Causes and consequences of within-host parasite communities

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Contents

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III) Sallinen Suvi, Susi Hanna, Halliday Fletcher & Laine Anna-Liisa (*In review*): Altered within-host and between-host transmission under coinfection underpin parasite co-occurrence patterns in the wild.

IV) Sallinen Suvi & Laine Anna-Liisa (*Manuscript*): Short-term fitness consequences of infection depend on host genotype and within-host parasite community.

Contributions

	I	II	III	IV
Original idea	SS, A-LL	HH, SS	SS, A-LL	SS, A-LL
Study design	HH, SS, A-LL	HH, SS	SS, HS, A-LL	SS, A-LL
Data collection	SS	HH, SS	SS, HS	SS
Data analyses	AN, SS	HH, SS	SS, FH	SS
Manuscript preparation	SS, AN, A-LL	HH, SS, A-LL	SS, HS, FH, A-LL	SS, A-LL

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A-LL Anna-Liisa Laine AN Anna Norberg

ABSTRACT

Multiple infection of individuals is rather the rule than the exception and surveys of wild populations have revealed that coinfections, where more than one parasite simultaneously infects an individual, are more common than would be expected by random. Most research on ecological and evolutionary consequences of parasite infections at the within-host level has focused on interactions between single-host single-parasite combinations or followed the effects of parasite species pairs. However, multiparasitism, where more than two parasites simultaneously exploit a host, can essentially alter the outcome of parasite-parasite interactions in ways that are not intuitively deductible from the effects of single parasites. Hence, it is necessary to experiment on how multi-parasite communities affect the growth, replication, and transmission of the parasites in order to predict parasite epidemics and to understand the evolution of parasites. Within-host parasite community dynamics are especially important because they have potential to alter parasite transmission which is among the most important factors explaining both within-host and population level dynamics.

In this dissertation, I used field, common-garden, and laboratory inoculation experiments to study the assembly and consequences of within-host parasite communities. I use the plant host *Plantago lanceolata*, and the fungal and viral parasites infecting natural *P. lanceolata* populations in the Åland Islands. I demonstrate that differences in within-host parasite communities are important for epidemics and may have potential consequences for evolution of parasites and their hosts. My experiments demonstrate that coinfection may affect both the coinhabiting parasites and hosts. The altered disease severity under coinfection can further lead to altered transmission and affect epidemics in host populations. My results also stress the importance of genotypic variation among hosts – I find that host genotype is an important determinant of virus community assembly during natural epidemics. Furthermore, the host is not only an important filter but a mediator of parasite dynamics, as demonstrated by the different directions of co-occurrence patterns of viruses. Finally, my results show that host growth and reproduction is affected by the within-host parasite community and host genotype. Jointly, my results highlight the importance of studying the causes and consequences of within-host parasite communities, as they may have far-reaching implications for disease epidemics and evolution.

TIIVISTELMÄ

Tutkimus on osoittanut, että yksilöt ovat usein eri loislajien isäntiä samanaikaisesti, mutta suurin osa loisten ekologisiin ja evolutiivisiin vaikutuksiin keskittyvästä tutkimuksesta käsittelee yhden loislajin ja isännän välistä suhdetta. Moniloisinta, jossa useampi kuin kaksi loislajia hyödyntää yhtä isäntää samanaikaisesti, voi vaikuttaa loisten lisääntymiseen ja leviämiseen, sekä muuttaa isäntään kohdistuvaa haittaa. Moniloisinnan seurauksia ei aina voi päätellä yksittäisten loisten vaikutuksista.

Väitöskirjassani tein kokeita heinäratamoa (*Plantago lanceolata*) ja luonnollisissa heinäratamopopulaatioissa Ahvenanmaalla esiintyviä loisia käyttäen loisyhteisöjen muodostumiseen vaikuttavia tekijöitä ja loismonimuotoisuuden vaikutuksia loisiin ja kasviyksilöihin. Testasin 1) millaisia virusyhteisöjä perimältään erilaisiin kasviyksilöihin muodostuu virusepidemian aikana, 2) miten kasvien kasvu, kukinta ja siementuotanto vaihtelevat, kun kasveihin tartuttaa erilaisia loisyhteisöjä, 3) miten *Phomopsis subordinaria* -sieniloisen leviäminen tartunnan saaneista kasveista uusiin vaihtelee loisyhteisöstä riippuen, ja 4) miten Plantago lanceolata latent virus vaikuttaa *P. subordinaria*n oireen leviämiseen ja itiötuotantoon.

Väitöskirjani päätuloksia on, että isännän sisäisellä loisyhteisöllä voi olla vaikutusta loisepidemioihin sekä loisten evoluutioon, koska loisten yhteisesiintyminen voi vaikuttaa loisten kasvuun ja leviämiseen. Sienioireen leviäminen hidastui, mutta itiörakenteiden tiheys kasvoi, kun kasvi oli tartutettu myös viruksella. Leviämiskokeessa sieniloisen leviäminen oli tehokkaampaa, jos samaan aikaan kasveja tartutti toinen sieni. Sieniloisten positiivinen yhteys oli havaittavissa myös luonnonepidemiasta kerätyssä aineistossa. Tulokseni korostavat myös, että isännän perimä on tärkeä osa moniloisinnan syiden ja seurausten kokonaisuutta, sillä virusyhteisöjen rakenne kenttäkokeessa riippui isäntäyksilön perimästä. Perimä myös vaikutti yhdessä loisten kanssa kasvuun, ja itsenäisesti kukintaan ja siementuotantoon puutarhakokeessa. Väitöskirjani tulokset korostavat isännän sisäisen loismonimuotoisuuden merkitystä luonnon loisdynamiikan ohjaavana voimana. Loisten yhteisesiintyminen voi johtaa muutoksiin epidemioissa vaikuttamalla loisten kasvuun ja leviämiseen, sekä vaikuttaa isäntien evoluutioon muuttamalla isäntäyksilön kasvua ja siementuotantoa.

SUMMARY

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1 INTRODUCTION

1.1 PARASITE COMMUNITIES

Parasites, organisms who's life style is to live off of another organisms resources [literally para - beside, sitos - food], are the most abundant group of organisms on Earth (Dobson et al. 2008). They serve important roles in ecosystems by, for example, promoting plant diversity (Bradley et al. 2008) and shaping the evolution of host populations (Rigaud et al. 2010, Thrall et al. 2012). Parasites include various taxa across the kingdoms of life, such as viruses, fungi, and helminths, and organisms with simple and multi-host life-cycles, as well as host-specialists and host-generalists have adopted a parasitic life style (Chernin 2000, Dobson et al. 2008). It is hardly a surprise that this highly diverse group of organisms form diverse communities throughout the orders of ecological organization; variation in parasite diversity and abundance is observed in host communities, host populations, and host individuals (Nieto-Rabiela et al. 2018, Bolnick et al. 2020, Norberg et al. 2021). Composition of parasite communities is often studied from the perspective that parasite-parasite interactions determine the outcome, and sometimes it is thought that the parasite community is formed through "filters", such as local abiotic conditions or host resistance, that define the occurrence of each parasite separately (Nieto-Rabiela et al. 2018, Bolnick et al. 2020). In reality, both of these processes operate simultaneously, as demonstrated by the metacommunity theory (Leibold et al. 2004, Mihaljevic 2012, Borer et al. 2016). Indeed, the community composition is determined differently on different spatial scales (Bolnick et al. 2020) but the scales are linked through feedbacks and processes, such as transmission (Borer et al. 2013, Strauss et al. 2019). Within-host dynamics can determine population level prevalence of parasites (Clay et al. 2019) and multi-parasite dynamics can affect the severity of disease outbreaks (Clay et al. 2020). Overall, a community approach is increasingly incorporated in parasite research (Auld et al. 2017, Abbate et al. 2018, Miller et al. 2018, Nieto-Rabiela et al. 2018).

Infections by multiple parasite species of host individuals is rather the rule than an exception (Taylor et al. 1997, Cox 2001, Rigaud et al. 2010, Telfer et al. 2010, McLeish et al. 2019). Surveys of wild populations have revealed that coinfections, where more than one parasite simultaneously infects an individual host, are more common than would be expected by random (Seabloom et al. 2013, Kamitani et al. 2016, Abbate et al. 2018, Norberg et al. 2021), and parasites tend to aggregate (Boag et al. 2001, Kendig et al. 2017). Observed parasite-parasites aassociations include both positive and negative co-occurrences (Telfer et al. 2010, Abbate et al. 2018, Dallas et al. 2019, Norberg et al. 2021), and some research suggests that parasite-parasite interactions are a major driver of spatial parasite occurrence patterns (Cattadori et al. 2008, McLeish et al. 2019). Others demonstrate that variation in host population resistance strongly affects spatial parasite occurrence patterns (Jousimo et al. 2014, Montes et al. 2021). Exploring which factors and processes determine parasite community composition on different scales is critical for understanding parasite dynamics.

While individuals with multiple parasites have been found in wild animal and plant systems for decades (Taylor et al. 1997, Cox 2001, Telfer et al. 2010, Balmer and Tanner 2011, McLeish et al. 2019), most research on ecological and evolutionary consequences of coinfection at the within-host level has concentrated on interactions between single host-single parasite combinations. Furthermore, most studies on coinfection address pairs of parasite species (Al-Naimi

et al. 2005, Wintermantel 2005, Alizon et al. 2013, Hoverman et al. 2013, Aguilar et al. 2017, Clay et al. 2019, Clerc et al. 2019), or strains within species (Taylor et al. 1997, Ben-ami et al. 2008, Choisy and Roode 2010, Karvonen et al. 2012). Multiparasitism, where more than two parasites simultaneously exploit a host, can essentially alter the outcome of parasite-parasite interactions in ways that are non-additive (Holt and Dobson 2013, Abbate et al. 2018, Marchetto and Power 2018, O'Keeffe et al. 2021). It is necessary to test how multi-parasite communities affect the growth, replication, and transmission of the parasites in order to predict parasite epidemic patterns and to understand the evolution of parasites (Rigaud et al. 2010, Lively et al. 2014).

1.2 CONSEQUENCES OF WITHIN-HOST PARASITE COMMUNITIES FOR THE PARASITES

Of the locally present parasites, only a fraction ends up infecting a host individual. Parasites are by definition, organisms that live by utilizing the host's resources (Chernin 2000, Rohde 2005, Mehlhorn 2016) and hence, parasites infecting a single host are sharing a limited resource and are expected to compete for it (Mideo 2009). At the beginning of an infection, parasites usually first grow within the host, and further during the infection, they replicate in order to increase the likelihood of successful transmission to another host (Anderson and May 1982, Chernin 2000). Hence, it is expected that parasite-parasite competition for the limited host resources would favour faster replicating parasites (Brown et al. 2002, De Roode et al. 2005, Choisy and Roode 2010). However, the outcome and optimal strategy of parasite-parasite competition can vary depending on many factors such as the type of competition, the relatedness of the parasites, parasite aggressiveness, and the order of arrival (Brown et al. 2002, Thomas et al. 2003, Choisy and Roode 2010, Rigaud et al. 2010, Caracuel et al. 2012, Alizon et al. 2013, Clay et al. 2019). Theory predicts and empirical evidence demonstrates that coinfection can significantly alter parasite growth and replication, and this may further cascade into altered epidemics (Anjos et al. 1992, Al-Naimi et al. 2005, Wintermantel 2005, Mideo et al. 2008, Alizon et al. 2013, Susi et al. 2015a, 2017b, Tollenaere and Brugidou 2017, Clay et al. 2019).

Indeed, within-host parasite community outcomes are especially important because they affect the replication of parasites, which is intimately linked with transmission (Anderson and May 1982, De Roode et al. 2005, Wintermantel et al. 2008). Transmission is among the most important factors explaining parasite within-host community assembly and population level dynamics (Dallas and Presley 2014, Handel and Rohani 2015). Transmission is not only a link between spatial scales of parasite communities but also a central parasite fitness trait (Anderson and May 1982), and hence it is a key step in building an understanding of parasite communities in the wild. Linking within-host parasite diversity to among-host transmission has been identified as an important research question, but in the context of multiparasitism, it has mainly been studied using mathematical models (Mideo et al. 2008, Strauss et al. 2019). Experiments comparing transmission from different parasite communities of multiple parasites are lacking. To understand the overall effects that parasite communities have on the different member s of the community, it is important to experimentally compare how transmission varies when the parasite community composition is altered.

It has been long hypothesized that parasites trade-off their ability to transmit with virulence (Anderson and May 1982), which is usually defined as host mortality or host fitness loss in evolutionary biology (Anderson and May 1982, Brown et al. 2002, Alizon et al. 2013). Indeed, ever increasing virulence would eventually lead to early host death and end transmission (Anderson and May 1982). Despite the established role of this hypothesis as a base for much of the parasite research, such a trade-off is not often confirmed (Acevedo et al. 2019), and multiparasitism has been suggested to be one reason for the lack of consistent support for the hypothesis (Rigaud et al. 2010, Alizon et al. 2013).

Parasite-parasite interactions range from synergistic, where one or both benefit, to antagonistic, where one or both parasites suffer negative effects (Behke et al. 2001, Syller 2012). The effect of parasites can be asymmetrical, where the first parasite positively affects the other, but the other parasite has a negative effect on the first one (Bush and Malenke 2008, Wintermantel et al. 2008, Clerc et al. 2019). Furthermore, theory predicts that adding a third species can lead to non-additive effects that are not intuitively deductible from the individual effects of the parasites (Holt and

Dobson 2013). Experiments testing how growth, replication, and transmission of parasites change when more than two species are added are rare. Some experiments have, however, shown that in two-parasites one-mutualist interactions the direction and magnitude of the effects can vary when a third species is added (Marchetto and Power 2018, O'Keeffe et al. 2021). Hence, experimental testing of parasite community outcomes is essential to gain knowledge of the consequences of parasite communities.

1.3 THE HOST – A DETERMINANT AND MEDIATOR OF PARASITE-PARASITE INTERACTIONS

Parasites can only exist where susceptible hosts exist. Parasite-parasite interactions can be direct or host mediated as parasites interact through resource competition and the changes induced in the host physiology, especially host immunity (Behke et al. 2001, Pedersen and Fenton 2007, Mideo 2009, Alizon and Lion 2011, Caracuel et al. 2012, Tollenaere et al. 2016, Kendig et al. 2020). Therefore, the host is presumably an important determinant of the within-host parasite community, and resistance is expected to act as a filter that determines which parasites infect a host individual (Borer et al. 2016). Parasite-parasite interactions have been shown to be mediated by the host immune system in various systems (Behke et al. 2001, Cox 2001, Al-Naimi et al. 2005, Bush and Malenke 2008, Halliday et al. 2018).

In plants, the within-host parasite community composition may especially be affected by two types of immune responses: 1. those that prevent infection and are often parasite-specific. These responses are often based on existence of specific genes or alleles and determine whether a plant can recognize and stop infection of a given parasite (Jones and Dangl 2006, Poland et al. 2009), and 2. Those that mitigate infection development. This can result from quantitative resistance or general stress responses, namely induction of plant hormones, and may be altered by coexisting parasites (Jones and Dangl 2006, Poland et al. 2009, Pieterse et al. 2012). Experiments show that the host genotype is a strong determinant of replication of individual parasites (Al-Naimi et al. 2005, Bruns et al. 2012). If the parasite is able to escape genetically based resistance and infect a host, the host will respond with altered plant hormones and attempt to reduce the growth and replication of the parasite (Poland et al. 2009, Pieterse et al. 2012).

The plant's hormonal signalling will change due to infection whilst changing the biotic environment of the parasite community (Bari and Jones 2009, Pieterse et al. 2012). Different parasites induce different hormonal responses. For example, a biotrophic fungi are expected to increase salicylic acid (SA) production whereas infection by a necrotrophic fungi causes jasmonic acid (JA) and ethylene-related responses, but fungi may also affect signaling of other hormones, such as auxin (Glazebrook 2005, Bari and Jones 2009, Ludwig-Müller 2015). Viruses often increase SA production and reduce JA production, manipulate auxin and gibberellin signaling, and possibly affect others, such as ethylene (ET) and brassinosteroids (Bari and Jones 2009, Thaler et al. 2012, Casteel et al. 2015, Aguilar et al. 2017). The hormonal signaling pathways further engage in cross-talk. JA and SA pathways often work antagonistically so that once one is up-regulated, the other one is down-regulated (Pieterse et al. 2012, Derksen et al. 2013, Llave 2016). Hence, a plant response against one parasite may hypothetically lead to increased or decreased possibility for another parasite to colonize the host. Much of the within-host parasite research has concentrated on how arrival order can affect the colonization success, growth, and replication of parasites, and the potential effects may cascade to affect population level parasite epidemics (Al-Naimi et al. 2005, Hoverman et al. 2013, Chávez-Calvillo et al. 2016, Clay et al. 2019, Karvonen et al. 2019).

Plant hormones are also induced by abiotic conditions (Nguyen et al. 2016), and coinfection outcomes can also be mediated by the host and depend on the abiotic conditions experienced by the host. For example, nutrient conditions can change the interaction and transmission of some viruses (Lacroix et al. 2014, Kendig et al. 2020) and the magnitude and direction of the parasite survival and replication can vary, as shown in a mouse host (Budischak et al. 2015).

The complexity of the plant immune system, together with other parasite-parasites interactions makes it is difficult to predict the consequences of multiparasitism. Hence, experimental inoculation

tests provide a useful tool to determine the outcomes of multi-parasite scenarios.

1.4 THE HOST – A BEARER OF CONSEQUENCES

The host is not only a determinant of parasite communities and interactions, but also the victim of parasite exploitation. Parasites may reduce host growth and reproduction and cause disease (e.g. (Maskell et al. 1999, Wintermantel 2005, Ben-ami et al. 2008, Caracuel et al. 2012, Gleichsner et al. 2018)). Parasitism may also be costly due to lowered resistance against further parasites (Desai et al. 2021). Infection may alter the allocation of resources towards growth or reproduction (Pagán et al. 2008, Gleichsner et al. 2018, Montes et al. 2020). A large body of empirical evidence has found coinfections to be more detrimental to hosts compared to single infections (Anjos et al. 1992, Ben-ami et al. 2008, Caracuel et al. 2012, Tollenaere and Brugidou 2017, Marchetto and Power 2018). While coinfection is often found to cause more severe disease (Wintermantel 2005, Meyer and Pataky 2010, Desai et al. 2021) parasite-parasite competition can also lead to less severe effects (Cassells and Herrick 1977, Ben-ami et al. 2008). On the other hand, negative effects on the host may be reduced under coinfection or the risk of serious illness is lower with another parasite (Abbate et al. 2018), possibly due to costly parasite-parasite competition (Ben-ami et al. 2008). However, even if a parasite provides protection against another stressor, it may still have negative effects. For example, growth could still be largely decreased despite the advantage of virus induced drought tolerance (Aguilar et al. 2017). Experimental testing of the consequences of varying within-host parasite communities will provide knowledge of the consequences of varying within-host parasite communities.

1.5 PLANT VIRUSES AND CROSS-KINGDOM PARASITE RESEARCH

While plants often simultaneously support a community of microbes involving members from multiple kingdoms (Tack et al. 2012, Borer et al. 2013), empirical testing of the consequences of cross-kingdom parasite communities remains scarce. The existing examples of pair-wise cross-kingdom parasite situations demonstrate that they have important consequences for both hosts and parasites (Tollenaere and Brugidou 2017). It is especially noteworthy that plant viruses are often studied separately from other types of parasites although limited evidence suggests that viruses may have significant effects on replication of fungal and bacterial pathogens (Zaiter et al. 1990, Marte et al. 1993, Bassanezi et al. 1998, Meyer and Pataky 2010, Abbate et al. 2018).

The development of methodology, especially the implementation of small-RNA sequencing techniques, in the past decades has helped to reveal how diverse virus communities wild plants support (Boonham et al. 2014, Bernardo et al. 2017). Viruses are highly abundant and they are found infecting large proportions of wild plants with up to 91 % prevalence (Raybould et al. 1999, Prendeville et al. 2012, Alexander et al. 2014, Seabloom et al. 2015, Kamitani et al. 2016). Although viruses move across the agro-ecological interface and hence, knowing the virus species and their biology in wild plants would improve prediction and identification of emerging threats, plant viruses have mainly been studied in agricultural plants. Some research also suggest that crop-associated viruses may spill over to wild plants and even threaten native plant communities (Malmstrom et al. 2005, Van Vianen et al. 2013). Hence, more research on the diversity and the consequences of viruses in wild plants has been called for (Cooper and Jones 2006, Alexander et al. 2014).

While viruses are traditionally viewed as pathogens, wild plant surveys often find virus infections in seemingly symptomless plants (Remold 2002, Prendeville et al. 2012, Kamitani et al. 2016). Indeed, viruses are not always pathogenic but may be commensal, mutualistic, or conditionally move along antagonistic-mutualistic continuum (Roossinck 2011a, Hily et al. 2016, Gleichsner et al. 2018). One of the objectives of this dissertation is to shed light on the role of these under-investigated parasites in wild plant populations. My experiments contribute to the knowledge gap of cross-kingdom parasites communities by testing the consequences of combining viruses and fungal parasites for growth, replication, and transmission of these parasites.

2 OBJECTIVES

Individuals are often infected by multiple parasites and these parasite communities are expected to affect epidemics and the evolution of hosts and parasites. However, we know remarkably little of how multi-parasite cross-kingdom communities affect the growth and transmission of the members of the community, and the growth and fitness of host plants. In addition, despite the obvious logic behind the expectation that host genotype is an important determinant of within-host parasite community assembly, the assumption has not, to my knowledge, been experimentally tested earlier. To contribute towards these knowledge gaps of the causes and consequences of within-host parasite communities, I combined experiments and survey data from natural host populations to test the following hypotheses:

H1. Host genotype determines virus community assembly **(I)**

H2. Coinfection with a virus alters life-history traits of a fungal parasite *Phomopsis subordinaria* **(II)**

H3. Transmission of fungal parasite *Phomopsis subordinaria* is affected by the coinfecting parasite community **(III)**

H4. Parasite co-occurrence in the wild is linked with altered parasite transmission **(III)**

H5. Host growth and short-term fitness is affected by the within-host parasite community **(IV)**

I have tested these hypotheses in my thesis chapters described in table 1.

Table 1. Summary of the chapters.

Chap	Objective	Methods	Results	Implications
I	Test hypothesis H1: Host genotype affects the assembly and composition of within-host virus communities	Field experiment in natural populations in the Åland Islands	Host genotype and local population context together drive the within-host virus community assembly	This result confirms the expected but previously untested importance of the host as a driver of within-host parasite communities
II	Test hypothesis H2: Does PlLV affect within-host spread and reproduction of *P. subordinaria*	Laboratory inoculation experiment	Prior infection by PlLV slows down spread but increases spore structure density	Cross-kingdom coinfections are important players for the evolution of parasites
III	Test hypothesis H3 and H4: within-host parasite community effects transmission of a *P. subordinaria*	Common-garden experiment, epidemic survey of natural populations	Coinfection with another fungus facilitates disease and transmission of *P. subordinaria*, but viruses do not. In natural populations, historical persistence of the facilitative fungus increases likelihood of presence and larger populations size of *P. subordinaria*	Coinfections are important drivers of epidemics through their effect on transmission, and facilitative interaction can lead to positive associations over time over-time
IV	Test Hypothesis H5: consequences of various within-host parasite communities on host growth and reproduction	Common-garden experiment	Within-host parasite community together with host genotype determine growth rate. Parasite community had a individual effect on seed production. Plant genotype is a strong determinant of life-history traits	Within-host parasite communities may affect the growth rates and reproduction of the hosts, and hence play an important role in the evolution of host populations

3 MATERIAL AND METHODS

I tested my research hypotheses on the plant host *Plantago lanceolata* and the parasites infecting *P. lanceolata* in the natural populations in the Åland Islands, southwest of Finland.

3.1 *PLANTAGO LANCEOLATA* PARASITE COMMUNITY IN THE ÅLAND ISLANDS

Plantago lanceolata (Fig. 1) is a herbaceous perennial plant that reproduces with self-incompatible, wind-dispersed pollen and vegetatively via side-rosettes (Sagar and Harper 1964). In the Åland Islands, *P. lanceolata* grows in ~4000 dry meadows forming a fragmented population network (Fig. 1) (Ojanen et al. 2013). The network has been monitored for *P. lanceolata* population size and locations since 1993 as part of the Melitaea cinxia butterfly surveys (Ojanen et al. 2013).

The *P. lanceolata* population network hosts a diverse parasite community consisting of fungi and viruses (Laine 2003, Jousimo et al. 2014, Susi et al. 2019). Two fungal parasites are often found in the *P. lanceolata* populations. *Phomopsis subordinaria* (Fig. 1) (Desm.) Trav. (telemorph Diaporthe adunca (Rob.) Niessl.) is an Ascomycota fungus mainly found infecting *P. lanceolata* (de Nooij and van der AA 1987). *Phomopsis subordinaria* spores are dispersed via water droplets but infection can only occur if the spores land on a wounded plant and can enter the plant tissue (de Nooij and van der Aa 1987, Linders et al. 1996). The infection typically begins under the ear of the flower, and weevils feeding on this area can help *P. subordinaria* spread (de Nooij and van der AA 1987, de Nooij 1988). Fungal infection causes a stalk disease as the infection proceeds in the flower stalks towards the leaf rosette while killing the tissue and producing spore structures (de Nooij and van der Aa 1987). Development of infected flowers is interrupted, developing seeds desiccate, and infection may eventually lead to host plant mortality (de Nooij and van der Aa 1987). In the Åland Islands, *P. subordinaria* only infects a small portion of plants within a population (Laine 2003). The genetic basis of *P. subordinaria* resistance in *P. lanceolata* has not been quantified in this system, but previous experiments indicate that *P. subordinaria* can infect most host genotypes (de Nooij and van der Aa 1987).

Podosphaera plantaginis (Fig. 1) is an Ascomycota fungus specialized in infecting *P. lanceolata* (Yarwood 1978). It is obligatory and requires a living host throughout its life cycle (Yarwood 1978). The relationship between *P. lanceolata* and *P. plantaginis* is genotype-specific whereby a given *P. plantaginis* strain is only able to infect some *P. lanceolata* genotypes but not all (Laine 2004). In the Åland Islands, *P. plantaginis* annually infects up to 20 % of *P. lanceolata* populations and the occurrence pattern follows classical metapopulation dynamics: some populations go extinct yearly while new populations

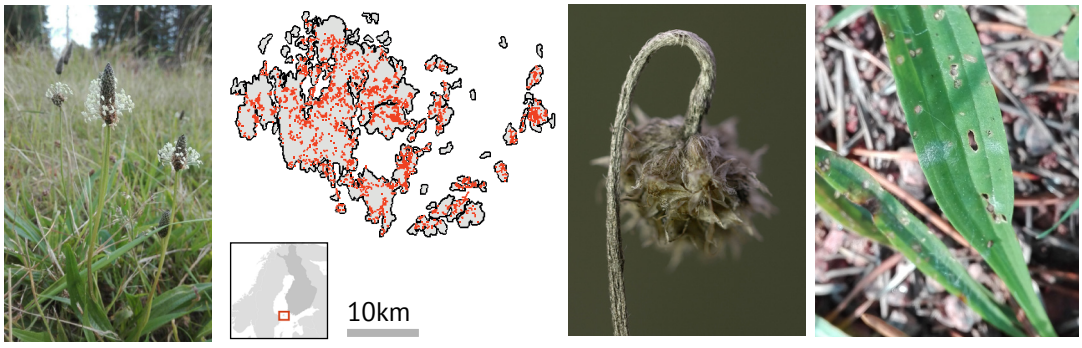

Figure 1.The host plant *Plantago lanceolata,* and the locations of *P. lanceolata* populations in the Åland Islands, fungal parasite *Phomopsis subordinaria,* and fungal parasite *Podosphaera plantaginis.*

are colonized (Jousimo et al. 2014). Infection can have a fitness cost on host individuals (Susi and Laine 2015) and can have negative effect population growth (Laine 2004, Penczykowski et al. 2014).

Sequencing of plant small-RNA has revealed a diverse virus community infecting *P. lanceolata* populations in the Åland Islands (Susi et al. 2019, Norberg et al. 2021). Small-RNA is a resistance response against virus infection called RNA-silencing, where the plant's immune system recognizes double-stranded RNA, a typical step in virus replication, and cuts them into small pieces of 21-24 nucleotide pairs, which are used as templates to recognize and cut more virus pieces (Baulcombe 2004, Kreuze et al. 2009). Hence, the recognized viruses are actively challenging the plant immune system. Five most common viruses are Plantago lanceolata latent virus, Plantago latent caulimovirus, Plantago betapartitivirus, Plantago enamovirus, and Plantago closterovirus (Susi et al. 2017a, 2019), and recently developed specific PCR primers and protocols can be utilized for their detection (Susi et al. 2019). These five viruses were selected to test virus-community related hypotheses (I, III, IV). These viruses infect plants across the *P. lanceolata* population network in varying frequencies and often without conspicuous symptoms, although yellowing of infected plants is encountered (Susi et al. 2019, Norberg et al. 2021).

3.2 PLANT AND PARASITE MATERIAL, AND INOCULATIONS

To test my hypotheses, I performed experiments in natural populations in the Åland Islands (I), in a common garden in Lammi biological station (III, IV), and in controlled climate chambers (II). Experimental plants were produced by cloning multiple parental plants into replicates. The parental plants originated from natural populations in the Åland Islands and were known to represent different *P. plantaginis* resistance genotypes. However, their susceptibility to *P. subordinaria* and the viruses was unknown. The experimental plants were cloned by placing a parental plant's pot on a pot filled with vermiculate and allowing the parental plant to grow roots through the lower pot. After one month, the roots were cut between to bottom of the parental plant's pot and the top of the vermiculate filling in the lower pot. After one month, the cut roots sprouted new leaves and these new rosettes were separated and planted into their own pots. The experimental plants were grown in an insect-free greenhouse before the start of the experiments for at least two months.

To test the effects of varying within-host parasite communities, parasites were collected from wild *P. lanceolata* populations in summers 2017, 2018, and 2019. *Phomopsis subordinaria* infected flower stalks were collected in 2017 into pergamin bags and inoculated onto living *P. lanceolata* plants in the laboratory. *Phomopsis subordinaria* was then maintained in living plants inside growth-cabins with constant conditions of 21'C and 16:8 light-dark cycle. Inoculations of *P. subordinaria* (II, III, IV) were done by mixing a piece of infected flower stalk into MilliQ-water and pipetting a droplet onto the flower stalks below the ear of the flower, and pushing a needle through the water droplet into the plant tissue.

PlLV was collected using sentinel plants that were placed into natural populations in the Åland Islands for two months in the beginning of summer 2017. The plants were transported to the laboratory in University of Helsinki and maintained in growth-cabinets with constant conditions of 21°C and 16:8 light-dark cycle, and infections were confirmed with PCR-detection (Susi et al. 2017a). Leaves of infected plants were used fresh (II, IV) or stored in the freezer before inoculations (III). More infected plants were produced, and experimental plants were inoculated by inoculations where plant SAP was first extracted form leaves of infected plants and the inoculated with a syringe. Leaves of infected plants were crushed with phosphate buffer and 200µl of the solution was pressed into the plant tissue with a syringe.

To increase the diversity of parasites in the common-garden experiments (III, IV), I collected *P. plantaginis* plants from populations in the Åland Islands that had typical virus symptoms (yellow, curly, or red leaves) (Susi et al. 2019). The plants were collected in summers 2018 and 2019. I extracted sap form these plants and pooled it into one "virus bulk inoculum". The diversity of virus infections was confirmed in the inoculated experimental plants with PCR using the primers for the five most common viruses (Susi et al. 2017a, 2019).

Figure 2 Field experiment testing hypothesis H1, that host genotype determines virus community assembly (chapter I). Cloned replicate plants of four genotypes were distributed among the natural vegetation in *Plantago lanceolata* populations in the Åland Islands. Plants were sampled for detection of the five most common viruses two weeks and seven weeks after the beginning of the experiment.

3.3 EXPERIMENTAL DESIGN

I conducted four experiments: one in the natural populations (I), two in semi-natural common garden conditions (III, IV), and one in controlled climate chambers (II). To test whether host genotype affects within-host virus communities (H1, I), I used 320 cloned individuals of four *P. lanceolata* genotypes and placed them into four natural . *lanceolata* populations in the Åland Islands (Fig. 2). Twenty replicates of each genotype were placed in each population. The plants were kept in the populations for twelve weeks. They were sampled for virus PCR-detections two and seven weeks after the beginning of the experiment, and the result from these two time points were pooled for the analyses. To tease apart some additional, potentially important explanatory factors, symptoms of herbivory (indication of potential virus-vector activity) and plant size (often found to affect parasite occurrence) were also measured and used as covariates in the statistical analysis.

The effect of coinfection with PlLV on *P. subordinaria* life-history traits (H2, II) was tested in laboratory by inoculating plants of a single genotype with PlLV and then with *P. subordinaria* one week later. To compare life-history traits between *P. subordinaria* alone and with PlLV, the plants were either inoculated with PlLV or mock-inoculated with the phosphate buffer. One week later, when the virus infection had established, the plants were inoculated with either *P. subordinaria* strain P43 or strain P29. Hence, there were four treatments: PlLV and P29, PlLV and P43, mock-inoculation with P29, and mock-inoculation with P43. The length of *P. subordinaria* lesions in the inoculated flower stalk were measured every week, and the density of spore-structures was determined in the final, fourth week. To asses infection load, the area under disease progress stairs (AUDPS, Simko and Piepho 2012) was calculated from the symptom data.

To test how within-host parasite community affects host growth and reproduction (H5, IV), I inoculated experimental host plants with four parasite treatments: *P. subordinaria*, *P. plantaginis*, PlLV, and bulk virus inoculum so that the experiment included 15 combinations of these four treatments, and measured the growth, flowering, and seed production of the host plants throughout summer 2018. The parasite treatments included all possible four-way, three-way, paired, and singe inoculations of *P. subordinaria*, *P. plantaginis*, PlLV, and virus bulk inoculum (please see "Plant and parasite material"). A control treatment without any parasites was also included. The treatments were grouped into four groups based on the type of community they represent: 1. single infections of *P. subordinaria*, *P. plantaginis*, and PlLV, 2. mixed fungus infection, 3. mixed virus infection, and 4. a mixed infection of fungi and viruses. The experimental plants were cloned replicates of three *P. lanceolata* genotypes and they grew in groups of five plants inside insect-proof net cages to obstruct transmission

of insect-transmitted parasites (Fig. 3). To measure plant growth rate, I counted the number of leaves, and measured the length and width of the longest leaf. These measurements were used to calculate plant leaf area, and growth rate was calculated between initial size in the beginning of the experiment and the data collection point in June or September. To measure reproduction, I counted the total number of flowers, and counted the number of seeds in up to six seed heads. To test whether the parasite community alters allocation of resources, I calculated the rate of flowers to growth rate, and seeds to growth rate.

I used the same plants growing in the common-garden after the 2018 experiment, as infection source plants to test whether *P. subordinaria* transmission is affected by the within-host parasite community in and experiment in 2019 (H3, III). All source plants were infected with *P. subordinaria* either through inoculation in the previous experiment or through natural transmission from the inoculated plants during fall and winter. The source plants represented eight parasite treatments that all included the focal parasite *P. subordinaria*: a three-way inoculation with *P. plantaginis*, PlLV, and a bulk virus inoculum, and pairwise inoculations. The plants had received the same parasite treatment in the previous year, and the inoculations were repeated in 2019 expect for *P. subordinaria* that was already present in the plants. Greenhouse grown cloned "recipient" plants of two *P. lanceolata* genotypes were placed inside the cages to measure transmission success. To measure transmission within-hosts, I counted the infected flowers in the source plants in the beginning and the end of the experiment. To measure transmission between hosts, I counted the number of infected flowers in the recipient plans in the end of the experiment. Because plant size may affect parasite establishment and the growth of *P. subordinaria*, and the number of flowers in the recipient was considered an important susceptibility factor (since the infections begin in the flower stalks), I measured the plant size of source plants and the number of flowers in the recipient plants and included them as covariates in the analysis.

3.4 SURVEY OF WILD *PHOMOPSIS SUBORDINARIA* POPULATIONS

To link experimental results with natural epidemics, we collected survey data from 261 *P. lanceolata* populations in 2018 (H3, II, III). We recorded *Phomopsis subordinaria* presence/absence and population size, measured on a categorical scale of 1) 1-10 infected plants, 2) 10-50 infected plants, 3) 50-100 infected plants, 4) 100-1000, infected plants, 5) >1000 infected plants, were recorded. We analyzed the overall *P. subordinaria* occurrence patterns in relation to population size and connectivity (II). To test the hypothesis that historical occurrence of *P. plantaginis* is related to current epidemics of *P. subordinaria* (III, H4), I used historical data on *P. plantaginis*, collected during annual surveys of the system (Laine and Hanski 2006, Ojanen et al. 2013, Jousimo et al. 2014). To include a robust estimate of how persistent *P. plantaginis* has been in the survey populations, I took the average of *P. plantaginis* population size, collected on the same scale as *P. subordinaria* population size data, over four years predating the *P. subordinaria* survey (2014-2017).

Figure 3 Common-garden set-up at the Lammi Biological station testing hypotheses H5, that host growth and short-term fitness is affected by the within-host parasite community (chapter IV). Plants of three genotypes were planted inside insect-proof net cages and inoculated with varying parasite communities to test how within-host parasite community affects host growth and reproduction.

3.5 VIRUS DETECTION METHODS

Within-host virus communities were determined and infections were tested using specific PCR primers (I, III, IV) published earlier (Susi et al. 2017a, 2019), as well as newly designed primers (I) (Sallinen et al. 2020), designed using small-RNA sequencing of natural *P. lanceolata* from the Åland Islands. Of the five focal viruses, three have an RNA-genome (Closterovirus, Betapartitivirus, Enamovirus) and two have a DNA-genome (PlLV, Caulimovirus). Hence, to detect RNA-viruses, RNA was first extracted and transcribed into cDNA with reverse-transcriptase enzyme. For DNA-viruses, DNA was first extracted. The cDNA and DNA were then used in the PCR-amplification of the virus specific sequence, and amplicons were resolved and interpreted using gel electrophoresis.

3.6 STATISTICAL ANALYSIS

Data were analyzed using a linear mixed effects model (LMM) (IV), generalized linear mixed effects models (GLMM) (II, III), a cumulative mixed link model (CLMM) (III), a path-model (III), and Bayesian joint species distribution modeling (I). Analyses were performed in R software and SAS software. Generalized linear models were fit using a log-link function and the Bernoulli error structure was selected for infected/uninfected response data (III), negative-binomial error structure was selected for over-dispersed count data (IV), and generalized Poisson error structure for under dispersed count data (IV). For zero-inflated negative-binomial model (IV) the model included zero-inflation of the response data. A path-model was used to test the causal relationship between within-host *P. subordinaria* disease and between-host transmission (III). We used joint-species distribution modeling to tease apart different drivers of virus community assembly (I) and calculated frequency-based co-occurrences-determine the co-occurrence patterns of the viruses (I).

4 MAIN RESULTS AND DISCUSSION

In this dissertation, I demonstrate that differences in within-host parasite communities have potential consequences for evolution of parasites and their hosts and may change epidemiological dynamics in the wild. I find that coinfection may affect growth and reproduction of both the coinfecting parasites and their hosts, and that the altered disease severity under coinfection can further lead to altered transmission (II, III) and affect epidemics in host populations (III). My results also highlight the importance of genotypic variation among hosts and show that host genotype is an important determinant of within-host parasite community composition (I). Finally, my results show that both host growth and reproduction are affected by the within-host parasite community and host genotype (IV).

4.1 PARASITE GROWTH AND REPRODUCTION ARE ALTERED IN COINFECTION

Coinfection is expected to alter growth and replication of parasites due to competitive interactions (Pedersen and Fenton 2007, Barrett et al. 2009, Alizon and Lion 2011, Alizon et al. 2013). My results further show that both coinfection and the identity of the parasites in a within-host community are important in determining parasite growth and replication (II, III). Firstly, prior inoculation with PlLV slowed down the spread of the disease symptoms and reduced the infection load of *P. subordinaria* but increased the density of spore structures (II) (Fig. 4). This result was similar for both tested *P. subordinaria* strains that differed in growth and reproduction (II). Similarly, a restricted number of empirical tests of plant virus-fungus interactions have found that virus infection can reduce the reproduction and disease symptoms of fungi (Zaiter et al. 1990, Marte et al. 1993, Bassanezi et al. 1998). Also, my results show that virus infection can have an effect on fungus spore structure production and demonstrate that viruses are potentially important members in the plant microbiome. The results support hypothesis H2.

Secondly, coinfection with *P. plantaginis* caused more severe disease, measured as stalk mortality, within hosts and increased transmission of *P. subordinaria* in the semi-natural common-garden experiment (III), also supporting hypotheses H2 and H3. Transmission is a central fitness component for parasites and altered transmission may have far-reaching consequences for epidemics, parasite evolution, and parasite community dynamics (De Roode et al. 2005, Mideo 2009, Susi

et al. 2015a, Strauss et al. 2019). While much of the coinfection literature has concentrated on the effects of parasite-parasite competition on transmission, these studies are mostly theoretical or focus on pairs of parasites or strains within a species (Mideo et al. 2008, Susi et al. 2015b, Strauss et al. 2019). My semi-natural common-garden experiment is a rare empirical effort to follow natural transmission from varied parasite communities to measure realized transmission.

While results of the laboratory experiment (II) suggested that virus infection may affect transmission of *P. subordinaria* through altered growth and spore production, I did not observe an increase nor decrease in transmission of *P. subordinaria* when the fungus shared the host with viruses (III). This could be because the virus simultaneously slowed down the spread of the fungus, i.e., the length of the symptom in the flower stalk, but increased sporulation, and hence, the output was not affected. It is also possible that the semi-natural abiotic conditions change the outcomes of parasite-parasites interactions compared to the controlled, constant conditions in the laboratory experiment (Lacroix et al. 2014, Hily et al. 2016). Jointly, these results demonstrate that the within-host parasite community can affect the growth and fitness of parasites, and the laboratory and common-garden experiments together highlight the importance of measuring transmission under natural conditions for accurate estimation of the impact of coinfection.

The laboratory inoculation experiment (II) and the transmission experiment (III) aimed to test how introduction of other parasites affects *P. subordinaria*. Hence, I did not measure the replication of the other parasites within hosts or their transmission among hosts. Parasites may interact through multiple mechanisms including host immunity (Pedersen and Fenton 2007, Mideo 2009, Caracuel et al. 2012, Tollenaere et al. 2016). The infections found in the subset of virus inoculated plants tested in the transmission experiment (III) suggest that the inoculum contained living virus particles. Inoculation of living virus particles should in minimum challenge host immunity which in some cases may have restricted the level of virus replication to undetectable levels. Hence, these experiments may have most power for testing host immunity mediated effects. Additionally, comparing the results of the experiments may be limited by the inoculation method which does not control for virus dosage in the inoculum. While I minimized variation in dosage within experiments by pooling the infected plant sap into a single pool in each experiment, doses may have varied among experiments. Testing how virus replication is affected by coinfection and how

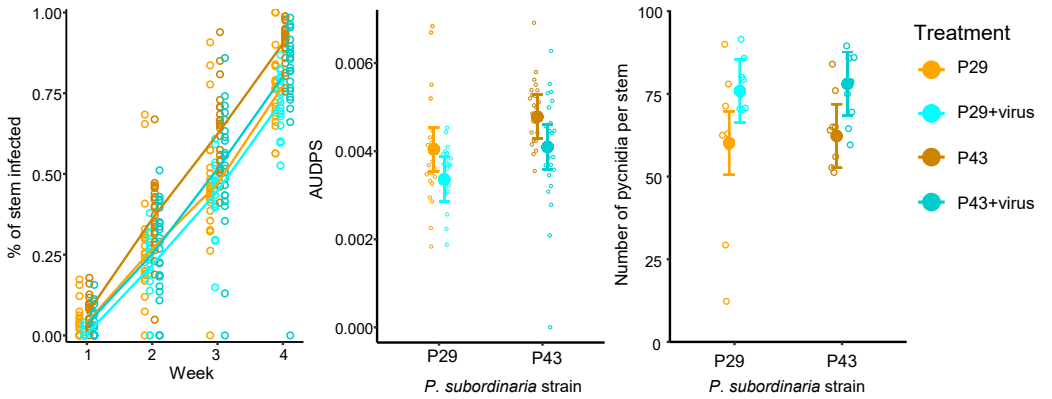

Figure 4 The impact of Plantago lanceolata latent virus (PlLV) infection on two strains of *Phomopsis subordinaria* performance on *Plantago lanceolata* in a laboratory experiment testing hypothesis H2 (chapter II). A) Proportion of each inoculated stalk (n = 90) with necrotic symptoms (empty circle) with and without PlLV coinfection over the four weeks of data recording. Means of each time point for each treatment are visualized 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.

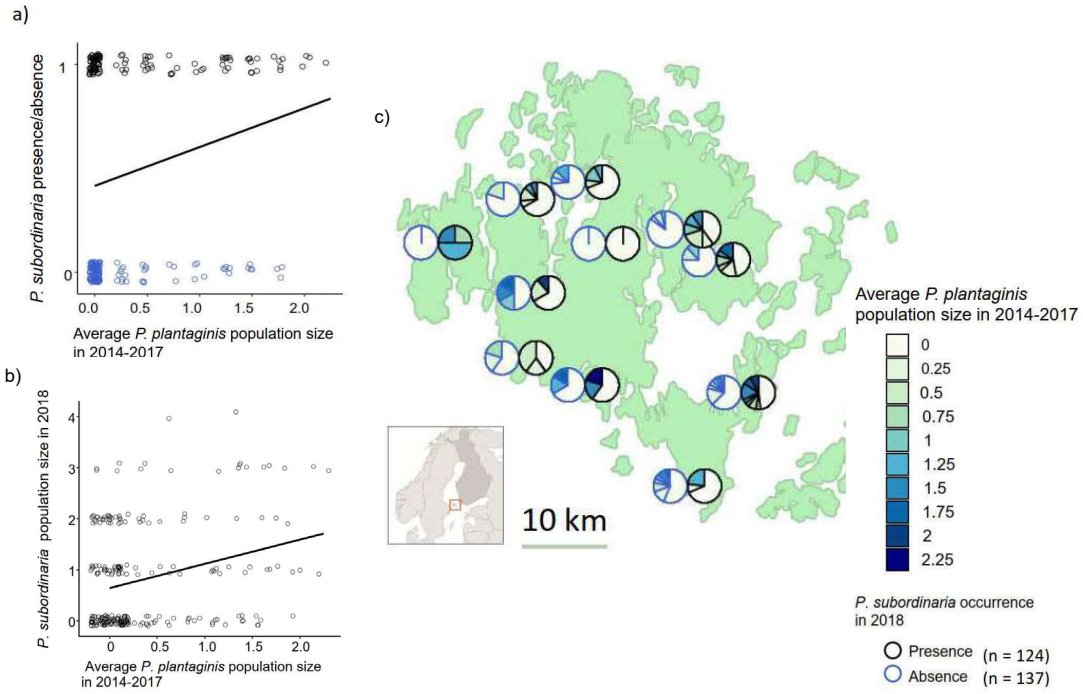

Figure 5 A positive relationship between *Phomopsis subordinaria* occurrence in 2018 and historical *Podosphaera plantaginis* infections in 261 *Plantago lanceolata* populations in the Åland Islands testing hypothesis H4 (chapter III). A) Relationship between *P. subordinaria* presence (n=124) and absence (n=137) and average historical *P. plantaginis* population size (calculated over years 2014-2017) in the surveyed *P. lanceolata* populations. Population size was measured on a categorical scale of five categories: 1) 1-10 infected plants, 2) 10-50 infected plants, 3) 50-100 infected plants, 4) 100-1000 infected plants, 5) >1000 infected plants. The line denotes a smoothed average. B) Relationship between *P. subordinaria* population size in 2018 and the average historical *P. plantaginis* population size, both measured on the categorical scale described above. The line denotes a smoothed average. C) Frequency of historical *P. plantaginis* population size in the surveyed *Plantago lanceolata* populations grouped to 11 spatial clusters of 10-49 populations each.

virus quantity affects parasite community dynamics are interesting avenue for further investigations.

4.2 NATURAL *PHOMOPSIS SUBORDINARIA* EPIDEMICS ARE AFFECTED BY A POSITIVE ASSOCIATION WITH *P. PLANTAGINIS*

My transmission experiment showed that coinfection with *P. plantaginis* increases both the disease severity, measured as stalk mortality, as well as transmission of *P. subordinaria* to recipient hosts (III) in support of hypothesis H3. I used epidemiological data collected from wild populations to test whether the positive association observed in the experiment also affects epidemics in the wild. More specifically, I tested whether the presence/absence and the population size of *P. subordinaria* in the survey 2018 is related to the historical *P. plantaginis* prevalence in the populations. The results show that *P. subordinaria* is more often present and that the *P. subordinaria* populations are larger in host populations with strong history of *P. plantaginis* (III) (Fig. 5), supporting hypothesis H4. The experimental and field-based results together suggest a positive association between *P. subordinaria* and *P. plantaginis*. The effects of this relationship may have accumulated over-time in wild populations as shown by the positive association between the two fungi (III). Co-existence can be promoted through many mechanisms (Clay et al. 2019), and the increased transmission under coinfection is one likely explanation for the pattern we observed in the wild populations.

4.3 ASSEMBLY OF VIRUS COMMUNITIES DEPENDS ON THE HOST GENOTYPE AND LOCAL POPULATION CONTEXT

Host genotype is expected to act as an important filter for the within-host parasite community through differences in resistance and vector preference, as well as be a potential mediator of parasite-parasite interactions (Borer et al. 2016, Hily et al. 2016). My field experiment using cloned replicates of *P. lanceolata* showed that different genotypes accumulate different combinations of viruses when placed into natural populations during the epidemic season (I). The results of the experiment confirm the previously untested expectation that genotype is important for parasite community assembly, supporting hypothesis H1. Furthermore, observed co-occurrence of the viruses, whether positively or negatively associated, depended on the genotype (I) (Fig. 6). Variation in the established virus communities among sites showed that the local population context is also important, and the relevance of different explanatory factors varied among the five viruses. These results suggest that virus occurrence and co-occurrence patterns are non-random and demonstrate that host population genetic variation is a major driver of parasite community structure.

4.4 HOST GROWTH AND REPRODUCTION IS DIFFERENTIALLY AFFECTED BY PARASITE COMMUNITIES

The host genotype does not only determine the composition of parasite communities, but host genotypes may vary in how they limit the effects of parasites on their own growth and reproduction (IV). My experiment testing how varying parasite communities affect host growth and short-term fitness shows that the within-host parasite community and genotype jointly modify plant growth and seed production (IV) (Fig, 7). Growth rate was affected by an interaction between parasite community and plant genotype, and the parasite communities caused both increased and decreased growth rate. Compared to

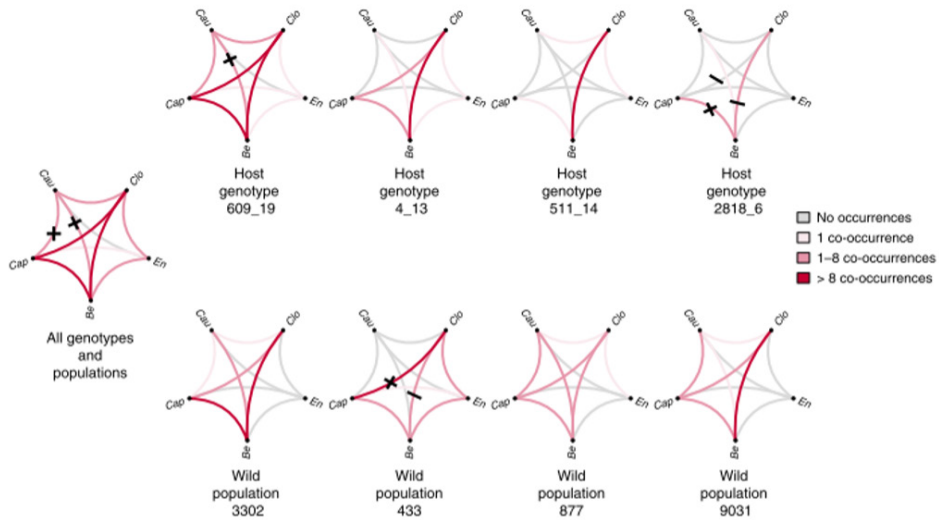

Figure 6 Co-occurrences between virus species observed in the field experiment testing hypothesis H1, that virus community assembly is determined by the host genotype (I). Co-occurrences are shown either in the whole data set (left, with total number of sentinel plants 320), or per plant genotype (upper panels, 80 plants per genotype), or by population (lower panels, 80 plants per population) as denoted by the horizontal axis. The genotypes and populations are ordered from left to right according to decreasing the overall frequency of infections. The plus (and minus) signs denote the pairs, for which the observed values were higher (or lower, respectively) than what would be expected based on their overall frequencies, and for which the probability of this difference was <0.1. The line colors denote the true numbers of co-occurrences between the species, as shown in the legend. 'Clo' refers to Plantago closterovirus, 'Be' to Plantago betapartitivirus, 'Cap' to Plantago lanceolate latent virus, 'Cau' to Plantago latent caulimovirus, and 'En' refers to Plantago enamovirus.

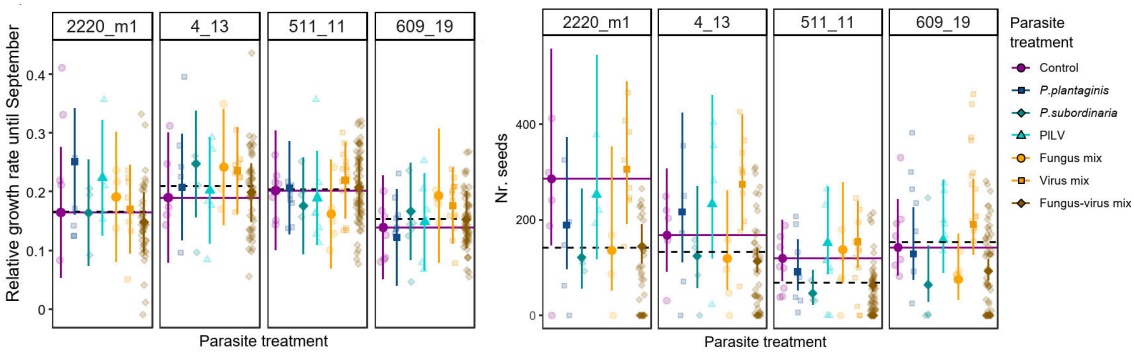

Figure 7 Relative growth rate from June to September varies according to parasite community and plant genotype (ID:s 2220_m1, 4_13, 511_11, 609_19) (chapter IV). Number of seeds at the end of the experiment varies by plant genotype but not by treatment. Each small circle is a single host plant. Large circle denote the predicted value of the model, and error bars are 95% confidence intervals.

single parasites, adding other parasites also caused both increase and decrease of growth rate, and the direction was not universal across genotypes. Surprisingly, the effect of parasites on the growth rate over the full course of the experiment was more often positive than negative (IV). There was not, however, evidence of a reallocation from reproduction towards growth (IV) as shown by the non-significant main and interaction effects of treatment on the calculated ratios (flowers to growth rate, seeds to growth rate). However, the direction of the effects shifted as the experiment continued (IV). Parasites often reduce the growth rate of their hosts (Maskell et al. 1999, Wintermantel 2005, Penczykowski et al. 2014) and as they live off of the hosts resources, they are expected to have a negative effect (Chernin 2000, Rohde 2005). During this experiment, the plants were watered, grew in fertile and ample soil, and were isolated from herbivores. Hence, it is possible that the abundant resources helped the plants buffer the possible negative effects on growth, masking any potential negative effects, or that the effects appear after a longer time (Maskell et al. 1999, Susi et al. 2015b, 2017b, Alexander et al. 2017). The results in this dissertation indicate that host is important and immune system is a likely driver of these findings, although measuring the mechanisms would be necessary to confirm this.

I also found an independent effect of parasite community on the number of seeds. Especially noticeable was that *P. subordinaria* reduced the number of seeds. Alone, *P. subordinaria* reduced seed production by 53 % compared to uninfected controls. This results is unsurprising given the nature of *P. subordinaria* infection: the infection damages and kills flower stalks and usually castrates the seeds (de Nooij and van der Aa 1987). Adding another fungus or viruses increased the seed production compared to *P. subordinaria* alone but was still lower than in treatments that did not have *P. subordinaria*. Virus treatment without fungal infection increased seed production. Limited evidence from multiple systems shows that a parasite may increase reproduction of the host, at least in short-term (Pagán et al. 2008, Susi et al. 2017b). I only measured flowering and seed production over one summer, but fitness of perennial plants, such as *P. lanceolata*, accumulate overtime and include survival (Alexander et al. 2017), and hence it would be interesting to follow the plants through their whole life cycle. The result of this experiment indicate that parasite communities have varying effects on host traits, and can change the outcome of the infection to unpredictable direction (Holt and Dobson 2013).

4.5 PLANT VIRUSES

Despite their prevalence, remarkably little is known about the role of viruses in wild plant systems (Cooper and Jones 2006, Roossinck 2011b, 2015, Alexander et al. 2014), and hence, one of the objectives in this dissertation was to investigate this. My results show that viruses indeed play a role for plant and parasite dynamics through their effect on growth, replication,

and transmission of other parasites (II) and effects on host growth and short-term fitness (IV). Moreover, these effects are not necessarily pathogenic for the host. I found neutral and positive effects on host growth and seed production (IV), and PlLV slowed down the spread of *P. subordinaria* within the host (II). However, concluding that the viruses are mutualistic or cause no harm for the hosts based on lack of negative effects in this experiment is not possible. In my experiments, I have protected the hosts from some abiotic and biotic stressors. Notably, viruses affect insect behavior by changing the attractiveness of plants (Mauck et al. 2010, Bosque-Pérez and Eigenbrode 2011). In my experiments, I have kept herbivores at minimum (II, III, IV) and hence it is possible that the plants have escaped indirect negative consequences of virus infection. Accumulating experimental evidence shows that viruses may move along an antagonist-mutualist continuum and the effects may change under abiotic stress (Hily et al. 2016). Viruses have been shown to improve drought tolerance, as well as tolerance of other abiotic stressors (Xu et al. 2008, Aguilar et al. 2017, Carr 2017, Bergès et al. 2020, González et al. 2020). However, these benefits might come at the cost of, for example, reduced growth rate (Aguilar et al. 2017). Measuring plant performance under varying abiotic and biotic stressors and following the effects over the entire life-cycle of the host would be key steps towards a more comprehensive view on the roles that viruses have in wild plant populations.

5 CONCLUSION AND FUTURE PROSPECTS

Multiparasitism is common in the wild and understanding the drivers of parasite community assembly and the consequences on parasites and hosts is a major question in parasite ecology. In this dissertation, I have investigated the causes and consequences of parasite communities on the level of individual hosts by testing the drivers of virus community assembly in the wild (I), by measuring the effect of coinfection on growth and replication (II) and transmission (III) of parasite *Phomopsis subordinaria*, and by measuring the consequences of varying parasite communities on host growth and reproduction (IV). I have demonstrated that the host genotype is an important driver of the within-host parasite community composition, disease severity and transmission dynamics (I, II, III, IV). Parasites have varying effects on disease severity (III), and growth and reproduction of their hosts (IV). I conclude that these results jointly demonstrate that parasite communities have important, but complex consequences for the evolution of hosts and parasites as well as epidemics, that are not necessarily easy to predict.

Throughout the experiments, the virus inoculum was drawn from virus infected plant tissue and the quantity of virus particles remains unknown. While I minimized variation in dosage within experiments by using a single pool of inoculum, it is unclear how much the dosage varied among experiments and how well it corresponded with natural transmission. This may limit the comparability and reproducibility of the results. I suggest that testing how altering the virus dosage affects the consequences for the hosts and the coinfecting parasites is an interesting avenue for further investigations.

In this dissertation, I have limited the studied cross-kingdom interactions to exclude insect herbivores and mutualist symbionts to disentangle some other aspects of within-host community dynamics. However, plant immune responses against herbivores resemble those against parasites and they are themselves important drivers of virus occurrence patterns (Blanc and Michalakis 2016, Kendig et al. 2017). I suggest that expanding the investigation of cross-kingdom within-host interactions to insects and mutualists would help build a comprehensive understanding of cross-kingdom interactions within plant hosts in the future (Marchetto and Power 2018, Miller et al. 2018, O'Keeffe et al. 2021). Likewise, testing whether the results obtained in these experiments are affected by changing abiotic conditions would improve our understanding of the parasite dynamics in the wild, where hosts are under constant stress.

While these results provide valuable insights into the causes and consequences of multiparasitism, much remains to be explored in this system. My results suggest that within-host parasite interactions affect population level parasite dynamics in the wild, and, as the plant genotype proved to be an important factor, linking host population genetic structure to parasite occurrence patterns in natural populations is an interesting future research avenue.

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7 LITERATURE

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