant (Multiple $R^2 = 0.1924$, $p = 0.01063$).
Figure 15. Comparison between damage and LMA, separated by photosynthetic pathway, with linear regressions. The equation for the C3 linear regression for this model is \( y = 86.816x - 133.86 \), with \( R^2 = 0.0475 \). The equation for the C4 linear regression for this model \( y = -210.25x + 690.78 \), with \( R^2 = 0.4889 \). This model is statistically significant (Multiple \( R^2 = 0.1627, p = 0.02529 \)).

Figure 16. Comparison between damage and leaf growth rate, separated by photosynthetic pathway, with linear regressions. The equation for the C3 linear regression for this model is \( 33.821x + 7.2679 \), with \( R^2 = 0.1136 \). The equation for the C4 linear regression for this model \( y = 132.43x - 109.61 \), with \( R^2 = 0.6189 \). This model is statistically significant (Multiple \( R^2 = 0.2936, p = 0.0003936 \)).
**Field Experiment**

**Figure 17.** Comparison of damage between species, with standard error bars. The differences in damage between the plant species are not statistically significant.

**Figure 18.** Comparison of damage between tribes, with standard error bars. The differences in damage between the plant tribes are not statistically significant.
Figure 19. Comparison of damage between plants of different origin, with standard error bars. The differences in damage between the plant origins are not statistically significant.

Figure 20. Comparison of damage between plants with different photosynthetic pathways, with standard error bars. The differences in damage between the plant photosynthetic pathways are not statistically significant.
Figure 21. Comparison between damage and photosynthesis rate, with linear regression. Photosynthesis rate does not significantly contribute to the variation in damage. The equation for linear regression for this model is $y = -0.0005x + 1.0787$, with $R^2 = 2E-06$.

Figure 22. Comparison between damage and leaf growth rate, with linear regression. Leaf growth rate does not significantly contribute to the variation in damage. The equation for linear regression for this model is $y = 0.036x + 1.0292$, with $R^2 = 0.0008$. 
Figure 23. Comparison between damage and LMA, with linear regression. LMA does not significantly contribute to the variation in damage. The equation for linear regression for this model is \( y = 23.878x + 1.0051 \), with \( R^2 = 0.0003 \).

Field Experiment by Tribe

Figure 24. Comparison between damage and photosynthesis rate, separated by tribe, with linear regressions. The equation for the Andropogoneae linear regression for this model is \( y = 0.0914x + 0.8821 \), with \( R^2 = 0.0469 \). The equation for the Poeae linear regression for this model is \( y = -0.0941x + 1.6919 \), with \( R^2 = 0.1008 \). The equation for the Danthonieae linear regression for this model is \( y = -0.0076x + 0.2315 \), with \( R^2 = 0.0196 \). The equation for the Paniceae linear regression for this model is \( y = -0.5948x + 4.5161 \), with \( R^2 = 0.4359 \). The equation for the Cynodoneae linear regression for this model is \( y = 0.2161x - 0.416 \), with \( R^2 = 0.8971 \).
**Figure 25.** Comparison between damage and LMA, separated by tribe, with linear regressions. The equation for the Andropogoneae linear regression for this model is $y = 70.203x + 1.2949$, with $R^2 = 0.0017$. The equation for the Poeae linear regression for this model is $y = 602.14x - 0.4256$, with $R^2 = 0.3958$. The equation for the Danthonieae linear regression for this model is $y = -425.16x + 1.8587$, with $R^2 = 0.2995$. The equation for the Paniceae linear regression for this model is $y = -1363.5x + 4.7544$, with $R^2 = 0.3584$. The equation for the Cynodonteae linear regression for this model is $y = -1385.6x + 5.0873$, with $R^2 = 0.8118$.

**Figure 26.** Comparison between damage and leaf growth rate, separated by tribe, with linear regressions. The equation for the Andropogoneae linear regression for this model is $y = -0.3596x + 2.0607$, with $R^2 = 0.0379$. The equation for the Poeae linear regression for this model is $y = 0.0197x + 1.1851$, with $R^2 = 0.0006$. The equation for the Danthonieae linear regression for this model is $y = 0.1652x + 0.0251$, with $R^2 = 0.4516$. The equation for the Paniceae linear regression for this model is $y = 0.5151x + 0.2843$, with $R^2 = 0.1054$. The equation for the Cynodonteae linear regression for this model is $y = -1.6515x + 3.1086$, with $R^2 = 0.7358$. 

Field Experiment by Origin

Figure 27. Comparison between damage and photosynthesis rate, separated by origin, with linear regressions. The equation for the Americas linear regression for this model is $y = 0.0756x + 0.5647$, with $R^2 = 0.0219$. The equation for the Eurasia linear regression for this model is $y = -0.0589x + 1.4419$, with $R^2 = 0.0662$.

Figure 28. Comparison between damage and LMA, separated by origin, with linear regressions. The equation for the Americas linear regression for this model is $y = -859.41x + 3.939$, with $R^2 = 0.1428$. The equation for the Eurasia linear regression for this model is $y = 569.72x - 0.3058$, with $R^2 = 0.3999$. This model statistically significant (Multiple $R^2 = 0.2128$, $p = 0.01381$).
**Field Experiment by Photosynthetic Pathway**

**Figure 29.** Comparison between damage and photosynthesis rate, separated by origin, with linear regressions. The equation for the Americas linear regression for this model is $y = 0.1991x + 0.8689$, with $R^2 = 0.0079$. The equation for the Eurasia linear regression for this model is $y = -0.0259x + 1.1224$, with $R^2 = 0.0013$.

**Figure 30.** Comparison between damage and photosynthesis rate, separated by photosynthetic pathway, with linear regressions. The equation for the C3 linear regression for this model is $y = -0.0909x + 1.499$, with $R^2 = 0.1128$. The equation for the C4 linear regression for this model is $y = 0.0738x + 0.6908$, with $R^2 = 0.0268$. 
Figure 31. Comparison between damage and LMA, separated by photosynthetic pathway, with linear regressions. The equation for the C3 linear regression for this model is $y = 260.99x + 0.1816$, with $R^2 = 0.083$. The equation for the C4 linear regression for this model is $y = -295.73x + 2.0621$, with $R^2 = 0.0255$.

Figure 32. Comparison between damage and leaf growth rate, separated by photosynthetic pathway, with linear regressions. The equation for the C3 linear regression for this model is $y = 0.0597x + 0.8661$, with $R^2 = 0.0052$. The equation for the C4 linear regression for this model is $y = 0.0077x + 1.1778$, with $R^2 = 2E-05$. 
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