Mycorrhizal fungi influence disease during epidemics in plant populations

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Beneficial, ‘mycorrhizal’ fungi in roots help plants grow and can protect them from disease caused by other, pathogenic microbes. This study shows that mycorrhizal fungi influence patterns of plant disease during pathogen outbreaks in a natural landscape.

Summary

- While pathogenic and mutualistic microbes are ubiquitous across ecosystems and often co-occur within hosts, how they interact to determine patterns of disease in genetically diverse host populations is unknown.
- To test whether microbial mutualists provide protection against pathogens, and whether this is influenced by host genotype, we conducted a field experiment in three naturally-occurring epidemics of a fungal pathogen, *Podosphaera plantaginis*, infecting a host plant, *Plantago lanceolata*, in the Åland Islands, Finland. In each population, we collected epidemiological data on experimental plants, originating from six allopatric populations, which had been inoculated with a mixture of mutualistic mycorrhizal fungi or a non-mycorrhizal control.
- Inoculation with mycorrhizal fungi introduced benefits to host from every population, such as increased host growth and reduced disease severity. Host genotypes that were more susceptible to the pathogen received stronger protective effects from mycorrhizal fungi. However, inoculation with mycorrhizal fungi also increased host infection risk.
- Our results show that mycorrhizal fungi introduce growth and defensive benefits, and shift infection risks in host populations under pathogen attack. Understanding how mutualists alter host susceptibility to disease will be important for predicting infection outcomes in ecological communities and in agriculture.

Introduction

Protective symbionts – species that provide defensive benefits to their hosts – help determine the outcome of species interactions and, thus, shape ecological and evolutionary dynamics between hosts and parasites (Brownlie & Johnson, 2009; May & Nelson, 2014; King *et al*., 2016; Sochard *et al*., 2020). Despite their importance, ecological studies examining the role of protective symbionts in influencing host-parasite interactions in natural populations and communities are rare (Oliver *et al*., 2014; Hafer-Hahmann & Vorburger, 2021). Protection against pathogenic disease by mutualistic microbes, such as mycorrhizal fungi, has been demonstrated under controlled laboratory conditions in several economically important agricultural plant species (Norman *et al*., 1996; Pozo *et al*., 2002; Li *et al*., 2010; Song *et al*., 2015; Berdeni *et al*., 2018). However, it has not been verified whether protective symbionts mediate infection risk under natural epidemics, which are characterized by repeated pathogen encounters, as well as by environmental and genotypic diversity in both hosts and pathogens (Newsham *et al*., 1995). Mycorrhizal associations are widespread among terrestrial plants (Öpik *et al*., 2006; van der Heijden *et al*., 2008), and have important impacts on plant fitness and population dynamics (Barea *et al*., 2002; Koide & Dickie, 2002), community composition (Hartnett & Wilson, 2002) and ecosystem functioning (Rillig, 2004). Understanding how mycorrhizal fungi and other mutualists influence patterns of plant disease is essential given that disease is a major factor shaping the abundance, diversity, and distribution of species in plant communities (Bever *et al*., 2015), and affecting food production (Johansson *et al*., 2004; Gosling *et al*., 2006; Pretty *et al*., 2011; Hohmann & Messmer, 2017). While both plant-associated pathogenic and mutualistic microbes are ubiquitous across ecosystems, how they interact to determine disease risk in natural, genetically diverse populations is not known.

Mycorrhizal fungi produce a suite of growth, nutritional, and/or defensive benefits that may help protect plants from co-occurring antagonists, such as pathogenic microbes and herbivores (Delavaux *et al*., 2017). Association with mycorrhizal fungi often improves plant nutrient and water uptake (Smith & Read, 2008), though there is both intra- and interspecific variation among plants in their affinity to form and benefit from mycorrhizal associations (Thrall *et al*., 2011; Rasmussen *et al*., 2019). Increases in host size and nutritional status due to mycorrhizal association can improve host tolerance against antagonists and abiotic stress (Azcón-Aguilar & Barea, 1996). Mycorrhizal fungi can also influence host defenses directly, by

upregulating defense gene expression in their host plants (Azcón-Aguilar & Barea, 1996; Pozo & Azcón-Aguilar, 2007; Jung *et al*., 2012). This form of protection, known as defense priming, allows a more efficient activation of defense mechanisms in response to attack by potential enemies, and has been shown to reduce the negative effects of interactions with a wide range of antagonist species (Jung *et al*., 2012; Delavaux *et al*., 2017). Though mycorrhizal associations occur belowground (at the root-soil interface), the induced resistance response in the host is systemic (Cameron *et al.*, 2013), meaning that even strictly aboveground antagonists may also be affected (Koricheva *et al*., 2009). It is unclear how often mycorrhizal growth and defensive benefits are conferred together in hosts, and how they operate simultaneously to determine the incidence and outcome of interactions between hosts and parasites.

Association with mycorrhizal fungi or other mutualists can also present risks to hosts that may influence infection dynamics (Polin *et al*., 2014). Ecological costs can occur when combinations of host and mutualist species or genotypes are mismatched (Klironomos, 2003; Hoeksema *et al*., 2010), resulting in inefficient mutualisms that fail to convert host resources into necessary benefits (Johnson *et al*., 1997; Jones & Smith, 2004; Grman, 2012). Furthermore, unfavorable abiotic conditions could reduce or negate potential mutualist benefits (Hoeksema *et al*., 2010; Qu *et al*., 2021). In addition, defensive benefits from mutualists may not be effective against all antagonist species (e.g., depending on pathogen life history) (Pozo & Azcón-Aguilar, 2007), or be durable to changes in pathogen traits and/or composition in the environment. Hence, the potential ecological risks and/or benefits of mutualist associations with regards to disease may depend on host genotype and could vary among or within host populations and environments; however, this has remained largely unexplored.

In addition, it is unclear how mutualism-derived protection acts alongside innate host resistance to determine the outcome of host-pathogen interactions. Host genetic resistance can vary widely among and within natural populations (Salvaudon *et al*., 2008, Laine *et al*., 2011) - potentially due to costs associated with its maintenance (Brown, 2003; Susi & Laine, 2015) - and may be under a different set of selection pressures than mutualism (Thompson, 1994). Whether mutualism presents benefits or costs to host individuals and populations could depend on their level of resistance. In resistant hosts, resources provided to mutualists in return for defensive benefits could represent an unnecessary metabolic cost. However, in susceptible hosts, mutualist-derived protection could compensate for lack of genetic resistance, presenting a viable alternate

strategy for coping with pathogens. Mutualism-derived protection could be especially beneficial when genetic resistance is costly to maintain, or when disease is ephemeral, as it often is in natural populations (Burdon & Thrall, 2014). How much of host resistance is derived from innate genetic defenses versus derived from protective symbionts (e.g., defense priming, or improved pathogen tolerance), and the relative importance of each type of defense in different species combinations and environmental contexts, remains to be seen.

Despite general recognition for the impact of mycorrhizal fungi on plant fitness, how mycorrhizal association may impact host infection risk – and to what extent this varies among plant genotypes and populations – is poorly understood in natural populations. To examine this, we conducted a field experiment to test whether inoculation with arbuscular mycorrhizal fungi affects infection dynamics by a fungal pathogen in a shared host under natural epidemic conditions. Specifically, we ask: 1) Do growth effects due to mycorrhizal inoculation vary among host populations and maternal genotypes? 2) Does inoculation with mycorrhizal fungi influence host infection risk? 3) Upon infection, does prior inoculation with mycorrhizal fungi affect disease severity? and 4) Are disease susceptibility and defensive effects from mycorrhizal inoculation linked in host genotypes? To answer these questions within an ecologically relevant context, we placed mycorrhizal-inoculated and non-mycorrhizal-inoculated plants in wild host populations during a natural pathogen epidemic. The experiment was conducted in the long-term Åland Islands study site (Finland), where infection by a fungal pathogen (powdery mildew) has been surveyed on a large host population network of *Plantago lanceolata* L. (Plantaginaceae) since 2001 (Jousimo *et al*., 2014). From prior studies in this pathosystem, we know that several factors, such as spatial context (Laine, 2006; Soubeyrand *et al*., 2009; Jousimo *et al*., 2014), pathogen genetic diversity (Eck *et al*., 2022), local adaptation of pathogen strains to sympatric host populations (Laine, 2005; Laine, 2007a), and abiotic conditions (Laine, 2007b; Laine, 2008) are all critical in determining infection, but the impact of mutualistic interactions on infection dynamics has not been determined. To our knowledge, this is the first study of the impact of mycorrhizal fungi on infection risk by a plant pathogen under natural epidemics and across different host populations and genotypes.

Methods

Study system

Our study is focused on a fungal pathogen, *Podosphaera plantaginis* (Castagne) U. Braun & S. Takam., infecting *Plantago lanceolata* L. (Plantaginaceae) in the Åland Islands (60°08'53" N, 19°47'18" E), Finland. We carried out an experiment in three natural *Pl. lanceolata* populations that are part of a network of > 4000 mapped populations (Hanski, 1999). These populations have been surveyed for infection by *Po. plantaginis* since 2001 (Laine & Hanski, 2006). *Plantago lanceolata* (ribwort plantain) is native to Åland and much of Eurasia; it occurs mainly in small meadows and disturbed areas in Åland. It is monoecious, self-incompatible, and reproduces either sexually (via wind-dispersed pollen and seeds; Bos, 1992) or asexually (via clonally-produced side-rosettes) (Sagar & Harper, 1964). *Plantago lanceolata* associates commonly with mycorrhizal fungi and has been found in association with a wide variety of mycorrhizal species in Åland (Rasmussen *et al*., 2018; Eck *et al*., unpublished data).

Podosphaera plantaginis, a powdery mildew fungus (order Erysiphales), is an obligate biotroph of foliar tissue, and is host-specific in Åland, infecting only *Pl. lanceolata* (Laine, 2004). Fungal hyphae grow on the surface of *Pl. lanceolata* leaves, producing localized infections that mitigate host growth and reproduction (Bushnell, 2002), and may lead to mortality in the presence of abiotic stress, such as drought (Laine, 2004; Susi & Laine, 2015). Infections are transmitted via asexually-produced, wind-dispersed spores (conidia) that are produced cyclically (~ every two weeks) throughout an epidemic season (~ June to September in Åland) (Ovaskainen & Laine, 2006). Resting structures (chasmothecia), produced via haploid selfing or outcrossing between strains (Tollenaere & Laine, 2013), allow the pathogen to overwinter (Tack & Laine, 2014). In Åland, *Po. plantaginis* persists as a metapopulation, with frequent colonization and extinction events (Jousimo *et al*., 2014). Resistance in *Pl. lanceolata* against *Po. plantaginis* is strain-specific (Laine, 2004; Laine, 2007a). Previous studies have demonstrated high levels of variation in resistance within and among host populations (Laine, 2004; Laine 2007a, Jousimo *et al*. 2014).

Mycorrhizal inoculation of experimental plants

To measure the effects of association with mycorrhizal fungi in genetically diverse host plants, seeds were collected from six geographically-variable populations of *Pl. lanceolata* in the Åland Islands (Fig. S1) in August 2007. Seeds were collected from five haphazardly chosen maternal plants in each population and were stored separately in paper envelopes (seeds from

one maternal plant are half or full siblings). In April 2008, seeds from each of the 30 maternal plants were planted in separate 6 x 6 x 7 cm pots in a greenhouse at the University of Oulu (Oulu, Finland) in sterilized sand. Ten healthy two-week-old seedlings from each maternal genotype were transferred to individual pots (one seeding per 6 x 6 x 7 cm pot) filled with experimental substrate (a 5:4:1 mixture of heat-sterilized sand : heat-sterilized garden soil : perlite, combined with 1 g of bone meal and 3 g of dolomite per liter substrate). At the time of transfer, five of the seedlings from each maternal genotype were inoculated with mycorrhizal fungi, while the other five received a non-mycorrhizal control treatment. The mycorrhizal inoculum consisted of a mixture of spores of three arbuscular mycorrhizal fungal species native to Finland (*Glomus hoi*, *Claroideoglomus clareoideum*, and *Gl. mosseae (BEG 29)*), as *Pl. lanceolata* is colonized naturally by several mycorrhizal symbionts (Johnson *et al*., 2003). Allopatric isolates of each species (originating from central Finland, 61º10 N, 24º40 E, rather than Åland) were used so as not to confound the experiment with potential local adaptation between mycorrhizal fungi and their host populations (Hoeksema *et al*., 2010). Spore inocula were produced by growing the mycorrhizal species with non-experimental *Pl. lanceolata* in a soil substrate identical to the experimental one. Spores were washed out of the non-experimental substrate with water, and 15 spores of each species (45 spores in total) were pipetted on to the roots of each seedling in the mycorrhizal treatment in 2 mL of water (6 mL in total); seedlings in the non-mycorrhizal control treatment received 2 mL of filtered spore washing water from each fungal species (6 mL in total). Hereafter, plants in the mycorrhizal-inoculated treatment are abbreviated as ‘AMF’ (i.e., arbuscular mycorrhizal fungi), and plants in the non-mycorrhizal control treatment as ‘NM’ (i.e., non-mycorrhizal). All experimental seedlings were also inoculated with bacteria at this time: bacteria were filtered from a soil mix collected from the six Åland seed source populations to restore the native, non-mycorrhizal soil microbial community. Seedlings were fertilized weekly with a dilute nitrogen-based solution. Natural light in the greenhouse was supplemented with Osram HQI lamps to provide a photoperiod of 18 h day / 6 h night. At six weeks of age (four weeks post-inoculation), the plants were moved to an outdoor area at the University of Oulu and grown on tables under a transparent plastic roof for an additional six weeks, to acclimatize to field conditions (as *Pl. lanceolata* and *Po. plantaginis* do not inhabit this region, infection at this stage was highly unlikely).

Natural epidemic field experiment

Ten weeks post-inoculation (mid-July 2008), 288 healthy twelve-week-old experimental plants were placed in to three naturally-occurring populations of *Pl. lanceolata*, infected by populations of *Po. plantaginis*, in Åland, to gain infections naturally from the surrounding epidemic. The three field epidemic populations were allopatric to the six seed origin populations (see Fig. S1 for a map). Thus, they represent common garden field sites, in which the host genotypes are not locally adapted to the local environmental conditions or to the local pathogen population. Presence of *Po. plantaginis* at the sites was confirmed by surveying the populations for visible signs of infection prior to the placement of the experimental plants. Each field site received 96 experimental plants: 16 plants from each of the six seed origin populations (eight AMF plants and eight NM plants). Within these subgroups, the five maternal genotypes within each seed origin population were allocated as evenly as possible to the three field sites; the individuals placed in each site were selected at random (full, pairwise replication of every maternal genotype × mycorrhizal treatment combination within every field site was not possible due to lack of plants). At each field site, experimental plants were randomly fitted in to one of 96 plastic containers (14 x 10.5 x 4.5 cm) that were placed on the ground in a random array in the proximity of naturally-infected, wild *Pl. lanceolata* individuals inhabiting the field population. The containers prevented contact between the bottom of the pots and the soil, reducing the likelihood that the experimental plants would acquire soil microbes from the environment.

At the time of placement in the field epidemic populations, we measured the initial size of each experimental plant: the total number of leaves, as well as the length and width (in cm) of the longest leaf were recorded. All experimental plants were uninfected (i.e., had zero leaves infected by *Po. plantaginis*) at the beginning of the experiment. Infection surveys were then conducted every three days at each field site, on a rotating survey schedule (1st day field population 9051, 2nd day field population 3484, and 3rd day field population 9066). During each infection survey, the number of healthy leaves and the number of leaves infected by *Po. plantaginis* were measured (leaves that withered during the experiment were not counted). The plants were also re-randomized into a new position in the experimental array (this was done to minimize the effect of spatial positioning, with respect to distance to infected individuals and prevailing wind direction, on infection risk and severity), and were watered if necessary. Seven infection surveys were conducted in each field population. At the end of the experiment (in late

August 2008) a haphazardly-selected subset of 21 AMF plants and 20 NM plants were harvested, and their oven-dried aboveground biomass was measured. We created a metric approximating the leaf area of each plant at the beginning of the experiment by first applying the length and width of the plant's longest leaf to the equation yielding the area of an oval, then multiplying the area of the longest leaf by the total number of leaves on the plant. Heavy rains during the 4th and 5th surveys resulted in missing infection data for some field populations (identifying symptoms caused by *Po. plantaginis* on wet leaves is challenging) and may have influenced infection in the 6th and 7th surveys in all populations (as heavy rain washes spores away from infected leaf tissue and damages spore viability; Sivapalan, 1993). Because of this, we focus here on infection data from the 3rd survey (coinciding with peak infection rates and the completion of one 14-day initial pathogen infection and reproduction cycle), as well as the final, 7th survey at the end of the experiment (coinciding with re-infections because of pathogen reproduction and incorporating variable abiotic conditions). Using data from these two surveys (peak epidemic and end-of-experiment), we quantified the infection status (0 / 1, infected or uninfected) and calculated the infection severity (i.e., the proportion of infected leaves) of each experimental plant. Plants were considered infected if one or more leaves showed signs of infection. One plant that died during the experiment was excluded from all analyses.

Statistical methods

Do growth effects due to mycorrhizal inoculation vary among host populations and maternal genotypes?

To test whether host growth was explained by inoculation with mycorrhizal fungi, host genetic origin, or an interaction between these factors, we built a series of multiple linear regression models. To estimate the effect of genetic origin on host growth, seed origin population (abbreviation: POP) and maternal genotype (abbreviation: GEN) were each included as explanatory factors in separate models. Host growth was modelled both prior to pathogen exposure (i.e., initial host size at the time of placement in the field epidemics) and post-pathogen exposure (i.e., final host size during the final survey of the experiment). In each model, mycorrhizal treatment (abbreviation: MYC) was included as an explanatory factor, as was an interaction term between MYC and the host genetic origin term (POP or GEN). The log-transformed metric estimating total leaf area was used as the response variable in models of host

size prior to pathogen exposure. The log-transformed total number of leaves on the plant was used as the response variable in models of final host size (as data on leaf area was not available). Field epidemic population (abbreviation: SITE) and log-transformed initial host size (abbreviation: SIZE) were also included as explanatory factors in the end-of-experiment models. We also tested whether a small subset of AMF and NM plants varied in biomass at the end of the experiment using a linear regression model (POP and/or GEN and SITE were not included in this model due to low replication). Non-significant interaction terms were removed from the models. All statistical tests were conducted in the R statistical environment (R Core Team, 2021).

Does inoculation with mycorrhizal fungi influence host infection risk?

To test whether host infection risk upon exposure to natural epidemics is explained by inoculation with mycorrhizal fungi, host genetic origin, or an interaction between these factors, we built two generalized logistic multiple regression models. Host infection status was used as the binomial response variable in each model. Infection risk was analyzed at the peak of the epidemic and at the end of the experiment (in separate models). In each model, MYC, POP, SITE, and SIZE (at the corresponding time point) were included as explanatory factors, as well as two interaction terms of interest: 1) MYC × POP, and 2) MYC × SIZE. Similar models examining the effect of genotype on host infection risk could not be analyzed due to insufficient sample sizes within each infection category.

Upon infection, does prior inoculation with mycorrhizal fungi affect disease severity?

To test whether disease severity in infected hosts is influenced by prior inoculation with mycorrhizal fungi, host genetic origin, or an interaction between these factors, we built a set of four generalized logistic and/or linear multiple regression models. These models explored two measures of disease severity (i.e., the proportion of leaves infected and the number of infected leaves in infected individuals) at two time points (i.e., at the peak of the epidemic and at the end of the experiment). In each model, weights were set to the total number of leaves on the individual; family was set to binomial in models of the proportion of leaves infected, and to poisson in models of the number of leaves infected. In each model, we included MYC, POP, SITE, and SIZE (at the time point) as explanatory factors, as well as three interaction terms of interest: 1) MYC × POP, 2) MYC × SIZE, and 3) POP × SIZE.

Are disease susceptibility and defensive effects from mycorrhizal inoculation linked in host genotypes?

We then tested for a relationship between host disease susceptibility and defensive effects received due to inoculation with mycorrhizal fungi in the host genotypes. To do this, we first quantified baseline disease susceptibility in infected, NM plants (i.e., in the absence of the mutualist) in each maternal genotype. Specifically, disease susceptibility in each genotype was quantified using the estimated coefficients from the models examining disease severity at the epidemic peak (i.e., from two of the four models described in the prior paragraph; mycorrhizal defense effects could not be estimated at the end of the experiment due to low numbers of infected plants in some genotypes). A set of genotype-level coefficients representing each disease severity metric (proportion of leaves infected and number of infected leaves) were obtained using the lsmeans package (Lenth, 2016) and were averaged over the levels of field epidemic population. Second, defensive effects due to inoculation with mycorrhizal fungi were quantified for each maternal genotype as the effect size due to mycorrhizal inoculation (i.e., the change in the coefficients estimating each disease severity metric when the genotype had been inoculated with mycorrhizal fungi) from these same models. Finally, we built two linear regression models (one model per disease severity metric) linking defense effect sizes (as the response variable) and disease susceptibility (as the explanatory variable) in the genotypes. We then also tested for a relationship between the growth and defensive effects received from inoculation with mycorrhizal fungi in the host genotypes (see Supplementary Information for full details).

Results

Do growth effects due to mycorrhizal inoculation vary among host populations and maternal genotypes?

Mycorrhizal inoculation led to growth benefits in host plants of every genetic origin; differences among host populations and maternal genotypes in the magnitude of these benefits appeared early on but disappeared after pathogen exposure in field conditions. Prior to pathogen exposure, there was a significant interaction between mycorrhizal inoculation and host genetic origin in determining host growth, both among seed origin populations (Fig. 1 & Table S1; POP

× MYC: $p = 0.006$, $n = 287$ plants) and among maternal genotypes (Fig. 1 & Table S2; GEN × MYC: $p < 0.001$). AMF plants grew much larger than NM plants in all six seed origin populations (Fig. 1 & Table S1; MYC: $p < 0.001$), and in all 30 maternal genotypes (Fig. 1 & Table S2; MYC: $p < 0.001$). In addition, growth varied among the seed origin populations and maternal genotypes prior to pathogen exposure (Table S1; POP: $p < 0.001$; GEN: $p < 0.001$). After pathogen exposure in the field epidemic experiment, host size continued to be linked tightly to mycorrhizal inoculation (Fig. S2 & S3, Table S3 & S4; MYC: $p < 0.001$ (seed origin population model); $p < 0.001$ (maternal genotype model); $n = 287$ plants) and to host genetic origin, i.e., seed origin population (Fig. S2 & Table S3; POP.: $p = < 0.001$) and maternal genotype (Fig. S3 & Table S4; GEN: $p = 0.004$), but the interactive effects of these factors disappeared (Table S3 & S4). Host size at the end of the experiment was similar among the three field epidemic populations (Table S3 & S4; SITE: $p = 0.74$ (seed origin population model); $p = 0.58$ (maternal genotype model)). At the end of the experiment, AMF plants had higher biomass than NM plants (Fig. S4 & Table S5; $p < 0.001$, $R^2 = 0.66$, $n = 41$ plants).

Does inoculation with mycorrhizal fungi influence host infection risk?

Upon pathogen exposure in the field epidemics, host infection risk was influenced by several factors whose importance shifted over time. Infection rates reached 73 % at the peak of the epidemic but fell to 34 % by the end of the experiment. Though mycorrhizal inoculation did not strongly influence host infection risk during the peak of the epidemic (Fig. Table S6; MYC: $p = 0.09$, $n = 287$ plants), at the end of experiment AMF plants were more likely to be infected than NM plants (Fig. 2A & Table S7; MYC: $p = 0.03$, $n = 287$ plants). Host size was positively associated with host infection risk: larger plants experienced higher infection risks than smaller plants (Table S6 – S7; SIZE: $p = 0.05$ (peak of epidemic), $p = 0.02$ (end of experiment)). Furthermore, mycorrhizal inoculation and host size interacted to explain host infection risk during the peak of the epidemic: infection risk increased with increases in host size in NM plants, but not in AMF plants (Fig. 2B & Table S6; MYC × SIZE: $p = 0.01$). However, this interaction disappeared by the end of the experiment (Table S7; MYC × SIZE: $p = 0.56$). Host infection risk also varied among the three field epidemic populations (Fig. S5 - S6 & Table S6 – S7; SITE: $p = 0.02$ (peak of epidemic), $p < 0.001$ (end of experiment)).

Upon infection, does prior inoculation with mycorrhizal fungi affect disease severity?

Among infected individuals, disease severity was influenced by several factors, including mycorrhizal inoculation and the genetic origin and size of the host. AMF plants had lower proportions of their leaves infected relative to NM plants (Figs. 3A & S6 & Tables S8 – S9; MYC: $p < 0.001$, $n = 210$ plants (peak epidemic), $p = 0.003$, $n = 99$ plants (end experiment)). Mycorrhizal-inoculated plants also had fewer infected leaves than NM plants at the end of the experiment (Fig. S7 & Table S11; MYC: $p < 0.001$), but this effect was not yet present at the peak of the epidemic (Table S10; MYC: $p = 0.32$). The proportion of leaves infected also varied among seed origin populations during the peak of the epidemic (Fig. 3A & Table S8; POP: $p = 0.005$) but was similar among populations by the end of the experiment (Table S9; POP: $p = 0.24$). The effect of mycorrhizal inoculation on the number of infected leaves also varied among seed origin populations (Figs. 3B & S7 & Tables S10 – S11; MYC × POP: $p < 0.001$ (peak epidemic), $p < 0.001$ (end experiment). Host size also influenced disease severity: at the peak of the epidemic, the number of infected leaves on an individual increased more strongly with increases in host size in NM plants than in AMF plants (Fig. 3C & Table S10; MYC × SIZE: $p = 0.004$); this interaction weakened by the end of the experiment (Table S11; MYC × SIZE: $p = 0.13$). In addition, the extent to which increases in the number of infected leaves on an individual were influenced by increases in host size also varied among seed origin populations (Figs. 3D & S8, Tables S10 – S11; POP × SIZE: $p < 0.001$ (peak epidemic & end experiment). Finally, hosts in different field epidemic populations experienced differences in their proportion of infected leaves during the peak of the epidemic (Fig. S9 & Table S8; SITE: $p = 0.04$), as well as differences in their number of infected leaves throughout the experiment (Table S10; SITE: $p < 0.001$ (peak epidemic & end experiment).

Are disease susceptibility and defensive effects from mycorrhizal inoculation linked in host genotypes?

We found a strong, positive relationship between baseline host susceptibility to infection and the magnitude of the defensive effect received from mycorrhizal inoculation in the maternal genotypes. This relationship was apparent when examining both metrics of infection severity, including changes in the number of infected leaves (Fig. 4 & Table S12; $p < 0.001$, $n = 30$ maternal genotypes) and the proportion of leaves infected (Fig. S12 & Table S13, $p < 0.001$, $n =$

30 maternal genotypes) in infected individuals at the peak of the epidemic. Maternal genotypes that suffered more severe infections without mycorrhizal fungi experienced greater protection against infections when inoculated with mycorrhizal fungi (Fig. 4). (Fig. 4). There was no relationship between the growth and defensive effects conferred by inoculation with mycorrhizal fungi across the host maternal genotypes (Figs. S13 & S14, Tables S14 & S15; see Supplementary Information for full details).

Discussion

While both plant-associated pathogenic and mutualistic microbes are ubiquitous across ecosystems, how they interact to determine disease risk in natural, genetically diverse populations is not known. In a natural epidemic field experiment, we found that inoculation with a root microbial mutualist influenced infection by an aboveground pathogen in wild host populations. Inoculation with mycorrhizal fungi provided an array of growth and defensive benefits to host populations, altering patterns of host growth, infection risk, and infection severity with a foliar fungal pathogen. Mycorrhizal fungi have been shown to protect agricultural plants against belowground (Azcón-Aguilar & Barea, 1996) and foliar (Fiorilli *et al*., 2018, Pozo de la Hoz *et al*., 2021) pathogens in controlled laboratory conditions, but this is the first evidence of mycorrhizal effects on pathogen dynamics in a genetically diverse wild host, and under natural ecological and epidemic conditions. In addition to benefits, we found that inoculation with mycorrhizal fungi introduced risks to host plants, such as increased likelihood of infection. Mycorrhizal fungi also altered the underlying relationships between growth and pathogen susceptibility in the host individuals, allowing plants to grow larger without increasing their infection risk or load. Moreover, disease susceptibility and mycorrhizal-derived defense effects were linked in the host genotypes: more susceptible host genotypes received stronger protection against disease when inoculated with mycorrhizal fungi. These results underscore how interactions between hosts, microbial mutualists, and pathogens are shaped by host genotype and combine to determine host fitness in genetically diverse natural populations.

Inoculation with mycorrhizal fungi provided growth benefits to hosts from every population and maternal genotype. The magnitude of these benefits varied among host genetic origins prior to pathogen exposure but became more homogenous over time in the field conditions. Due to the positive relationship between plant size and survival, growth is often

intrinsically linked to host fitness (Harper & White, 1974). More specifically, our results point to the importance of mycorrhizal fungi in determining host fitness (Rasmussen *et al*., 2017, Qin *et al*., 2021) within and among genetically variable plant populations. In addition, pathogen susceptibility varied among hosts of different genetic origins in our study, consistent with other studies in this pathosystem (Laine, 2005, Laine, 2007a, Tack *et al*., 2014, Susi & Laine, 2015) and with the broader wild plant disease literature (Carlsson-Granér, 1997, Price *et al*., 2004). Together, these results provide evidence for the importance of genotype in mediating host-microbe interactions (Eck *et al*. 2019, Sallinen *et al*., 2020), through more generalist interactions can also certainly occur (Gilbert & Webb, 2007, Halbritter *et al*., 2012, Hersh *et al*., 2012).

Building upon such studies, we show evidence of changes in host-parasite interactions due to prior inoculation with a microbial mutualist. Protective symbionts are known in the plant and animal literature and are thought to play an important role in determining host fitness (Brownlie & Johnson, 2009; May & Nelson, 2014; King *et al*., 2016; Sochard *et al*., 2020). Studies using agricultural plant species have shown that association with mycorrhizal fungi can reduce the negative effects of pathogens (Norman *et al*., 1996; Pozo *et al*., 2002; Li *et al*., 2010; Song *et al*., 2015; Berdeni *et al*., 2018), and theoretical studies speculate as to how mycorrhizal benefits might counteract reductions in host fitness due to pathogens and affect diversity in natural communities (Bachelot *et al*., 2015). However, symbiotic relationships in cultivars may differ from those of wild plants (Xing *et al*., 2012); thus, it is not straightforward to predict responses in wild populations from controlled agricultural trials. In this study, mycorrhizal inoculation generally increased infection risk in wild hosts, but then protected against further development of established infections. The strength and directionality of protective effects from mycorrhizal inoculation varied among hosts from different genetic origins, in line with other experimental studies showing that mycorrhizal defense benefits may vary among host genotypes (Mark & Cassells 1996; Steinkellner *et al*. 2012).

In many free-living organisms, an evolutionary trade-off exists between growth and defense (Herms & Mattson, 1992). In our experiment, insights into how microbial mutualists may affect this trade-off can be gained by examining how growth influences infection patterns in hosts with and without mutualists inoculated. When not inoculated with mycorrhizal mutualists, larger hosts were more likely to become infected and to develop severe infections – this could occur if increased surface area increases the likelihood that hosts encounter wind-dispersed

pathogen spores – and this relationship varied among host populations. In contrast, though mutualist inoculation caused plants to grow larger, AMF plants grew larger without further increasing their infection risk or load. Molecular studies have shown that mycorrhizal fungi can elicit the induction of defence mechanisms (priming) in the absence of pathogens (Goddard *et al*. 2021), followed by a faster and stronger activation of pathogenesis-related genes upon pathogen infection (Pozo & Azcón-Aguilar, 2007) reducing infection (Hao *et al*. 2005). Mycorrhizal protective effects in this wild pathosystem best correspond to the latter, rather than the former, mechanism: AMF plants were infected, but less repeatedly than NM plants (i.e., the pathogen did not spread to new leaves as readily, or additional infections were better resisted). Another potential mechanism that could explain our results include improvements in host tolerance to pathogens due to mycorrhizal association, such as could be expected if mycorrhizal fungi improve host nutritional status or increase leaf toughness, making foliar pathogen spread more difficult (Meier & Hunter, 2018).

In addition to offsetting the costs of increased infection risk from growing large, our findings indicate that mycorrhizal fungi could help compensate for a lack of innate host resistance. We found that the magnitude of defense effects following mycorrhizal inoculation was linked to pathogen susceptibility in the host genotypes: host genotypes that were more susceptible to severe infections by the pathogen (in the absence of the mutualist) received stronger disease protection effects when inoculated with the mutualist. In contrast, more resistant host genotypes, which exhibited less severe infections when not inoculated with mycorrhizal fungi, sometimes experienced slight increases in disease load when inoculated. This could indicate that host genotypes experience a trade-off in investment in genetic resistance vs. mycorrhizal-mediated resistance. Thus, mycorrhizal association may have been selected for and maintained in host populations partially because it increases the fitness of susceptible host genotypes, while not causing devastating infection gains in well-defended genotypes. In this way, mycorrhizal protective effects could contribute to the maintenance of diversity in host genetic resistance within and among populations, by helping to maintain susceptible host genotypes, which have not invested heavily in defense. In contrast, the magnitude of the growth-enhancing and pathogen-defense effects conferred by inoculation with mycorrhizal fungi were not linked in the host genotypes, suggesting that the delivery of these two types of benefits does not necessarily create a resource allocation conflict. These results highlight the range and variety

of potential mycorrhizal effects on hosts and suggest that different selection processes may act to determine the magnitude and direction of mycorrhizal growth vs. defensive effects.

Variation among host populations and genotypes in mycorrhizal benefits and risks could be due to several factors, such as intraspecific differences among hosts in their ability to form associations with and derive function from each mycorrhizal species, differences in mycorrhizal colonization rates or community composition, as well as environmental conditions. Prior studies demonstrate variation in mycorrhizal colonization rates among individuals within species – variation which is thought to have a partially genetic component (Plouznikoff *et al*. 2019; Pawlowski *et al*. 2020). Differences in mutualist function among mycorrhizal-associated hosts can have important effects on host fitness and could cascade to structure host populations and communities (Brown *et al*., 2001). Hence, though colonization levels from different mycorrhizal species were not quantified in this study, this will be an important avenue for further research. Environmental conditions may also impact plant-mycorrhizal interactions, though in our study, host-pathogen interactions were most clearly impacted by the environment: field infection rates were reduced in one field population relative to others and fell after a period of intense rainfall; in contrast, the effects of mycorrhizal inoculation on hosts, and host growth itself, did not differ among field populations. Future studies in which mycorrhizal composition, colonization intensity, and function are quantified in genetically diverse hosts and in variable environmental conditions will aid in further disentangling the effect of mycorrhizal growth and defensive effects from intraspecific variation in host growth and resistance.

Together, our results show that mutualism with mycorrhizal fungi produces benefits and alters infection risks from a pathogen during natural epidemics in genetically variable host populations. Altered growth and infection risk due to mutualist species may cascade to affect larger ecological patterns of abundance and distribution of the associated organisms, as well as ecosystem processes (Brown *et al*., 2001). Differences among host genotypes in pathogen-mutualist interactions also highlight the importance of maintaining genetic diversity within and among wild plant populations. Characterizing the frequency of specialized interactions between microbial pathogens and mutualists and their host organisms under natural conditions and temporal interaction sequences will also allow for more precise modelling of ecological patterns in natural communities. In addition, the generality of growth and defensive effects due to mutualism should also affect the co-evolutionary trajectories of the associated organisms,

because we expect organisms to be adapted to events that are common over evolutionary time spans (van Dam & Heil, 2011). Moreover, we are also beginning to acknowledge the importance of direct and indirect interactions among microbes within hosts in determining host fitness and parasite population dynamics (Kemen, 2014; Kemen *et al*., 2015; Kroll *et al*., 2017). Belowground soil and rhizosphere processes may affect aboveground interactions with pathogens and herbivores, and vice versa (van der Putten *et al*., 2001; Wardle *et al*., 2004; Frew, 2021), and mutualists and antagonists may act simultaneously to determine host fitness (Bezemer & van Dam, 2005). Understanding how mutualism-derived protection alters host susceptibility and parasite interactions will be important for understanding, predicting, and managing disease in both natural communities and in agriculture.

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Author Contributions

A-LL and M-MK designed and conducted the experiment. JLE and A-LL analyzed the data. JLE wrote the first draft of the manuscript. All authors contributed to and approved the final version.

Data Availability

The data that support the findings of this study are openly available in the Dryad Digital Repository at http://doi.org/[doi], reference number [reference number].

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Figures & Tables

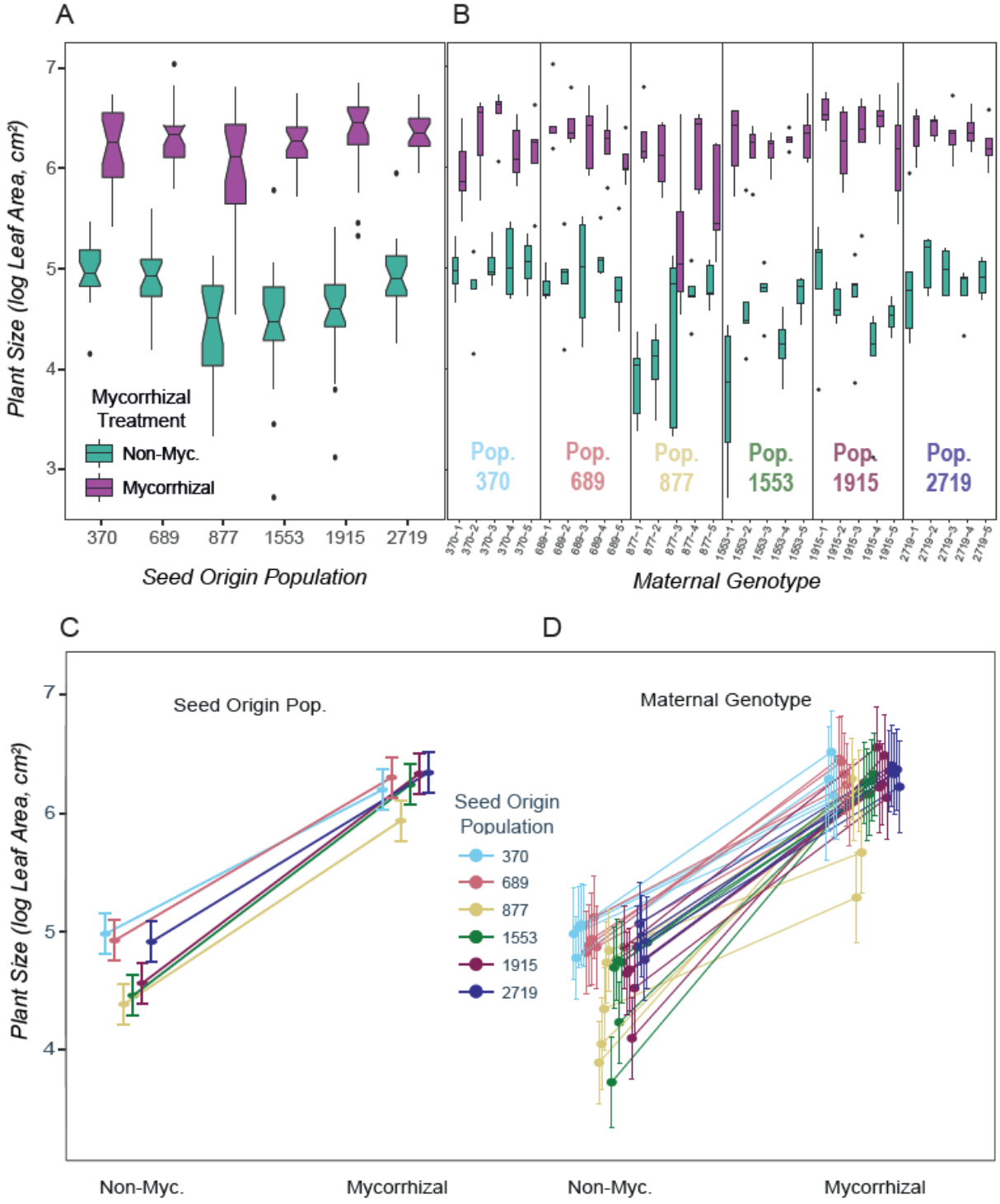

Fig. 1: Growth benefits due to mycorrhizal inoculation vary in magnitude among host populations and maternal genotypes. Inoculation with a mixture of three mycorrhizal fungal species increased the growth of experimental *Pl. lanceolata* plants originating from six seed origin populations (*Panel A*) and 30 maternal genotypes (*Panel B*) (Table S1; $p < 0.001$, $n = 287$

plants). Though growth effects due to mycorrhizal inoculation were always positive, these effects vary in magnitude among the seed origin populations (*Panel C*) and maternal genotypes (*Panel D*) (Table S1; POP × MYC: $p = 0.006$; GEN × MYC: $p < 0.001$; $n = 287$ plants). Here, growth is measured as the aboveground size of the plants (i.e., a log-transformed estimate of total leaf area) prior to pathogen exposure (i.e., at the start of the field epidemic experiment). In *A*, box notches represent a 95 % confidence interval for comparing medians. In *A* and *B*, box hinges correspond to the 1st and 3rd quartiles, while box whiskers extend to the largest and smallest value no further than 1.5 × the interquartile range from the hinges. In *C* and *D*, error bars represent a 95 % confidence interval.

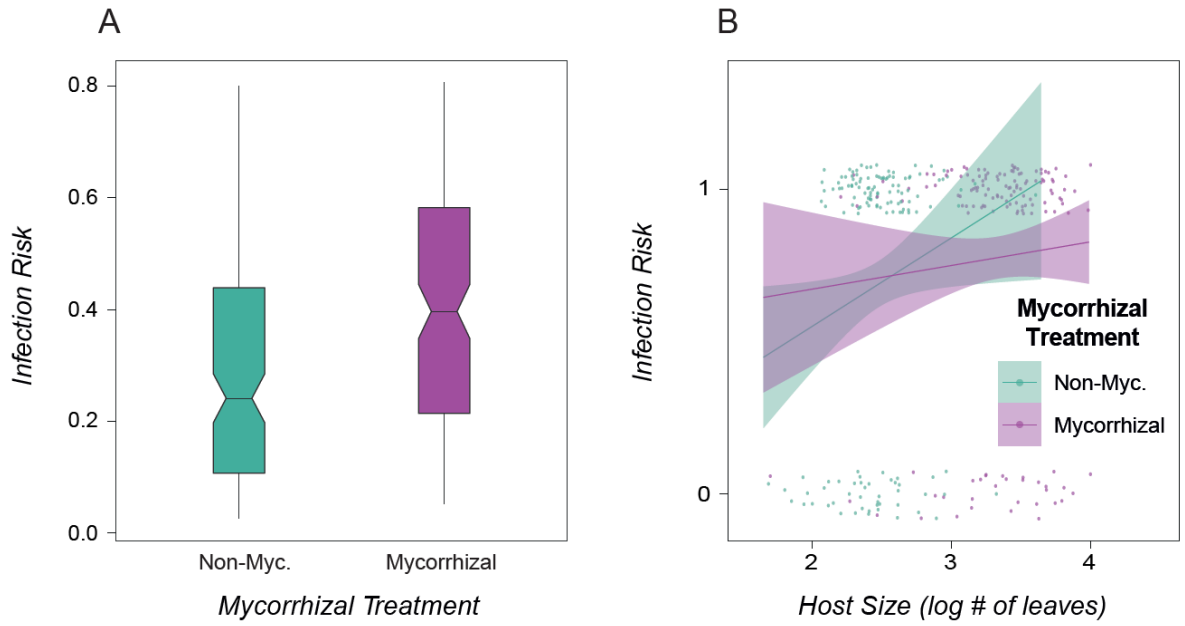

Fig. 2: Infection risk varied among mycorrhizal treatments and was related to host size. *Panel A*: Host infection risk at the end of the field epidemic experiment was increased in AMF plants relative to NM plants (Table S7; MYC: $p = 0.03$, $n = 287$ plants). *Panel B*: In addition, the size of host individuals influenced infection risk differently in AMF vs. NM plants: during the peak of the epidemic, the infection risk of NM plants increased with size, while AMF plants grew larger without changes in infection risk (Table S6; MYC × SIZE: $p = 0.01$, $n = 287$ plants). In *A*, box notches represent a 95 % confidence interval for comparing group medians. Box hinges correspond to the 1st and 3rd quartiles, while box whiskers extend to the largest and smallest value no further than 1.5 × the interquartile range from the hinges. In *B*, shaded areas represent a 95 % confidence interval.

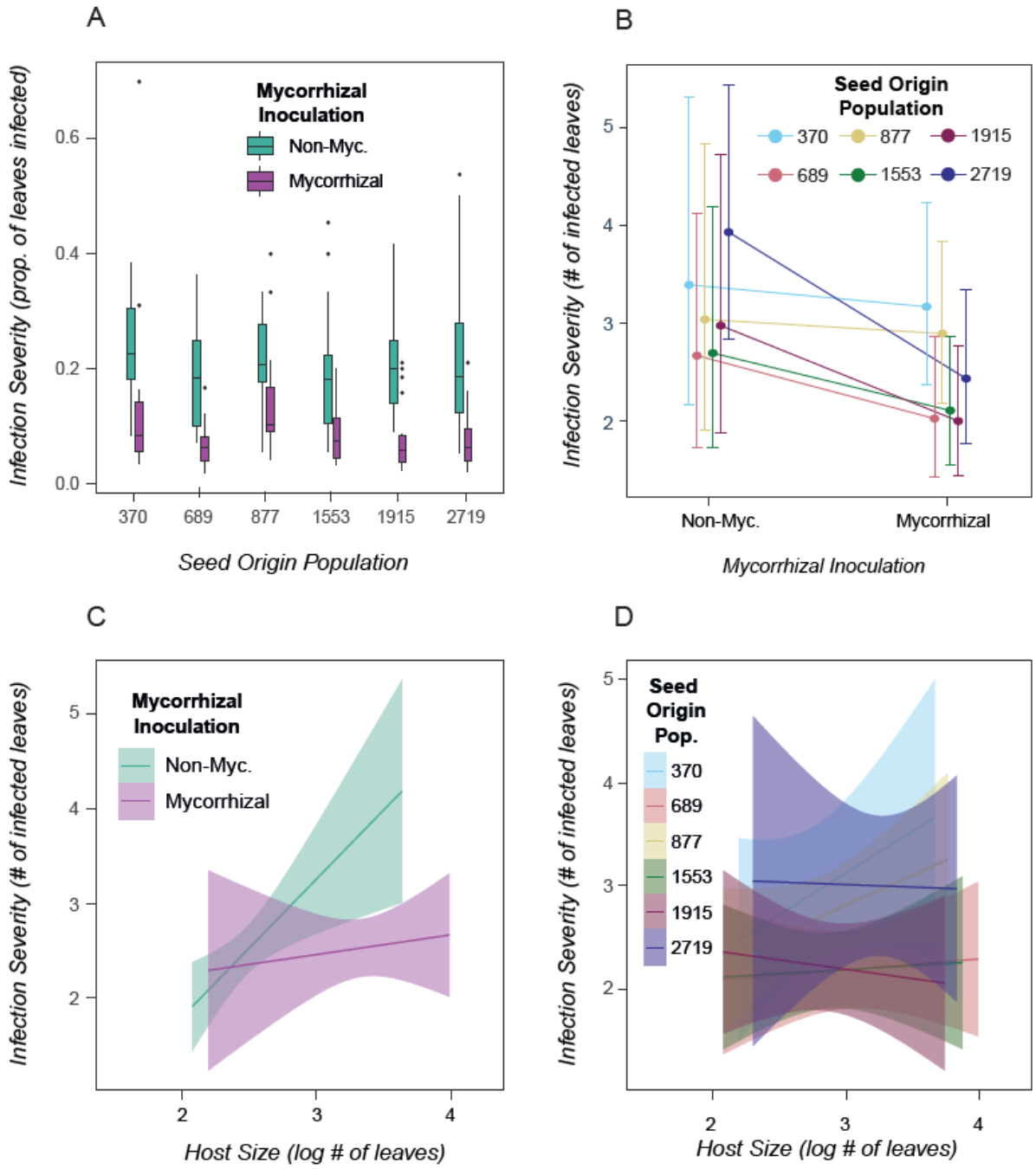

Fig. 3: Among infected plants, disease severity varied among mycorrhizal treatments and seed origin populations and was related to host size. *Panel A*: Mycorrhizal inoculation reduced disease severity (i.e., the proportion of leaves infected on infected individuals) during the peak of the field epidemic experiment, relative to NM plants (Table S8; MYC: $p < 0.001$, n = 210 plants); seed origin populations also varied in their proportions of leaves infected at this time (Table S8; POP: $p = 0.005$, n = 210 plants). Box hinges correspond to the 1st and 3rd quartiles, while box whiskers extend to the largest and smallest value no further than 1.5 × the interquartile range from the hinges. *Panel B*: Mycorrhizal inoculation affected the number of infected leaves in infected individuals differently among seed origin populations (Table S10; MYC × POP: $p <$

0.001, n = 210 plants). Error bars represent a 95 % confidence interval. *Panel C:* Host size increased infection severity more strongly in NM plants than in AMF plants at the peak of the epidemic (Table S10; MYC × SIZE: p = 0.004, n = 210 plants). *Panel D*: Host size also influenced infection severity differently among seed origin populations (Table S10; POP × SIZE: $p < 0.001$, n = 210 plants). In *C* and *D*, shaded areas represent a 95 % confidence interval.

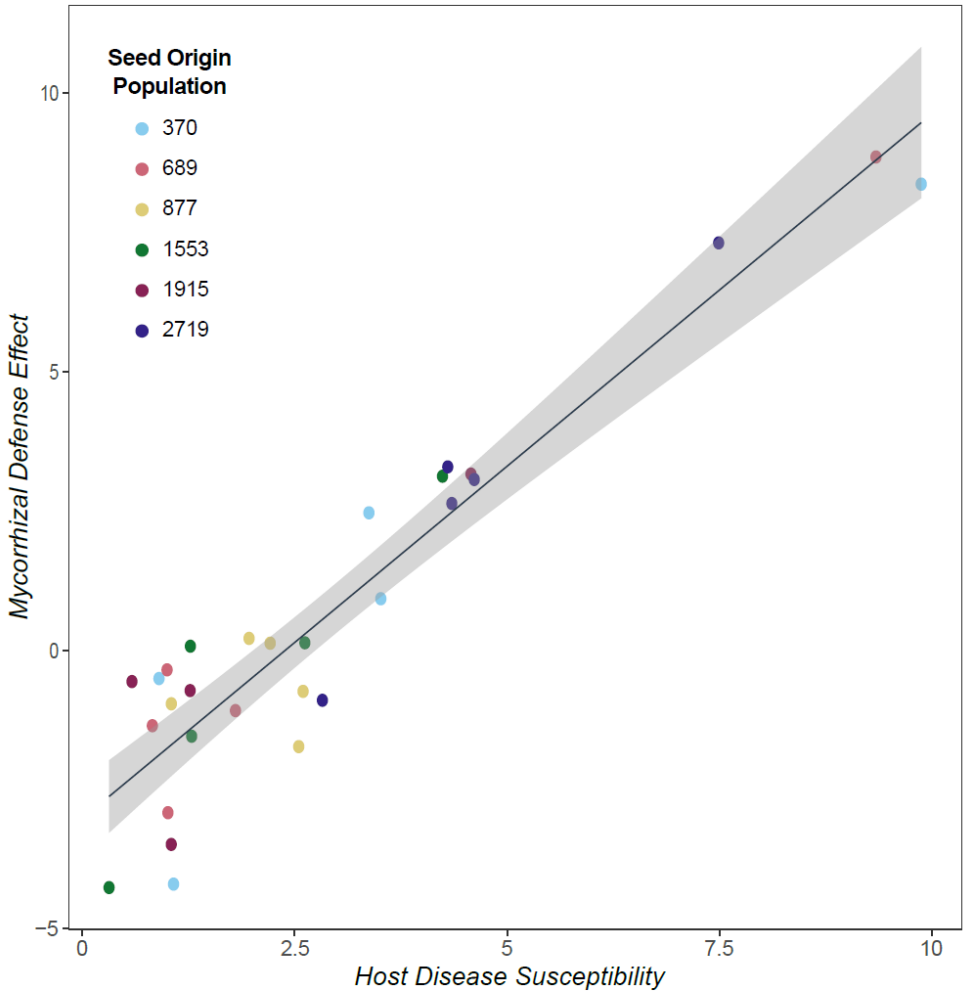

Fig. 4: Host susceptibility to infection and defensive effects due to mycorrhizal fungi are linked in the host genotypes. In a natural epidemic field experiment with plants from thirty maternal genotypes, host genotypes that were more susceptible to infection (when not inoculated with mycorrhizae) received stronger disease protection effects from inoculation with mycorrhizal fungi (Table S12). Here, each point represents one maternal genotype, originating from one of six seed origin populations. Infection susceptibility, on the x-axis, is the estimated coefficient representing the number of infected leaves in infected, NM plants at the peak of the epidemic: more positive x-axis values indicate more severe infections and thus, higher disease susceptibility. On the y-axis, mycorrhizal defense effects are the effect size representing the change in the number of infected leaves in the genotype due to mycorrhizal inoculation. More positive y-axis values indicate stronger mycorrhizal defensive effects (i.e., greater reductions in the number of infected leaves when the genotype was inoculated with mycorrhizae); negative y-axis values indicate increases in disease when the genotype was inoculated with mycorrhizal fungi. The shaded area represents a 95 % confidence interval.