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Multi-infections, competitive interactions, and pathogen coexistence

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Abstract

Multiple infections, either simultaneous or sequential, affecting a single plant or crop are now recognised to be common in plant disease epidemics. These multiple infections thus generate a range of competitive interactions (exploitation competition, apparent competition, or interference competition) which directly impact competitors life history traits, and hence fitness. While a theoretical framework, based primarily on three ecological theories - niche exclusion, the 'tragedy of the commons' and the virulence-transmission trade off, provides insights into potential outcomes of multiple infections on pathogen evolution and virulence and on disease dynamics, experimental observations, although rather rare so far, strongly suggest that unexpected outputs can also occur. This review therefore attempts to provide a comprehensive overview of both theoretical and empirical knowledge about multiple infection in plants. It highlights the need for a detailed assessment of pathogen life history traits and their modulation according to competition types, host receptivity, and pathogen life strategies for a better understanding and prediction of the evolutionary and demographic outcomes of multiple infections, as well as research questions still open in this emerging field of plant pathology.

Keywords: co-occurrence, disease complex, life history traits allocation, competition, sequential events, trade-off

Introduction

Diseases in both plants and animals have long time been considered to be the result of the interaction between one host and a single pathogen. However, with the development of molecular tools, a high prevalence of multi-infections has been demonstrated (Read & Taylor, 2001; Balmer & Tanner,

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2011; Tollenaere *et al.*, 2016). Indeed, a host individual is usually subjected, throughout its life, to simultaneous and/or successive pressure from several pathogenic species and/or genotypes. Such multiple genotypes of a parasite are present in many natural systems, and their simultaneous occurrence has major consequences on the evolution of disease severity. One of these is that different pathogens must coexist, *i.e.* share - at least temporarily - the same host and thus the resources necessary for their development, reproduction, transmission and survival. Under multi-infection, the parasite genotypes therefore compete for the limited resources provided by the host (Read & Taylor, 2001; Mideo, 2009; Balmer & Tanner, 2011; Susi *et al.*, 2015a; Tollenaere *et al.*, 2016; Abdullah *et al.*, 2017).

Under multi-infections, pathogens may interact either directly (mechanical or chemical interactions) or indirectly through host resources or defence. These direct or indirect interactions under multi-infections can change aggressivity, within-host pathogen accumulation, and transmission. The occurrence of multi-infections within hosts are therefore important to our understanding of diseases (Vaumourin *et al.*, 2015). Their interactions may be synergistic (*i.e.* the presence of one parasite may facilitate subsequent infections by other parasites), or antagonistic (*i.e.* the presence of one parasite may inhibit subsequent infections by other parasites).

To understand the evolution and epidemiology of pathogens, dynamics of pathogens within and between hosts must be linked (Mideo *et al.*, 2009; Susi *et al.*, 2015a). This is often done through theoretical evo-devo models, usually developed from the 'tragedy of the commons' theory, which proposes that multi-infections should favour the most virulent genotypes due to competition for limited resources (Nowak & May, 1994; Levin & Bull, 1994; Antia *et al.*, 1994). However, competitive interactions do not necessarily converge towards this result. Moreover, the coupling of intra- and inter-host dynamics approaches is only approached from a theoretical point of view (*i.e.* nested models) (Mideo *et al.*, 2009). The lack of knowledge of the life cycle of pathogens and the difficulties encountered in differentiating pathogens co-infecting the same host have long delayed the implementation of empirical studies. The development of powerful molecular tools has since filled these gaps, and confirmed that multi-infections are common in nature and may alter intra-host dynamics (López-Villavicencio *et al.*, 2007; López-Villavicencio *et al.*, 2011; Tollenaere *et al.*, 2012; Susi *et al.*, 2015a). For example, López-Villavicencio *et al.* (2011) showed that multi-infection of the plant *Silene latifolia* by many different genotypes of the fungus *Microbotryum violaceum* are extremely frequent in natural populations within single host plants. The fungal genotypes found in the different stems of single plants were, however, more related than expected by chance, suggesting that *M. violaceum* actively excludes dissimilar genotypes while tolerating closely related competitors. Thus, changes in intra-host dynamics can have profound consequences on inter-host

dynamics (Wintermantel *et al.*, 2008; Susi *et al.*, 2015a; Tollenaere *et al.*, 2016). Various empirical studies suggest a link between pathogen transmission and intra-host competition, leading to a potential change in disease transmission under multi-infection (de Roode *et al.*, 2005; Susi *et al.*, 2015a). The work of Susi *et al.* (2015a), for example, has shown an increase in propagule transmission and more devastating epidemics in plants co-inoculated with strains of *Podosphaera plantaginis* compared to single infections. However, only few traits were measured in this study and according to the trade-off theory (Stearns, 1989) the increased highlighted for transmission must be balanced by a decrease in other life history traits. The knowledge of these life history traits changes would allow a better comprehension of the disease dynamics observed. This example illustrates the importance to finely determine the life history changes and trait trade-offs that are at the origin of the epidemiological differences. Indeed, the evaluation of the life history traits of pathogens has been little considered in the study of the epidemiology of multi-infections.

The effects of multi-infection on epidemiology have mainly been studied in the case of human and animal diseases, based on models and empirical studies. However, this type of work is relatively uncommon for plant diseases (Tollenaere *et al.*, 2016). In this review, after showing how plant pathogens can coexist, we will highlight how *i*) multi-infection interactions regulate this coexistence and what are the consequences on survival and development, *ii*) the life history traits of pathogens evolve to allow this coexistence, *iii*) the sequentiality of host plant colonization may condition the interactions between pathogens colonizing the same host plant, and *iv*) the level and evolution of the physiological receptivity of the plant during the growing season may affect multi-infection interactions.

Niche differentiation and plant pathogens coexistence

The concept of ecological niche offers an interesting frame to analyse pathogen coexistence. This term is often mis-used to describe only the type of place where each individual lives, which in fact corresponds to the 'habitat' of the individual considered (Begon *et al.*, 2006). However, the ecological niche does not define a place: it covers both the set of resources required by an organism and what it tolerates in order to persist, grow and reproduce (Alley, 1982; Putman, 1994; Tilman, 2004; Begon *et al.*, 2006; Hirzel & Le Lay, 2008). The ecological niche is a well-documented concept, and many definitions have been proposed, including that of Hutchinson (1957) which considers the ecological niche as a hypervolume with n dimensions. Each dimension represents a resource or environmental condition that influences the survival or reproduction of individuals. For example, temperature limits the growth and

reproduction of all organisms; however, different individuals tolerate different temperature ranges. This 'range' then corresponds to a dimension of their niche (Leibold, 1995; Begon *et al.*, 2006).

The ecological niche of pathogens is highly dependent on the characteristics of their host plant. While a conducive abiotic environment is also necessary for the infectious success of pathogens, a major determinant of their niche is their host genotype (Al-Naimi *et al.*, 2005). The niche of a pathogen with a complex life cycle, including host alternation or saprophytic stages, is adjusting, changing and it is affected by biotic (including the presence of co-infected pathogens) and abiotic environment, within the limits of its genotype and phenotypic plasticity.

This can be illustrated by recent work on fungal diseases of annuals or trees. One of these is the discovery that four genetic lineages, probably cryptic species, cause oak powdery mildew in Europe. Testing the hypothesis of niche differentiation between the two most frequent ones (*Erysiphe althoides* and *Erysiphe quercicola*), by determining their relative prevalence at various times during the growing season, showed that *E. quercicola* was strictly associated with early, flag-shoot symptoms, while *E. althoides* predominated in most (70%) autumn samplings of the same natural oak stands. Time-course monitoring of powdery mildew in two natural regenerating oak populations confirmed the opposite patterns of relative abundance of these two species in early and late season (Feau *et al.*, 2012). Since oak powdery mildew fungi are widely thought to survive from one growing season to the next by generating chasmothecia, the sexual fruiting bodies (Marcais *et al.*, 2009), or by mycelia infecting shoot primordia under bud scales (Neger, 1915; Yarwood, 1957; Kerling, 1966; Junior *et al.*, 2014), the coexistence of these two closely related species may be due to the use of different strategies, resulting from a trade-off between overwintering (mycelium in buds vs. chasmothecia) and late-season performance. Indeed, several studies have already shown that different genetic groups of *Erysiphe necator* (biotypes A and B), the causal agent of grapevine powdery mildew, are associated with different ecological features (Delye *et al.*, 1997; Delye & Corio-Costet, 1998; Miazzi *et al.*, 2003) resulting in the temporal niche partitioning of these groups (Cortesi *et al.*, 2005; Montarry *et al.*, 2008). Biotype A strains overwinter in buds more frequently than biotype B strains, and are active only at the start of the growing season, whereas biotype B strains are found on leaves or berries in late summer (Montarry *et al.*, 2008; Montarry *et al.*, 2010).

Another example is the black spot disease complex of pea, previously known to be caused by three fungal pathogens (Le May *et al.*, 2009). However, four additional pathogens have been recently found in association with this disease. These new pathogens include *Phoma koolunga* (Davidson *et al.*, 2009), *Phoma herbarum* (Li *et al.*, 2011), *Boerema exigua* var. *exigua* (Li *et al.*, 2012), and *Phoma*

glomerata (Tran *et al.*, 2014). All these fungi are necrotrophic, generalist and polyphagous species, and these characteristics favour colonization of new environments. It is possible that these pathogens use synergism as a strategy to infect a large variety of plants, which might also explain why some pathogens occur more easily than others in a given environment or plant host (Lamichhane & Venturi, 2015).

Coexistence and multi-infection interactions

Pathogen coexistence involves commensalism, *i.e.* the mutual exploitation of at least one common limited resource, necessary for development and reproduction, by at least two individuals (different strains of the same species or several different species). It thus results in the establishment of interactions between these individuals (Pianka, 1981; Alley, 1982; Amarasekare, 2003; Mideo, 2009). The exploitation of the common resource by an individual, leads to a decrease in the availability of this resource for other. The presence of each organism consequently modifies the selective value and/or population size at equilibrium of the other (Pianka, 1981). Concepts of coexistence and competition are thus closely linked, and can be interpreted through the filter of niche theory, the competitive interactions between individuals being dependent on the degree of niche overlap (MacArthur, 1972; Pianka, 1981; Putman, 1986; Begon *et al.*, 2006).

Several studies have shown that resources allocation due to the coexistence of different pathogens can modify host exploitation strategies, change epidemic dynamics and also influence the evolution of the life history traits of individuals (Fitt *et al.*, 2006; Hellard *et al.*, 2015; Susi *et al.*, 2015b; Tollenaere *et al.*, 2016; Dutt *et al.*, 2021). These changes will depend on the type of interactions developed between the pathogens.

*** Types of multi-infection interactions between plant pathogens**

Three main types of multi-infection interactions are described in the literature: *(i)* resource-mediated (exploitation), *(ii)* host-mediated (apparent), and *(iii)* interference (Read & Taylor, 2001; Begon *et al.*, 2006; Mideo, 2009; Tollenaere *et al.*, 2016).

Resource-mediated interactions (exploitation). Resource-mediated interaction is a process in which a pathogen is affected by the amount and/or the quality of resource remaining as a result of the exploitation of the same resource by other pathogens. This type of competition can occur in the absence of contact between co-infected pathogens (Read & Taylor, 2001; Mideo, 2009; Balmer & Tanner, 2011; Tollenaere *et al.*, 2016; Sarrocco *et al.*, 2019a). Thus, the severity and the result of multi-infection will be

determined by the use of these time-limited resources (Chesson, 2000; Abdullah *et al.*, 2017). This form of indirect interaction is often associated with intraspecific competition, due to the important niche overlap between pathogens belonging to the same species. This type of interaction often leads to the fixing of population size (Balmer & Tanner, 2011), known as the environment carrying capacity. Although it is generally accepted that resource-mediated interaction can play an important role within plant pathogen communities, few empirical studies have been able to clearly demonstrate this type of interaction (Sarrocco *et al.*, 2019a, 2019b). Indeed, it seems that resource-mediated interaction is often associated to other mechanisms (Mercier & Reeleder, 1987; Le May *et al.*, 2009; Al-Askar & Rashad, 2010; Chatterjee *et al.*, 2016). For instance, Mercier & Reeleder (1987) showed that three fungi from the lettuce phyllosphere (*Trichoderma viride*, *Alternaria alternata* and *Epicoccum purpurascens*) significantly reduce the development of *S. sclerotiorum* on whole plants and significantly reduce the germ tube elongation from its ascospores on lettuce leaf discs. It seems that these fungi previously colonize senescent leaf tissue, limiting future infection by the pathogen. While the mechanisms related to this antagonism have not been studied, resource-mediated interaction seems to be at least partly involved. This interaction is thus dependent on the capacity of each individual to exploit the host resource (**Figure 1**).

Different adaptation strategies to overcome this type of interaction have been developed. One of these is for the pathogen to change the allocation of the host resource between different life history traits, when this resource is shared with competitors. Dutt *et al* (2021) observed that some strains belonging to the pea ascochyta blight complex respond to resource-mediated interaction by increasing their virulence (compared to the virulence observed for these same strains in single inoculations), allowing them to acquire the limited resource more rapidly than the competitor. However, this increase in virulence is associated with a decrease in the production of progeny (pycnidiospores). However, in some cases, the exploitation of the common host resource by a pathogen may modify the quality of this resource and thus favour the subsequent development of other pathogens (Zélé *et al.*, 2018). However, resource-mediated facilitation has rarely been observed for plant pathogens (Al-Naimi *et al.*, 2005). In that respect, Al-Naimi *et al.* (2005) showed that the inoculation of the pathogen *Pyrenophora tritici-repentis* on wheat plants before the pathogen *Puccinia triticina* promotes the production of conidia of the latter on the common host (compared to single inoculation). These authors hypothesize that the leaf exploitation by *P. triticina* alter host tissue and favour *P. tritici-repentis* development, which one developed better on senescent tissue (Al-Naimi *et al.*, 2005).

Host-mediated interaction (apparent). Host-mediated interaction is an indirect form of competition involving the stimulation of the host defences. The immune responses elicited by a pathogen alter the intra-host environment, and promote the host ability to modulate future infections by other pathogens by either suppressing or facilitating their development (Cox, 2001; Read & Taylor, 2001; Mideo, 2009; Tollenaere *et al.*, 2016). The mechanisms behind these interactions have sometimes been highlighted in empirical studies. Some infections can increase the plant defence capacity and activate responses against future pathogens. This induced resistance has been observed for multi-infection by species of the same genus, but also by distant genera affected by the same defence pathways. For instance, the inoculation of non-pathogenic *Fusarium oxysporum* into the root system of tomato plants leads to overexpression of plant defence genes - in particular the production of PR proteins, some of which could be involved in the salicylic acid defence pathway- reducing in turn the development of pathogenic strains of *F. oxysporum* (Aimé *et al.*, 2013). Similarly, Buxdorf *et al.* (2013) showed that *Pseudomonas aphidis* limits the development of the pathogenic fungus *Botrytis cinerea* on *Arabidopsis thaliana*, though the activation of the PR gene of the jasmonic and salicylic acid defence pathways, leading to a systemic reduction of *B. cinerea* infection. The mechanisms involved in the suppression of competitor development are varied and not all are known, except the increase expression of the plant defence genes (Abdullah *et al.*, 2017). It is likely that pathogens have evolved to adapt to the host responses, or even to manipulate their host for their own benefit. Thus, theoretical studies based on ecological models suggest that some pathogens may be able to elicit an immune response in their host, to which they are adapted but which will be detrimental to other pathogens already present. Excluding the original pathogen would then allow the newcomer to infect the host (Brown *et al.*, 2008; Mideo, 2009).

Conversely, certain infections may facilitate the development of secondary infections. This is the case of infection by the plant pathogen bacteria *Pseudomonas syringae* which, by activating the salicylic acid defence pathway, makes plants more susceptible to the plant pathogenic fungus *Alternaria brassicola* due to the suppression of the jasmonic acid pathway (the result of a compromise in the different plant defence pathways) (Spoel *et al.*, 2007; Abdullah *et al.*, 2017). Saunders & Kohn (2008) have shown that the maize pathogen *Fusarium verticilloides* can facilitate the development of endophytes (*Nigrospora oryzae*, *Acremonium zeae* and *Periconia macrospinosa*). This facilitation is to be related to the production of secondary antimicrobial metabolites (MBOA and BOA) by the host plant. Indeed, *F. verticilloides* is able to suppress the production of these secondary metabolites, allowing less tolerant individuals to colonize the host plant.

Interference. Interference corresponds to a direct interaction between the co-infecting pathogens, which can develop strategies to modulate, chemically or mechanically, the host colonisation, reproduction and transmission of co-infecting pathogens (Mideo, 2009). These interactions can be positive or negative, and can result from either the production by the pathogen of molecules affecting other pathogens positively (i.e. siderophores facilitating host exploitation) or negatively (i.e. production of compounds toxic to co-infecting pathogens, or from direct interactions between the respective proteins of the two protagonists, modifying their infection capacities (**Figure 1**). Recent studies were reported in multi-infection of tomato by two viruses, the Iris yellow spot virus and the Tomato spotted wilt virus, where viral proteins (the nucleocapsid and motion-associated proteins) interact, promoting replication and dispersal of both viruses within the host (Tripathi *et al.*, 2015; Tollenaere *et al.*, 2016).

Various experimental studies have been able to highlight chemical modes of action that can modulate the development of competitors. For instance, co-inoculation on wheat and rice seeds by the fungi *Trichoderma gamsii* and *Fusarium oxysporum* reduces the development and production of mycotoxins by the fusarium head blight agent *F. graminearum* through secondary metabolites affecting the development of the pathogen (Sarrocchio *et al.*, 2019b). Multi-infection experiments carried out on culture media and on detached bean leaves revealed an antagonism between *Ascochyta fabae* and *Botrytis fabae*, which could be related to the antimicrobial activity of ascochylin produced by *A. fabae* (Madeira *et al.*, 1993). Mousa *et al.* (2015) extracted antifungal compounds produced by a *Phoma* species, endophytic in *Eleusine coracana*. They demonstrated that each of these purified compounds can disrupt *F. graminearum* hyphae. Mechanical modes of action were also demonstrated, with some pathogens being able to modify host cell membranes by forming several layers of physical barriers, thus limiting the growth and infectious success of their competitors (Abdullah *et al.*, 2017). Mousa *et al.* (2016) have observed such a mechanism in the endophytic bacteria (*Enterobacter* sp.) that limits the development of *F. graminearum*. In the presence of *F. graminearum*, *Enterobacter* sp., which colonizes plant roots, is able to produce physico-chemical structures at the interface between the root and the rhizosphere, that prevent the entry of *F. graminearum* into the root or trap it and kill it later. These structures consist of dense, stacked layers of absorbent hairs (whose growth is stimulated by the endophyte) and colonies of the bacteria.

These types of interactions may often be associated. Although some may be predominant, theoretical and empirical studies often point to the simultaneous or successive existence of these different types of interaction, making their relative importance change over the course of the epidemic (Mideo, 2009; Tollenaere *et al.*, 2016). For example, Al-Asakar & Rachad (2010) highlighted a significant effect of

arbuscular mycorrhizal (AM) fungi on the control of pathogens responsible for bean root rot. Root colonization by AM, by triggering a range of defence mechanisms (host-mediated interaction), improves nutrient uptake by the plant and causes biochemical and physiological changes. However, interference (via the modification of the root system or the production of secondary metabolites) and resource-mediated interaction (pathogenic fungi and AMs exploit the same resources) can also intervene. Moreover, it is often difficult to define precisely the type(s) of multi-infection interaction(s) involved. In interactions between the wheat pathogens *Blumeria graminis* f. sp. *tritici* (biotroph) and *Zymoseptoria tritici* (necrotroph), a compatible interaction between *Z. tritici* and the wheat leaf leads to a reduction in the number and size of *B. graminis* colonies but also in their reproductive capacity (compared to single inoculation of *B. graminis*), whereas a compatible reaction between the host plant and *Z. tritici* has no effect on the development of *B. graminis* (Orton & Brown, 2016). These authors consider a possible elicitation of the host plant defence mechanisms by the necrotrophic agent, reducing its sensitivity to the biotroph. The restriction of the development of *B. graminis* to areas not colonized by *Z. tritici* also suggests a resource-mediated and/or interference interaction. Moreover, in cases where different types of interaction are envisaged, the question arises as to the sequence of action of these mechanisms and their respective importance during the epidemic (Orton & Brown, 2016).

*** Consequences of multi-infection interactions on pathogen development and survival**

Adaptations developed by pathogens to face interactions with their commensals can lead to different types of social strategies, ranging from sacrifice (*i.e.* altruistic suicide) to competitive exclusion and cooperation. These types of social behaviour, which have been widely studied in mammals, birds and insects, are also found in microorganisms. Social strategies between pathogens are then likely to impact their virulence, and thus govern epidemics. However, few studies have focused on these social strategies in the case of multi-infections between pathogenic microorganisms (Leggett *et al.*, 2014).

Competition and competitive exclusion. One of the basic principles of ecology is competitive exclusion (Gause, 1934). This principle states that when two species have identical ecological niches, they cannot coexist stably and indefinitely, with the more competitive species taking over and eventually excluding the second (Begon *et al.*, 2006; Fitt *et al.*, 2006; Hirzel & Le Lay, 2008) (**Figure 1**). Numerous studies have highlighted cases of competitive exclusion (change of niche or extinction) between species (DeBach & Sundby, 1963; DeBach *et al.*, 1978; Maherali & Klironomos, 2007; Hirzel & Le Lay, 2008). For example, Tan *et al.* (2015) showed that prior inoculation of tomato seedlings with the biocontrol agent *Bacillus amyloolusfaciens* prevents the development of the pathogenic bacterium *Ralstonia solanaceum*. This exclusion would probably be the result of resource-mediated interaction, as the biocontrol agent

colonizes the roots of the host plant more efficiently (Tan *et al.*, 2015). The principle of competitive exclusion is still being discussed today, particularly because of observations such as the "paradox of the plankton". This study conducted by Hutchinson (1961) reveals that in the same ecosystem, it is possible to observe many different species of phytoplankton (of the order of several thousand), even though the resources are limited, thus contradicting the principle of competitive exclusion. Subsequent investigations have pointed out that some pathogens occupying similar niches are able to coexist (Fitt *et al.*, 2006; López-Villavicencio *et al.*, 2007; Dutt *et al.*, 2021). Competitive exclusion is thus one of the possible consequences, but by no means the only one, of multi-infection interactions. In fact, it only occurs in the absence of differentiation of the competitors' realized niches (Begon *et al.*, 2006). Gold *et al.* (2009) studied the multi-infection of pathogenic fungi of the genus *Microbotryum* responsible for anthrax on *Silene vulgaris*. From sequential inoculation on host plants, they found that the pathogen inoculated first does not systematically exclude the competitor. They found that competitive exclusion is greater when individuals are closely related than with individuals belonging to different genotypes. Maherali & Klironomos (2007) made the same observation from communities of arbuscular mycorrhizal fungi colonizing the roots of *Plantago lanceolata*. Indeed, they found competitive exclusion of subsequently inoculated strains, but only when they were related to the initially inoculated strains. The authors therefore suggest that coexistence between more genetically distant AM species is allowed by niche differentiation.

Spiteful behaviour. In some cases, multi-infection may lead to a reduction in the development of all coexisting pathogens. This type of response is observed when a pathogen negatively impacts its own development by seeking to further limit that of the co-infecting pathogens through mechanical or chemical actions (Buckling & Brockhurst, 2008). Spiteful behaviour has been shown to occur mainly in the case of pathogenic bacteria, which are able to produce toxins targeting unrelated commensals (Buckling & Brockhurst, 2008; Bashey *et al.*, 2012). The production of bacteriocins by bacteria is a good example of 'spiteful behaviour'. Bacteriocins are expensive to produce (often involving the lysis of cells for their release), and only eliminate or limit the growth of co-infecting pathogens that are sensitive to them (Riley & Wertz, 2002; Buckling & Brockhurst, 2008; Mideo, 2009). Multi-infections with bacteriocin-producing bacteria can lead to a decrease in host exploitation, and thus a decrease in virulence (Mideo, 2009). Thus, Bashey *et al.* (2012) have demonstrated from 5 sympatric strains of *Xenorhabdus bovienii* that the production of bacteriocin by these strains reduces the host mortality rate.

Cooperation (or cooptation). Multi-infection can also lead to cooperative strategies. It is interesting to distinguish two types of cooperation: prudence, and the production of "public goods". Cooperation is

beneficial to both co-operators, but it is likely to lead to the emergence of 'cheats', *i.e.* individuals who do not cooperate but who benefit from those who do. Thus, if cooperation between pathogens exists, it must have some advantage, such as: (i) a direct benefit on the fitness of the actor (*i.e.* in the case where the actor can choose to establish cooperation only if it is reciprocal) or (ii) an indirect benefit on the fitness of the actor, by favouring the transmission of copies of his genes carried by related individuals with whom he cooperates (theory known as kin selection) (Buckling & Brockhurst, 2008; Abdullah *et al.*, 2017).

➤ *Prudence*

Prudence is a form of cooperation in which individuals limit their use of resources for the benefit of others. This cooperative strategy aims to prioritize the transmission of all pathogens by leaving a fraction of the total available resources for other pathogens. Since virulence can have a negative impact on the success of transmission, particularly in cases where pathogens are transmitted only from living hosts, or where this transmission is reduced on weakened hosts, pathogens will then reduce their virulence to promote their own transmission (**Figure 1**). As a consequence of this trade-off, intermediate levels of host exploitation (corresponding to optimal virulence) will maximize the transmission success of the pathogen population (Buckling & Brockhurst, 2008). Such an increase in transmission of individuals was observed during the co-inoculation of two strains of *Podosphaera plantaginis* on *Plantago lanceolata* in semi-natural conditions. Although the authors did not study the effect of this increase in transmission on the virulence of the strains, at the inter-host level, this increased transmission increases the number of infected leaves and thus leads to larger epidemics (Susi *et al.*, 2015b). Such trade-offs are easier to detect in less complex study systems. Indeed, Barret *et al.* (2021) recently evaluated the effect of intra-host competition during different stages of the infection cycle for the highly diverse plant pathogenic fungus *Z. tritici*. Their results indicated that competition for resources and cross-reaction of the immune system might lead to the observed reduction in transmission potential for the pathogen in mixed infections and that the effects of competition were host- and strain-specific. Strains that suppressed the growth of competitors during host colonization did not always produce more offspring than the suppressed strains. Their results suggested that mixed infections in *Z. tritici* did not necessarily lead to selection of more virulent strains as no association was observed between virulence (lesion development) in single infections and transmission potential (proportion of pycnidiospores produced) in mixed infections. Dutt *et al.* (2021) also observed this response pattern when co-inoculating sympatric strains belonging to the pea ascochyta blight complex on detached pea stipules. In response to competition, some strains reduced their development (the proportion of necrotic area in infection is lower than in single infection) but increased their progeny production, thus favouring their transmission.

In some cases, involving closely pathogens, this type of cooperation can lead to the altruistic suicide of one of the co-infecting pathogens. The least competitive pathogen will experience a reduction in its reproduction rate, preserving the resource for the reproduction of other individuals. This extreme case is based on kin selection, maximizing inclusive fitness including the adaptive success of the direct progeny of an individual but also of his relatives according to the degree of genetic relationship (Hamilton, 1963). Hamilton (1963) describes this cooperation as genetic nepotism. Indeed, closely related individuals have many alleles in common; when these individuals coexist, each can expect to transmit a high fraction of its own genetic heritage through the reproduction of other individuals, which is not the case when the host is shared with a distantly related individual. It is therefore in the interest of closely related individuals to cooperate, or at least to limit competition (Buckling & Brockhurst, 2008; Leggett *et al.*, 2014). In the case of the pathogenic fungus *Microbotryum lychnidis-dioicae* growing on *Silene latifolia*, López-Villavicencio *et al.* (2007) observed a reduction in competition when *Microbotryum* strains are strongly related. This theory thus implies that it does not matter who transmits the genes, what matters is the success of transmission of these genes (Maynard-Smith, 1964).

➤ *The production of public goods to share.*

In response to multi-infection interactions, some pathogens may produce resources ("public good") that can be used by other pathogens with which they coexist. Cooperation between pathogens to produce these resources can thus lead to mutual benefit and thus promote host exploitation (Hoek *et al.*, 2016; Abdullah *et al.*, 2017). This production of "public goods" is therefore beneficial for the population, but costly at the individual level (Buckling & Brockhurst, 2008). These "public goods" can interact directly with the host (*i.e.* production of compounds necessary for infection), promoting host colonization. However, these "public goods" may also enable the survival and multiplication of coexisting pathogens within the host (West *et al.*, 2007) (**Figure 1**). Thus, in the case of bacteriophages infecting *Pseudomonas syringae*, it has been shown that the replicative enzymes produced can be used by all virions (provided they are sufficiently related), leading to an increase in their reproduction rate (Turner & Chao, 1999). Harrison *et al.* (2006) also showed that cooperation of siderophore (pyoverdine) producing strains of *Pseudomonas aeruginosa* results in faster host exploitation compared to non-pyoverdine producing strains. Although this production of "public goods" is mainly referenced in the case of pathogenic bacteria, it has also been observed in the pathogenic fungus *Magnaporthe oryzae* growing on rice crops. Indeed, Lindsay *et al.* (2016) have shown that the invertase produced by a strain of *M. oryzae* can be used by a mutant strain with significantly reduced invertase production. The results of this study also suggest that other "public goods" may be exchanged between the co-infecting strains. Reciprocal exchange of these resources is a

common strategy through which pathogens establish stable cooperation (Hoek *et al.*, 2016; Abdullah *et al.*, 2017). However, in the presence of 'cheats', this production of 'public goods' benefits the 'cheats' and leads to a decrease in virulence compared to multi-infection situations involving only cooperators (Mideo, 2009). For example, the bacterium *Pseudomonas aeruginosa* is able to produce siderophores, which are beneficial when iron is limiting but metabolically expensive for the bacterium. In natural populations, some bacteria are able to use the siderophores produced without paying the cost, allowing them to increase their prevalence in the population (Griffin *et al.*, 2004; Harrison *et al.* 2006; Mideo, 2009).

Life history traits changes in multi-infection

*** Evolution of virulence in multi-infections.**

The various interactions described above result in a modification of the host exploitation dynamics (Alizon *et al.*, 2013). Most studies on multi-infections evaluated the evolution of virulence, defined as a parameter reflecting this exploitation of the host by the pathogen and leading to a decrease in the fitness of the host (Read, 1994). Evolutionists initially hypothesized that the virulence of a pathogen (in single infection) was only transient, leading to an evolution towards interactions of commensalism or mutualism. It seems counter-intuitive that pathogens can harm their host when their survival depends on it (May & Anderson, 1983; Alizon *et al.*, 2013). Following these studies, many theories have sought to explain the evolution and maintenance of virulence in pathogens. The concept emerged of virulence as an adaptive process, with an increase in virulence being linked to an increase in transmission between hosts (Ewald, 1983; May & Anderson, 1983). Indeed, pathogens need to grow sufficiently on a host to ensure their inter-host transmission. Virulence is therefore an unavoidable cost of host exploitation, necessary for transmission to new hosts. Thus, if the costs of increased host exploitation outweigh the benefits of inter-host transmission, natural selection should favour an intermediate level of host exploitation (*i.e.*, optimal virulence), highlighting a positive correlation between virulence and transmission (Alizon *et al.*, 2013; Leggett *et al.*, 2014). Various empirical studies have highlighted such a correlation (Kover & Clay, 1998; Salvaudon *et al.*, 2005; Héraudet *et al.*, 2008; Doumayrou *et al.*, 2013; Laine & Barrès, 2013). Doumayrou *et al.* (2013) evaluated under controlled conditions the transmission, virulence and intra-host accumulation of nine strains of Cauliflower mosaic virus after their inoculation on *Brassica napa*. They observed a positive correlation between virulence (assessed as the proportion of the host leaf area impacted by virus development) and virus transmission, suggesting the existence of an intermediate level of virulence. If we now consider the case of multi-infections, the presence of several pathogens

corresponds to an additional level of selection (beyond that of transmission between hosts) acting on the evolution of the virulence of the pathogens. Thus, when different pathogens share the same resources, the optimal level of virulence may not be the same as in single infections (Alizon *et al.*, 2013). This is why the evolution of virulence into multi-infections is often studied in the light of Hardin's (1968) 'tragedy of the commons' theory: in competition, individuals maximize their profits by quickly and selfishly exploiting their host to the detriment of all individuals. Theoretical studies therefore predict an increase in virulence in cases of multi-infections, with intra-host selection favouring the most rapidly developing and therefore the most virulent pathogens (Hamilton, 1972; Nowak & May, 1994; Mideo, 2009; Choisy & de Roode, 2010; Alizon *et al.*, 2013). Some empirical work supports the idea that virulence increases in multi-infections. Thus, Rochow & Ross (1995) have shown that co-inoculation of viruses X and Y or successive inoculation of potato viruses Y and X on plants of *Nicotiana tabacum* L. leads to an increase in virulence (defined as the severity of leaf symptoms). These authors were able to link this increase in virulence with the increased multiplication of virus X in multi-infection situations.

Dutt *et al.* (2021) also observed this response pattern when co-inoculating strains belonging to the pea ascochyta blight complex. They showed that some strains respond to resource-mediated interaction by increasing their virulence (compared to the virulence observed for the same strains in single inoculation) and by reducing their progeny production, while other strains respond inversely by lowering their virulence and increasing their progeny production. This last behaviour was also observed in greenhouse experiments by inoculating two wheat lines with three genotypes of the pathogenic fungus *Mycosphaerella graminicola* alone or in mixtures (Tollenaere *et al.*, 2016). However, for some mixtures, the virulence was lower than that observed for the same genotypes inoculated alone (Schürch & Roy, 2004). Similarly, the virulence of the pathogen responsible for black rust (*Puccinia graminis*) (defined as the number of rust pustules on leaves) decreased when inoculated on barley plants previously inoculated with the powdery mildew (*Erysiphe graminis*) (Round & Wheeler, 1978).

As detailed above, different types of interaction can develop in response to multi-infections (exploitation, apparent, interference), and thus impact the evolution of virulence (Turner & Chao, 1999; Buckling & Brockhurst, 2008; Leggett *et al.*, 2014). For example, Dutt *et al.* (2021) were able to demonstrate from 4 different strains of the pea ascochyta blight complex that virulence in multi-infection can decrease or increase depending on the combinations tested. These variations were best explained by different types of interactions between the different combinations, although the types of interactions involved were not highlighted during this work. Bashey *et al.* (2012) showed that interference, leading to spiteful behaviour, in strains of *Xenorhabdus bovienii* leads to a decrease in overall virulence, while

interference with cooperative behaviour in *Pseudomonas aeruginosa* strains leads to an increase in overall virulence (Harrison *et al.*, 2006). Moreover, at the scale of an epidemic, if we link the 'tragedy of the commons' theory to the virulence -transmission trade-off theory, a strategy optimal at the intra-host level would not necessarily be optimal at the inter-host level (*i.e.* it does not allow for optimal transmission) (Alizon *et al.*, 2013). Multi-infections are thus the cause of changes in virulence, but current theories do not allow us to unequivocally predict the direction of these changes (Tollenaere *et al.*, 2016).

In evolutionary ecology, virulence is a composite trait associated with the modification of host fitness in response to interaction with a pathogen (Bull, 1994; Alizon *et al.*, 2009). It thus corresponds to the set of negative effects induced by the presence of a pathogen on host development, fecundity, survival and competitiveness (Lively, 2001; Thomas *et al.*, 2016). However, this overly broad definition has led to a high degree of variability in the measurement methods used. Empirical studies have attempted to understand virulence by estimating the proportion of symptomatic tissue, host life span, relative reproduction, relative growth rate or relative mortality (Shaner *et al.*, 1992; Alizon *et al.*, 2009; Thomas *et al.*, 2016). These studies therefore often summarize virulence as a measure of aggressiveness, whereas other traits of interest must be considered to understand the coexistence of pathogens. It thus appears more relevant to study the consequences of multi-infection interactions on the life history of pathogens, making it possible to adapt the traits measured to the life cycle of each pathogen and to the specific characteristics of each pathosystem.

*** Characterizing pathogens coexistence by measuring life history traits.**

The evaluation of the life history traits is essential to understand the biological and epidemiological differences at the origin of their niche differentiation and thus their coexistence. This importance of trait assessment in understanding coexistence was initiated by MacArthur (1972), who drew up a dichotomy between species according to their life histories, based on the theory of trade-offs between traits (Stearns, 1989). This author thus distinguishes between two distinct strategies, known as "r" and "K". The "r" strategy is that of pioneers: these individuals develop in an environment with a high availability of resources and a low rate of competition. Individuals with an r-strategy are characterized by a high developmental capacity, high fertility and low longevity. In fact, this strategy is associated with a notion of productivity. By contrast, "K" strategists develop in an environment where resources are more limited and competition is intense, leading to a strong need for efficiency in the exploitation of these resources. The "K" strategy is therefore associated with highly competitive species, with a high longevity but a much lower rate of population growth.

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These two strategies constitute the two ends of a continuum along which each individual can be positioned (Pianka, 1970). The concept of "r" and "K" strategies has often been associated with the coexistence of species. Indeed, species sufficiently distant along the continuum will present biological and epidemiological characteristics sufficiently disjointed to allow niche differentiation. For example, Montarry *et al.* (2008) used the theory of "r" and "K" strategies to evaluate the coexistence of groups A and B of *Erysiphe necator* strains, the agent responsible for grapevine downy mildew, showing that group A strains present an "r" type strategy and group B strains a "K" type strategy. Similarly, Dutt *et al.* (2020) observed three different life history strategies in cryptic species of the pea ascochyta blight complex: pioneer colonisers, scavengers and intermediates (renamed opportunists in Dutt *et al.*, 2021). They used the theory of "r" and "K" strategies to understand how these three strategies allow the coexistence of these strains. The pioneer colonisers would present an "r" type strategy and would be good colonisers of empty ecological niches, with the capacity to persist in their niche over time and with weak responses to competition. By contrast, scavengers would present a "K" strategy and use their strong competitive ability to develop on old tissue already colonized by strains presenting one of the other strategies. Finally, opportunists would be positioned between the two strategies described above and would be able to develop in much more variable environments, giving them a low sensitivity to competition. This example illustrates how the measurement of life history traits, and their aggregation into life history strategies, can allow a better understanding of the coexistence of these pathogens during their development (Fitt *et al.*, 2006).

*** Multi-infection and resources allocation.**

Life history traits of pathogens are likely to vary so as to adapt to any changes in their environment (particularly the presence of competitors for access to a limited resource). Thus, the life history traits measured for pathogens in single infection situations, although necessary to understand the life history of these individuals, are likely to change in multi-infection situations (Round & Wheeler, 1978; Rochow & Ross, 1995; Schürch & Roy, 2004; Bashey *et al.*, 2012; Dutt *et al.*, 2021). Multi-infections will therefore have an impact on the pattern of resource allocation between different biological functions. In addition, the trade-offs between life history traits that pathogens face in single infections can also be altered in multi-infections. Although empirical studies have evaluated some relationships and trade-offs between life history traits in single infections (Susi & Laine, 2012; Laine & Barrès, 2013), the evaluation of these trade-offs under multi-infections, which seems to be of crucial importance in understanding and thus controlling epidemics, has been the subject of only very few studies to date. Laine & Mäkinen (2018) demonstrated, from single and co-inoculations of *P. plantaginis* strains on detached leaves of their host

plant (*P. lanceolata*), that the weak negative correlation between the pathogen amount (DNA concentration quantified by qPCR) and the time required for sporulation becomes significantly positive in multi-infection situations. These trade-offs between life history traits are directly dependent on the life history of the pathogens (Alizon *et al.*, 2013). For example, in the case of pathogens with a saprophytic phase, allowing them to survive during the off-season, trade-offs between traits related to pathogenicity and those related to the survival phase are likely, but do not apply to obligate parasites (Montarry *et al.*, 2008). Finally, pathogen responses to multi-infections are dependent on multi-infection interactions, and thus on the co-infected pathogen (Wargo *et al.*, 2007, Dutt *et al.*, 2021). Indeed, in the case of pea ascochyta blight, multi-infection between different sympatric strains of the complex reveals variable responses to co-infection depending on the competitor. This response always seems to be part of a trade-off between transmission and necrotic development (Dutt *et al.*, 2021).

*** Life history traits, good indicators to evaluate coexistence strategies.**

Work addressing issues of coexistence and competition usually use ecological concepts (fitness, virulence), and focus on defining a measure for a given pathosystem. However, fitness is a universal concept aimed at quantifying the evolutionary changes in a population subject to natural selection (Darwin, 1859; Lenormand *et al.*, 2016), which may lead to changes in their life history traits (Roff, 1992; Stearns, 1992). Virulence, on the other hand, is associated with changes in host fitness in response to interaction with a pathogen (Bull, 1994; Alizon *et al.*, 2009). However, such universal measures do not allow the life history of individuals to be summarized as a simple number. An exhaustive study should be based on measures of traits related to each of the different stages of the epidemic phase (*i.e.* development, reproduction, transmission ...), but also include measures affecting the survival phase, which will condition the initiation of the disease (Agrios, 2005; Tack & Laine, 2014) as well as the interactions of the species with its environment. Thus, the correct definition of the necessary and measurable life history traits in a multi-infection studies is not trivial. The evaluation of life history traits also implies a reflection on the allocation of resources between these different traits, thus allowing a better understanding of the host exploitation strategies of individuals (**Figure 2**). However, such an evaluation requires a sufficient number of individuals and life history traits to highlight correlations that reflect a real functional antagonism (Lenormand *et al.*, 2016). Thus, since the development and reproduction of pathogens is conditioned by the availability of living host tissue (Agrios, 2005), it is better to perform measurements of these traits under conditions where the host resource is finite and measurable. This is the case, for example, with tests on detached pea leaves to assess the life history

traits of pathogenic strains responsible for pea ascochyta blight. This method consists of inoculating one drop of a spore suspension (or 2 for co-infection studies) on pea leaf stipules maintained in survival on water (Onfroy *et al.*, 2007; Le May *et al.*, 2009; Davidson *et al.*, 2012, Dutt *et al.*, 2020, 2021). It allows simultaneous and repeatable measurements of life history traits. However, regardless of the experimental design, the accurate measurement of a dynamic process such as reproduction remains delicate (Marçais *et al.*, 2009). Indeed, numerous studies based on different pathosystems have shown that the duration of sporulation varies according to the pathogen species (Pariaud *et al.*, 2009). Moreover, Declerck *et al.* (2001) have shown that spore production by mycorrhizal fungi follows a logistic law. The dynamics of spore production results in a linear increase, followed by a stable phase of sporulation and finally a decrease phase, the duration of each of these phases may vary according to the pathogen under consideration (Declerck *et al.*, 2001). An accurate estimate of progeny production should therefore ideally be based on temporal monitoring, which can be tricky to implement. It is therefore essential to determine in advance the number of measurements needed to estimate progeny production, and the time interval between these measurements. In addition, for many plant pathogenic fungi, the phases of sexual and asexual reproduction follow one another during the epidemic, with asexual reproduction occurring early in the epidemic and sexual reproduction occurring later in the season (Agrios, 2005). As a result, this investment in sexual reproduction is expected to result in a trade-off with other life history traits (Roff, 1992; Stearns, 1992; Michalakis, 2009; Hamelin *et al.*, 2016), potentially altering the host exploitation strategy of the pathogen of interest and impacting species coexistence strategies. In addition, infection efficiency, sporulation rate, latent period and infectious period are not necessarily similar to those measured for spores derived from asexual reproduction (Gilles *et al.*, 2001; Pariaud *et al.*, 2009). For example, *Ascochyta rabiei*, a pathogen of chickpea, has been demonstrated to have faster germination rate and germ tube length for ascospores compared to pycnidiospores (Trapero-Casas & Kaiser, 2007).

Beyond the measurement of traits related to the epidemic phase, the survival phase is often considered as a "black box" in view of the difficulties of measurement associated to it. However, several studies have highlighted the existence of trade-offs between traits related to epidemic development and those associated with the survival phase (Stearns, 1992; Montarry *et al.*, 2008; Hamelin *et al.*, 2016). The implications of multi-infections on these trade-offs have, however, been the subject of few experimental studies to date (Laine & Mäkinen, 2018), in particular because of the complexity of the experimental protocols to be implemented. Thus, although the approach aimed at studying multi-infection through the filter of life history traits is delicate to implement, it appears indispensable for a better understanding of

coexistence and competitive interactions and for predicting the impact of this coexistence on epidemic dynamics (**Figure 2**).

Sequential development of multi-infections by plant pathogens

Today, research on multi-infections often deals with simultaneous inoculations of pathogen species (or ignores their order of arrival), although, in reality, the arrival of pathogen species co-infecting a host is in most systems sequential. The chronology of infections can sometimes be predictable (*i.e.* due to phenology or season), but in many cases it is unpredictable and results from random contact between hosts and pathogen propagules present at the same time in the environment. Since multi-infection is a temporal process, it is more likely that two pathogenic species will affect the same host successively rather than simultaneously. Moreover, the period between these two infections may be short or very long, depending on the life span of the host (Laine, 2011; Marchetto & Power, 2017; Karvonen *et al.*, 2019). Under natural conditions, such sequential events are difficult to predict, and are conditioned by many environmental factors. The identity and number of pathogen species on the same host plant may thus depend on whether early infecting pathogens induce changes in the host plant, and affect the establishment and development of later infecting species (Le May *et al.*, 2009).

Host life-history traits could also influence the occurrence of multi-infections and sequential development of pathogens in numerous ways. At the individual host level, life history traits, such as parental investment, have been shown to favour multi-infection (Christe *et al.*, 2012). Other studies showed that lifespan, and more particularly longer-lived hosts, by investing more in anti-parasite defences, were less frequently multi-infected (Arriero *et al.*, 2008).

Changes in competitive interactions in sequential infections. The time between the arrival of the different pathogenic species can vary from a few seconds to several years. The host plant corresponds to a resource where the first pathogen arriving will be served first (Tollenaere *et al.*, 2016; Karvonen *et al.*, 2019). The sequentiality of arrival on the host plant will then give a competitive advantage to the earliest pathogen, allowing it to acquire the resources first (resource-mediated interaction), but also to trigger responses from the host immune system (host-mediated interaction) that can modulate the development of the pathogen that arrive later (Laine, 2011; Susi *et al.*, 2015a; Karvonen *et al.*, 2019). Le May *et al.*

(2009) demonstrated that the simultaneous inoculation of two plant pathogenic fungi (*Peyronella pinodes* (*P. pinodes*) and *Phoma medicaginis* var. *pinodella* (*P. pinodella*)) associated to the Ascochyta blight disease complex limits disease development and their reproduction; however, when plants pre-inoculated with one pathogen were then inoculated with the other, there was a marked increase in severity of the disease. In contrast to this report, Sagar & Sugha (1997) reported a decrease in necrotic symptoms of pea caused by *P. pinodella* previously inoculated with the vascular root pathogen *F. oxysporum* f. sp. *pisi*. The order/succession of host infection by each pathogen in a complex disease and its trophic level might affect the nature of interaction that these pathogens eventually develop during disease occurrence. Such temporal effects could also be related to the notion of ecological niche (Lamichhane & Venturi, 2015), as the niche constituted by an infected host is clearly different than that offered by a healthy one.

In extreme cases, sequential inoculation may result in competitive exclusion, with little effect on the performance of the first invader (Alizon *et al.*, 2013; Karvonen *et al.*, 2019). This is for example the case of sequential inoculation of different strains of *Oidium neolycopersici*, responsible for tomato powdery mildew: a first inoculation with an avirulent strain allows to activate a hypersensitive response, protecting the plant against the virulent strains inoculated afterwards (Seifi *et al.*, 2011). However, in most scenarios, the first pathogen limits the development of the second pathogen without achieving complete exclusion, leading to a decrease in virulence compared to simultaneous infection (Karvonen *et al.*, 2019). This decrease in virulence in sequential infection has been observed by Goodman & Ross (1974) with potato viruses X and Y. Indeed, simultaneous inoculation favours the accumulation of both viruses. However, as soon as the inoculation delay exceeds 24 hours, this synergistic effect on accumulation is lost, regardless of which virus was inoculated first.

In other cases, removal of the first pathogen via the host defence system may also result in facilitation of infection by pathogens arriving later (Graham, 2008). The first pathogen may also promote the development of the later pathogen, for example by causing immunosuppression of the host, allowing better replication for the second pathogen (Karvonen *et al.*, 2019). Olesen *et al.* (2003) observed that the early arrival of a virulent strain of *Blumeria graminis* suppressed host resistance, allowing penetration of other strains and promoting their development.

Impact of sequential development of pathogens on their life history traits. The importance of the sequence of arrival of pathogens has mainly been approached from models of multi-infections (coexistence of different strains and/or pathogenic species) and superinfection (total exclusion of a

pathogenic individual without coexistence) (Alizon *et al.*, 2013). The arrival sequence would have a significant impact on the life history traits of pathogens and the trade-offs between these traits. A few empirical studies have thus been able to highlight such a variation. For example, Le May *et al.* (2009) focused on the sequential inoculation of *P. pinodes* and *P. pinodella* on pea seedlings. They observed that simultaneous inoculation limits the development of symptoms of these two pathogens compared to sequential inoculations. Moreover, when *P. pinodella* was inoculated 6 days after *P. pinodes*, the size of its lesions increased and the number of pycnidia decreased, compared to simultaneous inoculation or to inoculations only 3 days apart. In another study, Al-Naimi *et al.* (2005) investigated the effect of the inoculation sequence on the production of spores of *Puccinia triticina* and *Pyrenophora tritici-repentis*. In the case of simultaneous inoculation, they found that *P. tritici-repentis* is a better competitor than *P. triticina* and produces more spores in competition. This facilitation is exacerbated when *P. triticina* is inoculated three days later. These different results demonstrate that the arrival sequence of pathogens is likely to modify their intra-host dynamics and thus their inter-host transmission.

Host plant resistance, receptivity and multi-infection processes.

Plant resistance (intrinsic resistance) limits the development of a pathogen by excluding it altogether or by modifying some of its life history traits (Agrios, 2005). The level of plant resistance can have an unequal impact on the different life history traits related to the epidemic phase of the pathogen. Susi *et al.* (2015b) demonstrated a change in the relationship between the proportion of infected leaves and the number of spores released by *Podosphaera plantaginis* and the resistance level of its host plant (*Plantago lanceolata*). Indeed, their results showed that strength of this correlation varies according to the strain and host genotype considered, confirming that the outcome of multi-infections is well dependent on the host plant genotype (Fellous *et al.*, 2012; Susi *et al.*, 2015b; Susi & Laine, 2017). More recently, Mariette *et al.* (2018) explored whether a negative relationship between progeny size and number occurred in the late blight oomycete, *Phytophthora infestans*, and whether the trade-off was impacted by potato cultivar or host of origin of the pathogen (tomato and potato). They showed that this trade-off was not changed by these biotic factors, suggesting that the observed polyphenism for these traits in *P. infestans* populations will favour the coexistence of distinct reproductive strategies. In another study, Dutt *et al.* (2020), by measuring different life history traits on two pea genotypes (susceptible and partially resistant), showed that all the life history traits were negatively impacted by the level of resistance.

Infection is a complex process involving reciprocally both the intensity of host exposure to a pathogen as well as the host intrinsic “receptivity”, or permissiveness to infection. Disentangling their

respective contributions is currently seen as a fundamental gap in our knowledge. Receptivity may vary not only with the host plant genotype, but also with the age of the tissues (Al-Naimi *et al.*, 2005).

The receptivity of host tissues to plant pathogens is also likely to alter intra-host dynamics. In particular, according to the age of tissues, nutrient availability as well as defense levels may vary significantly, with opposing effects on the success of infection (Kus *et al.*, 2002; Al-Naimi *et al.*, 2005). Recently, by studying ontogenic resistance against powdery mildew (*Erysiphe necator*) on *Vitis vinifera*, Calonnec *et al.* (2018) showed that variation of three pathogenic traits (infection efficiency, sporulation and mycelium growth) was strongly correlated with leaf age. Sporulation was more closely correlated with variations in sugar contents and the infection efficiency with leaf water content, suggesting that ontogenic resistance on grapevine leaves seems to be an immutable physiological process that *E. necator* is able to circumvent by restricting its development to sink tissue. Similarly, Maupetit *et al.* (2018) showed that *M. larici-populina* was more aggressive on more mature leaves as indicated by wider uredinia and a higher sporulation rate. In contrast, phenolic contents (flavonols, hydroxycinnamic acid esters, and salicinoids) were negatively correlated with uredinia size and sporulation rate suggesting that pathogen's fitness appeared to be more constrained by the constitutive plant defense level than limited by nutrient availability, as evident in the decrease in sporulation.

Although many studies have shown that plant receptivity changes with age, the mechanisms behind it have been relatively little studied. In the case of cereal rusts, such responses are assumed to be dependent on a single resistance gene expressed in older plants (Kus *et al.*, 2002). Lazarovits *et al.* (1981) observed a positive correlation between the increase in the age of the plant, the production of glyceroline, a phytoalexin, and resistance to *Phytophthora megasperma* var. *sojae* on soybean. A similar correlation was observed in cotton with an accumulation of phytoalexin in response to the development of *Verticillium albo-atrum* (Bell, 1969). Similarly, the increased susceptibility of pea plants to *P. pinodes* is thought to be due to a decrease in phytoalexin (pisatin) concentration in the tissues, limiting or inhibiting defence responses (Toyoda *et al.*, 1995; Le May *et al.*, 2009). These different studies therefore suggest that this modulation of receptivity is controlled by the development of the plant. In some cases, this response may also be associated with the accumulation of toxic compounds during plant development (Kus *et al.*, 2002). Several genes associated with plant defence functions are expressed late in the development of the plant, suggesting an involvement of these genes in the observed change in tissue receptivity. For example, some genes coding for pathogenesis-related (PR) proteins are overexpressed during flower development and senescence, leading to an increase in resistance to pathogens (Fraser, 1981; Kus *et al.*, 2002). Different reports have shown for instance that the epidemic development of the main agent of pea ascochyta blight (*P. pinodes*) was impacted by the age of leaf tissues (Zimmer &

Sabourin, 1986; Richard *et al.*, 2012), the earliness at flowering (Bretag & Ramsey, 2001; Prioul *et al.*, 2004) or the age of the plant or pea canopy (Hwang *et al.*, 2012). Indeed, the receptivity of pea leaves and pods to *P. pinodes* increases sharply during the transition to senescence, and is partly responsible for the decreasing disease gradient generally observed between the base and the top of the canopy (Richard *et al.*, 2012). However, despite some hypotheses on the link with the differential accumulation of a phytoalexin (pisatin) (Zimmer & Sabourin, 1986), the underlying mechanisms, particularly in terms of defence, to this increased susceptibility of older tissues remain unexplained, and their impact on the dynamics of the disease complex (at least 3 fungal species, and several ecologically distinct groups; Dutt *et al.*, 2020) of the ascochyta blight is unknown. Similar observations have been made in potatoes with respect to late blight (*P. infestans*), but also with respect to alternaria (*Alternaria solani*), to which susceptibility is strongly conditioned by the change in nitrogen status occurring during the transition to senescence and the remobilisation of assimilates to newly formed tubers (Barclay *et al.*, 1973). Various QTL controlling senescence in potato are also described (Malosetti *et al.*, 2006; Hurtado *et al.*, 2012), and some are potentially close to defence or resistance genes. Similar results have been obtained in other pathosystems, including those involving the model species *Arabidopsis thaliana* (Kus *et al.*, 2002), highlighting the genericity of the interdependence between development, susceptibility and defence.

Where to now?

It is evident that the interactions of different diseases occurring on one host are a very complex phenomenon. Indeed, as this review has shown, several factors will modify the result of interactions between different pathogens and may affect the outcome of the disease. These factors include sites and timing of inoculations, level of infection, host age or plant receptivity (Hyde, 1981). Therefore, vast challenges still lay ahead for a more comprehensive understanding of multi-infections and their consequences on plant disease epidemics and on the ecology of plant disease complexes.

The first of these challenges is methodological. There has been considerable technical progress in recent years allowing to investigate multi-infections under controlled conditions and accurately measure some major life history traits in pathogens. The development of high throughput phenotyping and the automation of measurements from image analysis, coupled with more efficient genotyping methods, allows a more precise assessment of the contribution of co-infectors to disease severity and pathogen interactions. However, some major gaps remain to be filled. This is the case for some essential, inter-related but fastidious life history traits, such as progeny quality, infection efficiency, inoculum survival, or

sexual reproduction. Indeed, the sexual stage is notoriously difficult to study, and thus for most pathogens we lack sufficient insight into the production and maturation of sexual structures. This is non-trivial, given that the sexual stage plays a key role in generating variation in pathogen populations (Nemri *et al.*, 2012), and is also ecologically important because many pathogens survive unfavourable conditions via sexually produced resting spores (Agrios, 2005; Schmid *et al.*, 2011; Numminen *et al.*, 2019).

The second challenge is to bridge the gap between scales – spatial and temporal. On the spatial side, most experimental work on multi-infections is currently done either under controlled conditions in the laboratory, on a few plants or plant organs, or result from direct observations under outdoor, field conditions. Connecting the two is far from obvious, and often relies on either inference based on ecological theories (notably the ‘tragedy of the commons’ and the ‘virulence-transmission trade-off’), that sometimes is contradicted, or from model simulations based on the same concepts. Unfortunately, disease development in agroecosystems is strongly conditioned by the agronomic characteristics of these agrosystems, and by the local pedoclimatic conditions (Willcoquet *et al.*, 2004). The choice of agronomic practices applied on the crops, but also to the surrounding landscape (Plantegenest *et al.*, 2007) are critical in the constitution of the disease complex infecting a crop: they drive the dynamics of inoculum reservoirs, the connectivity between hosts, and hence the local disease pressure and kinetics. This implies that detailed and documented epidemic surveys data are essential for a correct appreciation of the setup and dynamics of disease complexes.

On the temporal scale, two elements are of utmost importance for future work: the state of the host canopy, as influenced by the crop developmental stages, and the dynamics of primary inoculum sources. As highlighted above, the physiological and ecophysiological status of hosts (senescence; nutritional and defence status, etc...) are key in shaping the plant receptivity to infection, but also in modulating the various types of competitive interactions that take place within disease complexes. To this date, and despite the availability of various sensors, we know of no practical means for reliably assess the host status *in situ*. It is likely that the rapid progress in multispectral image analysis might provide such means in the near future.

The dynamics of primary inoculum, also remains poorly accessible. Most experimental studies still consider this as a ‘black box’, because of the intrinsic difficulties in sampling, quantification and identification of small, fragmented and often cryptic populations (Agrios, 2005; Tack & Laine, 2014). Progress in this respect might come by measuring ‘proxy’ traits for the survival ability of pathogens between epidemics. Among these rank the number /spatial density of resting structures, extinction rates (estimated from genotype frequencies), or the infectious capacity of resting spores (Tack & Laine, 2014).

The third challenge is to connect the ecology and evolutionary dimensions of the issue of multi-infections. This review highlighted the importance of life history traits as predictors and markers for multi-infection performance, and selection both within and between epidemic stages is crucial for the evolution of pathogen populations (Montarry *et al.*, 2008; Castel *et al.*, 2013). However, the genetic determinism behind most life history traits remains a mystery. Fortunately, the advent of genome scan approaches now gives easier access to the genomic regions under selection, and to their polymorphism. These approaches will facilitate the understanding of the total microbial species involved in plant diseases as well as the underlying mechanisms. Hence, studies of complex diseases now need to benefit from culture-independent analyses (high-throughput sequencing for example). This approach does not have the limitations of the classical culture-based approach, which is often lengthy and costly (Nikolaki & Tsiamis, 2013). In the modern era of biodiversity surveillance, techniques such as next-generation sequencing (NGS) have enabled high-throughput analyses of complex microbial populations (reviewed by van Dijketal *et al.*, 2014). This has transformed microbiology and has revealed that microbial diversity is vastly underestimated based on classical cultivation-based techniques (Gilbert & Dupont, 2011). In the last decade, metagenomic projects have been combined with NGS technologies and boosted studies in microbial ecology (Venter *et al.*, 2004; Tringe & Hugenholtz, 2008; Lamichhane & Ventyri, 2015). Now is the time to apply them to the understanding of multi-infections and of their evolutionary and ecological consequences.

Because co infections are an integral part of plant disease epidemics, and since they impact the health status of the crops in ways beyond the simple additive effects of their individual components, risk prediction models and decision support systems should take multi-infections into full account. Indeed, there can't be real IPM systems without an efficient management of multi-infections and disease complexes.

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Authors contributions

AD, DA, and CLM coordinated and supervised the manuscript writing and the experiments. All authors approved the final draft of the manuscript.

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Figure caption

Figure 1. Non-exhaustive synthesis of the multi-infections interactions which might occur between two plant pathogens and possible results of these interactions. Blue drops and orange octagon correspond to the co-infecting pathogens, while the leaves represent the within-host environment shared by these pathogens. Blue stars symbolize molecules that can be produced by one of the pathogens and affecting the development of the other.

Figure 2. Representation of the importance of life history traits evaluation in multi-infection studies. (a), (b) and (c), the sizes of the blue and orange circles symbolize the aggressiveness of the pathogens. (a) schematic representation of the life cycle of a pathogen species (in blue) including disease development and reproduction within its host (green circle), transmission intra and inter-host and disease initiation the following year, when alone on its host plant. (b) progressive reduction in epidemic severity due to the virulence-transmission trade-off. In co-infection with another pathogen (orange), aggressiveness within host increases, but the production of propagules and survival structures decrease leading to a weaker intra and inter host transmission and also to a weaker disease initiation the following year. These decreases may lead to less devastating epidemics over time compared to single inoculation. (c) In co-infection with another pathogen (orange), aggressiveness within host decrease, but the blue pathogen reproduced earlier, which probably allow more secondary infections and thus more devastating epidemics compared to single inoculation.

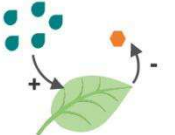
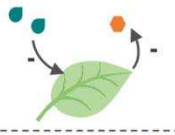
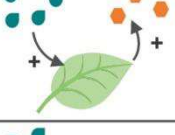
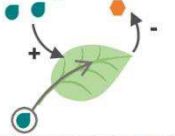
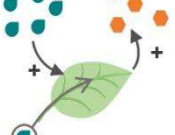


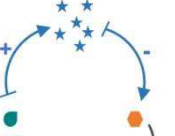
	Multi-infection interaction	Possible result of multi-infection interaction	Examples of multi-infections
Resource-mediated (exploitation)		Competition : the development of the pathogen (●) reduces host resources availability for the pathogen (●) leading to a limitation of its development. In some cases this limitation can lead to competitive exclusion.	<ul style="list-style-type: none"> • <i>Trichoderma viride</i>, <i>Alternaria alternata</i>, <i>Epicoccum purpurascens</i> / <i>Sclerotinia sclerotiorum</i> (Mercier & Reeleder, 1987) • Strains of <i>Peyronalle pinodes</i> and <i>Phoma medicaginis</i> var. <i>pinodella</i> (Dutt et al. 2021) • <i>Bacillus amyloliquefaciens</i> / <i>Ralstonia solanaceum</i> (Tan et al., 2015)
		Cooperation : both pathogens (● and ●) reduce their development on their common host in order to favour their transmission to other hosts.	<ul style="list-style-type: none"> • Strains of <i>Peyronalle pinodes</i> and <i>Phoma medicaginis</i> var. <i>pinodella</i> (Dutt et al. 2021) • <i>Microbotryum lychnidis-dioicae</i> (Tan et al., 2015)
		Cooperation : for its development the pathogen (●) produces components altering the host. This host modified tissue favours the development of the co-infected pathogen (●).	<ul style="list-style-type: none"> • <i>Pyrenophora tritici-repentis</i> / <i>Puccinia triticina</i> (Al-Naimi et al., 2005)
Host-mediated (apparent)		Competition : the development of the pathogen (●) on its host can enhance host plants defense responses (●→) against the other pathogen (●) leading to a limitation of its development, reproduction and/or transmission. In some cases this limitation can lead to competitive exclusion.	<ul style="list-style-type: none"> • Non pathogenic <i>Fusarium oxysporum</i> / pathogenic <i>Fusarium oxysporum</i> (Aimé et al., 2013) • <i>Pseudomonas aphidis</i> / <i>Botrytis cinerea</i> (Buxdorf et al., 2013)
		Cooperation : the development of the pathogen (●) on its host can inhibit host plants defense responses (●→) against the other pathogen (●) favouring its development, reproduction and/or transmission on the common host.	<ul style="list-style-type: none"> • <i>Pseudomonas syringae</i> / <i>Alternaria brassicola</i> (Spoel et al., 2007) • <i>Fusarium verticilloides</i> / <i>Nigrospora oryzae</i>, <i>Acremonium zeae</i>, <i>Periconia macrospinoso</i> (Saunders & Kohn, 2008)
Interference		Competition : the pathogen (●) produces toxins (★) which may directly inhibit the development, the reproduction or the transmission of the co-infected pathogen (●) on the common host. In some cases this toxins production can lead to competitive exclusion.	<ul style="list-style-type: none"> • <i>Trichoderma gamsii</i>, <i>Fusarium oxysporum</i> / <i>Fusarium graminearum</i> (Sarocco et al., 2019b) • <i>Ascochyta fabae</i> / <i>Botrytis fabae</i> (Madeira et al., 1993) • <i>Phoma</i> sp. / <i>Fusarium graminearum</i> (Mousa et al., 2015) • <i>Enterobacter</i> sp / <i>Fusarium graminearum</i> (Mousa et al., 2016)
		Cooperation : the pathogen (●) produces 'public goods' (★) which may be directly used by the co-infected pathogen (●) favouring its host colonisation and/or its multiplication on the common host plant.	<ul style="list-style-type: none"> • <i>Iris yellow spot virus</i> / <i>Tomato spotted wilt virus</i> (Tripathi et al., 2015) • <i>Pseudomona aeruginosa</i> strains (Harrison et al., 2006) • <i>Magnaporthe oryzae</i> strains (Lindsay et al. 2016)
		Spiteful behaviour : the pathogen (●) produces toxins (★) which may directly inhibit the development, the reproduction or the transmission of the co-infected pathogen (●) on the common host. However, this toxins production alters the development of the producer (●) on the host.	<ul style="list-style-type: none"> • 5 strains of <i>Xenorhabdus bovienii</i> (Bashey et al., 2012)

Figure 1

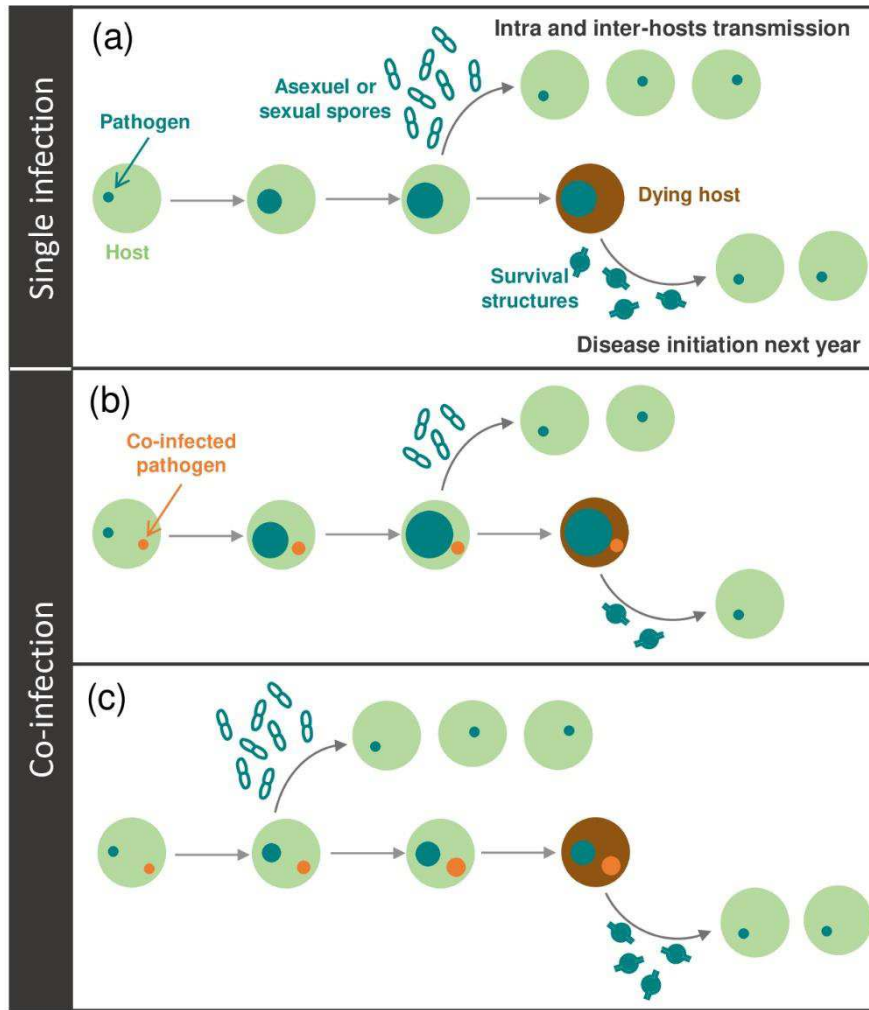


Figure 2