Arbuscular mycorrhizal fungi influence host infection during epidemics in a wild plant

pathosystem

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Plain Language Summary (2-3 sentences):

Beneficial, 'mycorrhizal' fungi in roots help plants grow and may protect them from diseases

caused by pathogenic microbes. This study shows that arbuscular mycorrhizal fungi can

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 wild populations is unknown.

• To test whether microbial mutualists provide protection against pathogens, and whether
this varies among host genotypes, 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 from six allopatric populations that
had been inoculated with a mixture of mutualistic arbuscular mycorrhizal fungi or a non-
mycorrhizal control.

• Inoculation with arbuscular mycorrhizal fungi increased growth in plants from every
population, but also increased host infection rate. Mycorrhizal effects on disease severity
varied among host genotypes and strengthened over time during the epidemic. Host
genotypes that were more susceptible to the pathogen received stronger protective effects
from inoculation.

• Our results show that arbuscular mycorrhizal fungi introduce both benefits and risks to
host plants, and shift patterns of infection 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 infectious 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 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 effects 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 ability 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 to parasites and abiotic stress (Azcón-Aguilar & Barea, 1996). Arbuscular 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 parasites may 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.

Empirical studies in controlled environments have shown that host association with

mutualists may also present risks that may influence infection dynamics (Polin *et al*., 2014). For

example, ecological costs may 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 growth or defensive benefits (Johnson *et al*.,

1997; Jones & Smith, 2004; Grman, 2012). Furthermore, unfavorable abiotic conditions may

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 parasite species

(e.g., depending on their life history) (Pozo & Azcón-Aguilar, 2007), or durable to changes in

parasite traits and/or composition in the environment. Finally, mutualists could also affect

patterns of host infection indirectly, e.g., via changes in host size that influence parasite contact

rates. Hence, the potential ecological risks and/or benefits of mutualist association in the

presence of parasites may depend on host genotype and vary among or within host populations

and environments; however, this has remained largely unexplored in natural populations.

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

symbiosis 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 whether these types of defenses are linked 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 – 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 inoculation with arbuscular

mycorrhizal fungi vary among host populations and maternal genotypes? 2) Does inoculation

with arbuscular mycorrhizal fungi influence host infection rate? 3) Upon infection, does prior

inoculation with arbuscular mycorrhizal fungi affect disease severity? 4) Are growth and

defensive effects from inoculation with arbuscular mycorrhizal fungi linked in host genotypes?

and 5) Are disease susceptibility and defensive effects due to inoculation with arbuscular

mycorrhizal fungi 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 by a plant pathogen under natural epidemics and across

different host populations and genotypes.

Materials and 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 (c. every two wks) throughout an epidemic season (c. 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 arbuscular mycorrhizal fungi in genetically

diverse host plants, seeds were collected from six geographically variable populations of *Pl.*

lanceolata in the Åland Islands (Fig. 1A) 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 glasshouse 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, 61°10 N, 24°40 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 glasshouse was supplemented with Osram HQI lamps to provide a photoperiod of 18 h : 6

h light : dark. At 6 wks of age (4 wks 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 12-wk-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 (Fig. 1A).

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 before 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. Plants were considered infected if any leaf showed powdery,

white spot(s) (i.e., characteristic signs of infection with *Po. plantaginis* in this region; Supporting

Information Fig. S1) forming a blotch or lesion of any size upon visual inspection (all leaves

were inspected for signs of infection). 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 leaves infected by *Po. plantaginis* on each
plant were counted (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 rate 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.
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Statistical methods
Do growth effects due to inoculation with arbuscular mycorrhizal fungi vary among host
populations and maternal genotypes?
To test whether host growth was explained by inoculation with arbuscular mycorrhizal
fungi, host genetic origin, or an interaction between these factors, we built a series of generalized

linear models (GLMs). All statistical tests were conducted in the R statistical environment (R
Core Team, 2021). Negative binomial GLMs were constructed with the MASS package counter
overdispersion (Venables & Ripley, 2002). To explore the effect of genetic origin on host growth
at two levels of biological organization, seed origin population (abbreviation: POP) and maternal
genotype (abbreviation: GEN) were included as explanatory factors in separate models. Host
growth was modeled before epidemic exposure (at the time of placement in the field experiment)
and post-epidemic exposure (during the last survey of the experiment) using the number of
leaves on each host. When modeling initial host size, the explanatory factors were mycorrhizal
treatment (abbreviation: MYC), POP/GEN, and their interaction. When modeling final host size,
field epidemic population (abbreviation: SITE), initial host size (abbreviation: SIZE), and a four-
way interaction term between all factors were also included as explanatory factors whenever
possible (without compromising model fit). We also tested whether a subset of AMF and NM
plants varied in aboveground dry biomass at the end of the experiment using a Mann-Whitney U
test to counter non-normality in the data. Throughout our study, non-significant interaction terms
were removed from all final models. To address non-independence of the mycorrhizal treatment
and host size variables during the field experiment, all models including size were also tested
with this variable omitted, and the results were compared.
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Does inoculation with arbuscular mycorrhizal fungi influence host infection rate?
To test whether host infection rate upon exposure to field epidemics is explained by
inoculation with arbuscular mycorrhizal fungi, host genetic origin, field epidemic population, or
an interaction between these factors, we built a series of generalized logistic regression models.
Host infection status was used as the binomial response variable in each model (with family set
to quasibinomial to counter overdispersion). Infection data were analyzed at the peak of the
epidemic and at the end of the experiment. In each model, MYC, POP/GEN, SITE (and SIZE at
the corresponding time point, in applicable models) were included as explanatory factors, as well
as all interaction terms.
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Upon infection, does prior inoculation with arbuscular mycorrhizal fungi affect disease severity?
To test whether disease severity in infected hosts is influenced by prior inoculation with
arbuscular mycorrhizal fungi, host genetic origin, field epidemic population, or an interaction

between these factors, we built a series of generalized logistic 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

quasipoisson in models of the number of leaves infected (to counter overdispersion). In each

model, MYC, POP/GEN, SITE (and SIZE at the corresponding time point, in applicable models)

were included as explanatory factors, as well as all interaction terms.

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

To test for a relationship between growth and defensive effects following inoculation

with arbuscular mycorrhizal fungi in the maternal genotypes, we first used the negative binomial

GLM examining differences in host size (i.e., the model built in the first section of the statistical

methods) to calculate the estimated effect of mycorrhizal inoculation on host growth in each

maternal genotype. Host growth effects were estimated at the beginning of the experiment to

avoid the effects of pathogen exposure and variable field conditions. Effect sizes were obtained

using the lsmeans package (Lenth, 2016) and were averaged over the levels of field epidemic

population. Second, we used the generalized logistic regression model examining differences in

the number of infected leaves in infected hosts (i.e., the model built in the third section of the

statistical methods) to calculate the estimated effect of mycorrhizal inoculation on host defense

in each maternal genotype. Defensive effects were quantified at the epidemic peak to capture the

highest infection levels. We then built a linear regression model linking defense effects (as the

response variable) and growth effects in each genotype.

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

genotypes?

We tested for a relationship between host disease susceptibility and defensive effects

following inoculation with arbuscular mycorrhizal fungi in the host genotypes. First, we

quantified disease susceptibility in each genotype in the absence of the mutualist (i.e., in NM

plants only) using the estimated coefficients from the model examining the number of infected

leaves at the epidemic peak (i.e., the same model as used in the section above). Genotype-level

coefficients 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 of mycorrhizal

inoculation from these same models (i.e., as the change in the coefficient when the genotype was

inoculated). Finally, we built a linear regression model linking defense effect sizes (as the

response variable) and disease susceptibility in each genotype.

Results

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

maternal genotypes?

Inoculation with arbuscular mycorrhizal fungi produced growth benefits in experimental

plants from nearly every host genetic origin. Before pathogen exposure in field conditions, host

growth was determined by mycorrhizal inoculation and seed origin population (Supporting

Information Fig. S2 & Table S1; MYC: $p < 0.001$, POP: $p < 0.001$, $n = 287$ plants), as well as by

an interaction between mycorrhizal inoculation and host maternal genotype (Fig. 1A &

Supporting Information Table S1; MYC $\times$ GEN: $p = 0.002$). At the end of field epidemic

experiment, mycorrhizal inoculation (Fig. 1B – 1C & Supporting Information Tables S2 – S3;

MYC: $p < 0.001$), seed origin population (Fig. 1B & Supporting Information Table S2; POP: $p <$

0.001), and maternal genotype (Fig. 1C & Supporting Information Table S3; GEN: $p < 0.001$)

continued to be tightly linked to host growth, but the interaction between mycorrhizal inoculation

and maternal genotype had disappeared. Host initial size also predicted of host final size

(Supporting Information Table S2; SIZE: $p < 0.001$). Mycorrhizal inoculation also increased the

aboveground dry biomass of plants at the end of the experiment (Supporting Information Fig. S3

& Table S4; MYC: $p < 0.001$, $W = 11$, $n = 41$ plants).

Does inoculation with mycorrhizal fungi influence host infection rate?

Upon pathogen exposure in the field epidemics, host infection rate was influenced by

several factors whose importance shifted over time. Host infection rates reached 73 % at the peak

of the epidemic but fell to 34 % by the end of the experiment (Fig. 2A). Host infection rate also

varied among the three field epidemic populations, with the effect of field site strengthening over

time (Figs. 2B – 2D & Supporting Information Tables S5 – S8; SITE (peak of epidemic): $p =$

0.03 (POP) – 0.01 (GEN); end of experiment: $p < 0.001$ (POP/GEN). Though inoculation with

arbuscular mycorrhizal fungi marginally influenced host infection rate during the peak of the

epidemic (Fig. 2C & Supporting Information Tables S5 – S6; MYC: $p = 0.09$ (POP) - 0.11

(GEN), $n = 287$ plants), at the end of experiment AMF plants were slightly more likely to be

infected than NM plants (Fig. 2D & Supporting Information Tables S7 – S8; MYC: $p = 0.04$

(POP) – 0.05 (GEN), $n = 286$ plants). The effect of mycorrhizal inoculation on host infection

rates was weaker than the effect on host growth. Host size also influenced host infection at the

end of the experiment: larger plants were more likely to be infected (Supporting Information

Tables S7 – S8; SIZE: $p = 0.06$ (POP) – 0.04 (GEN)).

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 host genetic origin. AMF plants had lower proportions of their

leaves infected relative to NM plants throughout the experiment (Figs. 3A & 3B, Supporting

Information Tables S9 – S12; MYC: $p < 0.001$, $n = 210$ plants (peak epidemic); $p = 0.003$, $n =$

98 plants (end experiment)). The proportion of leaves infected also varied among seed origin

populations (Fig. 3A & Supporting Information Table S9; POP: $p = 0.004$) and maternal

genotypes (Supporting Information Table S10; GEN: $p = 0.02$) during the peak of the epidemic

but was similar among host genetic origins by the end of the experiment (Supporting Information

Tables S11 – S12). At the epidemic peak, mycorrhizal inoculation and maternal genotype

interacted to determine the number of infected leaves on infected hosts (Fig. 3C & Supporting

Information Table S14; MYC × GEN: $p = 0.05$), with both positive and negative effects

occurring. By the end of the experiment, AMF plants had higher numbers of infected leaves than

NM plants (Fig. 3D & Supporting Information Table S16; MYC: $p = 0.04$). Like the effect on

host infection rates, the effect of mycorrhizal inoculation on disease severity was weaker than the

effect on host growth. Field epidemic population also influenced the number of infected leaves in

hosts at the end of the experiment (Fig. 3D & Supporting Information Table S15; SITE: $p =$

0.02). Host size predicted the proportion of infected leaves at the peak of the epidemic

(Supporting Information Tables S9 – S10; SIZE: $p < 0.001$) and the number of infected leaves

throughout the experiment (Supporting Information Tables S13 – S16; SIZE: $p \leq 0.01$).

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

There was a marginally significant relationship between the growth and defensive effects conferred by inoculation with arbuscular mycorrhizal fungi across the host maternal genotypes at the end of the experiment (Fig. 4A & Supporting Information Table S17; $p = 0.11$, $n = 30$ maternal genotypes). Host genotypes that experienced more positive growth effects due to inoculation with mycorrhizal fungi (i.e., higher increases in leaf number in AMF relative to NM plants) experienced slightly more negative defensive outcomes (i.e., higher increases in the number of infected leaves in AMF relative to NM plants).

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

We found a strong relationship between host disease susceptibility and the magnitude of the defensive effect received from mycorrhizal inoculation in the maternal genotypes (Fig. 4B & Supporting Information Table S18; $p < 0.001$, $n = 29$ maternal genotypes). At the peak of the epidemic, host genotypes that suffered more severe infections (i.e., had more infected leaves) when not inoculated with mycorrhizal fungi experienced greater reductions in disease severity (i.e., in the number of infected leaves) when inoculated with mycorrhizal fungi.

Discussion

While both plant-associated pathogenic and mutualistic microbes are ubiquitous across ecosystems, how they interact to determine patterns of infection in genetically diverse host populations is not known. In a field experiment placing wild hosts of thirty maternal genotypes in naturally occurring pathogen epidemics, we found that inoculation with a three-species mixture of arbuscular mycorrhizal fungi produced benefits and risks that influenced aboveground patterns of host infection. Mycorrhizal inoculation increased growth in hosts of nearly every genotype, but also increased infection rates from a foliar fungal pathogen. The effects of mycorrhizal inoculation on disease severity varied over the course of the epidemic, with both protective and negative effects occurring among the host genotypes. Moreover, disease susceptibility and mycorrhizae-derived defense effects appeared to be linked in the host genotypes: more susceptible host genotypes (in the absence of the mutualists) received stronger protection against disease when inoculated with mycorrhizal fungi. Arbuscular 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 our results provide the first evidence of how microbial mutualists can

shift patterns of host infection in genetically diverse wild populations under pathogen attack.

Mycorrhizal fungi also appeared to be linked to a growth-defense trade-off in the hosts:

mycorrhizal-inoculated plants grew larger and became more infected by the pathogen, with the

host genotypes that obtained the greatest growth benefit from mycorrhizal fungi also suffering

the largest increases in disease. Together, our results underscore that under natural ecological

and epidemic conditions mycorrhizal fungi produce a complex and temporally variable array of

positive and negative effects on host growth and infection.

Inoculation with mycorrhizal fungi provided growth benefits to host plants from every

population and maternal genotype. The magnitude of these effects varied among hosts from

different genetic origins before exposure to the field epidemics but homogenized over time in

field conditions. Due to the positive relationship between plant size and survival, growth is often

intrinsically linked to host fitness (Harper & White, 1974). Our results are consistent with studies

reporting evidence for the importance of mycorrhizal fungi in determining host growth and/or

fitness and showing variability in such effects among host genotypes (Rasmussen *et al*., 2017,

Qin *et al*., 2021). In addition, susceptibility to the pathogen epidemics in the field varied among

hosts of different genetic origins, consistent with other studies in this pathosystem (Laine, 2005,

Laine, 2007a, Tack *et al*., 2014, Susi & Laine, 2015) and 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), although 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 microbial mutualists. Inoculation experiments with agricultural

plant species and their pathogens have shown that arbuscular mycorrhizal fungi can reduce

disease incidence or severity (Norman *et al*., 1996; Pozo *et al*., 2002; Li *et al*., 2010; Song *et al*.,

2015; Berdeni *et al*., 2018), 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 increased

infection rates in hosts of a wild plant species and produced variable effects on disease severity

in hosts of different genotypes and over the course of the epidemic. By the end of the

experiment, mycorrhizal inoculation was weakly linked to higher numbers of infected leaves in

diseased hosts (though variation among genotypes remained). Our results suggest that

environmental, temporal, and genetic contexts may alter the potential defensive effects related to

mycorrhizal association and are consistent with other experimental studies showing that

mycorrhizal effects on host infection may vary among host genotypes (Mark & Cassells 1996;

Steinkellner *et al*. 2012). However, in our experiment the effects of mycorrhizal inoculation on

infection were weaker than the effects on host growth. Additional studies where mycorrhizal

colonization is explicitly quantified are needed to more reliably assess the contribution of the

symbiont to host growth, infection, and fitness. The potential risks of mycorrhizal association in

wild plants are relevant for theoretical studies which speculate as to how mycorrhizal fungi

might influence host fitness in the presence of pathogens and affect plant population and

community dynamics (Bachelot *et al*., 2015).

In many free-living organisms, an evolutionary trade-off exists between growth and

defense (Herms & Mattson, 1992). In our experiment, additional insights can be gained by

examining whether changes in host growth due to mutualism are related to changes in host

defense across genotypes. In our experiment, mycorrhizal-inoculated hosts consistently grew

larger and were more likely to become infected by the pathogen. This could occur if increases in

host size increase pathogen encounter rates, as could be expected for pathogen species with

wind- or passively-dispersed spores (like *Po. plantaginis*). In addition, host genotypes that

experienced larger growth benefits from mycorrhizal inoculation suffered marginally larger

increases in disease severity (though there was considerable variation in this effect). That some

host genotypes grew larger and experienced reductions in disease severity following mycorrhizal

inoculation suggests that defense priming could occur occasionally. Infected AMF plants also

had lower proportions of infected leaves than NM plants (relative to their size), though it is

unclear whether this may offset the costs of having higher numbers of infected leaves in this

pathosystem. Changes in host tolerance to pathogens following mycorrhizal inoculation could

also explain increases in infected leaf numbers, though mycorrhizal fungi are generally expected

to improve host nutritional status or increase leaf toughness, making foliar pathogen spread more

difficult (Meier & Hunter, 2018). Thus, it is likely that differences in host infection between

AMF and NM plants in our experiment were mediated by increases in host size (as host size also

predicted some aspects of infection) or defense priming in some genotypes.

In addition, our findings indicate that arbuscular mycorrhizal fungi could help susceptible

host genotypes compensate for lack of innate resistance while placing costs on well-defended

genotypes. We found that the magnitude of defense effects following mycorrhizal inoculation

were linked to pathogen susceptibility in the host genotypes: host genotypes that were

susceptible to more severe infections received stronger disease protection effects when

inoculated with the mutualist. In contrast, more resistant host genotypes were likely to slightly

increase in disease load when inoculated. Thus, inoculation with mycorrhizal fungi tended to

equalize disease severity between more resistant and more susceptible host genotypes,

potentially reducing the relative importance of host genetic resistance in determining pathogen

effects. However, the linkages between disease susceptibility and mycorrhizal defense effects in

our study (as well as between host growth and these effects) were revealed post-hoc and should

be confirmed with experiments designed to test these hypotheses. If confirmed, it could indicate

that host genotypes may experience trade-offs in investment in genetic resistance vs.

mycorrhizal-mediated resistance. It could also suggest that 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 host genetic resistance within and among populations (Laine, 2004;

Laine 2007a, Jousimo *et al*. 2014).

Variation among host populations and genotypes in mycorrhizal benefits and risks could

be due to several factors. These factors include intraspecific differences in hosts' ability to form

associations with and derive function from different mycorrhizal species, differences in

mycorrhizal colonization rates or community composition, environmental variation, or

differences in host-pathogen population dynamics over time. Prior studies demonstrate variation

in arbuscular 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). Though we observed clear differences in host growth and infection as a result of the

mycorrhizal inoculation treatment, data on mycorrhizal colonization are needed to confirm

mycorrhizae as the mechanism underlying the observed effects. Environmental conditions may

also impact plant-microbe interactions (Santoyo *et al*., 2017). Consistent with other studies in

this pathosystem, infection rates varied among field populations and changed over time (Eck *et*
al., 2022). In contrast, the effects of mycorrhizal inoculation on hosts were similar among field
populations, though changes over time also occurred. Growth benefits following inoculation with
mycorrhizal fungi varied among host populations and genotypes before pathogen exposure but
became homogenous over time in the field conditions. Changes in host infection rate and disease
severity due to mycorrhizal inoculation were also similar among field populations but increased
over time. Together, these results suggest that mycorrhizal effects on hosts are more temporally
than environmentally sensitive. There is also some chance that environmental mycorrhizal spores
could have come in to contact with our experimental soils, such that true levels of mycorrhizal
colonization in the non-mycorrhizal treatment could be low or some (rather than none), and the
mycorrhizal communities in the experimental pots could contain species that were not
inoculated. The limited duration of the field experiment reduces the likelihood that this could
cause strong effects (Sanders & Sheikh, 1986); however, if it occurred, it should have occurred
evenly among treatments and seed sources. Future studies in which mycorrhizal composition,
colonization rates, and function are quantified in genetically diverse hosts and in variable
environmental conditions over time are necessary to corroborate this work and disentangle the
effect of mycorrhizal growth and defensive effects from intraspecific variation in host growth
and resistance.

Together, our results suggest that symbiosis with arbuscular mycorrhizal fungi produces
benefits and alters infection risks from a pathogen during natural epidemics in genetically
variable host populations. Altered patterns of host growth and infection due to mutualist species
may cascade to affect patterns of abundance, diversity, and distribution of the associated
organisms, as well as ecosystem processes (Brown *et al*., 2001). Moreover, we are beginning to
acknowledge the importance of direct and indirect microbial interactions 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 also 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 parasites may act simultaneously to determine host
fitness (Bezemer & van Dam, 2005). Our results also highlight the importance of characterizing
host-microbe interactions under natural conditions and temporal interaction sequences, which
will allow more precise modeling of epidemiological and ecological community dynamics. In

addition, growth and defensive effects due to mutualism should ultimately 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). Understanding

how mutualism alters host susceptibility and parasite interactions will be important for

understanding, predicting, and managing disease in ecological 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 supports the findings of this study are available in the supplementary material of this article

References

- **Azcón-Aguilar C, Barea JM. 1996.** Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanisms involved. *Mycorrhiza* **6**: 457-464.

- **Bachelot B, Uriarte,M, McGuire K. 2015.** Interactions among mutualism, competition, and predation foster species coexistence in diverse communities. *Theoretical Ecology* **8**: 297-312.

- **Barea JM, Azcón R, Azcón-Aguilar C. 2002.** Mycorrhizosphere interactions to improve plant fitness and soil quality. *Antonie van Leeuwenhoek* **81**: 343-351.

- **Bates D, Maechler M, Bolker B, Walker S. 2015**. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67(1)**: 1-48.

- **Berdeni D, Cotton TEA, Daniell TJ, Bidartondo MI, Cameron DD, Evans KL. 2018.** The effects of arbuscular mycorrhizal fungal colonization on nutrient status, growth, productivity, and canker resistance of apple (*Malus pumila*). *Frontiers in Microbiology* **9:1461**. doi: 10.3389/fmicb.2018.01461

- **Bever JD, Mangan SA, Alexander HM. 2015.** Maintenance of plant species diversity by pathogens. *Annual Review of Ecology, Evolution, and Systematics* **46**: 305-325.

- **Bezemer TM, van Dam NM. 2005**. Linking aboveground and belowground interactions via induced plant defenses. *Trends in Ecology and Evolution* **20(11)**: 617-624.

- **Bos M. 1992**. Gene flow characters and population structure in *Plantago lanceolata*. In: Kuiper, PJC, Bos, M eds. *Plantago: A Multidisciplinary Study.* Berlin, DE: Springer-Verlag, 222-231.

- **Brown JH, Whitham TG, Ernest SKM, Gehring CA**. **2001.** Complex species interactions and the dynamics of ecological systems: long-term experiments. *Science* **293**: 643-650.

- **Brown JKM**. **2003.** A cost of disease resistance: paradigm or peculiarity? *Trends in Genetics* **19(12)**: 667-671.

- **Brownlie JC, Johnson KN. 2009.** Symbiont-mediated protection in insect hosts. *Trends in Microbiology* **17(8)**: 348-354.

- **Burdon JJ, Thrall PH. 2014.** What have we learned from studies of wild plant-pathogen associations?—the dynamic interplay of time, space and life-history. *European Journal of Plant Pathology* **138,** 417–429.

- **Bushnell WR. 2002.** The role of powdery mildew research in understanding host-pathogen interaction: past, present, and future. In: Bélanger RR, Bushnell WR, Dik AJ, Carver LW

eds. *The Powdery Mildews – a Comprehensive Treatise*. St. Paul, USA: The American

Phytopathology Society, 1-12.

• **Cameron DD, Neal AL, van Wees SCM, Ton J. 2013.** Mycorrhiza-induced resistance:

more than the sum of its parts? *Trends in Plant Science* **18(10)**: 539-545.

• **Carlsson-Granér U. 1997.** Anther-smut disease in *Silene dioica*: variation in susceptibility

among genotypes and populations, and patterns of disease within populations. *Evolution*

51(5): 1416-1426.

• **van Dam NM, Heil M. 2010.** Multitrophic interactions below and above ground: *en route* to

the next level. *Journal of Ecology* **99(1)**: 77-88.

• **Delavaux CS, Smith-Ramesh LM, Kuebbing SE. 2017.** Beyond nutrients: a meta-analysis

of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. *Ecology* **98(8)**:

2111-2119.

• **Eck JL, Stump SM, Delavaux CS, Mangan SA, Comita LS. 2019.** Evidence of within-

species specialization by soil microbes and the implications for plant community diversity.

PNAS. doi: 10.1073/pnas.1810767116

• **Eck JL, Barrès B, Soubeyrand S, Sirén J, Numminen E, Laine A-L. 2022.** Strain

diversity and spatial distribution are linked to epidemic dynamics in host populations. *The*

American Naturalist **199(1): ** 59-74.

• **Fiorilli V, Vannini C, Ortolani F, Garcia-Seco D, Chiapello M, Novero M, Domingo G,**

Terzi V, Morcia C, Bagnaresi P, *et al*. 2018. Omics approaches revealed how arbuscular

mycorrhizal symbiosis enhances yield and resistance to leaf pathogen in wheat. *Scientific*

Reports **8:** 9625. doi: 10.1038/s41598-018-27622-8

• **Frew A, 2021.** Aboveground herbivory suppresses the arbuscular mycorrhizal symbiosis,

reducing phosphorus uptake. *Applied Soil Ecology* **168**: 104133. doi:

10.1016/j.apsoil.2021.104133

• **Gilbert GS, Webb CO. 2007.** Phylogenetic signal in plant pathogen-host range. *PNAS*

104(12): 4979-4983.

• **Goddard M-L, Belval L, Martin I, Roth L, Laloue H, Deglene-Benbrahim L, Valat L,**

Bertsch C, Chong J. 2021. Arbuscular mycorrhizal symbiosis triggers major changes in

primary metabolism together with modification of defense responses and signaling in both
roots and leaves of Vitis vinifera. *Frontiers in Plant Science* 12: 721614.

• **Gosling P, Hodge A, Goodlass G, Bending GD. 2006.** Arbuscular mycorrhizal fungi and
organic farming. *Agriculture, Ecosystems, and Environment* **113**: 17-35.

• **Grman E. 2012**. Plant species differ in their ability to reduce allocation to non-beneficial
arbuscular mycorrhizal fungi. *Ecology* **93(4):** 711-718.

• **Hafer-Hahmann NC, Vorburger C. 2021.** Positive association between the diversity of
symbionts and parasitoids of aphids in field populations. *Ecosphere* **12(1)**. doi:
10.1002/ecs2.3355

• **Halbritter AH, Carroll GC, Güsewell S, Roy BA. 2012.** Testing assumptions of the enemy
release hypothesis: generalist versus specialist enemies of the grass *Brachypodium*
sylvaticum. *Mycologia* **104(1)**: 34-44.

• **Hanski I. 1999.** *Metapopulation Ecology*. Oxford University Press, Oxford, UK.

• **Hao Z, Christie P, Qin L, Wang C, Li X. 2005.** Control of fusarium wilt of cucumber
seedlings by inoculation with an arbuscular mycorrhizal fungus. *Journal of Plant Nutrition*
28: 1961–1974.

• **Harper JL, White J. 1974.** The demography of plants. *Annual Review of Ecology and*
Systematics **5:** 419–463.

• **Hartnett DC, Wilson GWT. 2002.** The role of mycorrhizas in plant community structure
and dynamics: lessons from grasslands. In: Smith SE, Smith, FA eds. *Diversity and*
integration in mycorrhizas. Developments in plant and soil sciences **94**. Dordrecht, NL:
Springer. doi: 10.1007/978-94-017-1284-2_30

• **van der Heijden MGA, Bardgett RD, van Straalen NM. 2008.** The unseen majority: soil
microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology*
Letters **11**: 296-310.

• **Herms DA, Mattson WJ. 1992.** The dilemma of plants: to grow or defend. *The Quarterly*
Review of Biology **67(3)**: 283–335.

• **Hersh MH, Vilgalys R, Clark JS. 2012.** Evaluating the impacts of multiple generalist
pathogens on temperate tree seedling survival. *Ecology* **93(3)**: 511-520.

• **Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide RT, Pringle**
A, Zabinski C, Bever JD, Moore JC et al. 2010. A meta-analysis of context-dependency in
plant response to inoculation with mycorrhizal fungi. *Ecology Letters* **13**: 394-407.

• **Hohmann P, Messmer MM. 2017.** Breeding for mycorrhizal symbiosis: focus on disease
resistance. *Euphytica* **213(113)**. doi: 10.1007/s10681-017-1900-x

• **Johansson JF, Paul LR, Finlay RD. 2004.** Microbial interactions in the mycorrhizosphere
and their significance for sustainable agriculture. *FEMS Microbiology Ecology* **48**: 1-13.

• **Johnson NC, Graham JH, Smith FA**. **1997**. Functioning of mycorrhizal associations along
the mutualism-parasitism continuum. *New Phytologist* **135**: 575-585.

• **Johnson D, Vandenkoornhuyse PJ, Leake JR, Gilbert L, Booth RE, Grime JP, Young**
JPW, Read DJ. 2003. Plant communities affect arbuscular mycorrhizal fungal diversity and
community composition in grassland microcosms. *New Phytologist* **161**: 503-515.

• **Jones MD, Smith SE. 2004.** Exploring functional definitions of mycorrhizas: are
mycorrhizas always mutualisms? *Canadian Journal of Botany* **82**: 1089-1109.

• **Jousimo J, Tack AJM, Ovaskainen O, Mononen T, Susi H, Tollenaere C, Laine A-L.**
2014. Ecological and evolutionary effects of fragmentation and disease dynamics. *Science*
344(6189): 1289-1293.

• **Jung SC, Martinez-Medina A, Lopez-Raez JA, Pozo MJ. 2012.** Mycorrhiza-induced
resistance and priming of plant defenses. *Journal of Chemical Ecology* **38**: 651-664.

• **Kemen E. 2014.** Microbe-microbe interactions determine oomycete and fungal host
colonization. *Current Opinion in Plant Biology* **20**: 75-81.

• **Kemen AC, Agler MT, Kemen E. 2015.** Host-microbe and microbe-microbe interactions in
the evolution of obligate plant parasitism. *New Phytologist* **206**: 1207-1228.

• **King KC, Brockhurst MA, Vasieva O, Paterson S, Betts A, Ford SA, Frost CL,**
Horsburgh MJ, Haldenby S, Hurst GDD. 2016. Rapid evolution of microbe-mediated
protection against pathogens in a worm host. *The ISME Journal* **10**: 1915-1924.

• **Klironomos J. 2003.** Variation in plant response to native and exotic arbuscular mycorrhizal
fungi. *Ecology* **84(9)**: 2292-2301.

• **Koide RT, Dickie IA. 2002.** Effects of mycorrhizal fungi on plant populations. In: Smith SE,
Smith, FA eds. *Diversity and integration in mycorrhizas. Developments in plant and soil*
sciences **94**. Dordrecht, NL: Springer. doi: 10.1007/978-94-017-1284-2_30

• **Koricheva J, Gange AC, Jones T. 2009.** Effects of mycorrhizal fungi on insect herbivores:
a meta-analysis. *Ecology* **90(8)**: 2088-2097.

• **Kroll S, Agler MT, Kemen E. 2017.** Genomic dissection of host-microbe and microbe-
microbe interactions for advanced plant breeding. *Current Opinion in Plant Biology* **36**: 71-
78.

• **Kuznetsova A, Brockhoff PB, Christensen RHB. 2017.** lmerTest package: tests in linear
mixed effects models. *Journal of Statistical Software* **82(13)**. doi: 10.18673/jss.v082.i13.

• **Laine A-L. 2004.** Resistance variation within and among host populations in a plant
pathogen metapopulation: implications for regional pathogen dynamics. *Journal of Ecology*
92(6): 990-1000.

• **Laine A-L. 2005**. Spatial scale of local adaptation in a plant-pathogen metapopulation.
Journal of Evolutionary Biology **18**: 930-938.

• **Laine A-L. 2006.** Evolution of host resistance: looking for coevolutionary hotspots at small
spatial scales. *Proceedings: Biological Sciences* **273(1584)**: 267-273.

• **Laine A-L. 2007a.** Detecting local adaptation in a natural plant-pathogen metapopulation: a
laboratory vs. field transplant approach. *Journal of Evolutionary Biology* **20**: 1665-1673.

• **Laine A-L. 2007b.** Pathogen fitness components and genotypes differ in their sensitivity to
nutrient and temperature variation in a wild plant-pathogen association. *Journal of*
Evolutionary Biology **20**: 2371-2378.

• **Laine A-L. 2008**. Temperature-mediated patterns of local adaptation in a natural plant-
pathogen metapopulation. *Ecology Letters* **11**: 327-337.

• **Laine A-L, Hanski I. 2006.** Large-scale dynamics of a specialist plant pathogen in a
fragmented landscape. *Journal of Ecology* **94(1)**: 217-226.

• **Laine A-L, Tellier A. 2008**. Heterogeneous selection promotes polymorphism maintenance
in host-parasite interactions. *Oikos* **117**: 1281-1288.

• **Laine A-L, Burdon JJ, Dodds PN, Thrall PH. 2011.** Spatial variation in disease resistance:
from molecules to metapopulations. *Journal of Ecology* **99**: 96-112.

• **Lenth RV. 2016**. Least-squares means: the R package lsmeans. *Journal of Statistical*
Software **69(1)**: 1-33.

•   **Li Y, Yanagi A, Miyawaki Y, Okada T, Matsubara Y. 2010.** Disease tolerance and
changes in antioxidative abilities in mycorrhizal strawberry plants. *Journal of the Japanese*
Society for Horticultural Science **79(2)**: 174-178.

•   **Lo Iacono G, van den Bosch F, Paveley N. 2012.** The evolution of plant pathogens in
response to host resistance: factors affecting the gain from deployment of qualitative and
quantitative resistance. *Journal of Theoretical Biology* **304**: 152-163.

•   **Mark GL, Cassells AC. 1996.** Genotype-dependence in the interaction between Glomus
fistulosum, Phytophthora fragariae and the wild strawberry (Fragaria vesca). *Plant & Soil*
185: 233–239.

•   **May G, Nelson P. 2014.** Defensive mutualisms: do microbial interactions within hosts drive
the evolution of defensive traits? *Functional Ecology* **28**: 356-363.

•   **Meier AR, Hunter MD. 2018.** Arbuscular mycorrhizal fungi mediate herbivore-induction of
plant defenses differently above and belowground. *Oikos* **127**: 1759-1775.

•   **Newsham KK, Fitter AH, Watkinson AR. 1995.** Arbuscular mycorrhiza protect an annual
grass from root pathogenic fungi in the field. *Journal of Ecology* **83(6)**: 991-1000.

•   **Norman JR, Atkinson D, Hooker JE. 1996.** Arbuscular mycorrhizal fungal-induced
alteration to root architecture in strawberry and induced resistance to the root pathogen
Phytophthora fragariae. *Plant and Soil* **185**: 191-198.

•   **Oliver KM, Smith AH, Russell JA. 2014.** Defensive symbiosis in the real world –
advancing ecological studies of heritable, protective bacteria in aphids and beyond.
Functional Ecology **28**: 341-355.

•   **Öpik M, Moora M, Liira J, Zobel M. 2006.** Composition of root-colonizing arbuscular
mycorrhizal fungal communities in different ecosystems around the globe. *Journal of*
Ecology **94**: 778-790.

•   **Ovaskainen O, Laine A-L. 2006**. Inferring evolutionary signals from ecological data in a
plant-pathogen metapopulation. *Ecology* **87(4)**: 880-891.

•   **Pawlowski ML, Vuong TD, Valliyodan B, Nguyen HT, Hartman GL. 2020.** Whole
genome resequencing identifies quantitative trait loci associated with mycorrhizal
colonization of soybean. *Theoretical and Applied Genetics* **133**: 409–417.

- **Penczykowski R, Walker E, Soubeyrand S**, **Laine A-L**. **2015**. Linking winter conditions to large-scale disease dynamics in a wild plant-pathogen metapopulation. *New Phytologist*, **205**: 1142-1152.

- **Plouznikoff K, Asins MJ, Dupré de Boulois H, Carbonell EA, Declerck S. 2019.** Genetic analysis of tomato root colonization by arbuscular mycorrhizal fungi. *Annals of Botany* **124**: 933–946.

- **Polin S., Simon J-C, Outreman Y. 2014.** An ecological cost associated with protective symbionts of aphids. *Ecology and Evolution* **4(6)**: 836-840.

- **Pozo MJ, Azcón-Aguilar C. 2007.** Unraveling mycorrhiza-induced resistance. *Current Opinion in Plant Biology* **10**: 393-398.

- **Pozo MJ, Cordier C, Dumas-Gaudot E, Gianinazzi S, Barea JM, Azcón-Aguilar C. 2002.** Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomato plants. *Journal of Experimental Botany* **53(368)**: 525-534.

- **Pozo de la Hoz J, Rivero J, Azcón-Aguilar C, Urrestarazu M, Pozo MJ. 2021.** Mycorrhiza-induced resistance against foliar pathogens is uncoupled of nutritional effects under different light intensities. *Journal of Fungi* **7**: 402. doi: 10.3390/jof7060402

- **Pretty J, Sutherland WJ, Ashby J, Auburn J, Baulcombe D, Bell M, Bentley J, Bickersteth S, Brown K, Burke J et al. 2011.** The top 100 questions of importance to the future of global agriculture. *International Journal of Agricultural Sustainability* **8(4)**: 219-236.

- **Price JS, Bever JD, Clay K. 2004.** Genotype, environment, and genotype by environment interactions determine quantitative resistance to leaf rust (*Coleosporium asterum*) in *Euthamia graminifolia* (Asteraceae). *New Phytologist* **162**: 729-743.

- **van der Putten WH, Vet LEM, Harvey JA, Wäckers FL. 2001.** Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology and Evolution* **16(10)**: 547-554.

- **Qin J, Geng Y, Li X, Zhang C, Zhao X, von Gadow K. 2021.** Mycorrhizal type and soil pathogenic fungi mediate tree survival and density dependence in a temperate forest. *Forest Ecology and Management* 496: 119459. doi: 10.1016/j.foreco.2021.119459

•   **Qu L, Wang M, Biere A. 2021**. Interactive effects of mycorrhizae, soil phosphorus, and
light on growth and induction and priming of defense in *Plantago lanceolata*. *Frontiers in*
Plant Science **12**: 647372. doi: 10.3389/fpls.2021.647372

•   **R Core Team. 2021**. R: A language and environment for statistical computing. R Foundation
for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org

•   **Rasmussen PU, Amin T, Bennett AE, Karlssongreen K, Timonen S, van Nouhuys S,**
Tack AJM. 2017. Plant and insect genetic variation mediate the impact of arbuscular
mycorrhizal fungi on a natural plant-herbivore interaction. *Ecological Entomology* **42**: 793-
802.

•   **Rasmussen PU, Hugerth LW, Blanchet FG, Andersson AF, Lindahl BD, Tack AJM.**
2018. Multiscale patterns and drivers of arbuscular mycorrhizal fungal communities in the
roots and root-associated soil of a wild perennial herb. *New Phytologist.* doi:
10.111/nph.15088

•   **Rasmussen PU, Chareesri A, Neilson R, Bennett AE, Tack AJM. 2019**. The impact of
dispersal, plant genotype and nematodes on arbuscular mycorrhizal fungal colonization. *Soil*
Biology and Biochemistry **132**: 28-35.

•   **Rillig MC. 2004.** Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecology*
Letters **7**: 740-754.

•   **Sagar GR, Harper JL. 1964**. *Plantago major* L., *P. media* L. and *P. lanceolata* L. *Journal*
of Ecology **52(1)**: 189-221.

•   **Sallinen S, Norberg A, Susi H, Laine A-L. 2020.** Intraspecific host variation plays a key
role in virus community assembly. *Nature Communications* **11**: 5610. doi: 10.1038/s41467-
020-19273-z

•   **Salvaudon L, Giraud T, Shykoff JA. 2008**. Genetic diversity in natural populations: a
fundamental component of plant-microbe interactions. *Current Opinion in Plant Biology*
11(2): 135-143.

•   **Sanders FE, Sheikh NA. 1983.** The development of vesicular-arbuscular mycorrhizal
infection in plant root systems. *Plant and Soil* **71(1/3)**: 223-246.

•   **Santoyo G, Hernández-Pacheco C, Hernández-Salmerón J, Hernández-León R. 2017.**
The role of abiotic factors modulating the plant-microbe-soil interactions: towards
sustainable agriculture – a review. *Spanish Journal of Agricultural Research* **15(1)**, e03R01.

• **Sivapalan A. 1993.** Effects of water on germination of powdery mildew conidia.
Mycological Research **97(1)**: 71-76.

• **Smith SE, Read DJ. 2008.** *Mycorrhizal Symbiosis*, *3*rd *edn*. Cambridge, UK: Academic
Press.

• **Sochard C, Bellec L, Simon J-C, Outreman Y. 2020.** Influence of "protective" symbionts
throughout the different steps of an aphid-parasitoid interaction. *Current Zoology*. doi:
10.1093/cz/zoaa053

• **Song Y, Chen D, Lu K, Sun Z, Zeng R. 2015**. Enhanced tomato disease resistance primed
by arbuscular mycorrhizal fungus. *Frontiers in Plant Science* **6(786)**: 1-13.

• **Soubeyrand S, Laine A-L, Hanski I, Penttinen A. 2009.** Spatiotemporal structure of host-
pathogen interactions in a metapopulation. *The American Naturalist* **174(3)**: 308-320.

• **Steinkellner S, Hage-Ahmed K, García-Garrido JM, Illana A, Ocampo JA, Vierheilig
H. 2012.** A comparison of wild-type, old and modern tomato cultivars in the interaction with
the arbuscular mycorrhizal fungus Glomus mosseae and the tomato pathogen Fusarium
oxysporum f. sp. lycopersici. *Mycorrhiza* **22:** 189–194.

• **Susi H, Laine A-L. 2015.** The effectiveness and costs of pathogen resistance strategies in a
perennial plant. *Journal of Ecology* 103: 303-315.

• **Tack AJM, Laine A-L. 2014.** Ecological and evolutionary implications of spatial
heterogeneity during the off-season for a wild plant pathogen. *New Phytologist* **202**: 297-308.

• **Tack AJM, Hakala J, Petäjä T, Kulmala M, Laine A-L. 2014.** Genotype and spatial
structure shape pathogen dispersal and disease dynamics at small spatial scales. *Ecology*
95(3): 703-714.

• **Thompson JN. 1994**. *The Coevolutionary Process*. Chicago, USA: The University of
Chicago Press.

• **Thrall PH, Laine A-L, Broadhurst LM, Bagnall DJ, Brockwell J. 2011.** Symbiotic
effectiveness of rhizobial mutualists varies in interactions with native Australian legume
genera. *PLoS ONE* **6**: ge23545. doi: 10.1371/journal.pone.0023545

• **Tollenaere C, Laine A-L. 2013.** Investigating the production of sexual resting structures in a
plant pathogen reveals unexpected self-fertility and genotype-by-environment effects.
Journal of Evolutionary Biology **26**: 1716–1726.

• **Venables WN, Ripley BD.** 2002. Modern Applied Statistics with S. Fourth Edition.

Springer, New York. ISBN 0-387-95457-0

• **Wardle DA, Bardgett RD, Klironomos JN, Setälä H, van der Putten WH, Wall DH.**

2004. Ecological linkages between aboveground and belowground biota. *Science* **304**: 1629-

1633.

• **Xing X, Koch AM, Jones AMP, Ragone D, Murch S, Hart MM. 2012.** Mutualism

breakdown in breadfruit domestication. *Proceedings of the Royal Society B* **279**: 1122–1130.

 Figures Legends

Figure 1: Inoculation with arbuscular mycorrhizal fungi produced growth benefits in

experimental plants from most genetic origins. Before exposure to *Podosphaera plantaginis*

in field conditions, the magnitude of the growth benefits in experimental *Plantago lanceolata*

plants following inoculation with a mixture of three mycorrhizal fungal species varied among

thirty host maternal genotypes (*Panel A*; Supporting Information Table S2; MYC × GEN: $p <$

0.001, $n = 287$ plants). In *A*, colored dots represent seed origin populations and black dots

represent field epidemic populations. After pathogen exposure in the field epidemic experiment,

host growth continued to be linked to mycorrhizal inoculation (*Panels B & C*; Supporting

Information Tables S3 & S4; MYC: $p < 0.001$), seed origin population (*Panel B*; Table S3; POP:

$p < 0.001$), and maternal genotype (*Panel D*; Supporting Information Table S4; GEN: $p < 0.001$),

but growth benefits no longer varied among maternal genotypes. In *A*, error bars represent a 95

% confidence interval. In *B & C*, box notches represent a 95 % confidence interval for

comparing 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.

Figure 2: Host infection rate was increased in mycorrhizal-inoculated plants and varied

among field epidemic populations. Infection rates by *Podosphaera plantaginis* in experimental

Plantago lanceolata plants increased over time in each mycorrhizal treatment (*Panel A*) and field

epidemic population (*Panel B*) until the peak of the epidemic, but fell by the end of the

experiment. Though host infection rate was similar between mycorrhizal treatments at the peak

of the epidemic (*Panel C*; Supporting Information Tables S5 - S6), at the end of the experiment

host infection rates were increased in mycorrhizal-inoculated plants relative to NM plants in all

three field epidemic populations (Supporting Information Tables S7 – S8; MYC: $p = 0.04$, $n =$

286 plants). Host infection rates also varied among the three field epidemic populations

throughout the experiment (*Panels C & D*; Supporting Information Tables S5 – S8; SITE: $p =$

0.01 (peak), $p < 0.001$ (end), $n = 286$ plants). In *B*, missing data due to heavy rains on days c. 15

- 20 are indicated by light grey solid lines. In *C & D*, predicted values resulting from generalized

logistic models are plotted; 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 $1.5 \times$ the interquartile range from the hinges.

Figure 3: Among infected plants, disease severity varied among mycorrhizal treatments,

seed origin populations, and field epidemic sites. Prior inoculation with a three-species

mixture of arbuscular mycorrhizal fungi was linked to reductions in the proportion of leaves

infected by *Podosphaera plantaginis* in infected individuals of *Plantago lanceolata*, both at the

peak of the epidemic (*Panel A*; Supporting Information Tables S9 – S10; MYC: $p < 0.001$, $n =$

210 plants) and at the end of the experiment (*Panel B*; Supporting Information Tables S11 – S12;

MYC: $p = 0.003$, $n = 98$ plants). Mycorrhizal inoculation and maternal genotype also interacted

to determine the number of infected leaves on infected plants at the peak of the epidemic, with

both negative and positive effects of mycorrhizae on disease (*Panel C*; Supporting Information

Table S14, MYC $\times$ GEN: $p = 0.05$). At the end of the experiment, infected AMF plants had more

infected leaves than infected NM plants (*Panel D*; Supporting Information Table S16; MYC: $p =$

0.04). Seed origin population (*Panel A*; Supporting Information Table S9; POP: $p = 0.004$) and

field epidemic population (*Panel D*; Supporting Information Tables S14 – S16; SITE: $p < 0.02$)

also influenced host disease severity. In *A – B & D*, predicted values resulting from generalized

logistic models are plotted; box hinges correspond to the 1st and 3rd quartiles, while box whiskers

extend to the largest and smallest value no further than $1.5 \times$ the interquartile range from the

hinges. In *C*, error bars represent a 95 % confidence interval.

Figure 4: Growth and disease susceptibility are linked to defensive effects from arbuscular

mycorrhizal fungi in the host genotypes. *Panel A:* In a field epidemic experiment with

Plantago lanceolata individuals from thirty maternal genotypes, host genotypes that grew larger

when inoculated with a three-species mixture of arbuscular mycorrhizal fungi experienced

marginally more negative infection outcomes from exposure to *Podosphaera plantaginis*

(Supporting Information Table S17; $p = 0.11$, $n = 30$ genotypes). *Panel B*: In the same

experiment, host genotypes that were more susceptible to infection (when not inoculated with

mycorrhizae) received stronger disease protection effects from inoculation with mycorrhizal

fungi (Supporting Information Table S18; $p < 0.001$, $n = 30$ genotypes). In *A & B*, each point

represents one maternal genotype, originating from one of six seed origin populations. The y-

axis represents defensive effects due to mycorrhizae, i.e., the estimated change in the mean

number of infected leaves in each genotype due to mycorrhizal inoculation. The shaded area

represents a 95 % confidence interval. In *A*, the x-axis represents growth effects due to

mycorrhizae, i.e., the estimated change in mean host size (leaf number) in each genotype due to

mycorrhizal inoculation. Changes in host size were estimated before pathogen exposure in

variable field conditions. In *B*, the x-axis represents host disease susceptibility, i.e., the estimated

mean number of infected leaves in infected NM plants in each genotype at the peak of the

epidemic: more positive x-axis values indicate more severe infections in the absence of the

mutualist.

Legend of Supporting Information
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Dataset S1 Growth and disease data from the field epidemic experiment.

Fig. S1 Photograph of *Pl. lanceolata* showing signs of infection with *Po. plantaginis*.

Fig. S2 Growth of AMF plants relative to NM plants in each seed origin population at the

beginning of the field epidemic experiment.

Fig. S3 Biomass was increased in AMF plants relative to NM plants at the end of the field

epidemic experiment.

Table S1 Effects of mycorrhizal inoculation and host genetic origin on the growth of healthy

individuals at the start of the field epidemic experiment.

Table S2 Factors influencing the growth of six seed origin populations at the end of the field

epidemic experiment.

Table S3 Factors influencing the growth of thirty maternal genotypes at the end of the field

epidemic experiment.

Table S4 Effect of mycorrhizal association on final harvest biomass in a subset of the

experimental individuals.

Table S5 Factors influencing host infection rate in six seed origin populations during the peak of

the field epidemic experiment.

Table S6 Factors influencing host infection rate in thirty maternal genotypes during the peak of

the field epidemic experiment.

Table S7 Factors influencing host infection rate in six seed origin populations at the end of the

field epidemic experiment.

Table S8 Factors influencing host infection rate in thirty maternal genotypes at the end of the

field epidemic experiment.

Table S9 Factors influencing the proportion of infected leaves in hosts in six seed origin

populations during the epidemic peak.

Table S10 Factors influencing the proportion of infected leaves in hosts in thirty maternal

genotypes at the epidemic peak.

Table S11 Factors influencing the proportion of infected leaves in hosts in six seed origin

populations at the end of the field epidemic experiment.

Table S12 Factors influencing the proportion of infected leaves in hosts in thirty maternal

genotypes at the end of the field epidemic experiment.

Table S13 Factors influencing the number of infected leaves in hosts in six seed origin

populations during the epidemic peak.

Table S14 Factors influencing the number of infected leaves in hosts in thirty maternal

genotypes at the peak of the field epidemic experiment.

Table S15 Factors influencing the number of infected leaves in hosts in six seed origin

populations at the end of the field epidemic experiment.

Table S16 Factors influencing the number of infected leaves in hosts in thirty maternal

genotypes at the end of the field epidemic experiment.

Table S17 Relationship between host growth and defensive effects due to mycorrhizal

inoculation in the maternal genotypes.

Table S18 Relationship between disease susceptibility and defensive effects due to mycorrhizal

inoculation in the maternal genotypes.