Genotype-specific benefits and risks of a mutualist association during natural epidemics

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Summary

- 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.
- To test whether mycorrhizal fungi provide protection against infection, and whether this functions in a genotype-specific manner, 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 field epidemiological data on mycorrhizal-inoculated and non-mycorrhizal experimental plants originating from six allopatric populations.
- Mycorrhizal association caused host genotype-specific changes in growth and infection rates in the host populations, but reduced infection severity in hosts from every genetic origin. Protection occurred via changes in the relationship between growth and infection in the host individuals: mycorrhizal individuals grew larger without increasing their infection risk or load. More susceptible host genotypes received stronger protective effects from mycorrhizae.
- Our results show that mycorrhizal fungi produce host genotype-specific growth and defensive benefits and alter infection risks in wild host populations. Understanding how mutualism-derived protection alters 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 and other mutualisms 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 in to 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 the lifestyle and infection strategy of the pathogen) (Pozo & Azcón-Aguilar, 2007), or be durable to changes in pathogen traits or composition in the environment. Hence, the potential ecological risks and/or benefits of mutualist associations for disease protection may depend on 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 particular host individuals or populations could depend on their resistance status. 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 defenses derived from protective symbionts (e.g., via defense priming, or improved tolerance to pathogens), 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 potential 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 association 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 association vary among host populations and maternal genotypes? 2) Does association with mycorrhizal fungi influence host infection risk? 3) Upon infection, does association with mycorrhizal fungi affect disease severity? 4) Are growth and defensive effects from mycorrhizal association linked in host genotypes? and 5) Are host disease susceptibility and mycorrhizal-derived defensive effects linked in host genotypes? To answer these questions within an ecologically relevant context, we placed mycorrhiza-inoculated and non-mycorrhizal plants in wild 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*., accepted manuscript), local adaptation between host and pathogen genotypes (Laine, 2004; Laine, 2005; Jousimo *et al*., 2014), and abiotic conditions (Laine, 2007; 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/or 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 larger network of > 4000 mapped populations in Åland (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, and 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 and initiate new infections in the next growing season (Tack & Laine, 2014).

In Åland, *Po. plantaginis* persists as a metapopulation, with frequent colonization and extinction events (~ 3 – 17 % of the *Pl. lanceolata* populations are infected in a given year) (Jousimo *et al*., 2014). Resistance in *Pl. lanceolata* against *Po. plantaginis* functions in the two-step manner typical of most plants (Jones & Dangl, 2006). The first step is strain specific, such that a host genotype expresses resistance against some strains (*i.e.* recognition) of the pathogen, while being susceptible to others (*i.e.* non-recognition) (Laine, 2004; Laine, 2007). Once a strain has successfully established, its development is affected by both pathogen and host genotype (Laine, 2007). Previous studies have demonstrated high levels of intraspecific variation in resistance within and among populations of the host (Laine, 2004; Laine 2007, Jousimo et al.

2014). Pathogen populations also contain variable levels of genetic diversity (Eck *et al*., accepted manuscript). Infected hosts individuals have reduced survival during drought periods compared to healthy hosts (Laine, 2004), and infection has a negative effect on host population growth rate (Penczykowski *et al*., 2015).

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 (a map of these populations is displayed in Fig. S1). In each population, seeds were collected from five haphazardly chosen maternal plants in August 2007. Seeds from each maternal plant were kept separate (seeds from one maternal plant are half or full siblings) and were stored in paper envelopes. In April 2008, seeds from each of the 30 maternal plants were germinated in separate 6 x 6 x 7 cm pots (one maternal genotype per pot) in a greenhouse at the University of Oulu (Oulu, Finland) in sterilized sand. At two weeks of age, ten seedlings from each maternal plant were transferred to identical, individual pots (one seeding per 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. Five of the seedlings in each maternal cohort 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).* The mycorrhizal inoculum consisted of allopatric isolates of each species (originating from central Finland, 61º10 N, 24º40 E, rather than Åland) so as not to confound the experiment with potential local adaptation between mycorrhizal fungi and their host populations (Hoeksema *et al*., 2010). A mixture of species was used as inoculum because *Pl. lanceolata* is colonized naturally by several mycorrhizal symbionts (Johnson *et al*., 2003). 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). All experimental seedlings

were also inoculated with bacteria filtered from a soil mix collected from the six seed source Åland populations to restore the natural, non-mycorrhizal soil microbial community. Seedlings were fertilized with a dilute nitrogen-based solution, and natural light was supplemented with Osram HQI lamps to provide a photoperiod of 18 h day / 6 h night. At six weeks of age, the plants were moved to an outdoor area (at the University of Oulu) and grown 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, healthy 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 from each of the six seed origin populations (eight of which had received mycorrhizal inoculum and eight of which were non-mycorrhizal). Within these subgroups, the five maternal genotypes within each seed origin population were allocated as evenly as possible to the three field sites; the particular 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 available plants). At each 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 *Pl. lanceolata* individuals inhabiting the field population. The containers prevented contact between the bottom of the pots and the soil, preventing the experimental plants from acquiring soil microbes from the field sites.

At the time of placement in the field epidemic populations, we measured the following for each plant: the number of total leaves, the number of leaves infected by *Po. plantaginis*, as well as the length and width (in cm) of the longest leaf. All experimental plants were uninfected (i.e., had zero infected leaves) 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 9061, 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 re-measured (leaves that withered during the experiment were not counted). Plants were also watered if necessary, and were re-randomized in to 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). Seven infection surveys were conducted in each field population. At the end of the experiment, a randomly-selected subset of 21 mycorrhizal and 20 non-mycorrhizal individuals were harvested, and their oven-dried total biomass was measured. To compare the size of the experimental plants at the beginning of the experiment (prior to pathogen exposure), we created a metric approximating the total leaf area of each plant. We did this by first applying the length and width of the plant's longest leaf to the equation yielding the area of an oval (*Pl. lanceolata* leaves resemble long, narrow, pointed ovals), 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 likely strongly 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, which coincides with the completion of one 14-day initial infection and reproduction cycle of the pathogen. Using data from this survey, we calculated the infection status (0 / 1, infected or uninfected) and 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.

Statistical methods

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

To test whether the growth of healthy individuals (prior to pathogen exposure) was explained by association with mycorrhizal fungi, host genetic origin, or an interaction between these factors, we built two multiple linear regression models. To estimate the effect of genetic origin on plant growth, seed origin population was included as an explanatory factor in one

model, while maternal genotype (without respect to population) was included in the second model. In each model, mycorrhizal treatment (mycorrhizal-inoculated or non-mycorrhizal control) was also included as an explanatory factor, as was an interaction term between mycorrhizal treatment and plant genetic origin. We used a log-transformed metric of plant size (i.e., an estimate of total leaf area prior to pathogen exposure) as the response variable. We also tested whether mycorrhizal and non-mycorrhizal individuals varied in size (i.e., in oven-dried total biomass) at the end of the experiment (i.e., post-pathogen exposure) using a separate linear regression model (this model was applied to the subset of plants for which these data were available; plant genetic origin was not included in this model due to low replication). All statistical tests in our study were conducted in the R statistical environment (R Core Team, 2021).

Does association with mycorrhizal fungi influence host infection risk?

To test whether host infection risk upon exposure to natural epidemics is explained by association with mycorrhizal fungi, plant genetic origin, or an interaction between these factors, we built a generalized linear mixed-effects model. In this model, we used plant infection risk (0 / 1) at the end of the initial infection cycle (i.e., ~14 days post-pathogen exposure) as the binomial response variable. We also included mycorrhizal treatment, seed origin population, and their interaction as fixed explanatory variables in the model (maternal genotype could not be examined in a similar manner due to low replication of each genotype × mycorrhizal treatment combination in each infection category). In addition, field epidemic population was included as a random effect in this model. All linear (and generalized linear) mixed-effects models in our study were performed using the lme4 package (Bates *et al*., 2015) in conjunction with the lmerTest package (Kuznetsova *et al*., 2017) in R.

We then tested the effect of host size (prior to pathogen exposure) on infection risk within each mycorrhizal treatment group independently, using separate generalized logistic regression models (to overcome strong multicollinearity between host size and mycorrhizal treatment). Two models were used to examine the interacting effects of host size and seed origin population on infection risks: first in mycorrhizal-inoculated individuals, and then separately in non-mycorrhizal individuals. In each model, plant infection status at the end of the initial infection cycle was used as the binomial response variable, while the log-transformed metric of

plant size (prior to pathogen exposure) was included as a fixed effect, as well as seed origin population, and an interaction term between these two factors. Field epidemic population was not included as a random effect in these models for reasons of model fit.

Upon infection, does association with mycorrhizal fungi affect disease severity?

To test whether disease severity in infected hosts is influenced by association with mycorrhizal fungi, plant genetic origin, or an interaction between these factors, we built a generalized linear mixed-effects model. In this model, we used the proportion of infected leaves on an infected individual as the response variable (with weights set to the total number of leaves on the individual, and with family set to binomial). We included mycorrhizal treatment, seed origin population, and their interaction as fixed explanatory variables (the effect of maternal genotype could not be examined in a similar model due to insufficient power). In addition, field epidemic population was included as a random effect in this model. Non-significant interaction terms were removed from the final model.

We then tested for the effect of host size on the disease severity experienced by infected individuals using two generalized linear mixed-effects models (to overcome strong multicollinearity between host size and mycorrhizal treatment). Two models were used to examine the interacting effects of host size and seed origin population on disease severity (i.e., the proportion of infected leaves), first in infected mycorrhizal-inoculated individuals and then in infected non-mycorrhizal individuals (to overcome strong multicollinearity between host size and mycorrhizal treatment). In each model, the log-transformed metric of plant size (prior to pathogen exposure) was included as a fixed effect, and field epidemic population was included as a random effect whenever possible (i.e., without compromising model fit). Non-significant interaction terms were removed from the final models. In the model examining infected mycorrhizal individuals, seed origin population was not a significant predictor and did not interact with size, so it was included instead as a random effect.

Are growth and defensive effects from mycorrhizal association linked in host genotypes?

To test for a relationship between the magnitude of the growth and defensive effects received in the host genotypes due to association with mycorrhizal fungi, we built a linear regression model. First, we quantified the magnitude of the growth effect due to association with

mycorrhizal fungi (in healthy plants, prior to pathogen exposure) for each maternal genotype. To do this, we extracted the estimated coefficients of the difference in host size (i.e., estimated log-leaf area, in cm^2) among the mycorrhizal and non-mycorrhizal treatments for each maternal genotype from the multiple regression model used to test for differences in host size among mycorrhizal treatments, maternal genotypes, and their interaction (i.e., the same model specified in the first section of the statistical methods). Second, we calculated the magnitude of the defense benefit due to association with mycorrhizal fungi for each genotype (when exposed to the pathogen in the field epidemic experiment). To do this, we extracted the estimated coefficients representing the difference in the proportion of infected leaves (in infected individuals only) among the mycorrhizal and non-mycorrhizal treatments for each maternal genotype from the generalized linear mixed-effects model used to test for differences among these factors (i.e., the model specified in the third section of the statistical methods; a similar model representing the difference in infection rates in the mycorrhizal and non-mycorrhizal treatments for each maternal genotype could not be fit due to convergence issues). Finally, we built a linear regression model that linked the coefficients for the difference in infection severity when the genotypes were associated with mycorrhizal fungi (as the response variable) with the coefficients for the difference in host size when the genotypes were associated with mycorrhizal fungi (as the explanatory variable). Here and in the section below, coefficient estimates, standard errors, and p-values for contrasts between mycorrhizal treatment groups within each genotype were obtained using the lsmeans package (Lenth, 2016).

Are host disease susceptibility and mycorrhizal-derived defensive effects linked in host genotypes?

To test for a relationship between the magnitude of baseline disease susceptibility in the host genotypes (in the absence of mycorrhizal fungi) and the magnitude of the defensive effects received in the host genotypes (due to association with mycorrhizal fungi), we built a linear regression model. First, we quantified the magnitude of baseline disease susceptibility as the estimated coefficient representing the proportion of infected leaves in infected individuals (in log odds ratio scale) when the genotype was not associated with mycorrhizal fungi. Next, defensive effects were quantified as the change in the proportion of infected leaves (in infected individuals) when the genotype was associated with mycorrhizal fungi (as in the section above). Thus,

baseline disease susceptibility and defensive effects due to mycorrhizal association were quantified using the effect size coefficients resulting from models contrasting the genotypes (with epidemic field site included as a random effect in both models). Finally, we built a linear regression model that linked the coefficients for the difference in infection severity when the genotypes were associated with mycorrhizal fungi (as the response variable) with the coefficients for the difference in baseline disease susceptibility when the genotypes were associated with mycorrhizal fungi (as the explanatory variable).

Results

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

We found that mycorrhizal association often provided growth benefits to hosts, but that these benefits varied in magnitude among host populations and genotypes. Prior to pathogen exposure, mycorrhizal individuals grew larger (i.e., had more total leaf area) than non-mycorrhizal individuals in all six seed origin populations (Fig. 1A, Table S1; $p < 0.001$, $n = 282$ plants), and in 29 of the 30 maternal genotypes (Fig. 1B, Table S1; $p < 0.001$, $n = 282$ plants). Yet, the strength of mycorrhizal growth benefits received varied among seed origin populations and maternal genotypes: there were significant interactions between mycorrhizal status and host genetic origin in determining host growth, at both the population (Fig. 1C & Table S1; $F = 3.31$, $p = 0.006$, $n = 282$ plants) and genotype (Fig. 1D & Table S1; $F = 3.06$, $p < 0.001$, $n = 282$ plants) level. Growth effects in the genotypes ranged from 8 – 67.9 % (among-genotype mean: 32.9 ± 12.9 %) increases in host size (i.e., estimated total leaf area) when associated with mycorrhizal fungi. In addition, host populations and maternal genotypes varied in their baseline levels of growth, regardless of mycorrhizal status (Table S1; $p < 0.001$ (seed origin population), $p < 0.001$ (maternal genotype); $n = 282$ plants). After pathogen exposure in natural field conditions, host size continued to be linked tightly to mycorrhizal association: the harvest biomass of mycorrhizal individuals was increased relative to non-mycorrhizal individuals (Fig. S2 & Table S2; $p < 0.001$, $R^2 = 0.66$, $F = 79.65_{(d.f.: 1, 39)}$, $n = 41$ plants).

Does association with mycorrhizal fungi influence host infection risk?

Upon exposure to naturally-occurring field epidemics, we found that infection risk was influenced by an interaction between the mycorrhizal status and genetic origin of the host. The effect of mycorrhizal association on infection risk varied among the six seed origin populations: mycorrhizal association greatly increased infection risk in one seed origin population (2719), while infection risk was similar among mycorrhizal and non-mycorrhizal individuals in the five remaining seed origin populations (Fig. 2A & Table S3; myc. assoc. × seed origin pop.: $p = 0.01$, $F = 1.33$, $n = 282$ plants). In addition, we found that host size (Fig. 2B) and seed origin population interacted to influence the likelihood of infection within groups of mycorrhizal and non-mycorrhizal individuals (Table S4). In non-mycorrhizal individuals, host size was linked to increases in infection risk in two out of the six seed origin populations (Pop. 877 and Pop. 1553) (Table S4 & Fig. S3A). In mycorrhizal individuals, host size was linked to increased infection risk in only one seed origin population (Pop. 370) (Table S4 & Fig. S3B; $p = 0.04$, $z = 2.09$, $n = 141$ plants).

Upon infection, does association with mycorrhizal fungi affect disease severity?

Among infected individuals, we found that the severity of disease experienced during the peak of the epidemic was influenced by both the mycorrhizal status and the genetic origin of the host. Mycorrhizal individuals had less severe infections (i.e., had reduced proportions of infected leaves) than non-mycorrhizal individuals (Fig. 3A & Table S5; $p < 0.001$, $F = 104.65$, $n = 205$ plants). In addition, seed origin population influenced the proportion of leaves infected on the host (Fig. 3A & Table S5; $p = 0.004$, $F = 3.59$, $n = 205$ plants). We also found that host size (just prior to pathogen exposure) affected disease severity differently in mycorrhizal versus non-mycorrhizal plants (Table S6). Larger mycorrhizal individuals had lower proportions of their leaves infected than smaller mycorrhizal individuals (Fig. 3B & Table S6; $p = 0.01$, $F = 7.52$, $n = 109$ plants), and this relationship was consistent across mycorrhizal plants from different genetic origins. In contrast, non-mycorrhizal individuals from most genetic origins had similar proportions of infected leaves regardless of their size (Fig. 3B & Table S6; $p = 0.06$, $F = 0.45$, $n = 96$ plants), though there was a positive relationship between size and disease severity in one seed origin population (Pop. 1915) (Fig. S4 & Table S6; $p = 0.04$, $F = 1.17$, $n = 96$ plants).

Are growth and defensive effects from mycorrhizal association linked in host genotypes?

We did not find a clear relationship between the growth and defensive benefits conferred by mycorrhizal fungi across the maternal genotypes (Table S7; $p = 0.39$, $F = 0.76$, $n = 30$ maternal genotypes). Growth effects due to mycorrhizal fungi (quantified in healthy individuals, prior to pathogen exposure; and which were positive in 29 out of the 30 genotypes) varied in magnitude independently of defensive effects (quantified from infected individuals, post-pathogen exposure); defensive effects varied in both strength and directionality among the genotypes (Table S7). Lack of a relationship between the two types of potential mycorrhizal effects (growth and/or defensive) was evident when examining changes in the proportion of infected leaves when the genotypes were associated with mycorrhizae (Table S7).

Are host disease susceptibility and mycorrhizal-derived defensive effects linked in host genotypes?

We found a strong, positive relationship between baseline host susceptibility to infection (measured in non-mycorrhizal individuals) and the magnitude of the defensive effect received from mycorrhizal association in the maternal genotypes (Fig 4 & Table S8; $p < 0.001$, $F = 22.65$, $n = 30$ maternal genotypes). In general, maternal genotypes that suffered more severe infections without mycorrhizal fungi experienced greater relief from infections with mycorrhizal fungi (Table S8). This pattern was evident when considering the change in the proportion of infected leaves when the genotypes were associated with mycorrhizae (Table S8).

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 association with a root microbial mutualist influenced infection by an aboveground pathogen in wild host populations. Mycorrhizal symbiosis provided an array of genotype-specific growth and defensive benefits to host plants, altering patterns of host growth and infection 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 association with mycorrhizal fungi introduced occasional risks to host plants, as we observed an increased likelihood of infection in some host populations. Association with mycorrhizal fungi altered the underlying relationships between growth and pathogen susceptibility in the host individuals, allowing them to grow larger without increasing infection risk or load. Moreover, the strength and directionality of mycorrhizal effects depended on host population and/or genotype, with more susceptible genotypes receiving stronger protective effects. Hence, this is some of the first evidence of genotype-specificity of host infection risk mediation by a third interacting species. These results underscore how genotype-specific interactions between hosts, microbial mutualists, and pathogens combine to determine host fitness in genetically diverse natural populations.

Though mycorrhizal fungi provided growth benefits to hosts of almost every genetic origin in our study, the magnitude of these benefits varied widely among host populations and maternal genotypes. 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, 2007, Tack *et al*., 2014, Susi & Laine, 2015) and in 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 also show evidence of changes in host-parasite interactions due to association with a microbial mutualist. Protective symbionts are known from 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 from 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 association mainly protected wild hosts against further development of established infections, rather than preventing infection by the pathogen entirely. Mycorrhizal association had little influence on infection risk in most host populations in our study, and even increased infection risk in one seed origin population (highlighting the genotype-dependency of such interactions). Instead, protective effects from mycorrhizal fungi, which resulted in reductions in disease severity in mycorrhizal hosts from all genetic origins, occurred by altering the underlying relationships between host growth and pathogen susceptibility.

In many free-living organisms, an evolutionary trade-off exists between growth and defense (Herms & Mattson, 1992). In our experiment, insights in to how microbial mutualists may affect this trade-off can be gained by examining how growth influences infection patterns in hosts with and without mutualist association. In the absence of mycorrhizal mutualists, larger hosts were more likely to become severely infected by the wind-dispersed pathogen (as increased surface area increases the likelihood that hosts encounter pathogen spores), and the strength of the relationship between growth and pathogen susceptibility varied among host populations. In contrast, though mutualist association caused plants to grow larger, mycorrhizal plants from most host populations grew larger without increasing their infection risk or load. Thus, mycorrhizal plants appear to be benefitting from a more quantitative than qualitative mechanism of pathogen resistance (Lo Iacono *et al*., 2012), which allows them to grow large while being relieved of the increased infection risks that normally accompany growth. Molecular studies have shown that mycorrhizal fungi can elicit induction of defence mechanisms (priming) in the absence of pathogens, followed by a faster and stronger activation of pathogenesis-related genes upon pathogen infection (Pozo & Azcón-Aguilar, 2007). Mycorrhizal protective effects in this wild pathosystem best correspond to the latter, rather than the former, mechanism: mycorrhizal hosts were infected, but not repeatedly (i.e., the pathogen did not spread to new leaves as readily as in non-mycorrhizal plants). Other potential mechanisms include improvements in host tolerance to pathogens due to mycorrhizal association, such as could be expected if mycorrhizal fungi, e.g., 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 association could help compensate for lack of innate host resistance. We found that the magnitude of mycorrhizal protective effects 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 from association with the mutualist. 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. In this way, mycorrhizal protective effects could contribute to the maintenance of diversity in pathogen genetic resistance within and among host populations, by helping to maintain susceptible genotypes, which have not invested heavily in defense. In contrast, the magnitude of the growth-enhancing and pathogen-protective benefits conferred by 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.

Together, our results show that mutualism with mycorrhizal fungi produces host genotype-specific benefits and alters infection risks from a pathogen during natural epidemics in 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). The presence of genotype-specificity in host-pathogen-mutualist interactions also highlights the importance of maintaining genetic diversity within and among wild plant populations. Characterizing the potential for and frequency of specialized interactions between microbial pathogens and mutualists and their host organisms under natural conditions, and using the natural temporal sequence at which the various associations are established, 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, E, 2014; Kemen, A, *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 agricultural settings.

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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

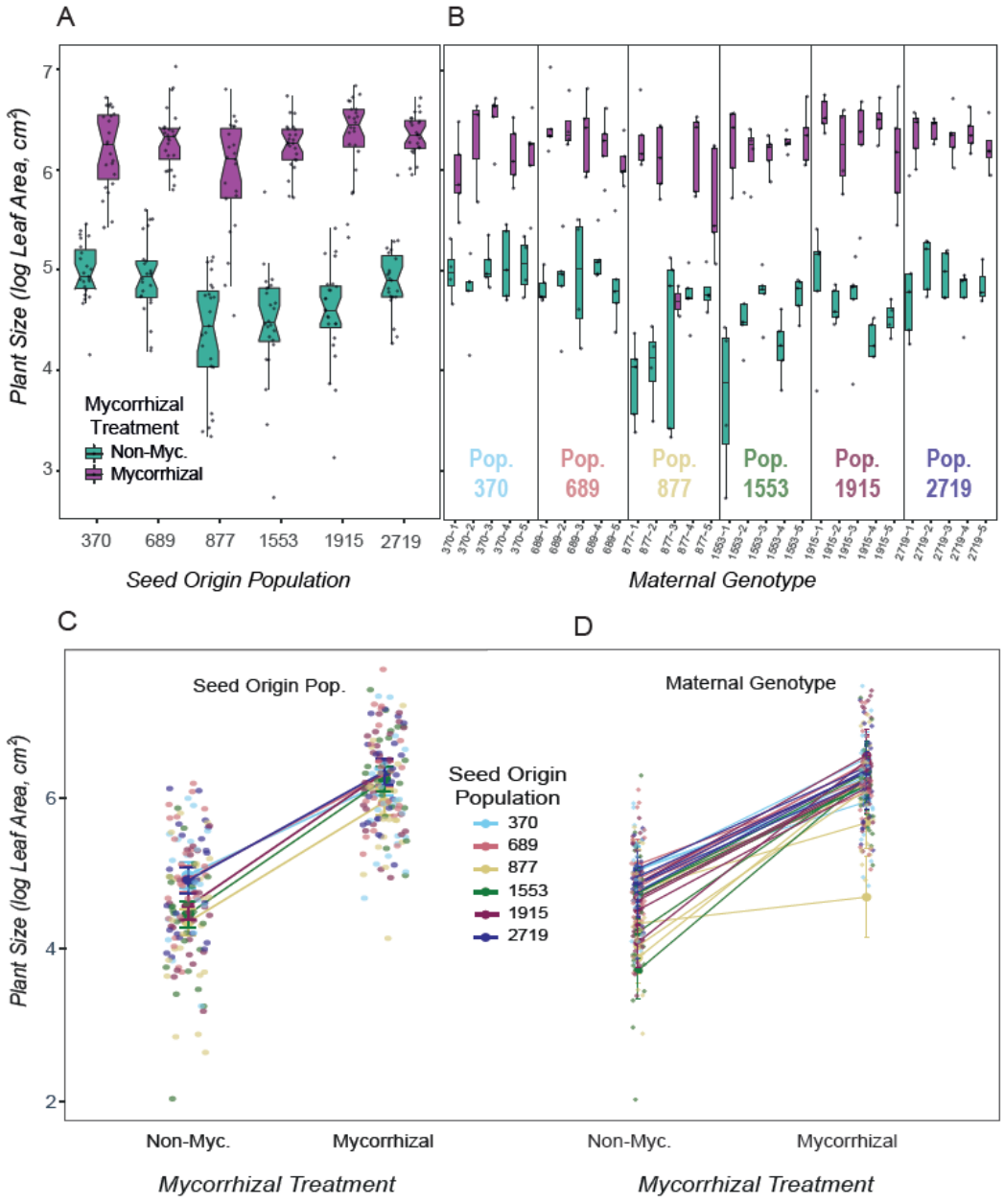

Fig. 1: Mycorrhizal growth benefits varied in magnitude among host populations and maternal genotypes. Association with mycorrhizal fungi conferred growth benefits to host individuals originating from six field populations (*Panel A*) and to 29 out of 30 maternal genotypes (*Panel B*) (Table S1). Growth effects were measured as aboveground size prior to pathogen exposure (i.e., a log-transformed estimate of total leaf area). Though host growth almost always increased in association with mycorrhizal fungi, the magnitude of the growth benefit received varied among the seed origin populations (*Panel C*) and among the maternal genotypes (*Panel D*), due to an interaction between mycorrhizal association and host genetic origin at both levels (Table S1).

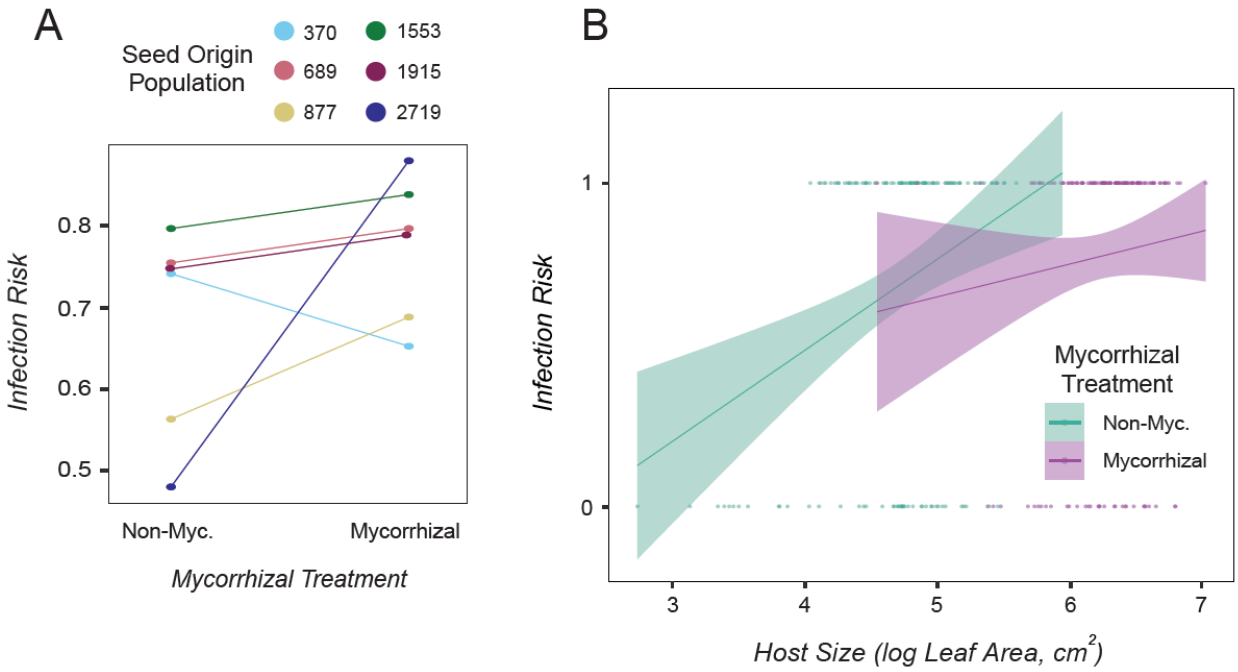

Fig. 2: Infection risk varied among mycorrhizal treatments and host populations, and depended on plant size. Host infection risk upon exposure to field epidemics were influenced by an interaction between mycorrhizal association and seed origin population: mycorrhizal association greatly increased infection risk in one population, 2719 (*Panel A*; Table S3). In addition, host size influenced infection risk differently in mycorrhizal versus non-mycorrhizal plants: while the infection risk of non-mycorrhizal plants increased with size, mycorrhizal plants experienced similar infection risk, regardless of size (*Panel B*; Table S4).

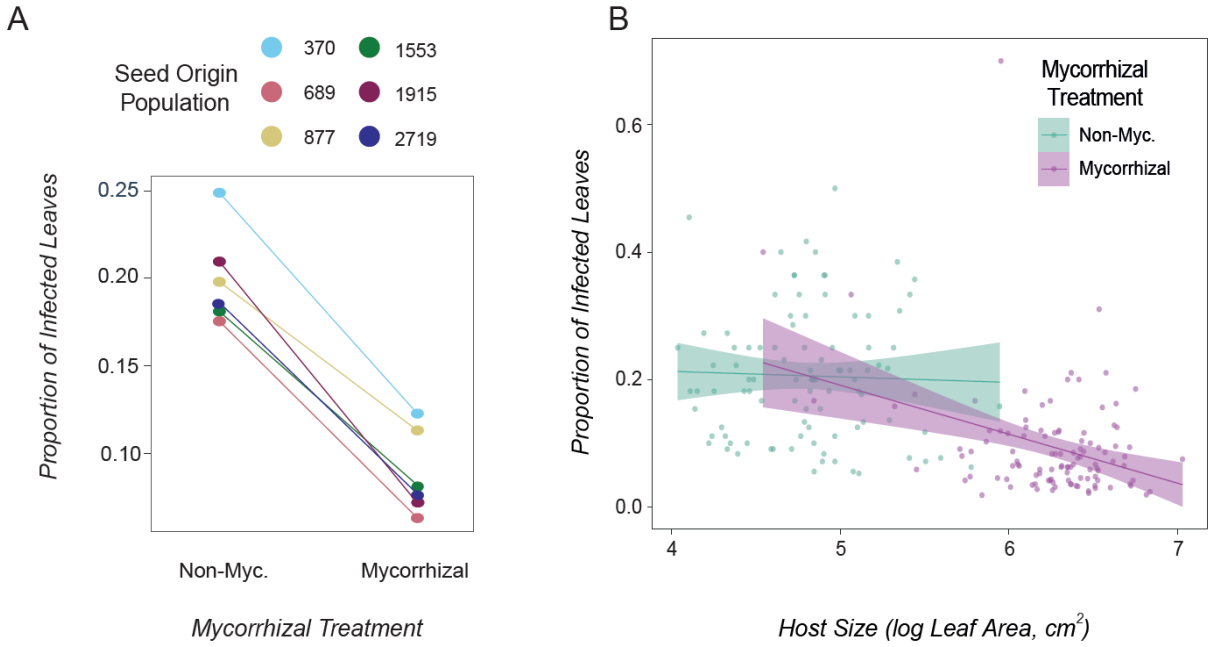

Fig. 3: Disease severity was reduced in mycorrhizal plants, while host size affected infection severity differently in mycorrhizal versus non-mycorrhizal plants. *Panel A*: In a natural epidemic field experiment, the disease severity experienced by infected plants was influenced by both mycorrhizal treatment and host genetic origin (though the two factors did not interact) (Table S5; n = 205 plants). Mycorrhizal plants in all six seed origin populations had reduced proportions of infected leaves, relative to non-mycorrhizal plants. Infection severity also differed among seed origin populations, suggesting baseline differences in disease susceptibility among populations. *Panel B*: Host size at the time of pathogen exposure was important in determining infection severity, but different dynamics were found in mycorrhizal versus non-mycorrhizal individuals (Table S6). Larger mycorrhizal individuals had reduced proportions of infected leaves relative to smaller mycorrhizal individuals (Table S6; p = 0.01, F = 7.52, n = 109 plants), while non-mycorrhizal individuals of all sizes had similar proportions of infected leaves (Table S6; p = 0.20, F = 0.45, n = 96 plants).

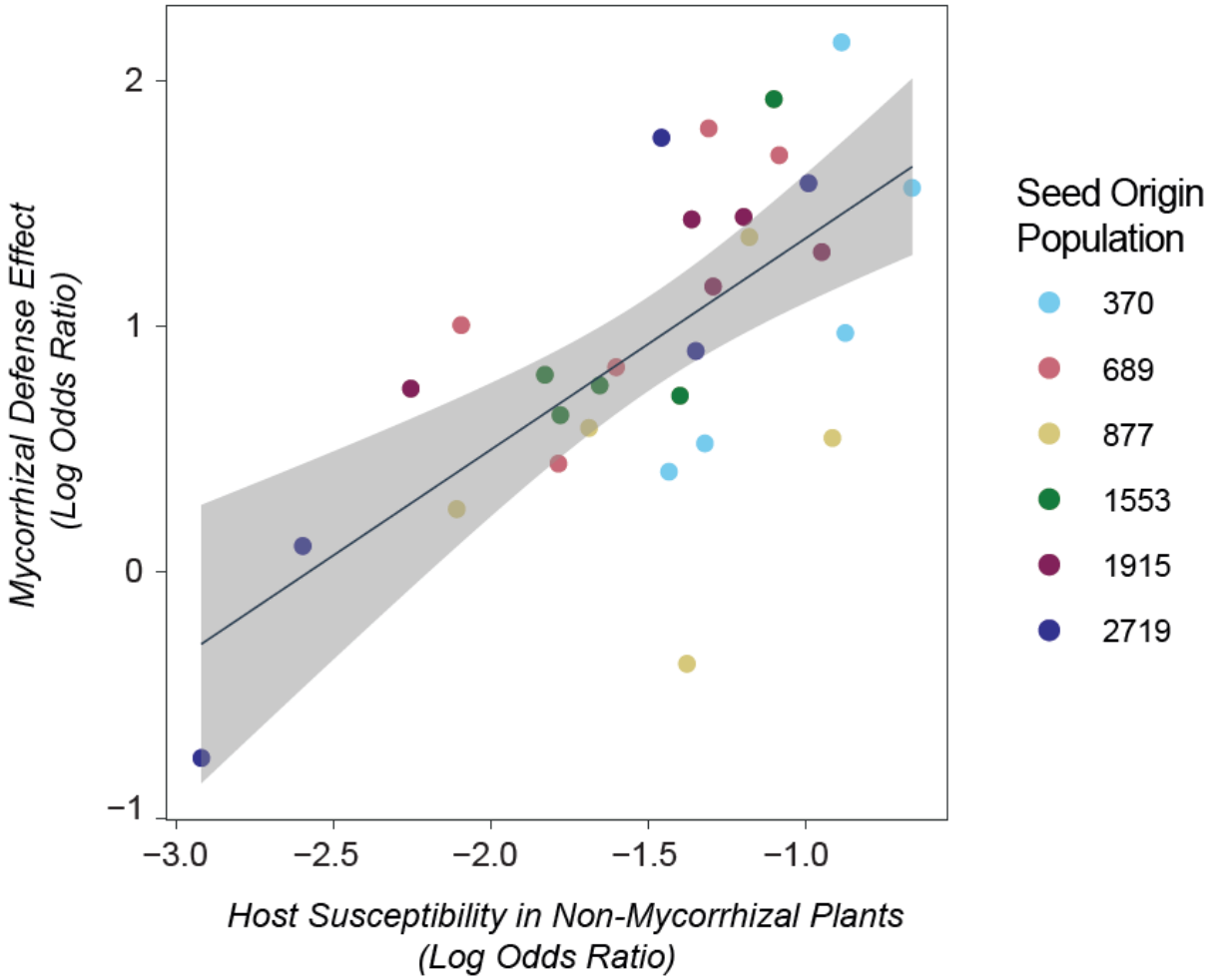

Fig. 4: Host susceptibility to infection and the defensive effects of mycorrhizal fungi are linked in the host genotypes. In a natural epidemic field experiment with plants from thirty maternal genotypes, we found that host genotypes that were more susceptible to infection (when not associated with mycorrhizae) received stronger disease protection effects from association with mycorrhizal fungi (Table S8). Here, each point represents one maternal genotype, originating from one of six seed origin populations (point colors indicate the seed origin population). Infection severity is examined as the effect size representing the proportion of infected leaves, calculated as a log odds ratio. On the x-axis, more positive values indicate more severe infections and thus, higher disease susceptibility. On the y-axis, more positive values indicate stronger mycorrhizal defensive effects (i.e., greater reductions in the proportion of infected leaves when the genotype was associated with mycorrhizae).