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Arthropod herbivory and fungal pathogen damage as determinants of distribution of tropical tree species *Lacistema aggregatum* across a Panamanian rainfall gradient

Martin, Lydia

Award date:
2023

Awarding institution:
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Arthropod herbivory and fungal pathogen damage
as determinants of distribution of tropical tree
species *Lacistema aggregatum* across a
Panamanian rainfall gradient

A thesis submitted to Bangor University by

Lydia J. Martin

In candidature of the degree of
Master of Science by Research

Supervised by

Dr Lars Markesteijn, Anita Weissflog

June 30th 2022

Declaration

I hereby declare that this thesis is the results of my own investigations, except where otherwise stated. All other sources are acknowledged by bibliographic references. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless, as agreed by the University, for approved dual awards.

Yr wyf drwy hyn yn datgan mai canlyniad fy ymchwil fy hun yw'r thesis hwn, ac eithrio lle nodir yn wahanol. Caiff ffynonellau eraill eu cydnabod gan droednodiadau yn rhoi cyfeiriadau eglur. Nid yw sylwedd y gwaith hwn wedi cael ei dderbyn o'r blaen ar gyfer unrhyw radd, ac nid yw'n cael ei gyflwyno ar yr un pryd mewn ymgeisiaeth am unrhyw radd oni bai ei fod, fel y cytunwyd gan y Brifysgol, am gymwysterau deuol cymeradwy.

Acknowledgements

I would like to thank Anita for her support, knowledge and our adventures in Panama whilst collecting data for this experiment. I thank Lars for letting me be a part of this project in which I've been able to connect with so many great people and learn from scientists from all over the world. I would like to thank all the people in Gamboa and Bangor who have offered their support, discussions, and friendship over the course of this degree, especially Kannan, Steph, James, Cameron, Amy and Sean. I thank everyone at home for their moral support, especially Mum, Jacob, Keira, Bailey and Niall. I thank STRI and its employees for the great work they do. I thank the Panama Canal Authority meteorology and hydrology branch, ForestGeo and STRI Physical Monitoring Program for making their data available for scientific use.

The BCI forest dynamics research project was made possible by National Science Foundation grants to Stephen P. Hubbell: DEB-0640386, DEB-0425651, DEB-0346488, DEB-0129874, DEB-00753102, DEB-9909347, DEB-9615226, DEB-9615226, DEB-9405933, DEB-9221033, DEB-9100058, DEB-8906869, DEB-8605042, DEB-8206992, DEB-7922197, support from the Center for Tropical Forest Science-Forest Global Earth Observatory, the Smithsonian Tropical Research Institute, the John D. and Catherine T. MacArthur Foundation, the Mellon Foundation, the Small World Institute Fund, and numerous private individuals, and through the hard work of over 100 people from 10 countries over the past three decades. The plot project is part the Center for Tropical Forest Science-Forest Global Earth Observatory (CTFS-ForestGEO), a global network of large-scale demographic tree plots.

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Abstract

In tropical forests, tree seedlings are subject to pressures from natural enemies and their abiotic environment. Their response to these pressures ultimately dictates their survival and the distribution of their species. Insect herbivores predate trees more than any other herbivore, with most predation occurring during the seedling stage of the plant's life. Fungal pathogens are also detrimental to seedling survival, sometimes causing complete cohort mortality. Whether the effects of insect herbivores and fungal pathogens on seedling performance can be changed by rainfall remains unresolved. This study aimed to measure the amount of fungal and herbivore damage upon seedlings in the field, and the subsequent response to this damage. I planted a total of 400 *Lacistema aggregatum* seedlings across four forest plots along a natural rainfall gradient in Panama. *L. aggregatum* naturally occurs at all of these sites, and thus, the seedlings were exposed to their natural predators and environmental conditions. At each site, 100 seedlings were planted in four pesticide treatment blocks with 25 seedlings per block. Pests were excluded by spraying these blocks of individuals at each site with one of four treatments: insecticide; fungicide; combination insecticide and fungicide; or water (control treatment). There were no consistent, significant trends for leaf gain, fungal damage or herbivory when compared between sites and treatments. This result suggests that neither the leaf gain of the seedlings of this widespread species, nor insect herbivory and pathogen damage are strongly affected by rainfall in our four sites. However, long term studies including more sites and treatment blocks, that consider more variables that may drive trends (such as soil type, nutrients, light availability, frequency of rainfall and pre-existing communities of natural enemies) are needed.

Key words: tropical tree distribution, *Lacistema aggregatum*, fungal pathogens, insect herbivores, rainfall gradient, pest pressure

INTRODUCTION

The biotic and abiotic factors a plant is exposed to in its early life as a seedling affect its probability of survival, thus determining the future distribution of the species (Swinfield *et al.* 2012, Bertacchi *et al.* 2016). Tropical forests house many species as the most biodiverse terrestrial biomes on Earth, comprising of up to 655 tree species per hectare (Coley *et al.* 2018). The generation and maintenance of biodiversity in the tropics is a topic that has been of great interest to ecologists for centuries and continues to be so in the present-day (Vandermeer, 2006). Due to the complexity of interactions within tropical forest habitats, there are always new avenues of research to explore. Furthermore, as human-driven threats intensify, understanding how biodiversity is maintained is the first step towards protecting it (Lee *et al.* 2011). Research proves human disturbance leads to lower biodiversity across most taxa, including insects (Lee *et al.* 2011). A large body of research is imperative to noticing how different taxa respond to human disturbance over time, allowing for the implementation of mitigations (Lee *et al.* 2011; Comita & Stump, 2020; Tonetti *et al.* 2022).

It has been suggested that arthropod herbivores and fungal pathogens play an important role in maintaining diverse tree communities by acting as natural enemies (Wright, 2002, Bagchi *et al.* 2014). The extent of damage caused to seedlings by natural enemies can be affected by climatic variables including rainfall (Gaviria & Engelbrecht, 2015, Weissflog *et al.* 2018). The volume of rainfall per year can have a direct effect on seedling performance (through water provision) (Segura *et al.*, 2002, Tyree *et al.* 2003, Gaviria & Engelbrecht, 2015) or a secondary effect by influencing the abundance and behaviour of natural predators (Weissflog *et al.* 2018). Understanding how seedlings respond to predation and environmental factors could further our understanding of how a species is able to maintain its presence in the vegetation, at both local and large scales.

Insects are a major taxonomic group showing disproportionately high diversity within the tropics (Basset *et al.*, 2012). Some ecologists suggest insect-plant interactions are responsible for maintaining high tree diversity by inciting strong selective pressure (Bagchi *et al.*, 2014; Becerra, 2015; Novotny *et al.*, 2006). The arms race between insect herbivory and plant defences has led to high levels of specialisation through host specificity (Becerra, 2015).

Insects are the main predators of leaves in tropical forests, responsible for 75% of the yearly leaf consumption (Coley & Barone, 1996). With over half of insects in forests opting for leaves

as their diet of choice (Odell *et al.*, 2019), it is no wonder that herbivory is an impactful factor upon the success of seedlings (Eichhorn *et al.*, 2010a). The overall loss of leaf area to herbivory is often low, in the region of 1%-8% (Anstett *et al.*, 2015; Kozlov *et al.*, 2015; Myers & Sarfraz, 2017; Schlinkert *et al.*, 2015). However, this has been shown to lead to reduced growth, fecundity and death (reviewed by Myers & Sarfraz, 2017).

Basset (2001) studied insect herbivores on *Pourouma bicolor* (Cecropiaceae) in an evergreen rainforest in Central Panama. Basset (2001) sampled invertebrates from saplings and mature trees and categorised the samples into taxonomic groups. The results give insight into the types of insect that are proven to conduct herbivory on trees and saplings, including leaf-chewing (e.g. Chrysomelidae and Lepidoptera larvae), sap-sucking (e.g. Cicadellidae) and wood boring (e.g. beetle larvae) (Basset, 2001).

Research shows that the herbivory (plant-eating) behaviour of insects is affected by gradients such as rainfall (Baltzer & Davies, 2012; Weissflog *et al.*, 2018). Insect herbivory of leaves reduces in drier forests (Andrade *et al.* 2020, Weissflog *et al.* 2018). There are several suggested reasons for this. Plants have evolved survival strategies for different rainfall levels, which alters susceptibility to herbivory (Santiago and Mulkey, 2005). In drier areas where leaves seasonally fall, plants grow thinner leaves to maximise photosynthesis, making them more vulnerable to leaf-eating pests (Santiago and Mulkey, 2005). In locations with more consistent rainfall, plants are able to retain their leaves and therefore have reliable, constant photosynthesis, allowing them to invest in thicker, defended leaves (Santiago and Mulkey, 2005). Drought stress also causes changes to plant nutritional content temporarily. Decreased rainfall increases nitrogen in leaves, which insect herbivores benefit from, allowing their populations to rapidly grow (Huberty & Denno, 2004). However, this applies only to shorter periods of drought; prolonged drought causes more detriment than benefit for the insects (Huberty & Denno, 2004). Rainfall can also make changes further up the food chain. In drier sites, folivores like caterpillars are predated upon less and so are more numerous (Andrade *et al.* 2020). It has been suggested that caterpillars are parasitised more in wetter forests by parasitoid wasps, whereas in drier forests wasp populations are controlled by desiccation (Connahs *et al.* 2011). Finally, tree species which are adapted to live in places with higher levels of damage from herbivores may have evolved stronger mechanical and chemical defences against these natural enemies (Coley & Barone, 1996). Insect herbivory upon trees in the crucial seedling stage is key in determining future tree distributions and biodiversity, since

species unable to survive the damage will be outcompeted by those with higher tolerance (Gaviria & Engelbrecht, 2015).

Fungal pathogens can also have strong mortality-inducing effects on seedlings, especially where seedlings occur at high densities (Augsburger & Kelly, 1984, Harms *et al.* 2000, Spear *et al.*, 2015, Bell, 2006, Bagchi *et al.* 2010, Bagchi *et al.* 2014). The degree to which fungal pathogens cause seedling mortality is co-determined by environmental factors, including humidity and rainfall (Jain *et al.*, 2019). Fungal pathogens significantly reduce survival of seedlings at higher volumes of watering (Swinfield *et al.* 2012). Additionally, frequent (daily) wetting of leaves increases mortality compared to just watering the soil (Swinfield *et al.* 2012). Although Swinfield *et al.* (2012) conducted their research in a shadehouse, their findings suggest a positive relationship between rainfall and fungal pathogen-induced mortality, especially in the wet season.

There are many suggested reasons for the association between increased water and higher fungal damage. It is possible that rainfall can impact fungal pathogen reproduction by triggering spore release (Swinfield *et al.*, 2012). Drought can cause plants to close the stomata on their leaves, meaning fungal pathogens are less able to infect them (Swinfield *et al.*, 2012). Leaf litter is another factor that influences fungal pathogen attacks on seedlings, with increased damage at sites with higher litter-cover (García-Guzmán & Benítez-Malvido, 2003). Higher leaf litter can occur at drier sites in Panama due to an increase in deciduous species (Condit *et al.* 2001).

However, density of plants is also an important factor. This has been attributed to the Janzen-Connell hypothesis, in which (conspecific) seedlings at higher densities are more likely to die as they contract fungal pathogens specific to their species (Janzen, 1970, Connell, 1971). Closer proximity to the species' parent tree increases seedling mortality (Johnson *et al.*, 2012; Terborgh, 2012, Downey *et al.* 2018). Bagchi *et al.* (2010) combined shadehouse and field experiments to test for the effects of fungal pathogen on seedling mortality of a tropical tree species. They found that fungal pathogens play a substantial role in seedling mortality related to negative density dependence. Without fungicide, after only four weeks, all field seedlings were dead and only 10% of shadehouse seedlings survived (Bagchi *et al.*, 2010). As this study focused only on the Janzen-Connell mechanism, it does not incorporate community-wide biotic and abiotic factors.

As the seedlings direct energy towards growth, they also have to survive predation, environmental risks like treefall and burial, differing light conditions and lack of nutrients (Augspurger & Kelly, 1984; Baltzer & Davies, 2012; Eichhorn *et al.*, 2010b; Fenner, 1985; García-Guzmán & Benítez-Malvido, 2003; Kitajima, 1996). To understand the links between abiotic conditions (like rainfall) and biotic interactions (like natural enemy damage) on the distribution of a species, I selected a tree species with a broad distribution for my study.

Lacistema aggregatum occurs across forests along the steep cross-Isthmus rainfall gradient in Panama. This is unusual as most species are naturally observed to have more restricted distributions, resulting in a large species turnover across the country (Condit *et al.*, 2010). The rainfall gradient is especially pronounced as Panama has a large difference in rainfall between coasts, from 1600mm to 4000mm per year (Engelbrecht *et al.* 2007). To successfully adapt to a relatively broad distribution across a country with a steep rainfall gradient, a species would have to survive a trade-off between drought, fungal pathogen infection and herbivory.

Whilst past researchers have investigated the relationships between rainfall, pests and pathogens, these studies used either two sites (one wet and one dry, with plant species exchanged from their natural habitat to the other where they did not naturally grow) or shadehouse-based studies, which can simulate rainfall but not associated habitat characteristics which would affect plant survival in the wild (Bagchi *et al.* 2010, Brenes-Arguedas *et al.*, 2009, Gaviria & Engelbrecht, 2015). By measuring both the pest damage upon the seedlings as well as their response to it, in four natural rainforest sites, this study may show how plants survive across rainfall gradients despite pest pressures varying, in a realistic setting. Pesticides for both insects and fungal pathogens have been used successfully in past studies to exclude these pests (Solé *et al.* 2019, Bagchi *et al.* 2014). Excluding insects, fungal pathogens or both simultaneously in blocks of seedlings allows for comparison between the damage types and the plants' response to different pests.

This study aims to compare how arthropod herbivory and fungal pathogen damage on seedlings varies across a rainfall gradient. It aims to provide a study where a species was grown in its own natural range and show how it is able to inhabit a large gradient, despite potential changes in pressure from insect herbivores and fungal pathogens. I assess natural enemy damage and the seedlings' response to it by measuring how their ability to gain and retain leaves is affected by the rainfall and damage.

I hypothesise that:

1. The effect of pesticide treatment on leaf gain will vary across the rainfall gradient
2. Fungal pathogen damage will increase with increased rainfall
3. Insect herbivory will decrease with increased rainfall

MATERIALS AND METHODS

Study area and research sites

Panama is a small country with 12,521 km² of land, 16.5% of which is national park (Autoridad Nacional del Ambiente 2000, Condit *et al.* 2001). Central Panama is covered with closed-canopy tropical lowland forest 20 –50 m tall (Condit *et al.* 2001). The topography varies between 30m-200m elevation above sea level (Karr, 1990).

The four tropical forest sites I used were close to the Panama Canal and covered the steep gradient of yearly rainfall along the Isthmus of Panama (Figure 1). From the drier Pacific coast to the wetter Caribbean coast, the site names and yearly rainfall were Parque Metropolitano

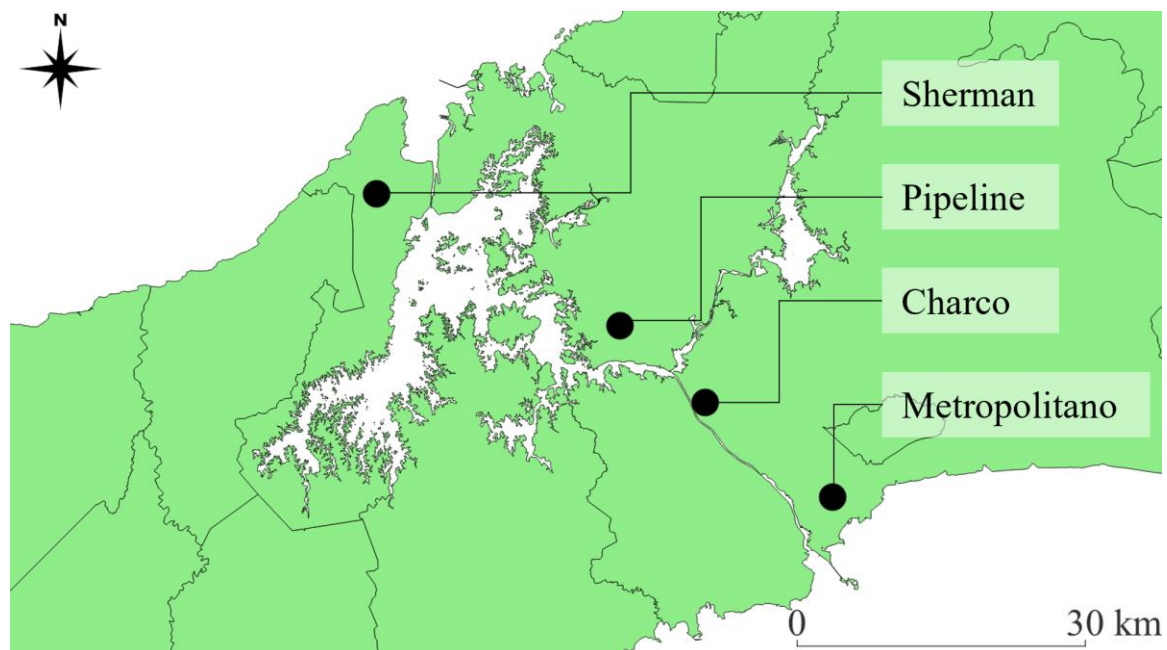


Figure 1. A map of central Panama showing each of the four field sites used, marked with a black point. Sites follow a steep rainfall gradient from the dry Pacific to the wet Caribbean site (Metropolitano: 1874mm⁻¹, Charco: 2050 mm⁻¹, Pipeline: 2311 mm⁻¹ and Sherman: 3203 mm⁻¹).

(1874 mm per year), El Charco (2050 mm per year), Pipeline Road (2311mm per year) and Fort San Lorenzo (also commonly called Sherman) (3203mm per year) (Condit *et al* 2013, Engelbrecht *et al* 2017). At the wetter Atlantic side, forests are 100% evergreen, which decreases slightly in a gradient towards the drier Pacific side of the country where forests are 75% evergreen and 25% deciduous (Condit *et al.* 2000, Condit *et al.* 2001). The research sites used were CTFS ForestGeo plots which provided us with historical species presence data and rainfall data from the Panama Canal Authority and STRI (Smithsonian Tropical Research Institute) sources (Condit *et al.* 2001, Engelbrecht *et al.* 2007, Panama Canal Authority, Physical Monitoring Program, STRI). *Lacistema aggregatum* is known to inhabit 98 out of 122 sites across central Panama with an occurrence probability of 94% at dry sites and 70% at wet sites (Engelbrecht *et al.* 2007, Supplementary Data 3 & 4). I planted 400 seedlings of *L. aggregatum* in four field sites along a rainfall gradient. At each site, I exposed 25 seedlings each to four different treatments: insecticide, fungicide, combination (insecticide plus fungicide) and water-sprayed control. I measured insect herbivory, pathogen damage, leaf gain and seedling survival for 4 months (December, January, February and March 2020). December was in the wet season, with January as the transitional month to the dry season in February and March.

Focal Species

Lacistema aggregatum (P.J. Bergius) Rusby is a tropical treelet, first named in the Bulletin of the New York Botanical Garden in 1907. In Panama, where this study was conducted, it is commonly known as ‘huesito’.

L. aggregatum's distribution covers Central and South America, as recorded in the Vascular Plants of the Americas project (Ulloa *et al.*, 2017). It seems to survive in a broad range of habitats, shown by its vast distribution in Central and South America and its spread across the environmentally diverse habitat of the Panamanian Isthmus (Condit *et al.*, 2010). It is a frequent species within tropical forests and can survive at a range of altitudes also (Lang & Knight, 1983; Sugiyama & Peterson, 2013). The seeds of *L. aggregatum* are dispersed by birds and small mammals (Sugiyama & Peterson, 2013).

Experimental Approach

Lacistema aggregatum seeds were collected directly from the branches of 12 mature trees in 12 distinct locations in May 2019. Mature trees were naturally growing, located inside forests and were more than 3km away from all field sites. All collected seeds were pooled and

thoroughly mixed. Undamaged seeds were cleaned from pulp and surface sterilised (70% ethanol, 10% bleach, distilled water). The seeds were then planted in shallow trays with steam-sterilized 50% soil and 50% sand on June 21st 2019. Seedlings were grown in the trays in a shade house at 40% ambient light for 5 months before transplantation to a small bag of soil per seedling. Seedlings were randomised to assure offspring from each parent tree were distributed throughout each field site and treatment. 400 seedlings were used in total, with any unhealthy in appearance discarded. Before transplantation to the field, each seedling was given a plastic label which was attached around its lower stem (Figure 2a). A unique alphanumeric code was assigned to track each seedling throughout the experiment. All plants were marked with small dots of permanent pen on the centre of the underside of each leaf. The number of leaves for each seedling was counted and recorded the day before transplantation to the field sites.

The seedlings were planted in the field sites on four consecutive days between 2nd – 5th December 2019. At each site, 100 *L. aggregatum* seedlings were planted directly into the soil in four treatment blocks of 25 seedlings in a uniform 4m² grid (5 x 5 seedlings, each spaced 50 cm apart) (Figure 2b). At each site, a small hole was dug for each seedling and it was planted directly into the ground.



Figure 2. A) An individual *L. aggregatum* seedling with a plastic identification tag around the base, planted in-field. B) One treatment block in-field, comprising of 25 *L. aggregatum* seedlings, marked with bright flagging tape.

A minimum of 3 m and maximum of 20 m space was ensured between treatment blocks. No treatment blocks were planted within 10 m of an adult *L. aggregatum* tree to avoid excessive or unequal exposure to species-specific fungal pathogen or insect herbivores. Equally, this lowered the likelihood of exposure to beneficial mycorrhizal fungi from the roots of adult *L. aggregatum* which would give nearby seedlings an unequal advantage. As there were other

trees in each site which could have been allelopathic species, there may have been an unseen advantage or disadvantage to seedlings. But, since there is little previous research on *L. aggregatum* and its allelopathic species are not known, it was not possible to factor this into the experimental design (Hierro & Callaway, 2021). Leaf litter was removed initially to facilitate planting, and then allowed to accumulate naturally throughout the experiment. This allowed a pathway for fungal pathogens to infect seedlings realistically, from the leaves of neighbouring tree species. Whilst the specific fungal pathogen species in this experiment are unknown, since they were naturally occurring, they were representative of those that occur in that level of rainfall. Other plants within the treatment blocks were removed to avoid effects on seedling performance through interspecific competition and possible conspecific negative density dependence effects in case *L. aggregatum* seedlings were present.

There were four blocks of 25 seedlings at each site (16 blocks in the whole experiment). At each site I used four treatments, one per block. The treatments were either insecticide, fungicide, combination insecticide and fungicide, or water (control treatment). All treatments were applied using a pressure spray pesticide applicator. There was a separate applicator for each treatment and the applicators were sanitised between each site using bleach solution (20% bleach mixed with 80% water).

ENGEO® 24.7 SC, a broad-spectrum insecticide, was applied to the ‘insecticide’ and ‘combination’ treatments on the day of transplantation to the field and once every fortnight thereafter. The insecticide consisted of neonicotinoid and pyrethroid active ingredients, ensuring systemic and contact action to exclude both sucking and chewing herbivores, according to the manufacturer. 0.25ml of insecticide was used per 1l of water. 0.4l of the diluted insecticide per application was used in each 4m² treatment block.

To exclude fungal pathogens, AMISTAR XTRA® 28 SC was applied to the relevant seedlings (‘fungicide’ and ‘combination’ treatments) on transplantation to the field and then at 8-week intervals thereafter. AMISTAR XTRA® 28 SC uses a combination of active ingredients Azoxystrobin and Ciproconazole. 1ml of fungicide was diluted in 1l of water. 200ml of diluted fungicide was applied per 4m² treatment block, per application. ENEGO and the precursor of AMISTAR XTRA have been used in field studies previously and no phytotoxic effects were reported (Solé et al. 2019, Bagchi *et al.* 2014).

Both pesticides were applied to the seedlings upon their transplantation to the field (on the same day as transplantation). Two weeks later, the first of four monthly data collections was

carried out. Insecticide was re-applied every two weeks from the initial transplantation date and fungicide was re-applied every 8 weeks from the initial transplantation date, as per manufacturers instructions. Subsequent data collection and pesticide application was halted due to the COVID-19 pandemic which restricted access to the sites.

Pesticide applicators, footwear and measuring equipment were sanitised between field sites with a 20% bleach and water solution. I used a different tape measure for each of the four treatment blocks.

Data collection

There were four data collection events in total, carried out once per month from December 2019 to March 2020. Plant height was measured every four weeks from the stem base to the apex of the central stem (not including leaves or leaf shoots). Deaths were recorded each month, defined by zero remaining leaves and a dry stem.

The production of leaves is an energetically costly process which is necessary for survival in seedlings as most leaf-loss due to insect herbivory happens in the seedling stage (Barton & Hanley, 2013). The number of leaves were counted every four weeks, and the loss of leaves and emergence of new leaves (recognisable as unmarked) were recorded. New leaves were then marked with permanent pen to avoid recounts. The mark was a small dot on the underside of the leaf to avoid impacting the plant's photosynthetic abilities. Because of the leaf-marking, it was possible to measure exactly how many leaves each plant had produced and how many it had shed or lost, rather than just a total number of leaves each month.

Arthropod herbivory of leaves was recorded every four weeks using percentage estimates of each leaf in 5% increments. A value was recorded for each leaf on each plant, to calculate the average standing level percentage area of herbivory the plant was suffering from at each 4-week interval. Individual leaves were not tracked, but individual seedlings were tracked using their ID tags. The same researcher made the estimates throughout the experiment and used a measured, transparent grid in-field to improve estimation accuracy.

Fungal pathogen damage was also recorded every four weeks using the same percentage categories as herbivory. The type of visible symptom was also noted.

Statistical Analysis

I analysed leaf gain, arthropod herbivory (the percentage of leaf area per plant removed) and fungal pathogen damage (the percentage of leaf area per plant visibly affected). The data from the fourth and final census were used for model analysis.

For leaf gain, a total was calculated from the four months of census data, to get the sum leaf gain for each individual seedling. I ran a generalised linear model (GLM) in R version 1.4.1717 (R Core Team 2021). To analyse leaf gain across the four sites, I used package lme4 with interactive effects between treatment and site, to show how excluding fungal pathogens and insects changed the overall leaf gain. I used Poisson family with log link for positive integer count data. I performed pairwise *post hoc* comparisons (package emmeans).

Interactive GLMs were made for herbivory and fungal damage, (package glmmTMB) and included a zero-inflated part of the models to mitigate the large amount of zero results in the datasets. The models analysed effects of rainfall on each damage type (herbivory and fungal damage) and showed how the effect changed depending on the third interactive variable, which was the pesticide treatment.

Two versions of each model were made for herbivory and fungal damage – one with and one without outliers. This was to check how much the outliers were driving the overall trends. Outliers were not removed from the analysis because they were true values and not erroneous. Outliers were detected using quantiles (under 2.5% and over 97.5%) for herbivory and fungal damage models.

Models have to fit a set of assumptions to be valid; these were checked using the qqnorm function. Residuals were plotted using package DHARMA with zero-inflation correction for herbivory and fungal damage. Odds ratios were made in order to show the probability of pest damage under different rainfall and treatment exposures compared to controls.

Post hoc pairwise comparisons were conducted for each model. Whilst the models tell if the variable (e.g. treatment) had an effect on the dependent variable, the *post hoc* test adds an extra level by showing which of the individual treatments had differences between them, in case just one treatment group is responsible for most of the variation. The pairwise comparisons were done in R using the ‘contrast’ function in the package ‘emmeans’, which corrects for the false-positive risk of multiple comparisons automatically, using the Tukey method. The pairwise comparisons with a p value under 0.05 are the ones driving the trend. Alternatively, the pairwise comparisons where the upper and lower 95% confidence intervals do not have zero in between

them are the ones that are significant, since a treatment cannot be increasing and decreasing a dependent variable simultaneously. Pairwise comparisons are produced using the ‘confint’ function and graphed using the ‘plot’ function.

RESULTS

Out of 400 seedlings, 50 had died by the end of the four-month experiment (12.5% mortality). Of the 50 deaths, 34 occurred in the fourth month. The most deaths over the four months occurred at Metropolitan, the driest site in this experiment. 64% of deaths occurred at Metropolitan, with 20% occurring at Sherman and 8% at Charco and Pipeline. The monthly mortality per site in relation to rainfall is shown in Figure 3.

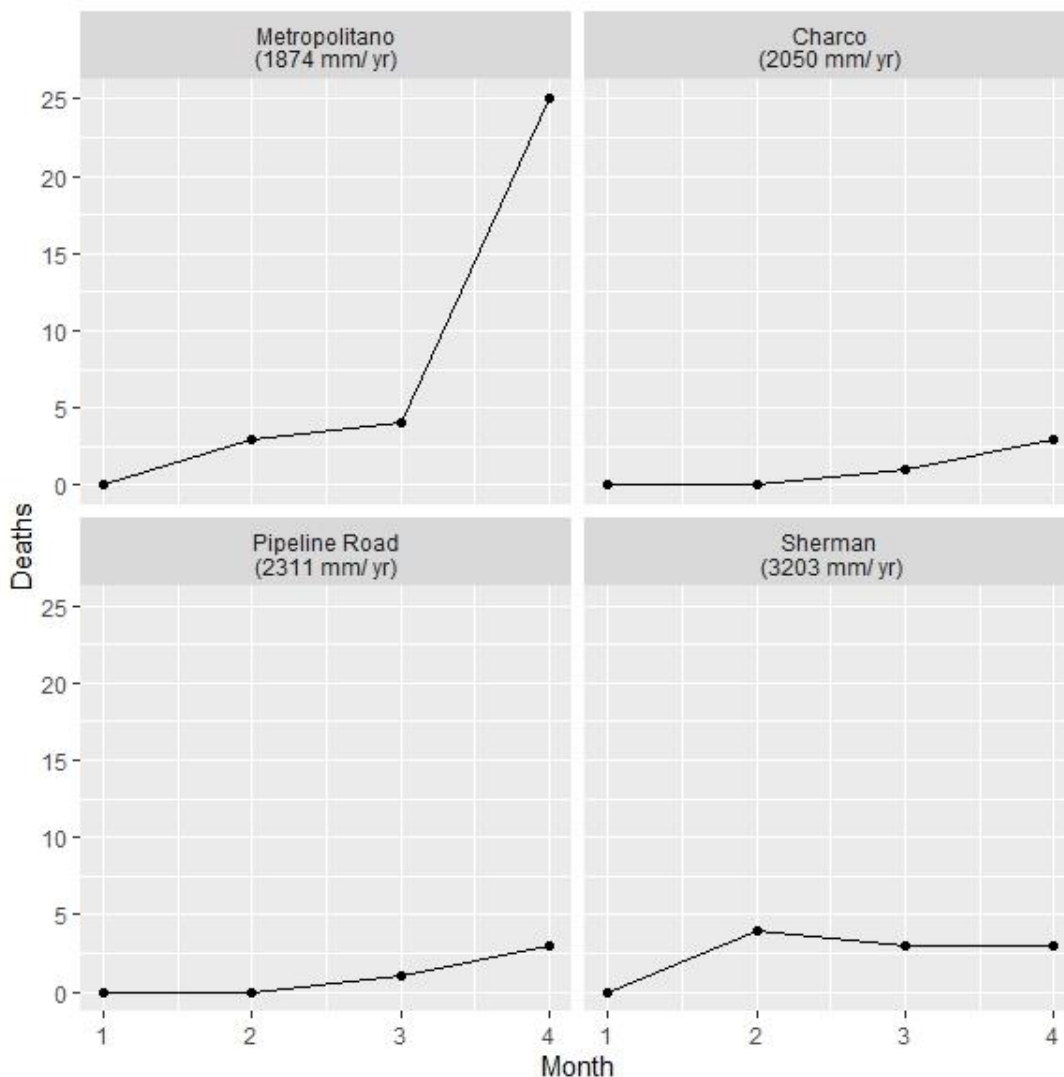


Figure 3. Sum of seedling deaths per month (1 = December 2019, 2 = January 2020, 3 = February 2020, 4 = March 2020). Each of the four graphs represents a different site, which is noted above each graph along with the yearly rainfall for that site in mm per year.

The total deaths per site is shown in Figure 3, split by site (rainfall). After no deaths at any site in month 1, the driest site (Metropolitano) experienced 3 deaths and the wettest site (Sherman) had 4 deaths in month 2, when the dry season was beginning. The two intermediate sites had no deaths in month two, and only one per site in month 3, continuing the trend for low deaths at these sites. Sherman deaths slightly reduced to 3 in month 3. Deaths at the driest site continued to rise into the third month with four deaths, before rapidly increasing to 25 deaths in the fourth month (March). This is half of all deaths in the experiment. Every other site had 3 seedling deaths in March.

203 new leaves grew across the whole experiment, which were identifiable by their lack of pen mark. As the plants were individually identifiable and the leaves on each plant were counted and marked each month, it was possible to calculate how many leaves each plant lost. In total, 1026 leaves were lost during the experiment. Each plant grew a mean of 0.5 leaves over the four months. Leaf gain varied slightly between sites and was on average 0.56 in Metropolitano; 0.44 in Charco; 0.72 in Pipeline and 0.31 in Sherman.

The seedlings were slow-growing. In the first month, mean height was 60.5mm (SE = 0.72) and had increased to 65.1mm (SE = 0.79) by month 4, meaning each plant grew on average 1.15 mm per month over the 4 month period.

The standing level of herbivory (percentage leaf area removed) in month four was a mean of 1.15% per plant. Most seedlings suffered no herbivory at all, with only 82 out of 400 seedlings (20.5%) having an occurrence of herbivory in month four.

The standing level of fungal pathogen damage (percentage leaf area visibly inflicted) in month four was slightly higher than herbivory, with a mean of 2.04% per seedling. Again, the majority of seedlings did not have any visible fungal damage, only 87 and thus 21.8% were recorded as affected in month four.

Hypothesis 1 – The effect of pesticide treatment on leaf gain will vary across the rainfall gradient

The highest leaf gain for any individual seedling in the experiment was $n = 3$ and the lowest was $n = 0$ (Figure 4). In the water-sprayed control, mean leaf gain varied across the rainfall gradient as follows: 1.08 in Metropolitano; 0.28 in Charco; 0.68 in Pipeline and 0.6 in

Sherman. In the same ascending order of sites from driest to wettest, seedlings in the insecticide treatment had mean leaf gain as follows: 0.48, 0.48, 1.08 and 0.2. For the fungicide treatment: 0.24, 0.52, 0.28 and 0.36. Finally, for the seedlings in the combination treatment: 0.44, 0.48, 0.84 and 0.08 (the lowest mean leaf gain of all the groups, Figure 4).

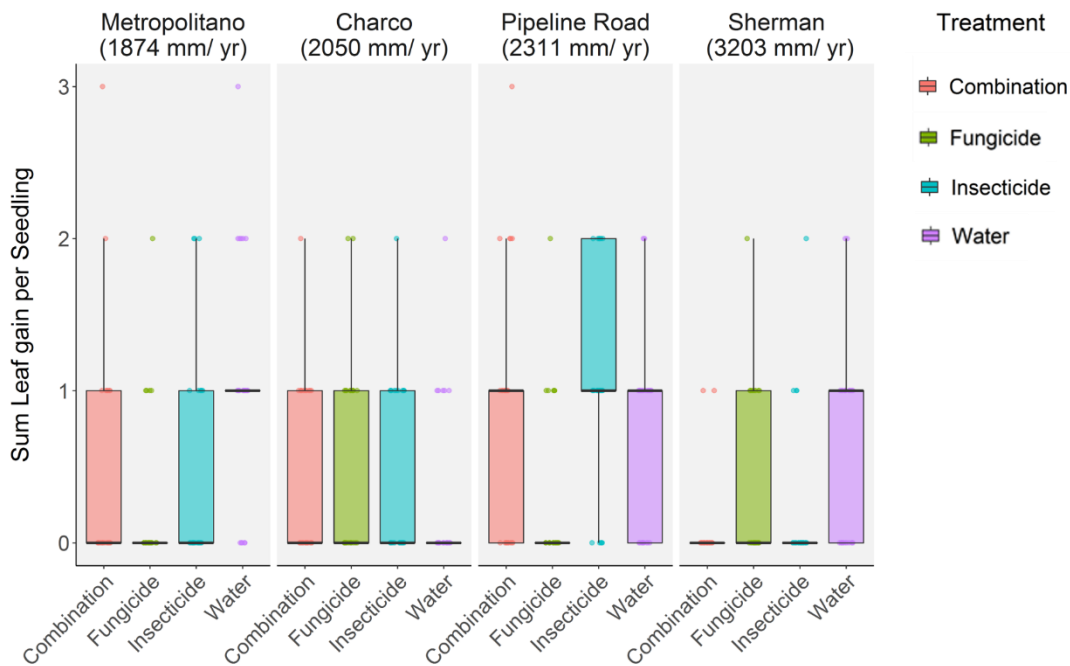


Figure 4. Histograms of raw data of leaf gain per seedling, split by treatment and site (rainfall per year). Boxes show interquartile range (IQR) from Q1 (25% of data points are below this value) to Q3 (25% of data points are above this value) with a bold line showing the median. Protruding lines show the upper and lower limits (1.5 times IQR above Q3 or below Q1). Dots are individual datapoints of leaf gain per seedling with some jitter applied to show there are multiple datapoints at some values of leaf gain. Dots laying outside the upper and lower limit lines are outliers.

Interactive effects of site and treatment

Not every seedling grew new leaves in the short time frame of the experiment (4 months). The incident rate ratios (Table 1) show the probability of leaf gain occurring in each treatment group in the given time. The intercept (water control treatment) had a ratio of 0.64 and a p value of 0.014 (Table 1). This means the likelihood of leaf gain occurring in control group was 0.64. The fungicide group was the only other treatment with a significant incidence rate ratio of 0.53 with a p value of 0.002 (Table 1). Therefore, plants in the fungicide group were slightly less likely to grow new leaves than the control group. The insecticide and combination groups did not have significant incidence rate ratios at 95% confidence interval (Table 1).

Table 1. Incident rate ratios of the effects of treatment groups on the leaf gain of seedlings, showing the likelihood of leaf gain during the experiment’s time span. CI is confidence interval.

Effects of Treatment on Leaf Gain

<i>Predictors</i>	<i>Incidence Rate Ratios</i>	<i>CI</i>	<i>p</i>
Intercept	0.64	0.44 - 0.91	0.014
Insecticide	0.85	0.59 - 1.21	0.364
Fungicide	0.53	0.35 - 0.80	0.002
Combination	0.70	0.48 - 1.01	0.059

Random Effects	
σ^2	1.11
τ_{00}	0.07
ICC	0.06
N_{Site}	4

Observations	400
Marginal R ²	0.045
Conditional R ²	0.104

Whilst only considering the effects of rainfall on leaf gain (removing the influence of treatment), the only statistically significant difference (Figure 6) across all sites after pairwise comparison was between Pipeline and Sherman (estimate = 0.978, $z = 3.63$, $p = 0.0016$) (Figure 6b). There were no significant differences in leaf gain between the different treatments overall when the influence of rainfall was removed (Figure 6a). However, some pairwise comparisons showed some significant differences (Figure 6d), meaning only combinations of certain sites and treatments caused a significant change to leaf gain, rather than an observable overarching trend. When split by site, there was a significant pairwise difference at Metropolitan (the driest site) for fungicide and water treatments (estimate = -1.50, $z = -3.33$, $p = 0.0048$). There were no significant differences between treatments at Charco. However, at Pipeline, the fungicide and insecticide treatments had a significant difference (estimate = -1.35, $z = -3.18$, $p = 0.008$). At Sherman there was a significant difference between the water control and combination pesticide treatments (estimate = -2.02, $z = -2.68$, $p = 0.037$).

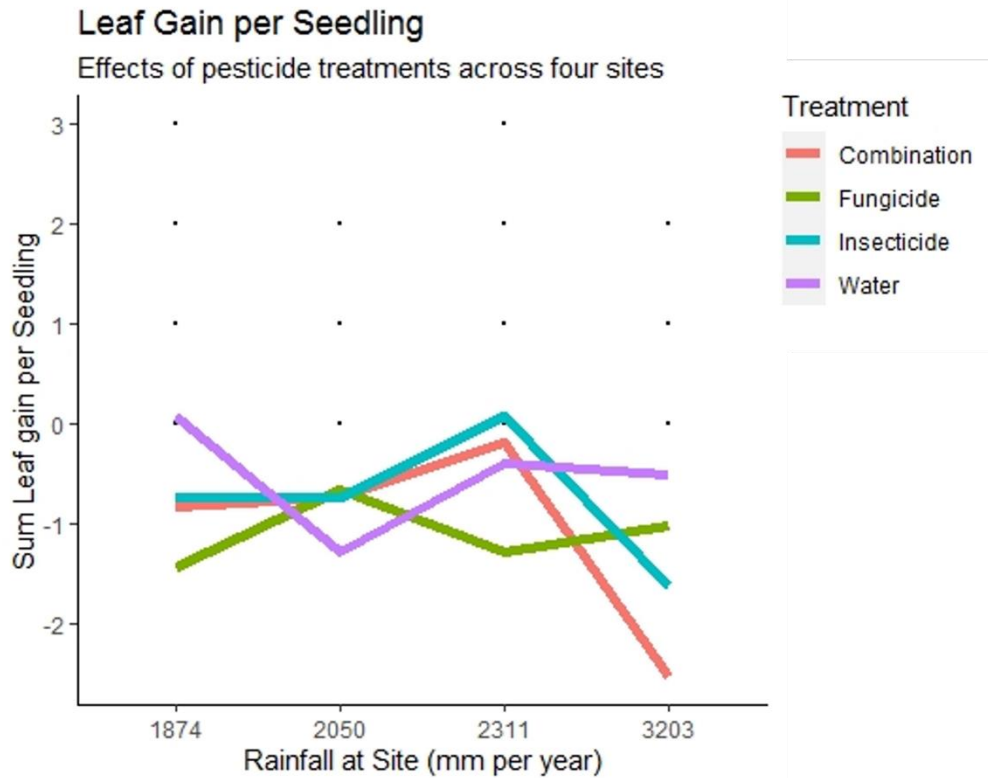


Figure 5. GLM showing leaf gain at each site, split by treatment. Lines show model gradients with colour to differentiate treatment, as shown in the key.

The intercept for the leaf gain model (Figure 5) was significant (estimate = -0.82, $z = -2.72$ $p = 0.006$). The effect of rainfall upon leaf gain was significant for the highest rainfall site, Sherman, which experiences $3203\text{mm}\text{y}^{-1}$ of rain (estimate = -1.70, $z = -2.21$ $p = 0.03$). Plants within the water treatment groups overall gained more leaves than those in the other treatment groups (estimate = 0.90, $z = 2.51$ $p = 0.01$). Some groups were responsible for driving these trends - within site Sherman (rainfall = $3203\text{m}\text{y}^{-1}$), seedlings within the fungicide treatment group were the only group with significantly different leaf gain (estimate = 2.11, $z = 2.26$ $p = 0.02$). Within the water (control) treatment group, the significant sites were Charco ($2050\text{mm}\text{y}^{-1}$) and Pipeline ($2311\text{mm}\text{y}^{-1}$), which were the two mid-rainfall sites. They both had a negative estimate suggesting lower leaf gain for plants with no pesticide protection at the mid-rainfall sites (Charco: estimate = -1.44, $z = -2.41$, $p = 0.02$; Pipeline: estimate = -1.12, $z = -2.29$, $p = 0.02$).

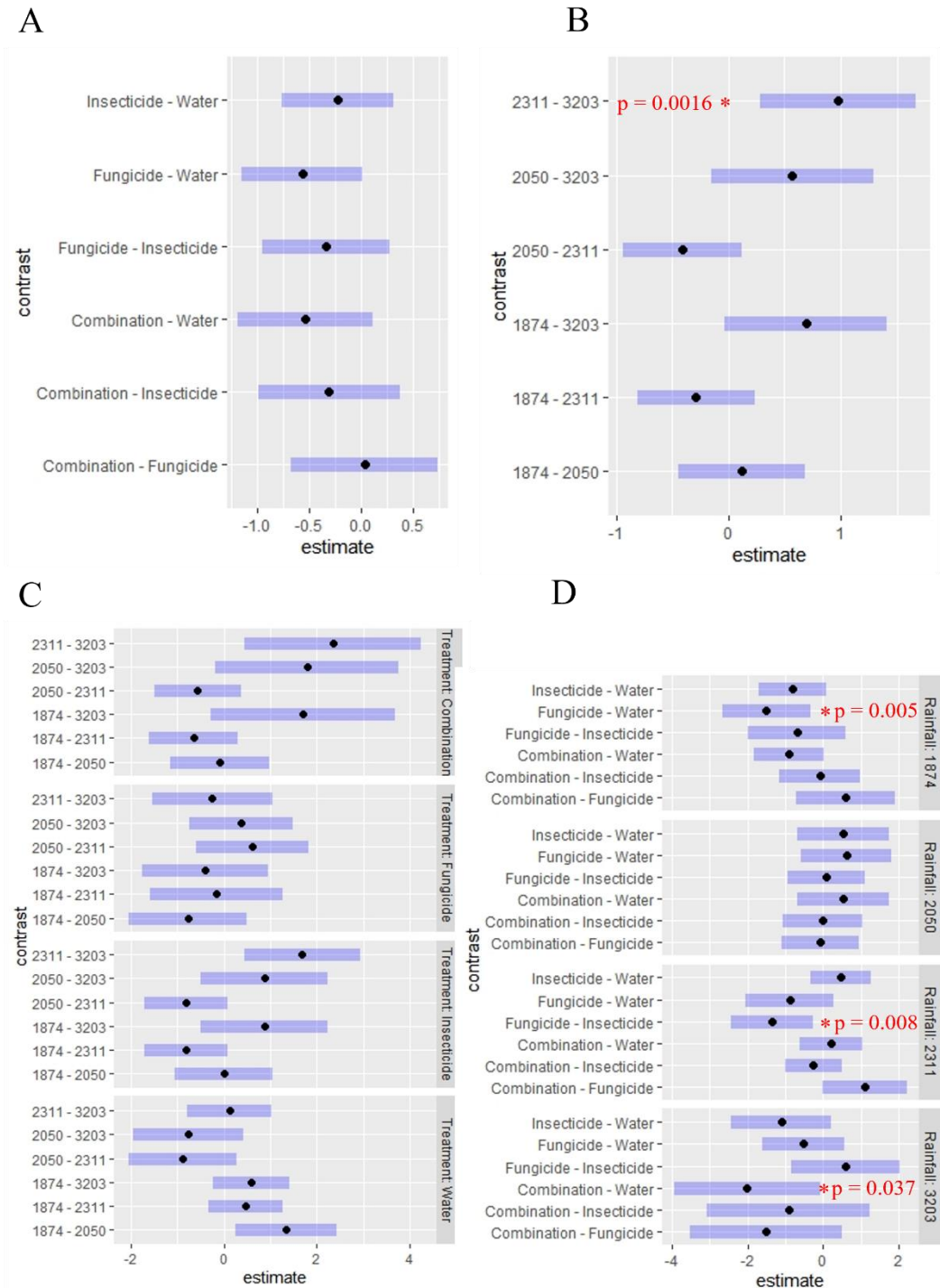


Figure 6. Pairwise comparisons of leaf gain between treatments and sites. Rainfall shown in mm per year. Significant pairwise relationships are indicated by a red asterisk with p value stated in red. **A)** Pairwise by treatment. **B)** Pairwise by Rainfall. **C)** Pairwise by rainfall within treatment. **D)** Pairwise by treatment within rainfall.

Hypothesis 2 – Fungal pathogen damage will increase with increased rainfall

Fungal damage was very low, with most seedlings experiencing 0% fungal damage (Figure 7). As presence of fungal damage was so low, most of the trends in the GLM (Figure 8) were driven by outliers (see Appendix Table A3 for outlier removed model). An interactive GLM with rainfall and treatment as interactive factors was used (Figure 8). The AIC (Akaike's Information Criterion) was 1133.6.

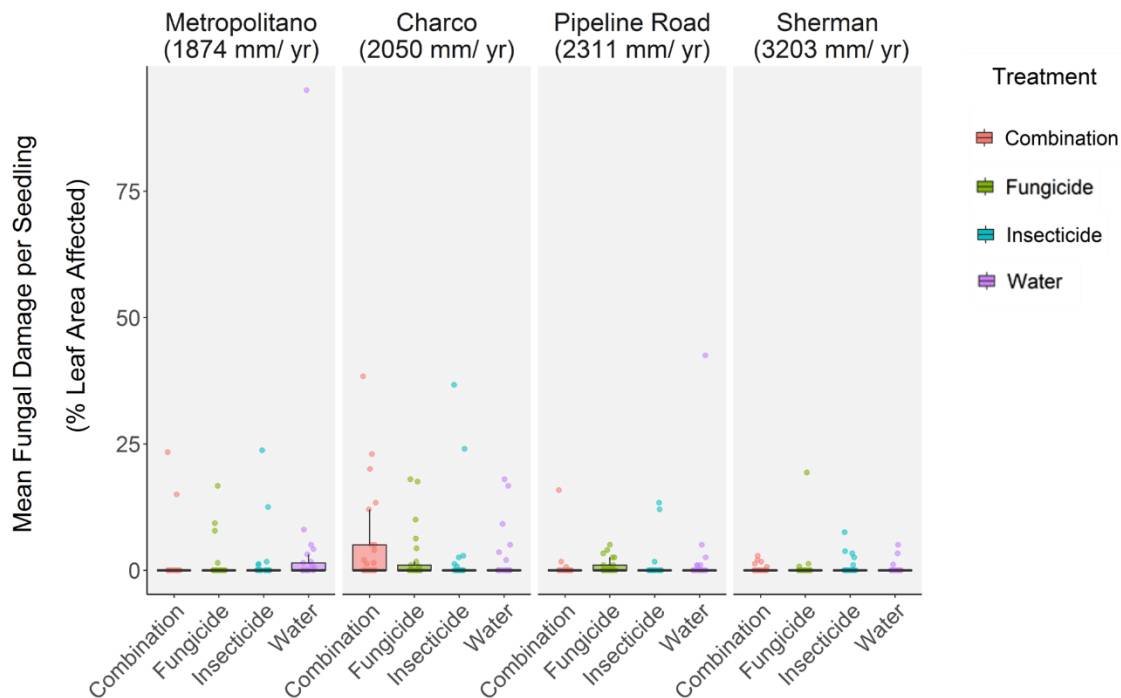


Figure 7. Histograms of raw data of fungal damage per seedling, split by treatment and site (rainfall per year). Boxes show interquartile range (IQR) from Q1 (25% of data points are below this value) to Q3 (25% of data points are above this value) with a bold line showing the median. Protruding lines show the upper and lower limits (1.5 times IQR above Q3 or below Q1). Dots are individual datapoints of mean fungal damage per seedling with some jitter applied to show there are multiple datapoints at some values of fungal damage. Dots laying outside the upper and lower limit lines are outliers. Most plants had no damage.

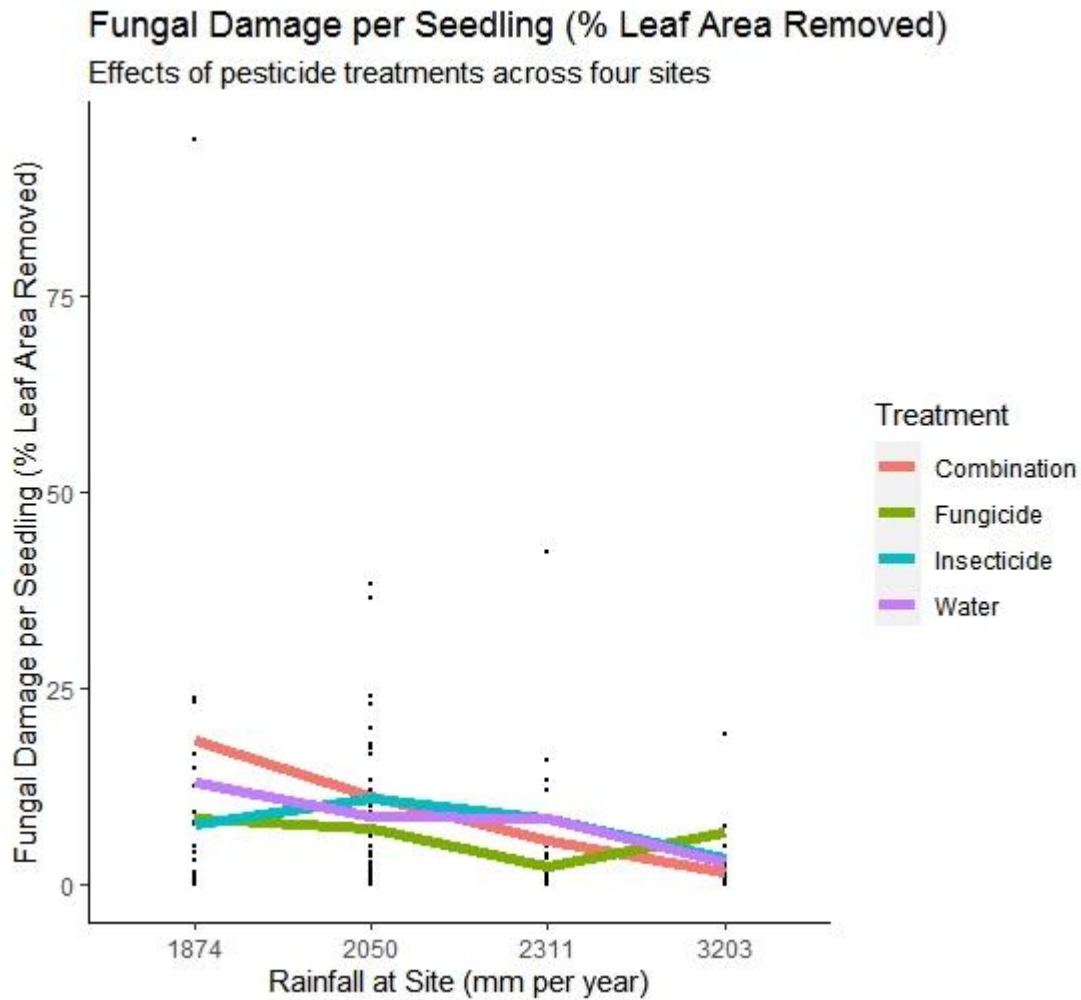


Figure 8. Interactive GLM showing fungal pathogen damage at each site, split by treatment. Coloured lines show the model gradients with treatments according to the key. Black dots are individual data points of fungal damage per seedling.

Odds ratios from the fungal damage model can be seen in Table 2. Odds ratios are a measure of likelihood, showing how likely an outcome is based on exposure to a certain treatment (Szumilas, 2010). An odds ratio of 1 would mean the predictor had no effect on the fungal damage (Table 2), with anything above or below 1 meaning higher or lower likelihood of fungal damage respectively (Szumilas, 2010). The intercept (Table 2) refers to the lowest rainfall site, Metropolitano, which is the only site with a significant effect on fungal damage according to the odds ratios (estimate = 18.54, $p = 0.035$). However, the confidence interval is relatively large, from 1.27 – 35.80 (Table 2). Whilst this shows an increase in fungal damage at this site, the large range is likely due to the raw data containing a lot of zero-values and a portion of comparatively extreme values or outliers, which can be seen in Figure 7 and Figure 8. No other sites, treatment groups or interactive effects were significant, as shown by the odds ratios in

Table 2 and estimates in Figure 9, which shows pairwise comparisons between treatments and rainfall (sites).

Table 2. Odds ratios of an interactive GLM with mean fungal damage per seedling as the response variable, and rainfall and treatment as interactive variables. CI is confidence interval.

Fungal Damage			
<i>Predictors</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
Count Model			
Intercept	18.54	1.27 - 35.80	0.035
Rainfall (2050)	-7.23	-25.94 - 11.47	0.449
Rainfall (2311)	-12.85	-34.74 - 9.05	0.250
Rainfall (3203)	-16.92	-37.12 - 3.28	0.101
Treatment (Fungicide)	-10.07	-31.00 - 10.85	0.345
Treatment (Insecticide)	-10.74	-30.99 - 9.51	0.298
Treatment (Water)	-5.34	-24.35 - 13.67	0.582
Rainfall (2050) * Treatment (Fungicide)	6.07	-17.59 - 29.74	0.615
Rainfall (2311) * Treatment (Fungicide)	6.83	-19.42 - 33.08	0.610
Rainfall (3203) * Treatment (Fungicide)	15.14	-11.88 - 42.16	0.272
Rainfall (2050) * Treatment (Insecticide)	10.55	-13.04 - 34.14	0.381
Rainfall (2311) * Treatment (Insecticide)	13.59	-14.27 - 41.45	0.339
Rainfall (3203) * Treatment (Insecticide)	12.61	-12.49 - 37.72	0.325
Rainfall (2050) * Treatment (Water)	2.90	-19.62 - 25.42	0.801
Rainfall (2311) * Treatment (Water)	8.25	-16.98 - 33.48	0.521
Rainfall (3203) * Treatment (Water)	6.67	-18.83 - 32.17	0.608
Intercept	12.18	10.51 - 14.11	
Zero-Inflated Model	1.25	1.01 - 1.49	<0.001
Intercept			
Observations	400		
Marginal R ²	NA		
Conditional R ²	0.947		

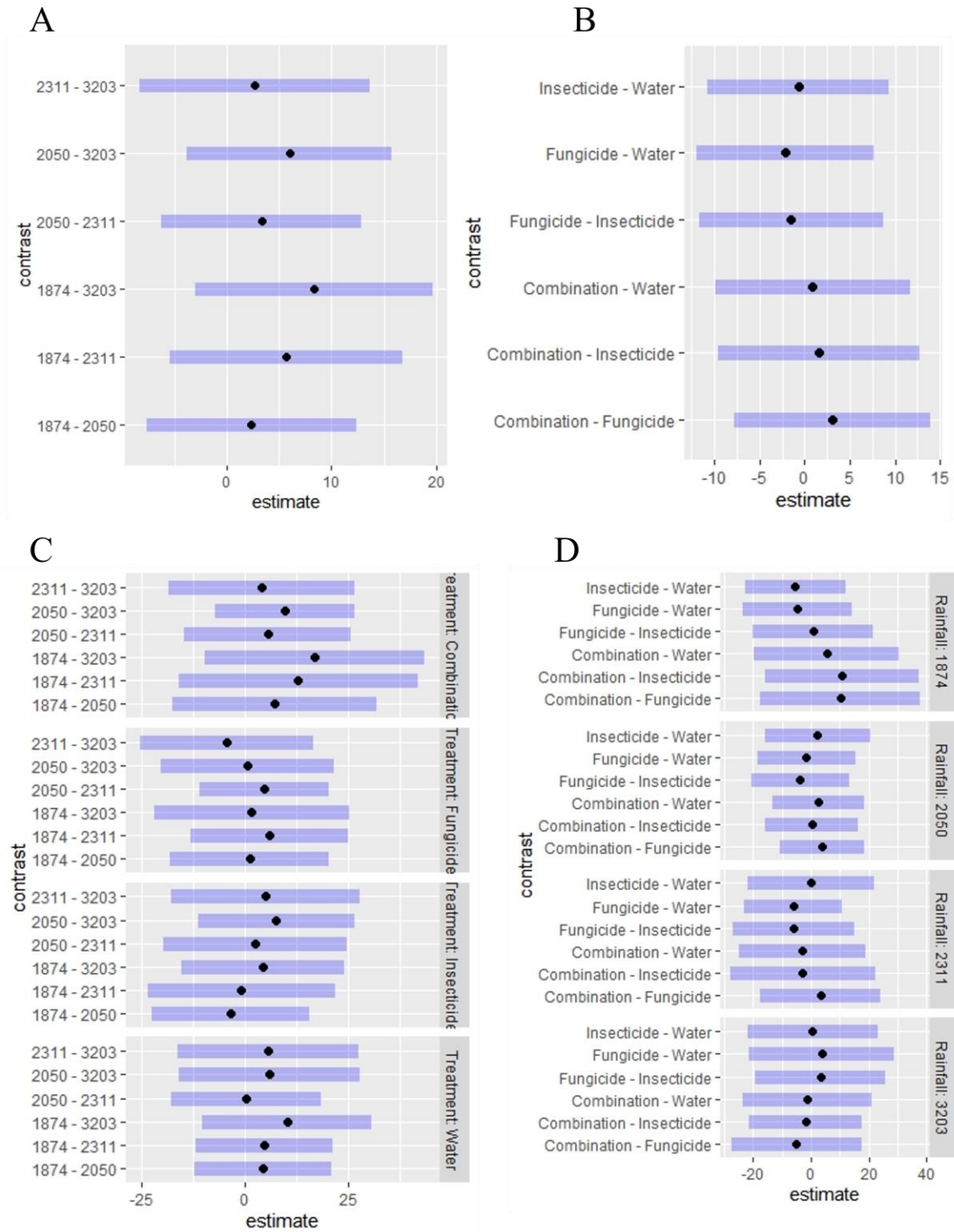


Figure 9. Pairwise comparisons for fungal damage between treatments and sites. Rainfall shown in mm per year. There were no significant pairwise comparisons for this response variable.

Hypothesis 3 – Insect herbivory will decrease with increased rainfall

Herbivory was also very low, generating a zero-inflated dataset (Figure 10). Most instances of herbivory were classed as outliers. An outlier-removed model for herbivory is shown in the appendix (Table A2).

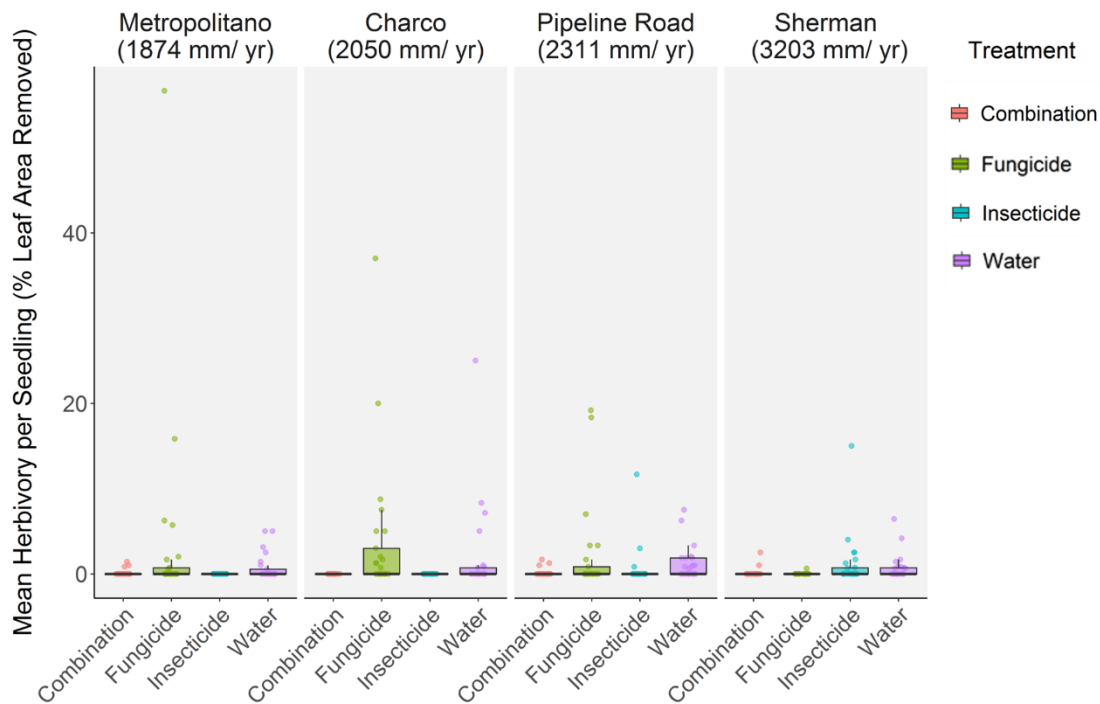


Figure 10. Histograms of raw herbivory damage per seedling, split by treatment and site (rainfall shown in mm per year). Boxes show interquartile range (IQR) from Q1 (25% of data points are below this value) to Q3 (25% of data points are above this value) with a bold line showing the median. Protruding lines show the upper and lower limits (1.5 times IQR above Q3 or below Q1). Dots are individual datapoints of mean herbivory per seedling with some jitter applied to show there are multiple datapoints at some values of herbivory. Dots laying outside the upper and lower limit lines are outliers.

Another GLM was made with mean herbivory per seedling as the dependent variable and rainfall and treatment as interactive variables (Figure 11). The intercept was estimated at 0.99 with a p value of 0.81. The AIC was 1002.8. None of the treatments or rainfall categories caused a significant change in the response variable except for the fungicide treatment, which had an estimate of 10.07 and p value of 0.04 (Table 3). This trend was driven by outliers as

shown by the outlier removed model, summarised in appendix Table A2. None of the pairwise comparisons were significant (Figure 12).

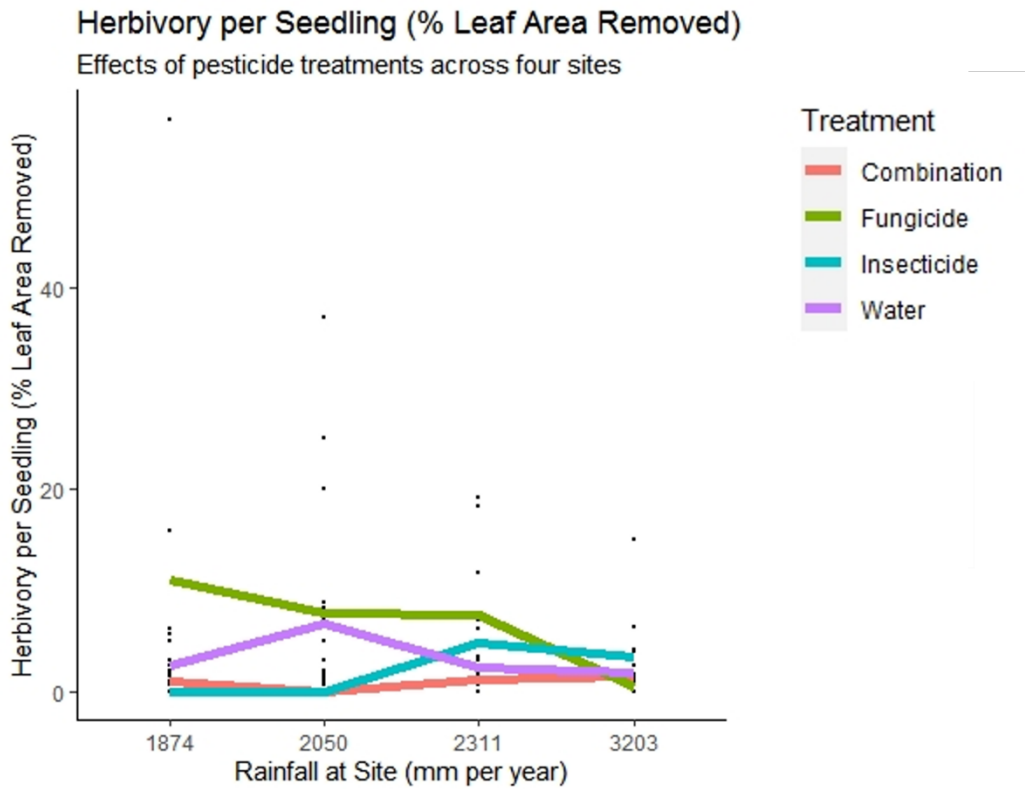


Figure 11. GLM showing herbivory at each site, split by treatment. Model gradients shown in coloured lines, with treatment specified by the colours in the key. Black dots show individual herbivory data points in percentage leaf area removed per seedling.

Table 3. Odds ratios of interactive GLM with mean herbivory damage per seedling as the response variable, and rainfall and treatment as interactive variables. CI is confidence interval.

Herbivory

<i>Predictors</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
Count Model			
Intercept	0.99	-7.22 - 9.20	0.814
Rainfall (2050)	-0.99	-27.39 - 25.41	0.942
Rainfall (2311)	0.20	-11.41 - 11.81	0.973
Rainfall (3203)	0.52	-12.28 - 13.33	0.936
Treatment (Fungicide)	10.07	0.30 - 19.85	0.043
Treatment (Insecticide)	-0.99	-27.39 - 25.41	0.942
Treatment (Water)	1.58	-8.33 - 11.50	0.754
Rainfall (2050) * Treatment (Fungicide)	-2.38	-29.65 - 24.89	0.864
Rainfall (2311) * Treatment (Fungicide)	-3.76	-17.71 - 10.20	0.598
Rainfall (3203) * Treatment (Fungicide)	-11.11	-30.05 - 7.82	0.250
Rainfall (2050) * Treatment (Insecticide)	0.99	-43.24 - 45.22	0.965
Rainfall (2311) * Treatment (Insecticide)	4.56	-24.33 - 33.46	0.757
Rainfall (3203) * Treatment (Insecticide)	2.92	-25.73 - 31.56	0.842
Rainfall (2050) * Treatment (Water)	5.11	-22.45 - 32.67	0.716
Rainfall (2311) * Treatment (Water)	-0.39	-13.95 - 13.81	0.955
Rainfall (3203) * Treatment (Water)	-1.21	-16.01 - 13.58	0.872
Intercept	7.61	6.55 - 8.85	
Zero-Inflated Model	1.30	1.05 - 1.54	<0.001
Intercept			
Observations	400		
Marginal R ²	NA		
Conditional R ²	0.913		

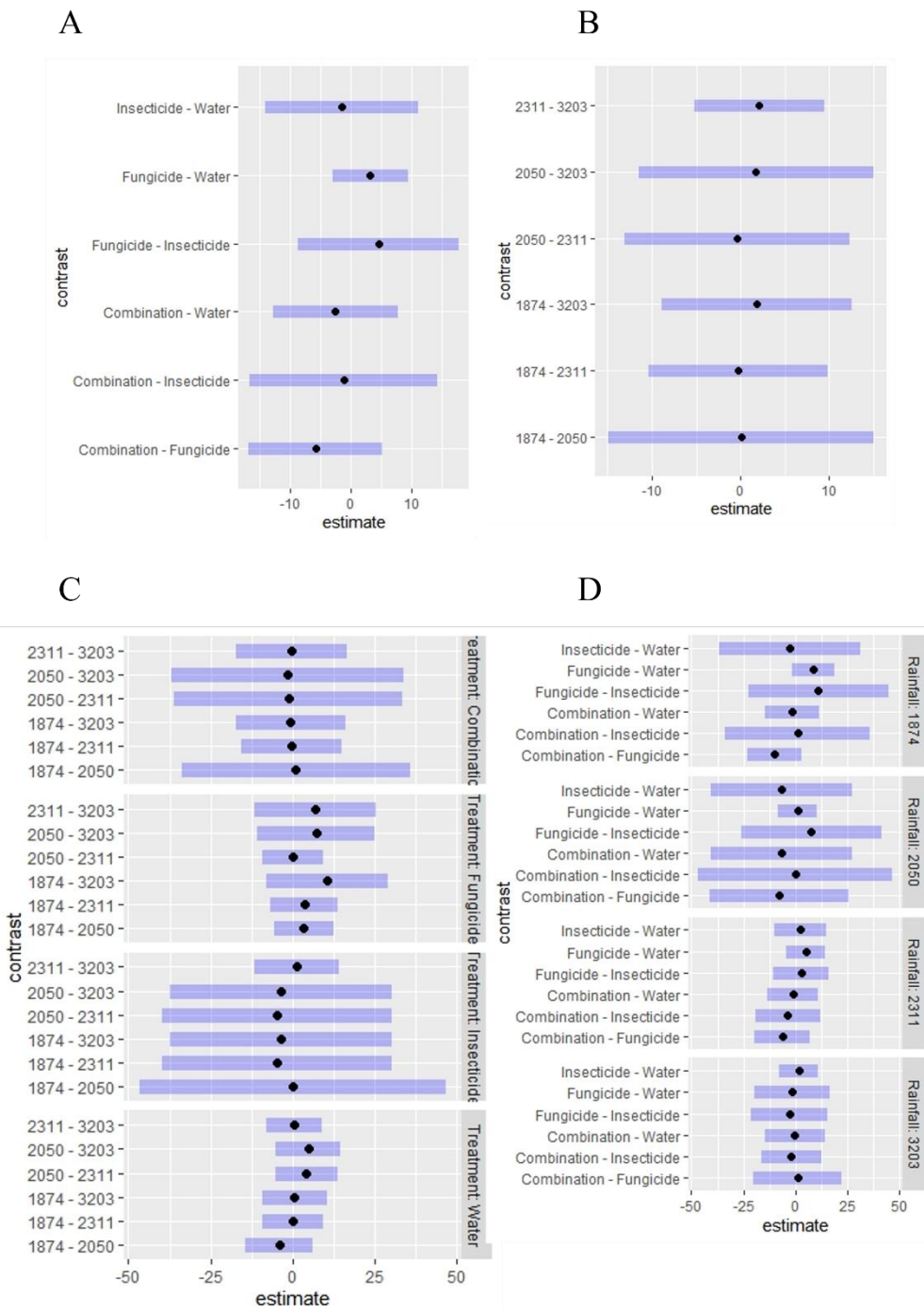


Figure 12. Emmeans-generated pairwise comparisons (for herbivory damage GLM) between treatments and sites (rainfall shown in mm per year). There were no significant pairwise comparisons for this response variable.

Outlier Removal

The leaf gain model had no outliers as all plants gained 0-3 leaves. For the herbivory model, AIC was 1002.8 with outliers and 755.1 without outliers. The trends were very similar except for some estimates in the fungicide treatment groups became negative when outliers were removed (they were previously positive) which led to the fungicide treatment group being statistically insignificant where it was previously significant. In the fungal damage model, the model with outliers included had an AIC of 1133.6, whereas the model excluding outliers had an AIC of 871.9. The trends remained similar but had stronger effects when outliers were removed with more interactions showing a significant p value.

Leaf Loss – Exploring Effects of Drought

In order to further investigate the seedlings' responses to treatments and rainfall, leaf loss was examined across the four months (Figure 13). As there was a shift from wet to dry season in the January of the experiment, it was important to consider the influence of drought on seedling success. The full model summary is available in the appendix (Table A6). The most leaves lost in a month was 8, which happened in the lowest rainfall site, Metropolitan, in January (Figure 13). Some plants retained all their leaves in any given month or treatment (Figure 13).

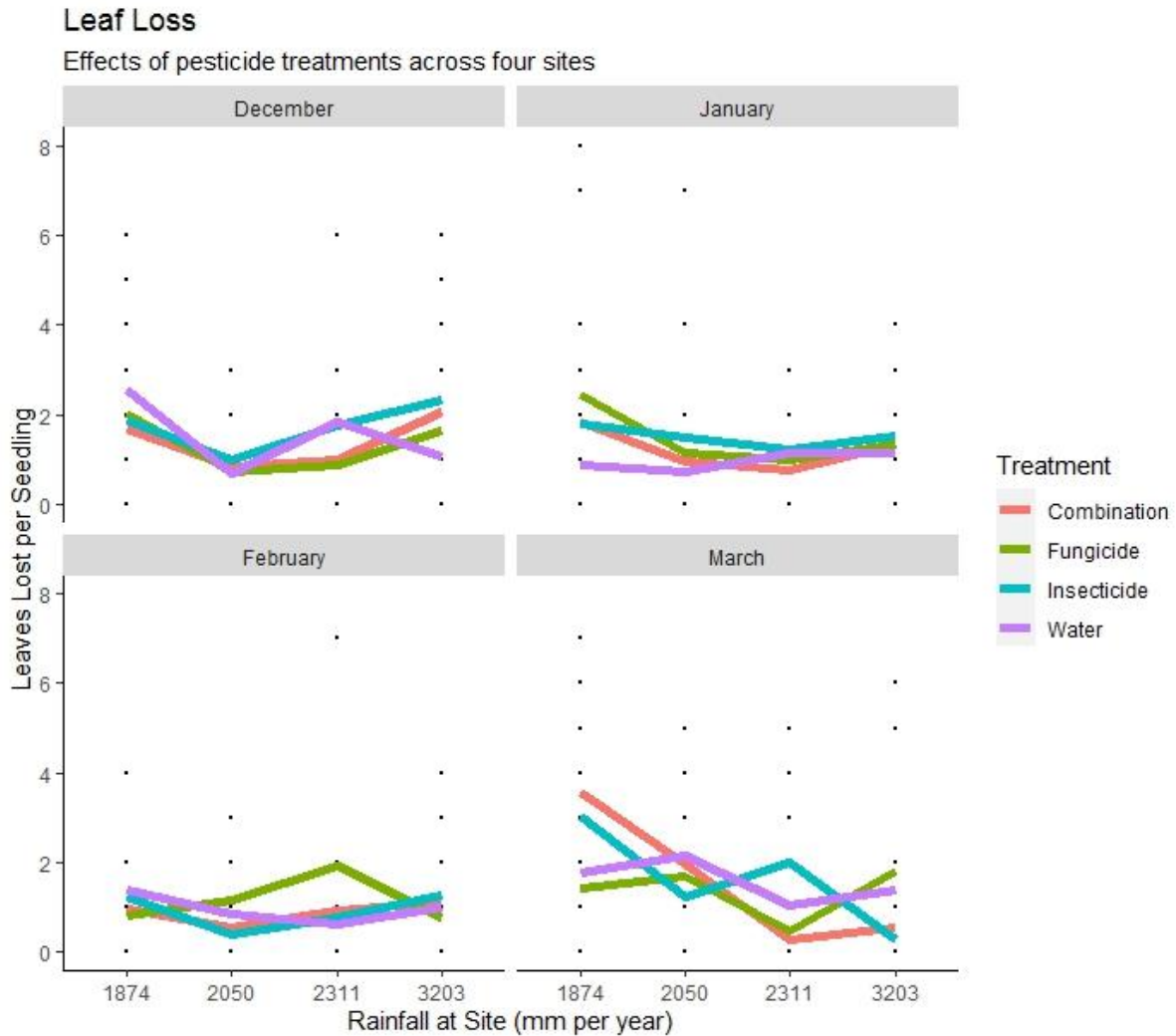


Figure 13. Visualisation of GLM for leaf loss per seedling, with treatment, rainfall and month as interactive terms. Model gradients are shown by coloured lines, split by treatment according to the key. Black dots show individual data points for leaf loss per seedling.

Pairwise comparisons were conducted for treatment on the model, which showed no treatment had a significant effect on leaf loss (Figure 14). As is visible in Figure 14, all estimates span zero. If an effect size spans zero, the result is insignificant, as a factor cannot cause a positive and negative effect on the outcome simultaneously. Therefore, pairwise comparisons were conducted for rainfall and month without treatment, to see if drought was responsible for the leaf loss (Figure 15).

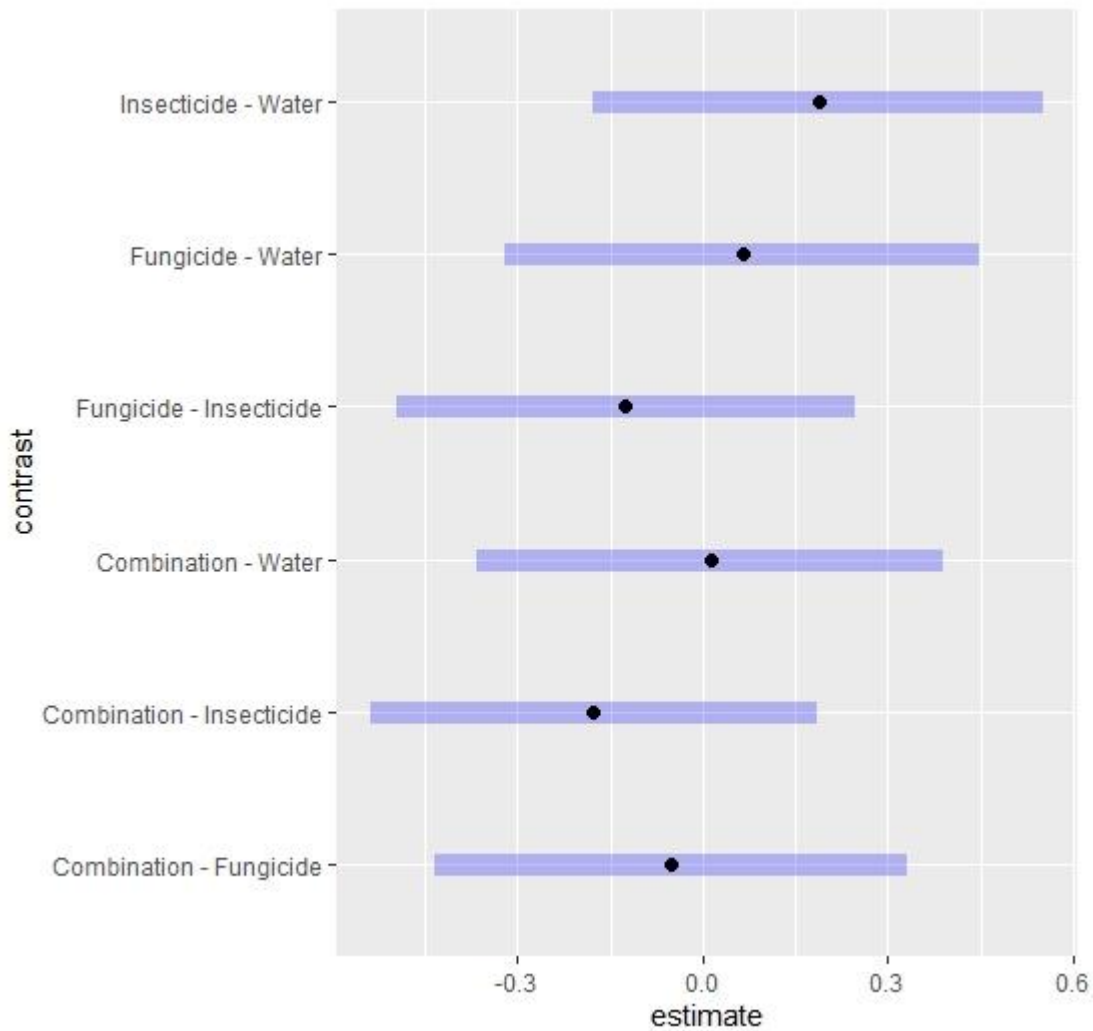


Figure 14. Pairwise comparisons for leaf loss between treatments. None have significant differences between the treatment groups.

Month 4 (March) had the most significant pairwise comparisons between rainfall (Figure 15), and also was the furthest month into the dry season. There was more difference between the two lower rainfall sites than the two highest ones (Figure 15), so rainfall differences had a stronger effect at the lower end of the scale. The biggest difference in leaf loss was when comparing the lowest rainfall site (Metropolitano) to the second-highest (Charco), with much higher leaf loss at the driest site. This supports the idea that drought played a role in leaf loss, however, the wettest site (Sherman) had slightly higher estimate for leaf loss than the second wettest site (Charco), so factors other than drought must be contributing to the leaf loss at the wetter sites (Figure 15).

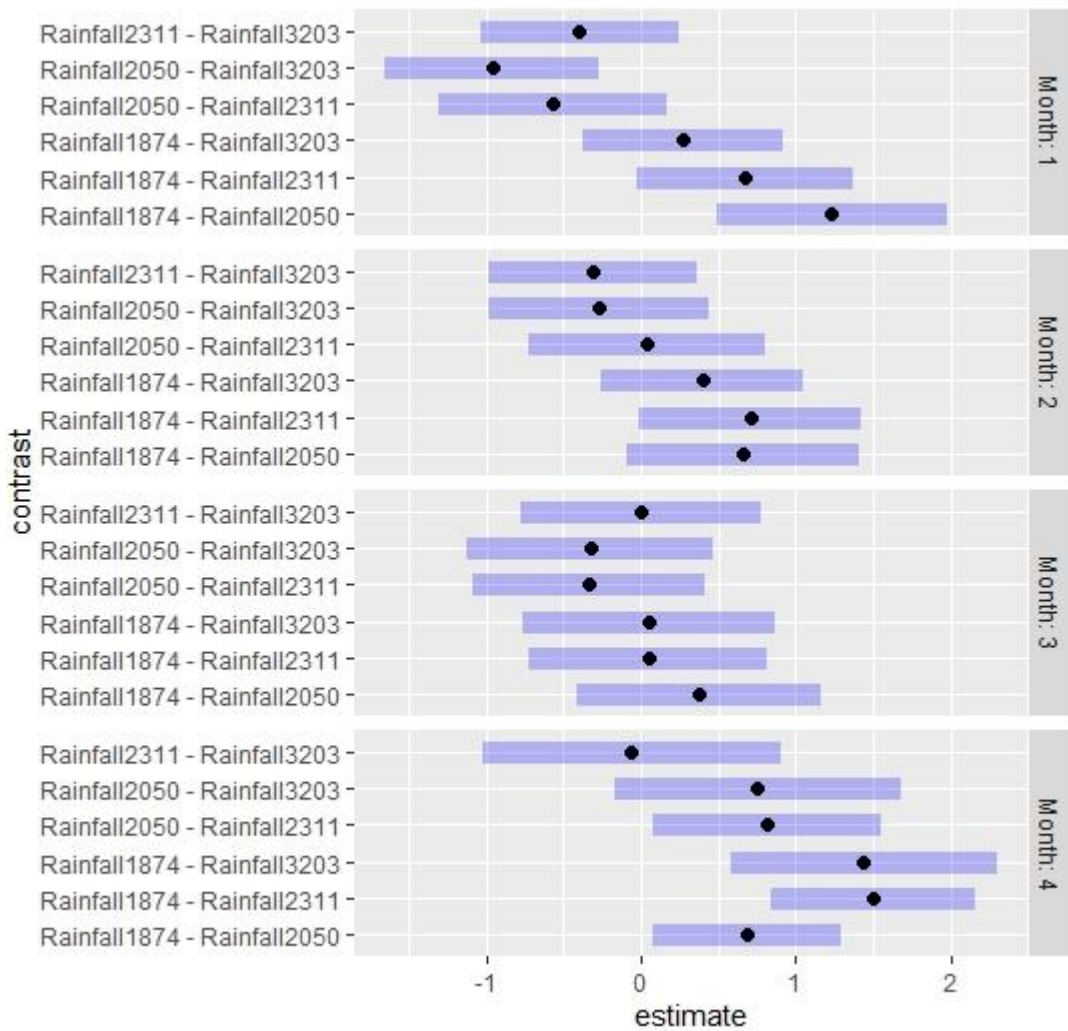


Figure 15. Pairwise comparisons for leaf loss between rainfall (sites) within each of the 4 months of the experiment.

DISCUSSION

Low herbivory damage was seen, averaging 1.15% per plant. Low levels of herbivory are consistent with previous studies on insect herbivory which range from 1% to 7.6% of damaged leaf area (Anstett *et al.*, 2015; Kozlov *et al.*, 2015; Myers & Sarfraz, 2017; Schlinkert *et al.*, 2015).

For the comparison of herbivory and fungal pathogen damage in different rainfall conditions, previous studies have traditionally been set up to compare one ‘wet’ and one ‘dry’ site, which does not take into account the gradient of rainfall which can occur across a species’ range (Brenes-Arguedas *et al.* 2009, Gaviria & Engelbrecht, 2015). The studies also place species in sites that they do not naturally grow in (reciprocal transplantation) (Brenes-Arguedas *et al.*

2009, Muehleisen *et al.* 2020). This study aimed to go beyond the previous studies by using multiple sites across the rainfall gradient, which provided us with the ability to compare how treatments affected the seedlings differently at a gradient of sites which varied in rainfall. Whilst this is more similar to how seedlings would naturally grow across a distribution, the stark contrast was lost in our study, meaning there were few significant differences between sites. This could be simply due to the short duration of the experiment, which was further cut short by the pandemic. Alternatively, there could be too many factors involved in the field too nuanced to be observable or measurable, which impact the overall trends. For example, in drier sites where more tree species are seasonal (Condit *et al.* 2000; Condit *et al.* 2001), light availability may change unevenly throughout the months as some trees drop their leaves. The effects of this could be positive, as the seedlings require light for photosynthesis, or negative as they would be more exposed and therefore vulnerable to drought. However, since it is known *L. aggregatum* naturally occurs at all four experimental sites, it is possible the study species could have adaptations that allow it to cope with a range of environments and rainfall, thus smoothing the trends of its response to challenges (Condit, R., 1998b, Santiago & Mulkey, 2005). In Brenes-Arguedas *et al.*'s study (2009), the seedlings were transplanted into a site that was not a part of their usual distribution and that they were not adapted to, which could be why the effects were stronger than in our study. Other researchers who have studied insect herbivory across rainfall gradients have used a longer time span, such as 1.5 years (Muehleisen *et al.* 2020). The limited timespan of our study (~4 months) may not have allowed enough time for insects to complete the full extent of herbivory that they did in previous experiments. This is supported by known distribution data for *L. aggregatum*, which occurs at 94% of dry sites and 70% of wet sites, converse to the mortality and leaf loss data in this study (Engelbrecht *et al.* 2007, Supplementary Data 3 & 4). Therefore, it is not unreasonable to assume the negative effects of drought must be counterbalanced during the rest of the year in order to make the focal species more successful in drier sites, which this study failed to observe.

Hypothesis 1 –The effect of pesticide treatment on leaf gain will vary across the rainfall gradient

In the control (water) treatment, where pests and pathogens were allowed to naturally predate the seedlings, the only significant difference in leaf gain was between the two lowest rainfall sites, Metropolitan and Charco. When insect herbivory was excluded, in the insecticide and combination treatments, there was almost no variation in leaf gain between the sites. This could

suggest that the insect herbivory was responsible for the change in leaf gain between the two lowest rainfall sites. Given that the only significant difference was between the two lowest rainfall sites, there may be different factors at play for seedlings growing at higher rainfall sites. For example, soil fertility increases tree diversity (Givnish, 1999), so the seedlings in this experiment may have benefitted from more fertile soils at some sites, which was unaccounted for. As this experiment was in the field, not every influence can be accounted for, so a limitation of this type of study is the inability to control every variable. In future studies, this could be overcome by using more plots at each site instead of just one plot per treatment. Additional variables could also be added to the analysis such as soil composition or light availability.

The pest exclusion treatments were not 100% effective despite being used to manufacturer instructions. It may be that they had been developed for farmland crops which do not experience the same range of predators as there would be in a biodiverse rainforest. An additional problem is the insecticide treatment would have been most effective in inhibiting leaf-residing chewing insects like lepidopteran larvae from carrying out long-term damage, as more mobile insects like leaf-eating beetles could have quickly eaten some of the leaf and then moved on before the insecticide took effect. Future experiments may benefit from different pesticides; pilot studies testing different application techniques; or utilising other pest-exclusion practices like netting. Unfortunately, in this experiment, netting could have protected seedlings from fungal pathogen infection as leaf litter would not have been able to fall on netted seedlings.

As only 25 seedlings were used per treatment block, the natural genetic variation of each plant could have caused less or more leaves to grow, regardless of environmental factors. Whilst this was controlled for by using multiple parent trees and randomised seeds, there would always be some seedlings naturally stronger than others. However, experiments in future could benefit from more seedlings and more treatment blocks.

Hypothesis 2 – Fungal pathogen damage will increase with increased rainfall

In this experiment, fungal pathogen damage was not significantly influenced by treatment, rainfall, or any interactions of the two. When comparing this to the results of older studies, it is apparent that this experiment's results do not conform to usual trends. Fungal pathogens usually function as a key role in seedling survival (Hersh *et al.* 2012). They have a major inhibitive effect on early tree growth (Hersh *et al.*, 2012; Schuldt *et al.*, 2017). Despite the effects of fungal pathogens changing across a rainfall gradient, a lot of fungal pathogens are

generalists (Spear, 2017, Weissflog *et al* 2018). Seasonality may play a role in fungal pathogen damage severity due to varying rainfall, and since this study only covered 4 months of data collection, it may have missed some of the divisive effects of the seasonality differences between wet and drier sites. This experiment was only running during one full month of the wet season (December) and so may have largely excluded fungal pathogens that need wetter conditions cause visible damage to seedlings. A study of at least one full year in length would be more effective in giving a full picture, as some outcomes may balance out throughout the seasons. Due to the slow-growing nature of the seedlings, multiple years with less frequent data collection may be more appropriate.

Hypothesis 3 – Insect herbivory will decrease with increased rainfall.

The insignificance of most results highlights the challenges of experiments in the field. Patterns previously observed by researchers have shown higher incidences of herbivory at higher rainfall sites (Brenes-Arguedas *et al.* 2009) whereas other research has observed more herbivory at drier sites (Weissflog *et al* 2018). Some of the limitations on pests at higher rainfall sites involve the physical restriction on movement (and therefore grazing) that heavy rainfall brings (Weissflog *et al* 2018). As our experiment covered a short time span of four months, during which the climate transitioned into the dry season, it could be possible the sites had little difference between them due to less rainfall overall, even at the wetter sites. Therefore, there would be less of a contrast in the insects' ability to predate the seedlings between wetter and drier sites. We know from past literature that pest pressure can alter the future distribution of a tree (Fine *et al.* 2004). However, different types of pests may be affected differently by the rainfall. Smaller insects are susceptible to desiccation but may have the advantage of only needing to eat from one plant for survival. Larger insects are less vulnerable to drying out but may need to move around more to forage enough food, making them more reliant on rain-free time to fly safely, for example. It is not currently known which specific pests usually target *L. aggregatum*. Finally, whilst the pesticide was not completely effective in preventing herbivory, any insect herbivores that visited plots with pesticide could have been killed by it, subsequently preventing them from visiting pesticide-free plots at that site.

Effects of Drought

In the limited timeframe of this study, the factor with the most conspicuous consequences was drought. As the months progressed into the dry season, a significant portion (50%) of seedling deaths occurred in the lowest rainfall site. Unfortunately, since the experiment was cut short by the COVID pandemic, it is not possible to see if the trend continued throughout

the dry season. However, the leaf loss analysis also supported the idea that drought was reducing the health of seedlings. Since the treatments had no effect on leaf loss, it is probable drought was the cause. In-field observations support this, as fallen leaves were often visible under the seedlings, completely brown in colour and dry in texture. Sometimes leaves were seen in this condition still attached to the plant, albeit insecurely. If drought was significant as suspected, there could have been secondary effects. The stomatal closure response of plants in drought that reduces their transpiration could also have inhibited fungal pathogen infections (Swinfield *et al.* 2012). However, plant health could also have been reduced as the seedlings would not have been able to carry out optimum gas exchange.

Limitations

Although there are benefits to growing the seedlings in a real-life, *in-situ* habitat, it removes some control over other factors in the environment. An example of this would be things like branch falls and large animals treading on the seedlings. Each damage occurrence was identified on a case-by-case basis, but there is possibility for misidentification of symptoms (e.g. fungal pathogens) without genetic analysis to confirm the damage source.

L. aggregatum was used because of its wide distribution across the rainfall gradient, which included the four sites used in the experiment. This was beneficial in providing a realistic view of how a plant species reacts to pressures in its environment. However, the downside of using only one species that is known to cope in these environments is that the adaptations the species has are not known. Future studies would benefit from analysis of the plant species for chemical adaptations that repel insects and fungal pathogens, and physical characteristics that make it succeed in fluctuating environmental conditions like rainfall.

The time frame of this study was limited by the length of the degree; a longer period of time may have provided further insight into how the seedlings coped with the pest pressures over time and shown different patterns of damage and growth in the wet and dry seasons. A longer time frame may also have reduced the number of plants which had zero herbivory or fungal damage, reducing the zero-inflation of the dataset. The time frame was further reduced by the start of the COVID-19 pandemic, in which entry to national parks was prohibited.

Height measurement was unable to be accurately measured due to soil washing into the experimental plots and settling around the base of the seedlings. This made height growth measurements inaccurate, in some cases making the height reduce rather than increase between months. This problem was further exacerbated by the slow growth of this species. However,

other metrics of growth (survival, leaf gain and loss) were able to be used instead. It would be recommendable for future researchers to use a brightly coloured, non-toxic but permanent mark on the base of each plant stem at the start of any height-related studies, so that subsequent height measurements can be taken from the mark to the plant apex, rather than relying on the soil level which has proven to be unreliable and subject to change.

The start of the pandemic and associated prohibited access of the field sites unfortunately prevented a planned portion of the data collection, including biomass assessments and more detailed pest damage measurements. It also meant studio photos of the seedlings and damage could not be taken.

CONCLUSION

This study has aimed to show how plant-pest interactions on the small-scale change across a large-scale abiotic rainfall gradient in four forest sites across Panama.

We planted 100 *L. aggregatum* seedlings in each forest site and treated them with either insecticide, fungicide, combination insecticide and fungicide, or water (control). We did a monthly data collection over four months. We measured seedling growth (height, leaf growth, leaf loss, mortality) for each seedling. We measured pest damage (herbivory and fungal damage) by visually measuring a percentage area estimate for each leaf in the field, to get a standing level of pest damage for each plant each month.

This study found no consistent difference in its hypotheses due to lack of significant trends. This could be due to multiple factors. Firstly, the short time span of the experiment reduced the available time for the seedlings to experience damage, leading to a dataset that mostly comprised of zeros. Secondly, the pesticide applications used were not fully effective in excluding pests and therefore treatment groups were similar to each other in damage levels of herbivory and fungal damage. Finally, in-field experiments may experience more random variation that can't be assigned to the measured variables because of influencing factors like small changes in soil type, insect communities, pre-existing fungal pathogens on nearby plants and disturbance from humans, large animals and branch falls.

This highlights the need for longer-term studies in future, especially those which take into account a larger number of measured factors which could affect seedling success such as humidity, soil type, nutrient and light availability and seasonality. There would also be advantages to using more sites with more treatment blocks replicated within each site to even

out the presence of random variables that may arise from the uncontrollably variable conditions within each site.

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APPENDIX

Leaf Gain Model

Table A1. R Summary output for interactive GLM for leaf gain with rainfall and treatment as predictor variables.

Call:

```
glm(formula = TotalLeafGain ~ Rainfall * Treatment, family = poisson(link = "log"),  
     data = junedata, na.action = na.exclude)
```

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.4697	-0.9485	-0.6325	0.5898	2.5293

Coefficients:

	Estimate	Std. Error	z value	Pr(> z)	
	-				
(Intercept)	0.82098	0.30151	-2.723	0.00647	**
Rainfall2050	0.08701	0.41742	0.208	0.83488	
Rainfall2311	0.64663	0.37219	1.737	0.08233	.
	-				
Rainfall3203	1.70475	0.76871	-2.218	0.02658	*
	-				
TreatmentFungicide	0.60614	0.50752	-1.194	0.23236	
TreatmentInsecticide	0.08701	0.41742	0.208	0.83488	
TreatmentWater	0.89794	0.3577	2.51	0.01206	*
Rainfall2050:TreatmentFungicide	0.68618	0.6464	1.062	0.28844	
	-				
Rainfall2311:TreatmentFungicide	0.49248	0.66937	-0.736	0.46189	
Rainfall3203:TreatmentFungicide	2.11021	0.93203	2.264	0.02357	*
	-				
Rainfall2050:TreatmentInsecticide	0.08701	0.58387	-0.149	0.88153	
Rainfall2311:TreatmentInsecticide	0.1643	0.50882	0.323	0.74676	
Rainfall3203:TreatmentInsecticide	0.82928	0.93501	0.887	0.37512	
	-				
Rainfall2050:TreatmentWater	1.43694	0.59509	-2.415	0.01575	*
	-				
Rainfall2311:TreatmentWater	1.10925	0.48414	-2.291	0.02195	*
Rainfall3203:TreatmentWater	1.11696	0.83343	1.34	0.18018	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for poisson family taken to be 1)

Null deviance: 386.64 on 399 degrees of freedom

Residual deviance: 323.98 on 384 degrees of freedom

AIC: 707.21

Number of Fisher Scoring iterations: 6

Outlier Included and Removed Models

Fungal Damage – Outliers Included

Table A2. R Summary output for interactive GLM for fungal damage with rainfall and treatment as predictor variables. Outliers were included in this model.

Family: gaussian (identity)

Formula: MeanFungal ~ Rainfall * Treatment

Zero inflation: ~1

Data: junedata

AIC	BIC	logLik	deviance	df.resid
1133.6	1205.4	-548.8	1097.6	382

Dispersion estimate for gaussian family (sigma²): 148

Conditional model:

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	18.538	8.81	2.104	*
Rainfall2050	-7.231	9.543	-0.758	0.4486
Rainfall2311	-12.846	11.17	-1.15	0.2501
Rainfall3203	-16.919	10.307	-1.642	0.1007
TreatmentFungicide	-10.075	10.675	-0.944	0.3453
TreatmentInsecticide	-10.743	10.331	-1.04	0.2984
TreatmentWater	-5.34	9.701	-0.55	0.582
Rainfall2050:TreatmentFungicide	6.073	12.073	0.503	0.6149
Rainfall2311:TreatmentFungicide	6.83	13.391	0.51	0.61
Rainfall3203:TreatmentFungicide	15.144	13.786	1.098	0.272

Rainfall2050:TreatmentInsecticide	10.549	12.035	0.876	0.3807
Rainfall2311:TreatmentInsecticide	13.593	14.213	0.956	0.3389
Rainfall3203:TreatmentInsecticide	12.614	12.809	0.985	0.3247
Rainfall2050:TreatmentWater	2.898	11.49	0.252	0.8009
Rainfall2311:TreatmentWater	8.255	12.873	0.641	0.5213
Rainfall3203:TreatmentWater	6.672	13.01	0.513	0.6081

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Zero-inflation model:

		Std.			
	Estimate	Error	z value	Pr(> z)	
(Intercept)	1.2467	0.1221	10.21	<2e-16	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Fungal Damage – Outliers Removed

Table A3. R Summary output for interactive GLM for fungal damage with rainfall and treatment as predictor variables. Outliers were not included in this model.

Formula: MeanFungal ~ Rainfall * Treatment

Zero inflation: ~1

Data: junefungoutrem

AIC	BIC	logLik	deviance	df.resid
871.9	943.3	-417.9	835.9	373

Dispersion estimate for gaussian family (sigma²): 19.2

Conditional model:

		Std.			
	Estimate	Error	z value	Pr(> z)	

(Intercept)	14.976	4.424	3.385	0.000711	***
Rainfall2050	-9.58	4.69	-2.043	0.041097	*
Rainfall2311	-9.367	5.129	-1.826	0.067797	.
Rainfall3203	-13.433	4.81	-2.793	0.00523	**
TreatmentFungicide	-6.343	4.964	-1.278	0.201334	
TreatmentInsecticide	-11.181	4.927	-2.269	0.023258	*
TreatmentWater	-11.977	4.682	-2.558	0.010533	*
Rainfall2050:TreatmentFungicide	8.282	5.434	1.524	0.127485	
Rainfall2311:TreatmentFungicide	3.125	5.806	0.538	0.590368	
Rainfall3203:TreatmentFungicide	11.519	5.926	1.944	0.051929	.
Rainfall2050:TreatmentInsecticide	7.442	5.578	1.334	0.182138	
Rainfall2311:TreatmentInsecticide	14.364	6.157	2.333	0.019655	*
Rainfall3203:TreatmentInsecticide	13.017	5.62	2.316	0.020546	*
Rainfall2050:TreatmentWater	15.565	5.259	2.96	0.00308	**
Rainfall2311:TreatmentWater	8.279	5.68	1.457	0.14498	
Rainfall3203:TreatmentWater	13.19	5.603	2.354	0.01856	*

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Zero-inflation model:

	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	1.3286	0.1283	10.36	<2e-16	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Herbivory Damage – Outliers Included

Table A4. R Summary output for interactive GLM for herbivory damage with rainfall and treatment as predictor variables. Outliers were included in this model.

Family: gaussian (identity)

Formula: MeanHerb ~ Rainfall * Treatment

Zero inflation: ~1

Data: junedata

AIC	BIC	logLik	deviance	df.resid
1002.8	1074.7	-483.4	966.8	382

Dispersion estimate for gaussian family (sigma²): 58

Conditional model:

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	0.986	4.1885	0.235	0.8139
Rainfall2050	-0.986	13.4699	-0.073	0.9416
Rainfall2311	0.1982	5.9248	0.033	0.9733
Rainfall3203	0.5233	6.532	0.08	0.9362
				0.0434
TreatmentFungicide	10.0723	4.9872	2.02	*
TreatmentInsecticide	-0.9862	13.4699	-0.073	0.9416
TreatmentWater	1.5841	5.0574	0.313	0.7541
Rainfall2050:TreatmentFungicide	-2.3825	13.914	-0.171	0.864
Rainfall2311:TreatmentFungicide	-3.7586	7.1207	-0.528	0.5976
	-			
Rainfall3203:TreatmentFungicide	11.1148	9.6604	-1.151	0.2499
Rainfall2050:TreatmentInsecticide	0.9864	22.5661	0.044	0.9651
Rainfall2311:TreatmentInsecticide	4.5617	14.743	0.309	0.757
Rainfall3203:TreatmentInsecticide	2.9156	14.6169	0.2	0.8419
Rainfall2050:TreatmentWater	5.1104	14.0607	0.363	0.7163
Rainfall2311:TreatmentWater	-0.3864	6.9213	-0.056	0.9555
Rainfall3203:TreatmentWater	-1.2146	7.5496	-0.161	0.8722

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Zero-inflation model:

	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	1.2955	0.1255	10.32	<2e-16	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Herbivory Damage – Outliers Removed

Table A5. R Summary output for interactive GLM for herbivory damage with rainfall and treatment as predictor variables. Outliers were not included in this model. The main groups changed by the removal of outliers are highlighted in yellow.

Family: gaussian (identity)

Formula: MeanHerb ~ Rainfall * Treatment

Zero inflation: ~1

Data: juneherboutrem

AIC	BIC	logLik	deviance	df.resid
755.1	826.5	-359.5	719.1	373

Dispersion estimate for gaussian family (sigma²): 6.84

Conditional model:

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	0.8492	1.3498	0.629	0.529
Rainfall2050	-0.8492	2.9367	-0.289	0.772
Rainfall2311	0.1756	1.9157	0.092	0.927
Rainfall3203	0.3872	2.098	0.184	0.854
TreatmentFungicide	1.7839	1.7206	1.037	0.3
TreatmentInsecticide	-0.8492	2.9367	-0.289	0.772
TreatmentWater	1.6431	1.6707	0.984	0.325

Rainfall2050:TreatmentFungicide	3.2837	3.2244	1.018	0.308
Rainfall2311:TreatmentFungicide	0.1953	2.4916	0.078	0.938
Rainfall3203:TreatmentFungicide	-2.7011	3.0097	-0.897	0.369
Rainfall2050:TreatmentInsecticide	0.8492	4.7147	0.18	0.857
Rainfall2311:TreatmentInsecticide	1.2065	3.6251	0.333	0.739
Rainfall3203:TreatmentInsecticide	1.3685	3.4841	0.393	0.694
Rainfall2050:TreatmentWater	2.0243	3.2841	0.616	0.538
Rainfall2311:TreatmentWater	-0.3205	2.2817	-0.141	0.888
Rainfall3203:TreatmentWater	-1.058	2.4718	-0.428	0.669

Zero-inflation model:

	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	1.2918	0.1341	9.633	<2e-16	***

Residuals of Models

Figure A1. Residuals plot for GLM fungal damage model.

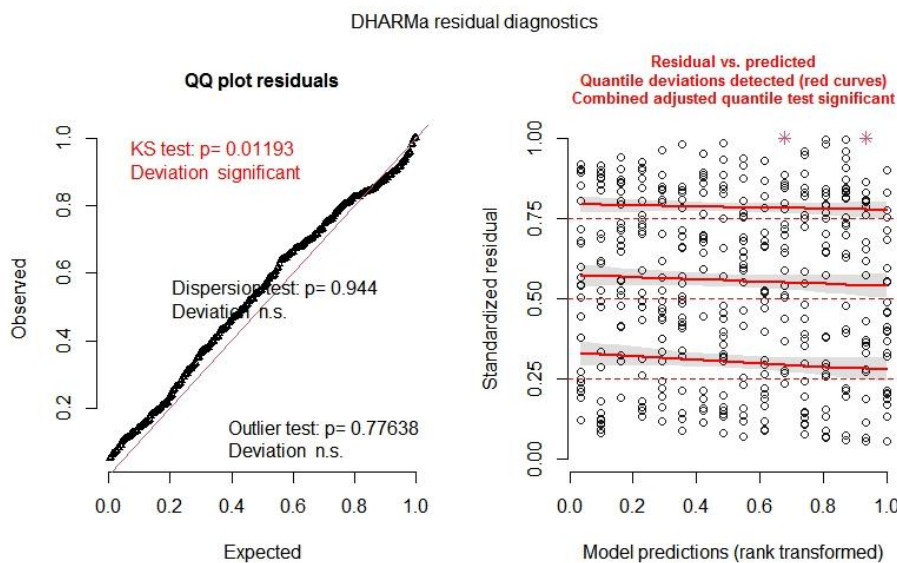


Figure A2. Residuals plot for GLM herbivory damage model.

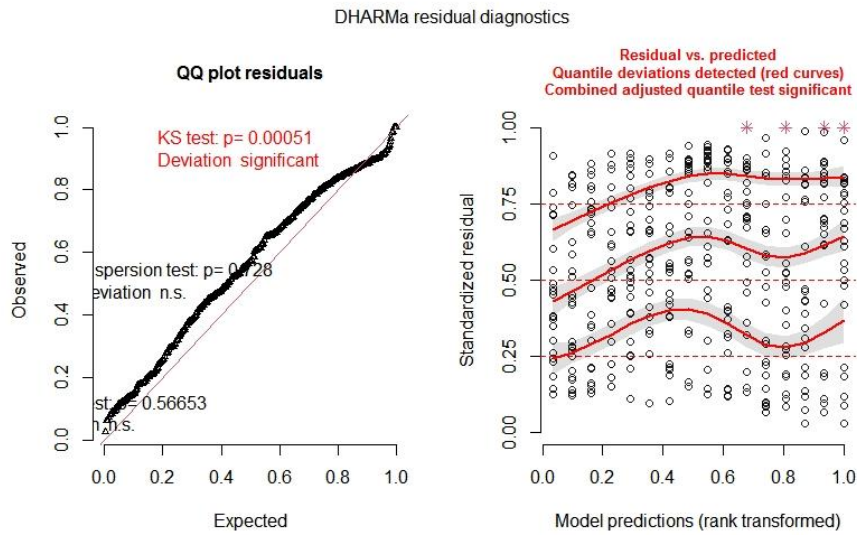


Figure A3. Residuals plots for fungal damage GLM.

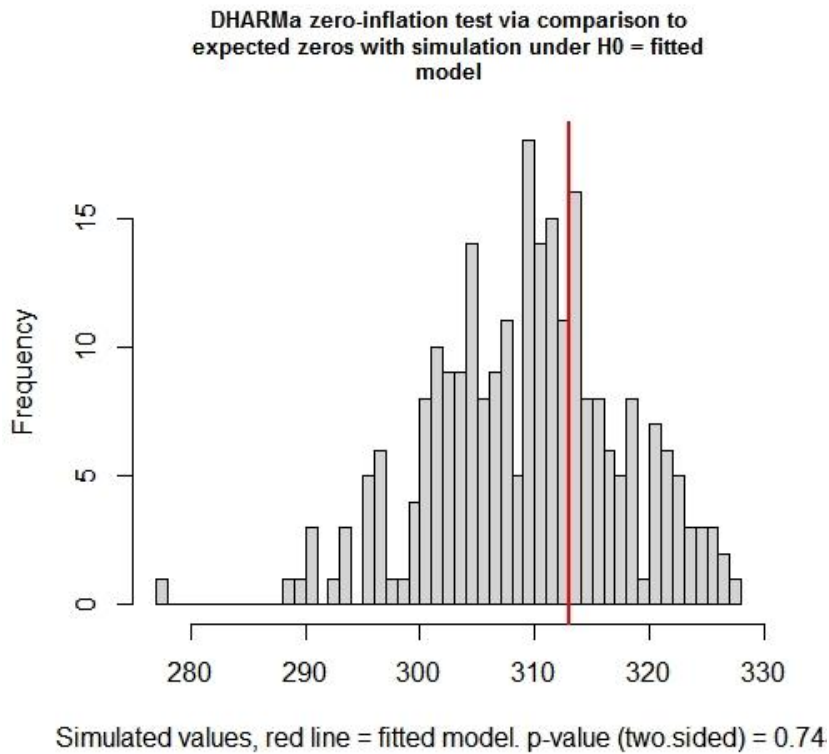
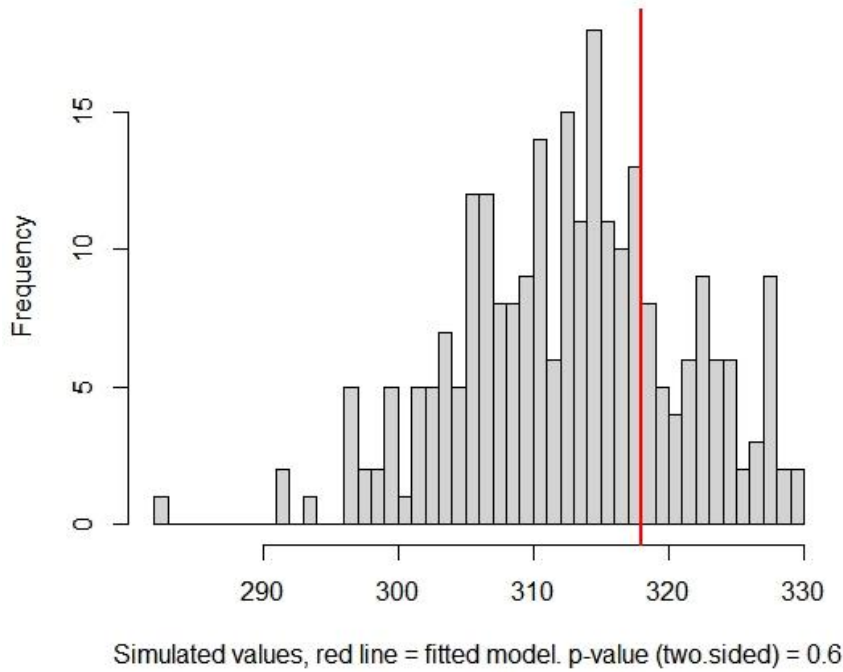


Figure A4. Residuals plots for herbivory damage GLM.

DHARMA zero-inflation test via comparison to expected zeros with simulation under H0 = fitted model



Leaf Loss Model

Table A6. Summary output for GLM for leaf loss with interactive effects of rainfall, treatment and month.

```
> summary(LeafLossModel2)
Family: gaussian ( identity )
Formula: LeafLoss ~ Rainfall * Treatment * Month
Zero inflation: ~1
Data: alldata
```

```
   AIC   BIC logLik deviance df.resid
3718.6 4073.5 -1793.3 3586.6   1534
```

Dispersion estimate for gaussian family (sigma²): 1.31

Conditional model:

	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	1.699346	0.447752	3.795	0.000147	***
Rainfall2050	-0.87405	0.62267	-1.404	0.160406	
Rainfall2311	-0.69762	0.621095	-1.123	0.261348	

Rainfall3203	0.356406	0.55851	0.638	0.523384	
TreatmentFungicide	0.315672	0.641312	0.492	0.622558	
TreatmentInsecticide	0.183323	0.530298	0.346	0.729569	
TreatmentWater	0.864506	0.556463	1.554	0.120286	
Month2	0.13658	0.579727	0.236	0.813748	
Month3	-0.71644	0.557512	-1.285	0.198768	
Month4	1.870641	0.512931	3.647	0.000265	***
Rainfall2050:TreatmentFungicide	-0.40001	0.870294	-0.46	0.645786	
Rainfall2311:TreatmentFungicide	-0.41827	0.867071	-0.482	0.629529	
Rainfall3203:TreatmentFungicide	-0.7377	0.794745	-0.928	0.353295	
Rainfall2050:TreatmentInsecticide	-0.03213	0.824034	-0.039	0.968898	
Rainfall2311:TreatmentInsecticide	0.564375	0.753319	0.749	0.453746	
Rainfall3203:TreatmentInsecticide	0.076681	0.688306	0.111	0.911295	
Rainfall2050:TreatmentWater	-1.0013	0.817967	-1.224	0.220903	
Rainfall2311:TreatmentWater	-0.03036	0.794551	-0.038	0.969517	
Rainfall3203:TreatmentWater	-1.85185	0.731494	-2.532	0.011354	*
Rainfall2050:Month2	-0.01869	0.873626	-0.021	0.982929	
Rainfall2311:Month2	-0.36423	0.853473	-0.427	0.669555	
Rainfall3203:Month2	-0.83502	0.764832	-1.092	0.274933	
Rainfall2050:Month3	0.438168	0.836226	0.524	0.60029	
Rainfall2311:Month3	0.66309	0.79125	0.838	0.402015	
Rainfall3203:Month3	-0.19398	0.849102	-0.228	0.819298	
Rainfall2050:Month4	-0.71415	0.743359	-0.961	0.336697	
Rainfall2311:Month4	-2.57872	0.805077	-3.203	0.00136	**
Rainfall3203:Month4	-3.37939	0.759216	-4.451	8.54E-06	***
TreatmentFungicide:Month2	0.311421	0.826027	0.377	0.706165	
TreatmentInsecticide:Month2	-0.21764	0.755152	-0.288	0.773188	
TreatmentWater:Month2	-1.83428	0.785056	-2.336	0.019465	*
TreatmentFungicide:Month3	-0.47787	0.81054	-0.59	0.555478	
TreatmentInsecticide:Month3	0.088428	0.724057	0.122	0.902797	
TreatmentWater:Month3	-0.4375	0.914999	-0.478	0.632551	
TreatmentFungicide:Month4	-2.4387	0.755738	-3.227	0.001251	**
TreatmentInsecticide:Month4	-0.70572	0.650189	-1.085	0.277739	
TreatmentWater:Month4	-2.66839	0.662898	-4.025	5.69E-05	***
Rainfall2050:TreatmentFungicide:Month2	-0.01477	1.181343	-0.012	0.990028	
Rainfall2311:TreatmentFungicide:Month2	-0.00644	1.19985	-0.005	0.995721	
Rainfall3203:TreatmentFungicide:Month2	0.088646	1.057145	0.084	0.933172	
Rainfall2050:TreatmentInsecticide:Month2	0.624327	1.170426	0.533	0.593744	
Rainfall2311:TreatmentInsecticide:Month2	-0.07056	1.078993	-0.065	0.947859	
Rainfall3203:TreatmentInsecticide:Month2	0.131759	1.014364	0.13	0.896651	
Rainfall2050:TreatmentWater:Month2	1.737192	1.20079	1.447	0.147979	
Rainfall2311:TreatmentWater:Month2	1.381566	1.127968	1.225	0.22064	
Rainfall3203:TreatmentWater:Month2	2.621048	1.042261	2.515	0.011911	*
Rainfall2050:TreatmentFungicide:Month3	1.165983	1.187606	0.982	0.326202	
Rainfall2311:TreatmentFungicide:Month3	1.551163	1.132431	1.37	0.170761	
Rainfall3203:TreatmentFungicide:Month3	0.53894	1.149387	0.469	0.639146	

Rainfall2050:TreatmentInsecticide:Month3	-0.38663	1.144102	-0.338	0.735412	
Rainfall2311:TreatmentInsecticide:Month3	-1.01039	1.070949	-0.943	0.345451	
Rainfall3203:TreatmentInsecticide:Month3	-0.19901	1.09942	-0.181	0.856355	
Rainfall2050:TreatmentWater:Month3	0.878518	1.234063	0.712	0.476532	
Rainfall2311:TreatmentWater:Month3	-0.72154	1.198974	-0.602	0.547308	
Rainfall3203:TreatmentWater:Month3	1.300734	1.236392	1.052	0.292781	
Rainfall2050:TreatmentFungicide:Month4	2.240568	1.104215	2.029	0.042448	*
Rainfall2311:TreatmentFungicide:Month4	2.735804	1.133208	2.414	0.015769	*
Rainfall3203:TreatmentFungicide:Month4	4.123408	1.287564	3.202	0.001362	**
Rainfall2050:TreatmentInsecticide:Month4	-0.1726	1.026944	-0.168	0.866531	
Rainfall2311:TreatmentInsecticide:Month4	1.679386	1.058261	1.587	0.112529	
Rainfall3203:TreatmentInsecticide:Month4	0.192385	1.008117	0.191	0.848654	
Rainfall2050:TreatmentWater:Month4	2.973691	1.019065	2.918	0.003522	**
Rainfall2311:TreatmentWater:Month4	2.577369	1.053405	2.447	0.014417	*
Rainfall3203:TreatmentWater:Month4	4.518636	1.130625	3.997	6.43E-05	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Zero-inflation model:

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	0.24298	0.06369	3.815	0.000136 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1