Host genotype and co-infection modify the relationship of within and between host transmission

Hanna Susi, Pedro F. Vale & Anna-Liisa Laine

1 Metapopulation Research Group, Department of Biosciences, PO Box 65 (Viikinkaari 1), FI-00014 University of Helsinki, Finland, Email: hanna.susi@helsinki.fi, anna-liisa.laine@helsinki.fi

2 Centre for Immunity, Infection, and Evolution, School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom, email: pferrei2@staffmail.ed.ac.uk

3 Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom.

Correspondence: E-mail: anna-liisa.laine@helsinki.fi
Phone: +358-2941-57750
Abstract

Variation in individual-level disease transmission is well documented, but the underlying causes of this variation are challenging to disentangle in natural epidemics. In general, within-host replication is critical in determining the extent to which infected hosts shed transmission propagules but which factors cause variation in this relationship are poorly understood. Here, using a plant host *Plantago lanceolata* and the powdery mildew fungus *Podosphaera plantaginis*, we quantify how the distinct stages of within-host spread (auto-infection), spore release, and successful transmission to new hosts (allo-infection) are influenced by host genotype, pathogen genotype and the coinfection status of the host. We find that within-host spread alone fails to predict transmission rates, as this relationship is modified by genetic variation in hosts and pathogens. Their contributions change throughout the course of the epidemic. Host genotype and coinfection had particularly pronounced effects on the dynamics of spore release from infected hosts. Confidently predicting disease spread from local levels of individual transmission therefore requires a more nuanced understanding of genotype specific infection outcomes. This knowledge is a key to a better understanding of the drivers of epidemiological dynamics and the resulting evolutionary trajectories of infectious disease.

Keywords: Disease transmission, epidemiology, host-pathogen interaction, *Plantago lanceolata*, *Podosphaera plantaginis*
Introduction

Understanding the determinants of disease emergence and spread is one of the major challenges in disease biology (Gandon et al. 2013; Lively et al. 2014; May et al. 2001; Yates et al. 2006). Like most biological invasions, pathogen spread is characterized by the movement of small numbers of individuals through a spatially heterogeneous environment (Hatcher et al. 2012; Schreiber and Lloyd-Smith 2009). Small numbers of invading individuals mean that the initial phases of pathogen epidemics are highly stochastic, making the likelihood of successful transmission greatly influenced by the host a pathogen happens to infect (Gandon et al. 2013; Hartfield and Alizon 2013; Schreiber and Lloyd-Smith 2009). From the pathogen’s perspective, not all hosts are equal and some hosts contribute more than others to the development of epidemics (Lloyd-Smith et al. 2005; Paull et al. 2012). Individual host heterogeneity in disease transmission is well recognized, and has been addressed both theoretically (Bifolchi et al. 2013; Lloyd-Smith et al. 2006; Matthews and Woolhouse 2005) and empirically (Courtenay et al. 2014; Cronin et al. 2010; Kilpatrick et al. 2006; VanderWaal et al. 2014; Woolhouse et al. 1997). However, while it is clear that host heterogeneity in transmission is common, we currently know little about the genetic and environmental conditions that may lead to some hosts to contribute disproportionally more to transmission (Fellous et al. 2012; Ferrari et al. 2004; Lass et al. 2013; Paull et al. 2012; Vale et al. 2013).

One facet of pathogen transmission that is currently poorly studied is the relationship between within-host colonization and between-host transmission, particularly how this relationship varies among individual hosts (Mideo et al. 2008). Once a pathogen has successfully infected a host, the process of within-host replication is expected to be critical for how much that infected host sheds transmission propagules that potentially leads to new infections. This link is a general feature of all host-parasite systems (Anderson and May 1982), regardless of the idiosyncrasies affecting exactly how
within-host infection progresses and how transmission is achieved (Bowen and Walker 2005; Ebert and Weisser 1997; Hughes et al. 2011; Quinn et al. 2000). In plant-pathogen epidemics, infection dynamics can be divided into two distinct phases. During auto-infection the pathogen spreads from the focal infection to the surrounding leaves within the same host plant. Variation in auto-infection leads to plants with varying numbers of infected leaves, and therefore auto-infection may also be seen as a measure of infection severity. During and following local spread within a host plant, allo-infection occurs, where the pathogen is transmitted to other hosts (Mundt 2009; Robinson 1976). A potentially important distinction between the auto- and allo-infection processes we describe here is that auto-infection involves the same pathogen transmission stage as allo-infection, while in most animal infections, they result from two different infection stages (for example in malaria merozoites spread infection within hosts and gametocytes transmit infection to other hosts; Bannister and Mitchell 2003; Schmidt-Hempel 2011).

Given that the window of time for a pathogen epidemic to develop is usually limited to certain environmental conditions (temperature, humidity) and host availability (Garrett et al. 2009), the optimal timing of auto- and allo-infection is crucial to the fate of the pathogen. Most epidemiological studies follow the rate of allo-infections, usually referred to as the transmission rate. Rates of auto-infection have typically received less attention, but some work has shown that host auto-infection can also vary (Lannou et al. 2008), with consequences for pathogen life history and virulence evolution (van den Berg et al. 2013). Measuring auto-infection is generally easier than quantifying the extent of allo-infection, and traditionally within-host pathogen replication is assumed to equate with transmission potential (Anderson and May 1982). Given that in most infections the symptoms arise as a consequence of the production of transmission propagules during auto-infection, we might expect hosts with severe disease symptoms to produce higher levels of transmission propagules (auto-infection), and ultimately
cause more disease transmission (during allo-infection; Beldomenico and Begon 2010). However, there are many reasons why this relationship may not always hold, and understanding them would help pinpoint which diseased individuals should be targeted in order to maximize the efficacy of disease control.

To date, the effect of host and pathogen genetic diversity on the spread of infectious disease has received little experimental exploration, and is currently one of the most pressing questions in disease ecology and evolution (Lively et al. 2014). Given that genetic variation (both of hosts and the pathogens that infect them) is well-known to affect all aspects of infection outcomes (Bergelson et al. 2001; Tack et al. 2012; Wolinska and King 2009), the relationship between auto- and allo-infection is also likely vary depending on the genetic composition both hosts and pathogens. First, pathogen populations are highly variable in their ability to infect and cause disease (Tack et al. 2012), and trade-offs between the different life-history stages of infection may also vary with genotype and genotype-by-genotype interactions (Laine and Barrès 2013). Hence, there may be considerable variation among strains in their ability to grow within an infected host, and transmit to new ones. Second, variation among hosts in how they resist becoming infected and mitigate infection development – as well as possible trade-offs between these traits - may further generate variation in auto-allo-infection dynamics (Bergelson and Purrington 1996; Susi and Laine 2015). Finally, hosts are often found to harbor mixed infections where the infection outcome cannot be predicted from those of single infections (Pedersen and Fenton 2007; Petney and Andrews 1998). Recognizing the variable nature of pathogen infections is important because mixed-genotype infections often result in increased parasite competition and virulence (Alizon et al. 2013; Choisy and de Roode 2010). Variation in the level of co-infection among individual plants is therefore likely to affect the extent of pathogen transmission propagule production during auto-infection, and influence between-host transmission. Therefore, in addition to commonly
studied genetic variation in host defences (Ayres and Schneider 2012; Laine et al. 2011; Råberg et al. 2009; Read et al. 2008; Roy and Kirchner 2000; Simms and Triplett 1994), the level of co-infection may also generate individual host heterogeneity in infectiousness (Lass et al. 2013), potentially modifying the dynamics of infectious disease (Streicker et al. 2013; Susi et al. 2014).

A clear understanding of what generates variation in the relationship between auto- and allo-infection calls for controlled experiments with two major features: 1) the level of auto-infection can be monitored in a non-destructive manner throughout the course of an infection across a large numbers of individual hosts, and 2) the resulting pathogen propagule shedding and spreading can be precisely measured for each individual host. In addition, a realistic understanding of auto- and allo-infection requires studying both single and mixed infections, as these are the conditions hosts will commonly experience in the wild. Fulfilling all these requirements is challenging, and so experiments such as the one we describe are understandably rare.

Here, we take a common garden approach using a plant-pathogen system to test how host genotype, pathogen genotype, coinfection and time affect 1) the level of auto-infection within the host, and 2) the relationship between auto-infection and allo-infection. The experimental work was carried out with powdery mildew, *Podosphaera plantaginis* infecting the host plant *Plantago lanceolata*. Our experiment used multiple host genotypes that were cloned into replicates, and inoculated with two pathogen strains, either singly or as a coinfection. This host-pathogen interaction is highly amenable to ecological studies, as infection is visually conspicuous on host surface and the disease cycle lacks extended latency periods. Hence, auto-infection can be visually directly quantified. With a combination of two spore trapping methods, spore traps and live susceptible leaves; we were able to disentangle the relationship between the shedding of transmission propagules and the actual spread of the infection. We monitored both auto- and allo-infection dynamics over the course of an epidemic to assess how this
relationship changes over time. By genotyping the resulting infections we were also able to identify the transmitted pathogen genotypes from co-infected host plants.

Material and methods

Host-pathogen interaction

*Podosphaera plantaginis* is a specialist powdery mildew naturally infecting *Plantago lanceolata* in the Åland archipelago, southwestern Finland. *Plantago lanceolata* is an obligate outcrossing perennial herb that reproduces both sexually and clonally via side-rosettes. The epidemiological dynamics of *Podosphaera* in its large host population network have been studied since 2001 in the Åland Islands, southwest of Finland. These studies have demonstrated that this pathogen persists as a highly dynamic metapopulation (Jousimo et al. 2014). Visible signs of infection appear in late June in those host populations in which the pathogen has successfully overwintered as resting spores. The epidemic begins from these initial disease foci as the pathogen is transmitted by wind both within and among hosts via clonally produced dispersal spores, conidia (Laine and Hanski 2006; Ovaskainen and Laine 2006). Some six to eight clonally produced generations follow one another in quick succession and as a consequence, infection spreads within (Ovaskainen and Laine 2006) and between host populations (Jousimo et al. 2014). By September weather conditions turn unfavorable to disease transmission, and the epidemic spread ceases.

In the interaction between *Plantago* and *Podosphaera* disease resistance is strain-specific with the same host genotype blocking infection by some strains of the pathogen while being susceptible to others. There is considerable variation among host individuals and populations in their degree of resistance. The obligate pathogen can only establish on susceptible hosts, and hence, variation in
resistance plays a fundamental role in determining disease dynamics (Jousimo et al. 2014). Local pathogen populations also support considerable genetic (Tollenaere et al. 2012) and phenotypic (Susi and Laine 2013) diversity. Coinfections, whereby two or more strains of *Podosphaera* simultaneously infect the same host, are common in the Åland metapopulation (Tollenaere et al. 2012).

**Spore trapping experiment**

To quantify the number of fungal spores released from each host plant, and the number of successfully established new infections (a measure of transmission), we carried out a spore trapping experiment. We used eight generally susceptible (i.e. wide range of *Podosphaera* strains including the strains used in this study are able to infect and sporulate in them) *Plantago* genotypes originating from five populations in Åland (IDs 4 (three plants), 511 (two plants), 1413 (one plant), 2220 (one plant), and 9031 (1 plant)) as focal plants. Host plants were collected as seed in August 2010 and stored in paper envelopes at room temperature. Seeds were germinated by placing them in 0.8 L pots in 50:50 sand–potting soil mixture in greenhouse conditions of 16 h of light and at +22 °C. Plants were cloned in the greenhouse according to the protocol described in Laine (2004), producing up to 24 ramets. Eight-week-old ramets were placed outside for two weeks of acclimation until the experiment was set up. We used two *Podosphaera* strains originating from Åland (strain 10 from population 2821 and strain 3 from population 877) that were infective on all host genotypes used in this experiment. The strains were purified and maintained on fresh susceptible *Plantago* leaves on Petri dishes in a growth chamber with 16:8 light: dark cycle at 20 ± 2° C.

To determine how host genetic background and pathogen treatment (single or co-infection) affect the relationship between auto-infection and allo-infection we performed a spore
trapping experiment under semi-natural conditions. The experiment was set up in 2013 at the Lammi
Biological Station (61°05’28’’N, 25°03’90’’E) where neither Plantago nor Podosphaera occur
naturally; hence environmental contamination by fungal spores was highly unlikely. In mid-July the
experimental plants were potted in 11 cm × 11 cm pots placed at a one meter radius from each other
and inoculated with strain 3, strain 10 or co-inoculation of strains 3 and 10. The amount of inoculum
(all spores brushed off from a 1 cm² ten-day old sporulating lesion onto one leaf of the receiving plant)
was the same for all plants, with the coinfected plants receiving half of the dose of the single genotype
inoculum (i.e. all spores brushed from a 0.5 cm² lesion of strain 3 and 0.5 cm² lesion of strain 10 on to
one leaf of receiving plant). We also included a control treatment with no pathogen spores to ensure
there was no contamination between the plants. Four replicates of each plant genotype × pathogen
treatment were used and two replicates of each plant genotype and control treatment were used,
resulting in 112 plants in total. The auto-infection rate of the infected plants was measured as the
number of leaves on a plant that were infected with powdery mildew.

To quantify spore release and allo-infection we conducted five spore trapping sessions at
20, 30, 40, 50 and 60 DPI. Our study was focused on quantifying short-distance transmission at 5 cm
distance from the infected host, as this is a relevant distance in the high density populations of Plantago
in Åland (Laine 2004), with most spores landing very close to the infection source (< 10 cm; Tack et al.
2014). We used two types of traps: petroleum jelly coated microscope slides (to quantify the number of
spores released) and live detached Plantago leaves (to measure the number of spores that landed on and
later germinated on a host, a measure of transmission potential). Four petroleum jelly coated
microscope slides were attached on wooden sticks at 5 cm distance from ground placed between the
infected leaves in radial design to quantify spore release. Sixteen detached live leaves of known
susceptible genotypes were attached to moist floral foam at 5 cm distance from the focal plant to
quantify allo-infection. These trap leaves were from five generally susceptible genotypes used in strain maintenance in the laboratory. The trapping period lasted 24 hours, after which the traps were removed. The petroleum jelly traps were then kept in 5°C and subsequently examined under a microscope using four transect lines to count the released spores. The live leaves were placed on moist filter paper in a Petri dish and kept in a growth chamber. After 14 days their infection status was monitored and the infected leaves were collected for subsequent genotyping. The level of auto-infection was assessed during each trapping session by counting the number of infected leaves. All control plants remained uninfected throughout the experiment. The infection status of the plants was monitored at 20 DPI on the leaf that had received the inoculation treatment. In total 75 plants (79.2%) become infected. Plants that did not show visible signs of infection at 20 DPI (20.8%) were excluded from the analyses. To avoid unnecessary handling of the plants during the experiment, the total number of leaves in each plant was counted at the end of the experiment.

**Genetic analyses**

We genotyped the trap leaves infected at time points 40-60 days post-infection (DPI) to ensure that there was no cross contamination between plants, and to determine which of the pathogen strains – or both - had successfully infected the trap leaves in the coinfection treatments. From each infected leaf, we cut the lesions, consisting of both host tissue and fungal material, into a 1.5 mL tube that was kept at -20°C until DNA was extracted using E.Z.N.A. Plant Mini Kit (Omega Bio Tek Inc. Norcross, GA, USA). Samples were genotyped using the 27 SNP panel as in Tollenaere et al. (2012). The pathogen lines used in the experiment can be distinguished from each other as they differ at eight loci used in the
genotyping panel. We classified leaves as coinfected if they showed presence of two alleles in polymorphic loci (Tollenaere et al. 2012).

Statistical analyses

To test how host genotype, pathogen genotype, coinfection and time affect on 1) auto-infection, 2) how auto-infection correlates with spore release, 3) how auto-infection correlates with infection establishment, and 4) how spore release correlates with infection establishment we performed four different analyses using Generalized Linear Mixed Models in SAS 9.2 (SAS Institute 2011) using the GLIMMIX procedure (Littell et al. 2006). To determine the factors that influence auto-infection, we analyzed the proportion of infected leaves as the response variable where number of infected leaves was used as defined as numerator and number of all leaves in a plant as denominator, with plant genotype and pathogen treatment as explanatory categorical variables; the number of leaves on a plant (counted at 60 DPI) and time (DPI) were included as covariates. Plant replicate, hierarchically nested under plant genotype, was defined as a random variable. The model was fitted with a binomial distribution of errors. In order to evaluate the relationship between auto-infection rate and spore release we analyzed the number of spores released from each plant (the number of spores in the four microscope slides) as the response variable with a Poisson distribution of errors, plant genotype and pathogen treatment as explanatory categorical variables, with number of infected leaves (auto-infection level) and time as days post-infection (DPI) as covariates. Plant replicate nested within genotype was treated as a random variable to control for possible variation due to the location of the plant in the study area.
To understand the relationship between auto-infection and allo-infection we analyzed the proportion of infected trap leaves (as a numerator) of all trap leaves (as denominator) around the focal plant as a response variable with a binomial distribution of errors. There was overdispersion in the data and we chose Complementary Log-Log link function to fit the model. The level of autoinfection was included as a covariate, otherwise the model variables are the same as described above. Finally, to understand the relationship between transmission potential and infection establishment, we analyzed how spore release affects the establishment of infection by having the proportion of infected trap leaves (as numerator) of all trap leaves (denominator) as a response variable, spore release (number of spores trapped on the four microscope slides) as a covariate, and plant genotype and pathogen treatment as explanatory categorical variables, and time as DPI as a covariate. We used binomial distribution of errors and Complementary Log-Log link function. To identify differences within significant main effects we used post hoc comparisons by computing least squared means of the main effects in SAS 9.2 Proc Glimmix (Littell et al. 2006). In all analyses non-significant interactions were excluded from the final models.

Results

Factors determining auto-infection

The fraction of infected leaves increased with time and there was weak negative correlation between number of leaves in a plant and the fraction of diseased leaves (Supplementary Figure 1; Supplementary Table 1). Plant genotype and pathogen treatment had a significant effect on the fraction of the diseased leaves (Supplementary Figure 1; Supplementary Table 1). By the end of the experiment,
none of the plants were saturated with infection: 40% or fewer of the leaves were infected (Supplementary Figure 1).

The dynamics of pathogen spore release

Spore release (estimated as the number of spores landing on the petroleum jelly traps) changed throughout the experiment, and across all treatments, peaking at 50 DPI (Figure 1, Table 1). The number of spores released depended on whether the hosts were infected singly or co-infected, with co-infected hosts shedding more pathogen spores (Figure 1A, Table 1). Post hoc comparisons revealed that while the overall number of spores released in the single infections did not differ, there were significant differences between the strains at 40 and 50 DPI (40 DPI; \( P < 0.0001 \); 50 DPI; \( P = < 0.0001 \)). This is especially clear at 50 DPI, where strain 3 released more spores than 10. The number of spores released from coinfected plants was highest at 50 DPI (Figure 1A). At 60 DPI the overall number of spores released decreased and the differences between treatments diminished (Figure 1A).

The amount of spores released depended also on the host genotype. Averaged across all single and coinfections, some host genotypes (G1, G3 and G6) produced more spores than others throughout the epidemic (Figure 1B, Table 1). The effect of host genetic variation on infectiousness is especially pronounced during the peak spore release at 50 DPI (Figure 1B) when the difference in spore release was the largest. Overall, the effect of pathogen treatment on spore release changed through time (significant interaction between time and treatment in Table 1; Figure 1A). The amount of spores released from coinfected plants was highest at 30 and 50 DPI, while at 40 DPI strain 10 had higher spore release than strain 3 and the coinfection treatment (Figure 1A). At the peak of epidemics strain 3
released more spores than strain 10 in single infections (Figure 1A). At 60 DPI the differences between treatments diminished (Figure 1A).

The relationship between auto-infection and spore release

One aim of our experiment was to explore how host genotype and coinfection status contributed to variation in the relationship between auto-infection and spore release. As expected, high auto-infection generally led to high levels of spore release (significant auto-infection term in Table 1). However, while auto-infection and spore release are clearly correlated, the strength of this relationship changed over the course of the epidemic, and was affected by both host and pathogen genotypes (Figure 2; Table 1). Specifically, in hosts infected singly with strain 10, we observed a positive correlation between the rates of auto-infection and spore release, while in plants infected with strain 3 this correlation was still positive, but weaker (Figure 2A-C; Table 1). In host plants coinfected with both strains the relationship between auto-infection and spore release was highly variable (Figure 2A-C; Table 1). Across all single infection and coinfection treatments, there is also variation in the relationship that arises from different host genetic backgrounds. In some host genotypes (e.g. G1 and G2) high auto-infection yielded high spore release whereas in other genotypes (e.g. G4, G5 and G6) the relationship was weaker (Figure 2D-F; Table 1).

The establishment of new infections

Consistent with what we observed for spore release, the establishment of infection by spores landing on trap leaves also peaked at 50 DPI (Figure 3A, Table 2). We found that high auto-infection rates led to
high rates of new infections becoming established, but we found no significant effects of host genotype and pathogen treatment (Table 2). The relationship between the number of spores that were released from the infected plant and the establishment of new infections varied according to which pathogen strain was involved (Spores × Treatment Figure 3B, Table 3). Generally, when hosts were infected with strain 10, shedding high numbers of spores led to the establishment of many new infections, while this relationship was noticeably weaker when hosts were co-infected with strains 3 and 10 (Figure 3B, Table 3). Genotyping of the infected trap leaves at 40-60 DPI from the coinfection treatment revealed that 40% were infected by strain 3, 40% by strain 10, and 20% of new infections consisted of both strains.

**Discussion**

Taken together, our results highlight the host’s heterogeneous contribution to the temporal dynamics of epidemics. We found that the contribution of a host individual to epidemics varies over time and correlates with its level of auto-infection as expected, but importantly, also depends on the host genetic background and whether it is coinfected or singly infected. We also tested a common assumption that rapid colonization of the host by the pathogen (auto-infection) is associated with the ability to spread infection between hosts (e.g. Robinson 1976). Our results indicate that host and pathogen genetic background can mediate the temporal dynamics of within- and between-host pathogen spread.

**The effect of pathogen strain on transmission dynamics**

In this study we found that, after controlling for the level of auto-infection in our models, the overall spore release and infection establishment rates between the singly infecting strains did not differ.
However, we observed significant differences between the strains at individual time points during the epidemics (Figure 1A), and importantly, differences in how many spores were released at different levels of auto-infection (Figure 2). In plants infected with pathogen strain 10 there was a stronger correlation than in the case of plants infected with strain 3. This may indicate a difference in the latent period of the two strains: the weaker correlation for strain 3 spores may result from a longer period between auto-infection and spore shedding. This suggests that different pathogen strains may have different optimization strategies between within-host and between-host levels. The timing of transmission is essential in the disease dynamics as the time window for infection spread is usually limited by the environment, host life span or behavior (Hartfield and Alizon 2013). The differences in transmission at the early stages of the epidemics may have profound consequences for disease epidemiology and pathogen evolution, as rapid transmission is expected to lead to a greater share of the prevailing pathogen population (Day 2003) and therefore higher pathogen fitness (Elena 2001). We also found that in hosts infected with strain 10 the amount of spores released accurately predicted infection establishment while in strain 3 this trend was not as clear, suggesting that there are differences between the strains in their spore quality (Figure 3B).

Host-mediated variation in within and between host dynamics

Auto-infection and allo-infection dynamics have been approached theoretically in epidemiological modelling (Mideo et al. 2008; Mundt and Leonard 1986; Willocquet and Savary 2004), but empirical studies remain rare and the host’s role in mediating the dynamics has been largely unexplored. We found heterogeneity in the contributions of different host genotypes to pathogen replication (Figure 1B), consistent with the idea that host genotype is critical for infection development (Salvaudon et al. 2003).
2008). Not only did host genotypes differ in the number of spores released, the relationship between spore release and infection establishment was also significantly determined by the host genetic background. One reason the relationship between auto-infection and spore release may vary is because hosts may differ in the types of defenses employed during infection, and in the level of nutrients available for the pathogen (Laine 2007). Hosts have evolved various strategies to reduce the harm caused by the pathogen (Ayres and Schneider 2012; Dodds and Rathjen 2010; Råberg et al. 2009; Read et al. 2008; Simms and Triplett 1994). One strategy is to resist the pathogen by mitigating its growth, leading to clearance and recovery (Alexander 1992; Schmidt-Hempel 2011). Another strategy is to minimize the negative fitness effects of infection through damage limitation mechanisms that allow hosts to tolerate the presence of pathogens (Ayres and Schneider 2012; Medzhitov et al. 2012; Roy and Kirchner 2000). It remains unclear how the interaction between different host defense strategies may shape the relationship between auto- and allo-infection spread. For example, hosts investing heavily in costly resistance mechanisms could clear pathogens, leading to reduced transmission, but could also experience severe symptoms due to immunopathology (Graham et al. 2005; Jones and Dangl 2006). Conversely, hosts investing heavily in damage limitation mechanisms may show only mild disease symptoms (Poland et al. 2009) but would still tolerate the production of transmission propagules during auto-infection and contribute considerably to transmission during allo-infection (Vale et al. 2014). We used host genotypes where mildew was able to establish and grow, but it is possible that host genotypes differed in their ability to tolerate infection. Infection tolerance has not been systematically explored in this system, but is an important line of future research. In general, an open question for future studies is how the relationship between auto-infection and spore release may vary between host genotypes with differential investment in resistance or tolerance strategies.
The effect of coinfection on transmission dynamics

We found that transmission dynamics were altered under coinfection; the overall highest spore shedding was observed in coinfected plants (Figure 1A). This result is in line with a study on mice where coinfection lead to higher helminth oviposition (Lass et al. 2013). Interestingly, in the early phase of the epidemic at 40 DPI, auto-infection rate was not a strong predictor of spore release in the coinfected plants – high spore release was observed at low auto-infection levels while host plants with high levels of auto-infection released a low number of spores (Figure 2). As the epidemic progressed, a positive correlation between auto-infection and spore release emerged. It has also been suggested previously that the relationship between host disease severity and pathogen transmission may be altered in co-infection (Bremermann and Pickering 1983). Here, we found that the correlation between spore release and allo-infection was weaker under coinfection than in the singly infecting strains. This finding suggests that the accelerated spore production we observe under coinfection comes at a cost of spore quality. These effects of co-infection are likely to be relevant more broadly, as hosts are frequently infected by more than a single pathogen strain (Balmer and Tanner 2011; Lopez-Villavicencio et al. 2007; Telfer et al. 2010) and, in some cases it has been shown that coinfection may lead to increased reproduction of the pathogen (Lass et al. 2013).

Hosts are bottlenecks of pathogen genetic diversity and can therefore shape pathogen epidemics (Fellous et al. 2012; Tack et al. 2014) and evolution (Cisarovsky and Schmid-Hempel 2014). High levels of mixed infections can therefore maintain pathogen genetic diversity at the level of individual hosts, but it is poorly understood if this diversity spreads to other hosts (Lively et al. 2014). Our genotyping revealed that 20% of the infections resulting from coinfected plants were also coinfectected suggesting the possibility of co-transmission, while the single infections established at equal probability from the coinfectected source (both strains found singly in 40% of the infected leaf traps).
Whether pathogens spread as single strains or are co-transmitted may have consequences for epidemiology as infection success has been reported to increase in co-transmission (Karvonen et al. 2012) and is assumed to have further consequences for the evolution of virulence (Alizon 2013). In the case of vector transmitted pathogens, simultaneous transmission of different strains and species is well documented (Pirone and Blanc 1996) but our study is among the few to investigate the frequency of co-transmission in airborne pathogens. Applied to natural populations, where multihost-multi-pathogen interaction networks are the norm (Pedersen and Fenton 2007), our results lend insight into a currently outstanding question in disease ecology and evolution (Lively et al. 2014): how pathogen interactions within hosts may regulate pathogen diversity at the population level.

Limitations of the current study

In this study we found that the relationship between spore release and infection establishment was mediated only by pathogen genetic background, but we detected no effect of the focal host genotype (Figure 3). However, it is important to emphasize that the overall proportion of infected trap leaves was low, 0.014. While this is comparable to the estimated success of single bouts of transmission (Ovaskainen and Laine 2006), it may have reduced our power to detect the effects of these potential sources of variation. The size of the current experimental design was determined by the need to study spore release, auto-infection and allo-infection. In order to increase statistical power to fully disentangle the sources of variation on successful disease spread during allo-infection, future studies should focus on the realized transmission on large numbers of host genotypes representing different resistance backgrounds and large numbers of pathogen stains with different infection profiles. Moreover, as previous studies have found evidence of pathogen local adaption to host resistance (Laine
2005; Laine 2008), it would be highly relevant to determine how coevolutionary dynamics may alter the relationship between auto- and allo-infection.

Conclusions

We detected important effects of host genotype and co-infection status on the temporal dynamics of spore release, which re-enforces the need to identify co-infection in wild-hosts as a potential risk factor of disease spread (Mideo et al. 2008; Susi et al. 2014). Our results also suggest that the degree to which a host plant sheds fungal spores onto its own leaves is not necessarily a good predictor of spread to neighboring host plants: we found the correlation between autoinfection and allo-infection to change over the course of our experimental epidemic, and to vary particularly in strength according to the host co-infection status. This result has important implications for the control of disease. In trying to delay the spread of infection, it would seem logical to remove or treat hosts showing the clearest signs of disease. While this may be true generally, our results suggest that timing is crucial, as a strong positive relationship between auto-infection and spore release only became apparent as the epidemic progressed. When designing disease management efforts, it is therefore important to understand the relationship between auto-infection, spore shedding and allo-infection, and how the relationship between them may vary according to the genetic and environmental context experienced by hosts.

Acknowledgements

Lammi Biological Station is acknowledged for hosting the experimental study, and P. Hyttinen and S. Hartikainen are acknowledged for their help in the field. L. Ruokolainen is acknowledged for statistical
advice. N. Mideo and R. Penczykowski are acknowledged for helpful comments on the earlier version of the manuscript. B. Barrès helped analyze the genetic samples and Institute of Biotechnology and Institute for Molecular Medicine at the University of Helsinki are acknowledged for carrying out the DNA extractions and genotyping of samples, respectively. HS is funded by LUOVA Doctoral School in Wildlife Biology Research and PFV is funded by a strategic award from the Wellcome Trust for the Centre for Immunity, Infection and Evolution (http://ciie.bio.ed.ac.uk; grant reference no. 095831), and by a Scottish Universities Life Sciences Alliance (SULSA) LEADERS mobility award. This work was supported by funding from the Academy of Finland (Grant Nos 250444, 136393, 133499) and the European Research Council (Independent Starting Grant PATHEVOL; 281517) to A.-L.L.

References


**Figure legends**

**Figure 1.** Variation in spore release (number of trapped spores; solid lines) and auto-infection (number of infected leaves; dashed line) of *Podosphaera plantaginis* throughout the experiment. Coinfected plants released more spores than singly infected plants (A). Spore release varied among the eight *Plantago lanceolata* host genotypes and through time (B). Standard error of the mean is shown.

**Figure 2.** The relationship between auto-infection and allo-infection of *Podosphaera plantaginis*. Relationship between proportion of infected leaves in the focal plant and the spore release of *Podosphaera* strains 3 and 10 singly and under coinfection (A-C). The relationship between the proportion of infected leaves and spore release on different *Plantago lanceolata* genotypes across all pathogen treatments (D-F).

**Figure 3.** Infection establishment on the live leaf traps varied through time (A). The relationship between spore release and infection establishment varied according to pathogen treatment (B). Standard error of the mean is shown.
**Tables**

**Table 1.** Sources of variation in *Podosphaera plantaginis* spore release analyzed with a Generalized linear mixed model. Statistically significant (*P < 0.05*) results are shown in bold.

<table>
<thead>
<tr>
<th>Effect</th>
<th>d.f.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>1,326</td>
<td>822.16</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Treatment</td>
<td>2,326</td>
<td>16.33</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Autoinfection</td>
<td>1,326</td>
<td>689.14</td>
<td>0.0006</td>
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<tr>
<td>Genotype</td>
<td>7,24</td>
<td>3.6</td>
<td>0.0086</td>
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<tr>
<td>Time × treatment</td>
<td>2,326</td>
<td>42.07</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Autoinfection × treatment</td>
<td>2,326</td>
<td>45.2</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Autoinfection × genotype</td>
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<td>Autoinfection × time</td>
<td>1,326</td>
<td>793.27</td>
<td>&lt; 0.0001</td>
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Table 2. Sources of variation in *Podosphaera plantaginis* infection establishment on trap leaves analyzed with a Generalized linear mixed model. Statistically significant \((P < 0.05)\) results are shown in bold. Akaike information criterion (AIC) value of the model was 433.45.

<table>
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<th>(P)</th>
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<td>(&lt; 0.0001)</td>
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<tr>
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<td>0.6765</td>
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<tr>
<td>Autoinfection × time</td>
<td>1,337</td>
<td>17.03</td>
<td>(&lt; 0.0001)</td>
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</tbody>
</table>
Table 3. The relationship between spore release and infection establishment in *Podosphaera plantaginis*. Results of spore trapping experiment analyzed with a Generalized linear mixed model. Statistically significant (P < 0.05) results are shown in bold. AIC value of the model was 441.19.

<table>
<thead>
<tr>
<th>Effect</th>
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<th>F</th>
<th>P</th>
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<tbody>
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<td>Treatment</td>
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<td>3.44</td>
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<td>Spores</td>
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<td>Treatment × spores</td>
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Figure 1.
Figure 3.