Coinfection with a virus constrains within-host infection load but increases transmission potential of a highly virulent fungal plant pathogen

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Abstract

The trade-off between within-host infection load and transmission to new hosts is predicted to constrain pathogen evolution, and to maintain polymorphism in pathogen populations. The life-history stages and their correlations that underpin infection development may change under coinfection with other parasites as they compete for the same limited host resources. Cross-kingdom interactions are common among pathogens in both natural and cultivated systems yet their impact on disease ecology and evolution are rarely studied. Host plant Plantago lanceolata is naturally infected by both Phompopsis subordinaria, a seed killing fungus, as well as Plantago lanceolata latent virus (PlLV) in the Åland Islands, SW Finland. We performed an inoculation assay to test whether coinfection with PlLV affects performance of two P. subordinaria strains, and the correlation between within-host infection load and transmission potential. The strains differed in the measured life-history traits and their correlations. Moreover, we found that under virus coinfection, within-host infection load of P. subordinaria was lower but transmission potential was higher compared to strains under single infection. The negative correlation between within-host infection load and transmission potential detected under single infection became positive under coinfection with PlLV. In wild populations, within-host infection load was positively associated with within-population disease prevalence. Jointly, our results suggest that the trade-off between within-host infection load and transmission may be strain specific, and that the pathogen life-history that underpin epidemics may change depending on the diversity of infection, generating variation in disease dynamics.

Introduction

In order to grow, multiply, and transmit, pathogens obtain resources from their host, and theoretically the resulting within-host infection load is expected to be proportional to the gained resources. However, too high rates of within-host infection load may come at the cost of increased virulence, thereby incurring a cost for the pathogen as decreased transmission (Blanquart et al., 2016). Thus, evolutionary theory and experimental studies (de Roode et al., 2008) have established that selection should favour intermediate levels of within-host infection load. This trade-off between within-host infection load and transmission has been proposed to maintain polymorphism in pathogen populations, and to prevent the rise of highly virulent pathogens (Frank, 1992). Trade-offs have been sought as an evolutionary solution to limit disease epidemics and the emergence of pathogen strains with extremely high within-host infection load (Zhan et al., 2015). However, insight on how pathogen within-host infection load links to transmission during epidemics where pathogens may encounter variation in both biotic and abiotic conditions (Susi & Laine, 2013, Blanquart et al., 2016, Dutta et al., 2021) has remained limited (Acevedo et al., 2019). In the wild, the limited evidence for trade-offs may be explained by spatial (Osnas et al., 2015) and host mediated processes (Kubinak et al., 2012). The trade-offs restraining within-host infection load may also occur between other traits i.e. adaptation to abiotic conditions (Mboup et al., 2012) or be context-dependent and become evident in stressful environments (Susi & Laine, 2013).

The drivers of disease evolution and epidemics are rarely limited to the interplay of one host and one pathogen, as in the wild most infections occur as coinfections whereby multiple pathogens are simultaneously infecting the same host (Tollenaere et al., 2016, Telfer et al., 2010). Coinfection may fundamentally change pathogen host exploitation strategy in order to outcompete other pathogens sharing the same limited resource (de Roode et al., 2005, Alizon et al., 2009, Alizon & van Baalen, 2008). Thus, it has been suggested that coinfection is an important driver of disease evolution (Alizon & van Baalen, 2008, Alizon et al., 2009). Experimental approaches have measured increased within-host infection load(Bell et al., 2006) and transmission(Susi et al., 2015b, Susi et al., 2015a) under coinfection but there are also exceptions to this trend (Orton & Brown, 2016). Overall, it is well established that the pathogen within-host infection load may change under coinfection, but studies explicitly testing trade-offs between within-host infection load and transmission under coinfection are rare, and evidence remains mixed (Suffert et al., 2016, Sacristan & Garcia-Arenal, 2008). Furthermore, experiments have often been conducted using strains of the same pathogen species, although interspecific interactions among pathogen species are likely to play an important role, as individual hosts often support diverse pathogen assemblages(Susi et al., 2019, Dallas et al., 2019, Telfer et al., 2010).

While intraspecific coinfection is a pre-requisite of outcrossing for many pathogens (Suffert et al., 2016), theory predicts the intensity of competition to increase as relatedness decreases (Alizon et al., 2013). Across plants (Tollenaere et al., 2016, Tollenaere et al., 2017), animals (Telfer et al., 2010) and humans (Lawn et al., 2006, Chen et al., 2020) inter-kingdom coinfections are common, and they are often suggested to have serious consequences in disease epidemics and disease severity. In particular, it is becoming increasingly clear that viruses are ubiquitous in nature (Munson-McGee et al., 2018, Bernardo et al., 2017), although their true diversity and prevalence in natural populations has been under-estimated for a long time (Wren et al., 2006, Roossinck et al., 2015). The ecological roles of viruses are still poorly understood (Roossinck, 2010, Alexander et al., 2014), but they have the potential to interact with other pathogen species via competition for shared host resources, and via shared effects on host immunity (Huang et al., 2019, Uehling et al., 2017). Thus, it is vital to test how coinfection with pathogens from distant taxa may influence within-host infection load and transmission and their potential trade-offs.

Phomopsis subordinaria is a castrating pathogen that infects its hosts through seed stalks. Here, we investigate trade-offs between within-host infection load and transmission by surveying 260 host plant (*Plantago lanceolata*) populations in the Åland Islands, south-west Finland, as well as in laboratory trials. In the laboratory, we challenged *P. subordinaria* strains with *Plantago lanceolata latent virus* (PlLV) in order to understand how cross-kingdom interactions affect within-host infection load and transmission potential, as well as their potential trade-off. Specifically, we ask 1) How common is *P. subordinaria* in the Åland Islands, and is there natural variation in within-host infection load in natural *P. lanceolata* populations, and 2) Is there a trade-off between within-host infection load and population size (potential proxy for among-host natural transmission) in *P. subordinaria* ? We hypothesize that high within-host infection load comes with a cost of lower transmission, measured as pathogen population size. In a laboratory experiment, we tested: 3) Is there a trade-off between laboratory measured within-host infection load and transmission potential of *P. subordinaria* ? We hypothesize that high within-host infection load is costly with respect to transmission potential. 4) Does coinfection with PlLV alter *P. subordinaria* within-host infection load and transmission potential? We hypothesize that coinfection increases both within-host infection load and transmission potential.

Material and Methods

Host plant and the pathogens

Plantago lanceolata is a perennial wind pollinated rosette-forming herb (Sagar & Harper, 1964) with a worldwide distribution. In the Åland Islands, it occurs as a network consisting of ca. 4000 meadows in a highly fragmented landscape (Ojanen et al., 2013). Annually in September, the 4000 *P. lanceolata* populations' size, area, and location are monitored and the presence of *Podosphaera plantaginis* fungus and *Melitaea cinxia* butterfly are surveyed. (Jousimo et al., 2014, Ojanen et al., 2013). In Åland *P. lanceolata* has been discovered to host two fungal pathogens (Laine, 2003, Jousimo et al., 2014) and five viruses (Susi et al., 2019) thus

far. *Phomopsis subordinaria* (telemorph *Diaporthe adunca*(Rob.) Niessl.) is a specialist fungal pathogen of *P. lanceolata*(Laine, 2003) transmitted by weevil *Trichosirocalus troglodytes*(de Nooji & van der Aa, 1987, Nieminen & Vikberg, 2015). The pathogen infects its host plant through a wound under the inflorescence of the plant causing the developing seeds to dry out (de Nooji & van der Aa, 1987). The pathogen kills the plant cells and feeds on dead tissue, subsequently causing death of the whole plant. It produces pycnidia in which its spores are formed. Here, we use pycnidia formation as a measure of transmission potential of the pathogen. *Plantago lanceolata latent virus* (PlLV) is a DNA virus belonging to Capulaviruses in Geminiviridae (Susi et al., 2017). The virus has been recently characterized (Susi et al., 2019, Susi et al., 2017) , and it is relatively common in populations of *P. lanceolata* in the Åland Islands (33% of populations infected)(Sallinen et al., 2020). Mode of transmission and potential host range of PILV are currently unknown.

Phomopsis subordinaria **field survey**

To characterize the distribution, and drivers, of *P. subordinaria,* and to measure the relationship between within-host symptom severity and epidemic size and within-population prevalence, we surveyed 260 *P. lanceolata* populations in early September 2018 for infection by *P. subordinaria* . These populations were selected to represent different areas in the Åland Islands. The field identification of *P. subordinaria* was confirmed by microscopy of field collected samples consisting of an infected floral stalk. In each population, the epidemic size (measured as number of infected plants), and within-population prevalence (measured as proportion of infected plants within-population) of *P. subordinaria* infected plants was counted. In each population, we assessed the within-host infection load of the pathogen by counting the proportion of infected flower stalks within each plant from ten infected plants. Host population size is expected to increase infection risk of populations (Parratt et al., 2016) and thus, we estimated the host population size as coverage of the host plant in square meters. Host connectivity was measured in order to understand how distances between host populations may impact pathogen prevalence (Jousimo et al., 2014). Host population connectivity was calculated as

$$S_i^L = \sum \exp(-\alpha d_{ij}) \sqrt{A_j},$$

.

where d_{ij} is the Euclidian distance between patches j and i and α is the parameter of the negative exponential dispersal kernel, which was set to 1 km^{-1} (see Jousimo et al. (2014) for more details).A_j , is the square root transformation of area (m^2) of habitat patch j .

The Åland archipelago is highly fragmented and consists of the main island and smaller islands that jointly form 16 regional districts of similar size. To capture possible spatial variation among the regions within the islands, we used regional district as a spatial unit. In our sampling area, there were 10 regional districts (Eckerö (12), Finström (41), Hammarland (38), Jomala (10) Lemland (37), Lumparland (49), Maarianhamina (11), Saltvik (19), and Sund (46), with the number of visited populations within each regional district given in parenthesis).

Inoculation experiment

In order to investigate potential trade-offs between within-host infection load and transmission potential, and whether coinfection with PlLV affects *P. subordinaria* infection or trade-offs, we performed a laboratory trial using two *P. subordinaria* strains (P29 and P43 originating from populations 1720 and 861, respectively) , one PlLV strain and one *P. lanceolata* genotype (511-14) originating from the Åland Islands. Altogether 32 plants were used in the experiment. To compare *P. subordinaria* performance and trade-offs alone and in coinfection with PlLV, we inoculated half of the plants with a PlLV strain originating from population

3301 and maintained in *P. lanceolata,* whereas half of the plants received mock inoculation with phosphate buffer. Sap from PCR confirmed (Susi et al., 2017) PlLV infected plants mixed with phosphate buffer was used for virus inoculation. Using a syringe, one leaf per plant was inoculated with 100 µl virus sap or phosphate buffer. After seven days from PlLV inoculation, the plants received *P. subordinaria* inoculation. To understand pathogen strain effect on trade-offs with PlLV, half of the plants received an inoculation with strain P29, and half received an inoculation with strain P43. The fungal strains were collected from field, purified on sequential inoculations on oat agar plates (de Nooji & van der Aa, 1987) and maintained on live *P. lanceolata* plants. The flower stalks were inoculated by wounding the stem just below the inflorescence with a scalpel and immediately pipetting 10^6 conidia/µl suspension to the wound. In the experiment, each treatment (PlLV coinfection yes/no with *P. subordinaria* strain P29 or P43) was replicated eight times. The plants were kept inside a growth chamber in 8:16 dark: light cycle at +20 °C. To prevent possible shelf effects, the locations of plants inside the chamber were moved daily. To quantify within-host infection load, the size of the *P. subordinaria* lesion in centimetres and the length of each flower stalk was measured once a week starting seven days post inoculation. Lesion growth measurements were then used for calculation of relative area under disease progress stairs (AUDPS; (Simko & Piepho, 2012)). To measure transmission potential, we observed the time and density of pycnidia formation. Pycnidia are the fruiting bodies of the fungus that contain the conidial spores that spread the fungus (de Nooji & van der Aa, 1987). The time when first pycnidia were observed on each plant was monitored. At the end of the experiment, we counted the pycnidia density within a square centimetre on one flower stalk from each plant using a microscope.

Statistical analyses

To analyse drivers of *P. subordinaria* epidemics in *P. lanceolata* populations in the Åland Islands, we ran Generalized linear models in SAS Proc Glimmix software (SAS Institute Inc.) with infection (0 = no infection, 1 = infection) as a binomial response variable, host population size, and connectivity as covariates, and regional district as categorical explanatory variable. Including host population connectivity allows controlling for spatial variation in putative gene flow among populations(Hanski, 1999), and including regional district allows controlling for possible regional variation in these data. To understand factors explaining *P. subordinaria* population size within-host populations, we fit a model defining the percentage of infected plants within-host populations as a response variable (1 [?] 10% infected plants; 2 = 11-25% infected plants; 3 = 26-50% infected plants; 4 = 51-100% infected plants) with host population size and connectivity as covariates and regional district as a categorical explanatory variable. To understand how within-host infection load and disease transmission are linked in natural populations, we included pathogen within-host infection load (as average proportion of infected stalks in a population) as a covariate in the model. A Gamma distribution of errors was assumed.

We then analysed the results from the laboratory trial measuring the performance of two *P. subordinaria* strains alone and in coinfection with PlLV using generalized linear mixed models implemented in SAS Proc Glimmix software (SAS Institute Inc.) To test whether PlLV coinfection influences *P. subordinaria* lesion development (within-host infection load), we used AUDPS as a response variable with a Gaussian distribution of errors. In this model, we used *P. subordinaria* strain, virus inoculation treatment (1 = PlLV inoculation; 0 = mock inoculation), and time as categorical explanatory variables. Flower stalk (n = 92) was nested under plant individual (n= 32) and used as random factor. To understand how virus coinfection and*P. subordinaria* strain identity affect pycnidia formation, we analysed the subset of stalks from which the pycnidia were counted (n = 32) using a generalized linear model. Number of pycnidia was used as a continuous response variable and PlLV coinfection and *P. subordinaria* strain as categorical explanatory variables. Poisson distribution of error was assumed.

To test for possible trade-offs between within-host infection load (measured as AUDPS), and transmission potential, measured as pycnidia number, we ran a model using the stalks (n = 92) from which pycnidia were counted as generalized linear models in SAS Proc Glimmix software (SAS Institute Inc.). We included AUDPS as a covariate, and virus inoculation treatment (1 = PlLV inoculation; 0 = mock inoculation) and *P. subordinaria* strain as categorical explanatory variables. The response variable in the model was the

pycnidia abundance as the pycnidia number counted in 1 cm^2 area on the lesions. A Poisson distribution of errors was assumed.

Results

Phomopsis subordinaria field survey

We found that *P. subordinaria* was widely spread in *P. lanceolata* populations in the Aland Islands as in 47.7% (124) of the sampled 260 populations; one or more infected plants were found (Figure 1A). Large populations were more commonly infected than small, and host population connectivity was positively associated with *P. subordinaria* infection (Table 1, Figs. 1B-C). We also found regional spatial variation in infection prevalence that was the highest in Hammarland, where 67.5% of the 37 surveyed host populations were infected, and lowest in Saltvik where 31.6% of the 19 surveyed host populations were infected (Table 1, Figs. 1A).

Within infected host populations, *P. subordinaria* population size and infection prevalence were typically low. In nearly half of the (48.4%) populations, we found 1-10 infected plants (Figure 1E). In 36.3% of the infected populations 11-100 infected plants were found, and in only 1.6% populations we found more than 1000 infected individuals (Figure 1E). Within populations less than 10% of the plants were infected in the majority of infected populations (87.9%), and in only 2.4% of the populations we observed more than 25% infected plants (Figure 1F). At the plant level, we found that *P. subordinaria* infections were highly virulent. On average 84.9% of stems were infected within infected plants (Figure 1D).

When we tested factors affecting pathogen within-population prevalence, regional districts differed in the proportion of plants infected and within-host infection load measured as proportion of infected stalks had small but significant positive effect on within-population prevalence (Table 1; Fig 1G). Neither host population connectivity nor size influenced *P. subordinaria* within-population prevalence (Table 1). None of the tested variables - regional district, host population connectivity and size, *P. subordinaria* epidemic size, or within-population prevalence – had a significant effect on within-host infection load (Table 1).

Coinfection with virus alters *Phomopsis subordinaria* performance

We investigated the effect of PlLV coinfection on *P. subordinaria* within-host infection load (measured as AUDPS), and transmission potential (measured as pycnidia density) in an inoculation experiment with two *P. subordinaria* strains (P29 and P43). The two strains differed significantly in their AUDPS with strain P43 outperforming strain P29 (Table 1; Fig 2B). For both strains, within-host infection load (measured as AUDPS) was lower under coinfection with PlLV than when *P. subordinaria* infected the host alone (Table 1; Figs. 2AB). Pycnidia density was significantly higher under coinfection than under single infection, while *P. subordinaria* strains did not differ in their pycnidia densities (Table 1; Fig. 2C).

Trade-offs between life-history traits

Finally, we tested whether there are trade-offs between within-host infection load and transmission potential, and whether *P. subordinaria* strain or PlLV coinfection have an effect on trade-offs. In this model, pathogen strain had significant impact on pycnidia formation, but PlLV coinfection did not (Table1). We found that while pycnidia formation was not directly correlated with AUDPS, the relationship between pycnidia formation and AUDPS was mediated by strain identity (significant interaction AUDPS x strain; Table 1; Fig. 3A) and by coinfection with PlLV (significant interaction AUDPS x PlLV coinfection; Table 1; Figure 3B). There was a negative correlation between AUDPS and pycnidia formation in strain P29 suggesting a trade-off, whereas in strain P43 there was no evidence of a trade-off (Figure 3A). Similarly, in mock inoculated plants trade-offs between pycnidia formation and AUDPS was observed, but not in PlLV infected plants (Figure 3B).

Discussion

Although the trade-off between within-host infection load and transmission is a central tenet of pathogen evolution (Alizon et al., 2009), remarkably little is understood of this association in natural populations, and under coinfection scenarios that are prevalent across pathosystems (Tollenaere et al., 2016, Alizon et al.,

2013). Here, we study within-host disease load and between-host transmission experimentally to understand how sensitive their association is to pathogen strain identity and coinfection with a virus. We conduct a survey of infection across 260 wild host populations to test whether our experimental results are reflected in epidemiological patterns in the wild.

Phomopsis subordinaria was detected in nearly half of the surveyed natural host populations. The other fungal pathogen studied in this same host population network - the powdery mildew fungus *P. plantaginis* - infects annually 2-20% of *P. lanceolata*populations (Jousimo et al., 2014), and hence, by comparison *P subordinaria* is relatively common. In few other wild plant pathosystems, similar disease incidence rates have been observed (e.g.*Triphragmium ulmariae* rust infected 29-69% *Filipendula ulmaria* host populations (Zhan et al., 2018), and *Uromyces valerianae* rust infected 43-73% of *Valeriana salina* populations (Ericson et al., 1999)). In our study, spatial structure was the main determinant for pathogen incidence as both host population connectivity and regional district explained variation in *P. subordinaria*distribution. Positive correlation between host connectivity and infection incidence suggests that host population connectivity increases between population transmission, as predicted by metapopulation theory (Hanski, 1999). Large populations were more likely to be infected than small populations, which is also in line with theoretical predictions (Hanski, 1999). Unlike in *P. plantaginis* (Jousimo et al., 2014), host population size did not have an effect on within-population infection prevalence. While airborne pathogens such as *P. plantaginis* are expected to be sensitive to host population size through density-dependent transmission, *P. subordinaria* is vector-transmitted and hence, is expected to be less responsive to variation in host population size (Thrall et al., 1995). This result highlights the importantance of the mode of transmission is for epidemics.

Our field survey releveled extremely high within-host infection load with 84% of host inflorescences being infected on average. While within-host infection load was at its upper limits, transmission was not, as the proportion of uninfected hosts was large. Majority of epidemics were limited to less than 10% of hosts being within populations. We did not find evidence of a trade-off between within-host infection load and transmission limiting the spread of *P. subordinaria* within its host populations. Instead, we found a positive correlation between within-host disease load and population infection prevalence. The observed low within-host populations prevalence of*P. subordinaria* could also result from a range of other factors unrelated to life-history correlations, including variation in host plant susceptibility or pathogen infectivity (de Nooij & Damme, 1988), constrained vector transmission (de Nooij, 1988, Pleydell et al., 2018), and interactions with other pathogens than PlLV (Susi et al., 2015a, Susi et al., 2015b).

Cross-kingdom coinfections are common (Lawn et al., 2006, Chen et al., 2020) (Tollenaere et al., 2016, Tollenaere et al., 2017, Telfer et al., 2010), and they are often suggested to have serious consequences in disease epidemics and disease severity. The trade-offs observed under single host–single pathogen scenarios may change under coinfection, as host exploitation rates are expected to change under diverse infections (Alizon et al., 2009). *Plantago lanceolata* is a host for a number of pathogens in the Aland Islands, and coinfections are frequently observed (Susi et al., 2015a, Susi et al., 2019). To understand how coinfections may shape life-history correlations and disease dynamics of*P. subordinaria* , we studied disease development under coinfection with a recently characterized virus, PlLV. We found that coinfection with virus had a profound impact on within-host infection load and transmission of *P. subordinaria* that can further impact evolution and epidemiology of the pathogen. Coinfection alleviated the harm caused for the host and increased transmission potential. Under natural epidemics such trade-offs could translate into low within-host disease load and increased among host transmission.

The two strains differed significantly in their within-host infection load, with strain P43 outperforming strain P29. Significant variation among pathogens strains in their life-history traits is commonly observed in natural pathogen populations (Tack et al., 2012). For both strains, within-host infection load was lower under coinfection with PlLV than when *P. subordinaria* infected the host alone, while transmission potential was significantly higher under coinfection than under single infection. The relationship between within-host infection load and transmission potential was mediated by both strain identity and coinfection. We observed a negative correlation between the measured life-history stages in strain P29 suggesting a trade-off, whereas

in strain P43 there was no evidence of a trade-off. This result is in line with previous research that found life-history correlations to be depending on the pathogen genotype (Clement et al., 2012, Bruns et al., 2014). The negative relationship between within-host infection load and pycnidia formation became positive under coinfection. This is in line with an earlier study testing coinfection with two strains of powdery mildew fungus *P. plantaginis* where the strains had higher performance and positive life-history trait correlations when challenged with a competing strain (Laine & Makinen, 2018). Contrary to other studies on coinfection where response to coinfection has been found pathogen strain specific, here the response of the *P. subordinaria* strains was similar. In bacteria –fluke coinfections on salmons (Louhi et al., 2015), genotype specific responses on coinfection were observed. Similarly, virus strain combinations resulted in different within-host growth rates of bacterial pathogen on rice (Tollenaere et al., 2017).

This study increases our understanding on the factors generating diversity in epidemics in natural populations. Furthermore, this is one of the very first reports addressing the knowledge gap on how pathogen life-history traits correlate in realized epidemics. By showing that pathogen coinfection and strain identity may alter life history correlations this study contributes to better understanding of disease evolution and epidemiology.

Data availability

Upon acceptance the data will be available in Dryad repository.

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Table 1. Results from Generalized Linear Models on*Phomopsis subordinaria* field survey across 260 natural*Plantago lanceolata* populations across the Åland Islands, and from a laboratory inoculation experiment testing the impact of virus coinfection and strain identity on performance of the pathogen, as well as life-history trade-offs.

Wild population disease dynamics	**Occurrence in populations**	**Occurrence in populations**	**Occurr**
	d.f.	*F*	*P*
Connectivity	**1, 247**	**9.81**	**0.0019**
Regional district	**8, 247**	**2.71**	**0.0071**
Host population size	**1, 247**	**8.95**	**0.0031**
Wild population disease dynamics	**Within-population prevalence**	**Within-population prevalence**	**Within**
	d.f.	*F*	*P*
Connectivity	1, 109	1.06	0.3057

Wild population disease dynamics	**Occurrence in populations**	**Occurrence in populations**	**Occurr**
Regional district	**8, 109**	**5.4**	**<.0001**
Host population size	1, 109	0.61	0.4353
Within-host infection load	1, 109	**4.03**	**0.0471**
Within-population prevalence	Within-population prevalence	Within-population prevalence	
Laboratory performance	**AUDPS**	**AUDPS**	
	d.f.	*F*	*P*
Virus coinfection	**1, 28**	**8.7**	**0.0064**
Strain	**1, 28**	**11.04**	**0.0025**
Laboratory measured	**Pycnidia formation**	**Pycnidia formation**	**Pycnid**
trade-offs	*d.f.*	*F*	*P*
AUDPS	1, 26	2.67	0.1145
Strain identity	**1, 26**	**9.98**	**0.004**
Virus coinfection	1, 26	1.07	0.3099
AUDPS × strain	**1, 26**	**5.11**	**0.0323**
AUDPS × virus coinfection	**1, 26**	**11.73**	**0.002**

Figure legends

Fig. 1. Variation in *Phomopsis subordinaria* infection prevalence in 260 *Plantago lanceolata* populations in the Åland Islands . A) The proportion of infected populations in the nine regional districts surveyed. The effect of host population B) size and C) connectivity on infection prevalence. D) The mean within-host infection load in infected populations measured as proportion of infected stalks within each infected plant. The pathogen within-population prevalence E) as the number of infected plants in the populations, and F) as the proportion of infected plants in the populations. G) The relationship between pathogen within-population prevalence in the population of infection and within-host infection load.

Fig. 2. The impact of *Plantago lanceolata latent virus*(PlLV) infection on two strains of *Phomopsis subordinaria*performance on *Plantago lanceolata* in a laboratory experiment.

A) Proportion of each inoculated stalk ($n = 90$) with necrotic symptom (empty circle) with and without PlLV coinfection over the four weeks of data recording. Means of each time point for each treatment are visualised with a line. B) Area under disease progress stairs per treatment (AUDPS). Empty circles represent each inoculated flower stalk ($n = 90$) and mean + standard error from a linear model are presented for each treatment. C) Area under disease progress stairs per treatment. Empty circles represent each inoculated flower stalk ($n = 90$) and mean + standard error from a linear model are presented for each treatment.

Fig. 3. The impact of strain identity and*Plantago lanceolata latent virus* (PlLV) coinfection on life-history trade-offs in *Phomopsis subordinaria* on*Plantago lanceolata* in a laboratory inoculation trial. The impact of strain identity (grey = strain P29; black = strain 43) relationship between A) pycnidia density and area under disease progress stairs (AUDPS). B) The impact of PlLV coinfection (blue) vs mock inoculation (orange) on the relationship between pycnidia formation and AUDPS.

Fig. 1.

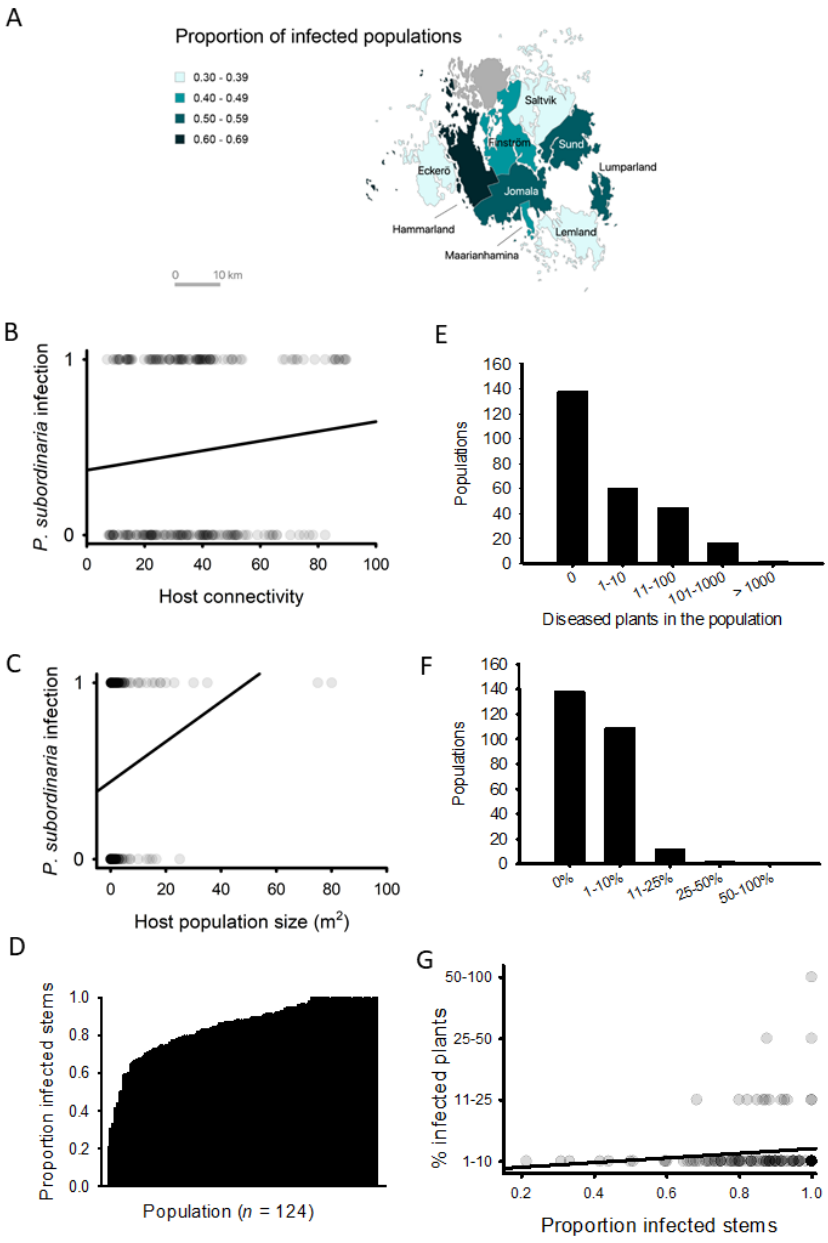

Fig. 2.

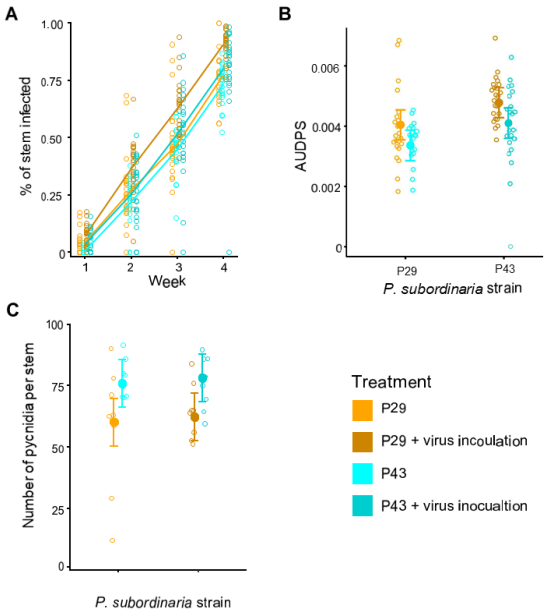

Figure 3.