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Factors driving susceptibility and resistance in aphids that share specialist fungal pathogens

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Pandora neoaphidis and *Entomophthora planchoniana* are widespread and important specialist fungal pathogens of aphids in cereals (*Sitobion avenae* and *Rhopalosiphum padi*). The two aphid species share these pathogens and we compare factors influencing susceptibility and resistance. Among factors that may influence susceptibility and resistance are aphid behavior, conspecific versus heterospecific host, aphid morph, life cycle, and presence of protective endosymbionts. It seems that the conspecific host is more susceptible (less resistant) than the heterospecific host, and alates are more susceptible than apterae. We conceptualize the findings in a diagram showing possible transmission in field situations and we pinpoint where there are knowledge gaps.

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Introduction

Species in the fungal subphylum Entomophthoromycotina (Zoopagomycota) play an important role in regulating host populations [1,2]. To understand the epizootic development of these fungi and their transmission within host species and between host species, we need to understand the abiotic and biotic factors affecting host susceptibility and resistance. Susceptibility to a disease can in general be defined as '*lack of ability to resist some extraneous agent (such as a pathogen or drug)*' [3] and resistance to a disease can in general be defined as '*the inherent ability of an organism to resist harmful influences (such as disease, toxic agents, or infection)*' [4]. Thereby, the two terms are somehow linked; a high susceptibility means a low resistance.

In this review, we focus on a host–pathogen system in aphids in cereals that includes two aphid pest species (the grain aphid etc. *Sitobion avenae* and the bird cherry etc. oat aphid *Rhopalosiphum padi*). Two specialist fungal pathogens are commonly reported on these two aphid species, namely *Pandora neoaphidis* (Syn. *Erynia neoaphidis*) and *Entomophthora planchoniana* (Tables 1 and 2). Since *P. neoaphidis* and *E. planchoniana* are shared between these two aphid species, this provides an excellent model system to understand which aphid species or morphs are most susceptible or resistant and which behaviors and environmental conditions favor or disfavor infections among these aphids. Information from other aphid-fungal systems is included when appropriate. Both fungal species have a global distribution and are found on many aphid species (Table 1). Indeed, several other entomophthoralean fungi occur on aphids in cereals but our review will focus on *P. neoaphidis* and *E. planchoniana*.

Epizootiological principles and life cycles of fungal pathogens in aphids in cereals

Epizootiology of insect diseases is the science of causes and forms of the mass phenomena of disease at all levels of intensity in a host population [5]. The primary factors that are involved in the cause, initiation, and development of epizootics of infectious diseases in insects are the pathogen population with its variable virulence and efficient means of transmission and the susceptibility of the host population to the pathogen [6]. We use the terms 'pathogenicity' and 'virulence' as defined by Refs. [7,8••]: pathogenicity is a qualitative character describing if a microorganism causes disease, while virulence is a quantitative expression of the power of a pathogen toward a specific host, for example, an aphid.

Host–pathogen interaction can be depicted as a struggle between competing species that develop adaptations and counter-adaptations against each other, resembling an arms race [7,9]. Some pathogens are able to colonize new hosts for example, if these hosts represent a resource similar to the original host and/or if host populations are genetically diverse allowing parts of the new host population to be susceptible at a given point in time [10••].

Table 1

Observational field studies on occurrence of fungal pathogens in a host–pathogen system consisting of aphids in cereals (*Sitobion avenae* and *Rhopalosiphum padi*) and entomopathogenic fungi (*Pandora neoaphidis* and *Entomophthora planchoniana*)

Observational field study	Aphid species studied	Fungus species found	Results	References
Occurrence in infected aphids on cereal crops	*S. avenae* and *R. padi*	*P. neoaphidis* and *E. planchoniana*	*P. neoaphidis* or *E. planchoniana* have a wide distribution and can develop epizootics	[54–60]
Occurrence in infected aphids in winter wheat fields with weeds	*S. avenae*	*P. neoaphidis* and *E. planchoniana*	Higher fungal prevalence in weedy plots than in herbicide treated plots	[61]
Occurrence in infected aphids on overwintering site, *Prunus padus*	*R. padi*	*P. neoaphidis* and *E. planchoniana*	Infections in *R. padi* in autumn much more common than in spring	[27]
Occurrence in soil in spring	*S. avenae*	*P. neoaphidis*	Inoculum present in soil samples, infective to *S. avenae* crawling on soil	[28]
Occurrence in infected aphids trapped from air	*S. avenae* and *R. padi*	*P. neoaphidis* and *E. planchoniana*	*P. neoaphidis* more common than *E. planchoniana*	[62]

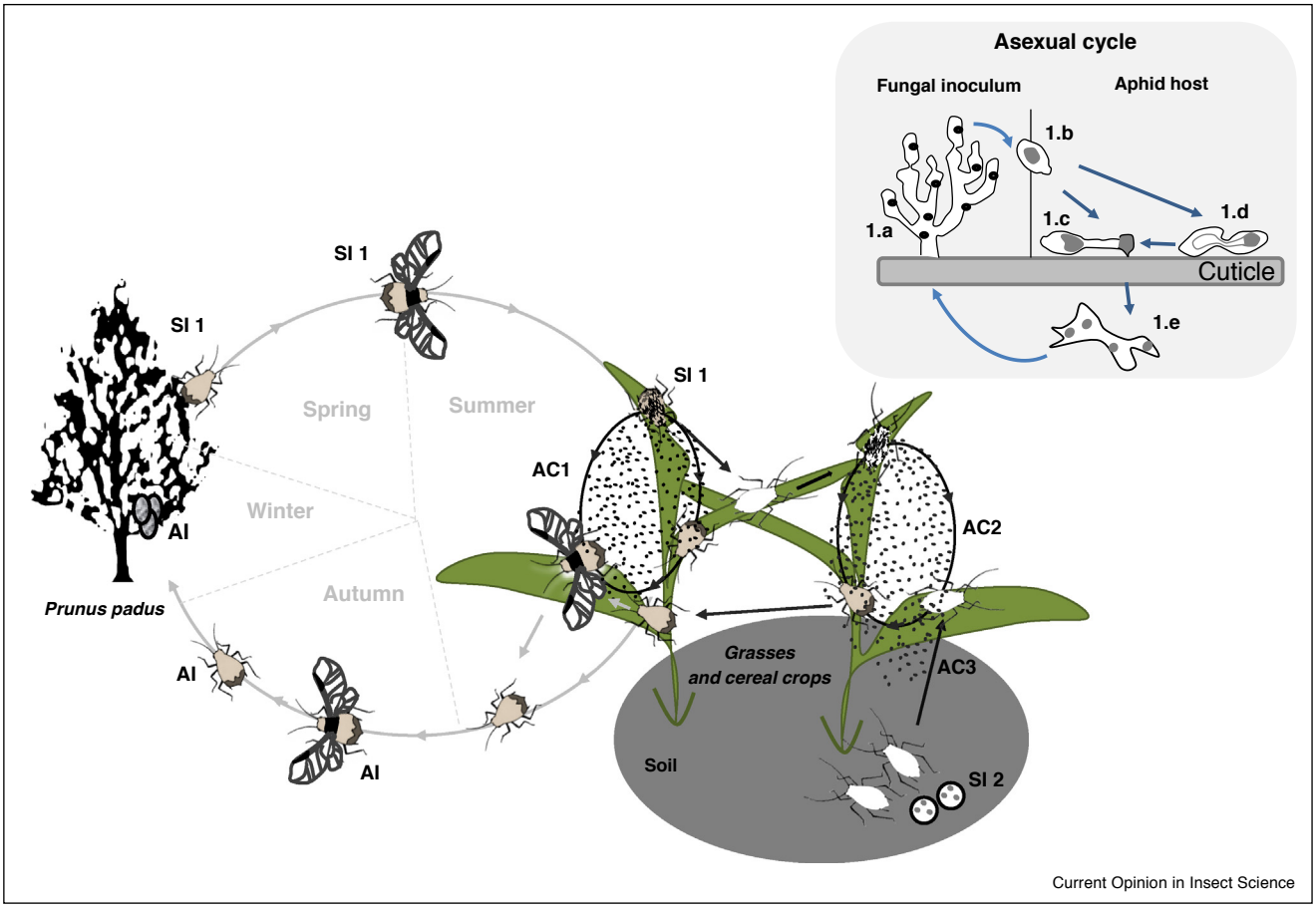
This might, in particular for obligate pathogens, result in a host driven divergence of genotypes, although this divergence may be specific to the host pathogen systems. In the case of the genus *Entomophthora*, a study [11] documented that the aphid pathogen *E. planchoniana* was much less prone to such divergence than the dipteran pathogen *Entomophthora muscae*. In other words, *E. planchoniana* from different host aphid species are rather similar with respect to genotype and we hypothesize that in a cereal field, *P. neoaphidis* and *E. planchoniana* are pathogenic to both aphid species (Table 1) and that transmission between hosts in cereals may take place (Table 2). The question is, whether one aphid species or one morph is in general more susceptible than the

Table 2

Experimental laboratory studies on factors influencing susceptibility in a host–pathogen system consisting of aphids in cereals (*Sitobion avenae* and *Rhopalosiphum padi*) and entomopathogenic fungi (*Pandora neoaphidis* and *Entomophthora planchoniana*)

Experimental laboratory study	Aphid species studied	Fungus species tested	Results	References
Aphid species	*S. avenae* *R. padi*	*P. neoaphidis,* several isolates	*S. avenae* more susceptible than *R. padi* but none of the isolates tested where conspecific with *R. padi*	[63]
Conspecific versus heterospecific hosts	*S. avenae* (conspecific) and *R. padi* (heterospecific)	*P. neoaphidis* and *E. planchoniana*	Conspecific host *S. avenae* more susceptible than heterospecific host *R. padi*. Fecundity was negatively affected in inoculated (but surviving) *S. avenae*	[37•,53]
Two heterospecific hosts	*R. padi* (heterospecific) and *Myzus persi-cae* (heterospecific)	*P. neoaphidis* (from *S. avenae*)	Heterospecific host *M. persicae* more susceptible than heterospecific host *R. padi*	[64]
Aphid morph	*S. avenae*	*P. neoaphidis*	Alate aphids more susceptible than apterous aphids	[40]
Aphid clone	*S. avenae*	*P. neoaphidis*	No effect of clones in susceptibility in laboratory experiments and in field data	[40,41]
Temperature	*S. avenae*	*P. neoaphidis*	Lethal time decreased with increasing incubation temperature, although not consistently. Increased temperature gave higher mortality but did not consistently affect lethal time or fecundity	[37•,65]
Fungal isolates	*S. avenae* *R. padi*	*P. neoaphidis* three isolates	No fungal isolate related difference in numbers of sporulating cadavers of *R. padi*. For *S. avenae,* a difference was found	[37•]

Figure 1



Life cycle of the insect pathogenic fungi from Entomophthorales (*Pandora neoaphidis* and *Entomophthora planchoniana*) in bird cherry-oat aphid *Rhopalosiphum padi* (with dark posterior) and grain aphid *Sitobion avenae* (without dark posterior). Dioecious holocyclic *R. padi* alternates between the winter host bird cherry (*Prunus padus*) and the summer hosts cereals and grasses.
Primary spring inoculum (**SI 1**) of *P. neoaphidis* or *E. planchoniana* will be brought via infected alates of *R. padi* upon migrating to the summer hosts. On the summer hosts, infected *R. padi* die and produce infective conidia, which are actively discharged. These conidia can infect alate or apterous *R. padi* and several asexual infection cycles (**AC1**) can occur during the cropping season. During autumn, *R padi* alates migrate to the winter host and infected individuals bring autumn inoculum (**AI**) to the winter host. If transmission of fungal disease from conspecific host *R. padi* to heterospecific host grain aphid *Sitobion avenae* or vice versa takes place in nature, such transmission may result in asexual infection cycles (**AC2**) in the heterospecific host and eventually back to conspecific host. Another source of spring inoculum (**SI 2**) is infective conidia produced by overwintering stages (most probably resting spores) in soil. This may in particular apply to *S. avenae*, which remains on grasses during autumn and winter and may become infected (**AC3**) during spring and initiate asexual infection cycles in the conspecific host *S. avenae.* We hypothesize that transmission to the heterospecific host *R. padi* can occur (**AC 3**), but probably with less success than transmission to the conspecific host (Table 2). Insert upper right shows infection process. Conidiophores (1.a) emerge through cuticle from killed host and primary conidia are forcibly discharged (1.b). Once the conidia land on the cuticle of a suitable aphid host, each will produce a germ tube that can penetrate the cuticle (1.c). Under unfavorable conditions, primary conidia may instead produce secondary conidia (1.d). Inside the aphid host, the fungus will develop as hyphal bodies (1.e), invade the host tissues, kill the host and finally produce conidiophores.

other, and whether the conspecific host (belonging to the same species as the inoculum source) is more susceptible than the heterospecific host (belonging to a different species than the inoculum source).

In northern Europe, the two main aphid species in cereals are the grain aphid *S. avenae* and the bird cherry-oat aphid *R. padi* from the family Aphididae (Hemiptera). They are serious pest insects in cereals and are two of the 14 aphid species considered the most important worldwide and share host plants in the Poaceae (grass) family, which includes crops like wheat [12]. In aphids, overwintering can be achieved by: diapausing eggs produced by mated sexual females in autumn, and/or persistent parthenogenetic viviparous females. When winters are cold, the first strategy is favored because sexual eggs are very cold-resistant [13]. *S. avenae* remains on grasses and overwinters as eggs or parthenogenetic viviparous females

on the stems, whereas *R. padi* migrates to its winter host bird cherry (*Prunus padus)* where it overwinters as eggs on the branches [12,14,15].

The anholocyclic life cycles of these two species of aphids during summer in cereal crops are similar but their niches differ. During the cropping season, *S. avenae* at first colonizes the underside of the leaves of cereal plants and later upper parts of the plant, for example the ears [16,17]. *Rhopalosiphum padi* shows another pattern: first, they position themselves on the cereal plant, but close to the soil surface. Later they colonize the more parts of the plant and position themselves mainly on the underside of the leaves [16,17]. Aphid populations in cereals are clonal during summer in Northern Europe, and some genotypes can be predominant throughout a growing season or year [18]. However, during only a week 20–60% of *S. avenae* colonies in a population may disappear and can be replaced by new colonies originating from airborne immigrants landing in the field [19]. Hence, the genetic structure of an *S. avenae* population may vary significantly throughout a growing season or between years and this may influence aphid resistance to fungal pathogens.

Pandora neoaphidis and *E. planchoniana* have been known as aphid pathogens since the 19th century [20]. They infect their aphid host by conidia (asexual spores) landing on and penetrating the aphid cuticle (Figure 1) initially developing inside the host as protoplasts/hyphal bodies [21]. Once the host is killed, the fungus breaks through the cuticle and produces primary conidia on conidiophores [22]. Primary conidia are actively discharged if conditions are favorable and initiate another infection cycle if they land on the integument of a suitable host, or they produce secondary conidia, which are also infective [23–25]. Thick walled resting spores [26] are produced in the dead host for winter survival. In autumn, infected alate *R. padi* migrate to the winter host, bird cherry, and may bring with them the pathogen to their overwintering site (Table 1), where conidia or resting spores are produced [27] (Figure 1). When *R. padi* eggs hatch in early spring, aphid nymphs may then become infected by overwintering fungal inoculum present on the bird cherry (S. Saussure., pers. obs.) and then probably transport it to the field via infected alates. For *S. avenae*, winter survival takes place in the cereal stubble or on grasses, and infected aphids will probably remain there and eventually drop to the soil surface. Inoculum of *P. neoaphidis* is therefore present on the soil surface in spring and can infect aphids exposed to soil and litter [28] (Figure 1 and Table 1). In Ref. [28], the authors provide a review on studies of winter survival of *P. neoaphidis*. Fungal inoculum may also be transported over long distances by infected alates (Table 1) or possibly also as air-borne conidia, and infect new individuals [29].

Host insect resistance against fungal pathogens

Insects have a complex hierarchy of defenses or resistance mechanisms that pathogens must overcome before a successful infection may occur. The main behavioral and physical barriers to infection are behavioral avoidance of the pathogen [30], morphological barriers to infection (cuticle, digestive system and tracheal system), or physiological responses to infection (distinguishing self from non-self or altered self, humoral responses, cellular responses, melanizaton, intracellular defenses) [31]. Aphids are not social insects and cannot perform social immunity against specialist fungi from Entomophthoromycotina like ants can do [32•]. However, aphid behavior may still be important. The different positions of *R. padi* and *S. avenae* on plants may be ecological traits leading to different susceptibilities. Differences in position on the plant are significant for insect pathogenic fungi, affecting their ability to target the host cuticle by their infection propagules. The more hidden the host, the lower the chances of spores landing on the cuticle and the lower the risk of infection. In arthropods, both attraction and avoidance of conspecifics infected with specialist entomopathogenic fungi have been noted in insect species [9,32•] and mite species [33]. The pea aphid, *Acyrthosiphon pisum*, seems to be indifferent to infected aphids and colonize new plants without regard to the presence of cadavers infected by *P. neoaphidis* [34]. An interesting behavioral resistance has been shown for milkweed aphid, *A. asclepiadis* [35]. Here, it is not the aphid itself having a behavior supporting resistance, instead ants (*Formica podzolica*) in the field quickly removed fungal killed aphids and in that way significantly lowered the possibilities of disease transmission among the aphids.

As almost all insect pathogenic fungi use the cuticle as their point of entry, the cuticle forms the first physical barrier for the pathogen to overcome. The resistance mechanisms in the cuticle may include both chemical compounds that inhibit germination of fungal propagule and/or hardness of the insect cuticle that inhibits penetration [31]. One study [36] aimed to discover fungal secretomes from field collected *S. avenae* and the authors discovered several fungal gene products involved with host cuticle penetration. The aphids themselves had, however, few genes involved in response to pathogen invasion. Even when the aphids received a high conidial dose, the host response by *S. avenae* was weak and probably of little significance in resistance.

A recent study [37•] found that *S. avenae* fecundity was reduced for aphids that were inoculated with but not killed by *P. neoaphidis*. This may be because some aphids escaped from becoming lethally infected, for example due to resistance responses. The loss in fecundity of infected but yet surviving *S. avenae* can be interpreted as energy losses of the host due to the immune response fighting the infection.

Aphids are polyphenic (multiple, discrete phenotypes that arise from a single genotype), and can exhibit different forms, or morphs, during the course of their seasonal life cycle. Among these, they produce apterae (lacking wings) or alate (with wings) adults in response to different conditions. Alates are probably subjected to higher disease pressure than apterae, because of their larger range of activity. Further, the energy cost of producing wings can be high and the energy allocated to resistance to pathogens may be lower. The high susceptibility of alate aphids is an advantage for the fungus, since migrating aphids may disperse the pathogen (Tables 1 and 2). The suggested advantage in fungal dispersal via alates finds further support in a study [38], which documented that infection of *A. pisum* with *P. neoaphidis* resulted in the production of a higher proportion of alates. In a study on *A. pisum* [39•] the authors showed that alates were more susceptible than apterae to infections by *P. neoaphidis*, fitting with the hypothesis of energy limitation. Similarly, in the case of *S. avenae* and *P. neoaphidis*, a bioassay study [40] showed that alates were more susceptible. Because of the migrating alates, cereal fields can contain several clonal lineages of aphids with potential differences in susceptibility, although results so far (Table 2) do not suggest major differences. Results from a two-year study in a winter wheat field in Denmark suggests that neither *P. neoaphidis* nor *E. planchoniana* affected the clonal distribution of *S. avenae* [41].

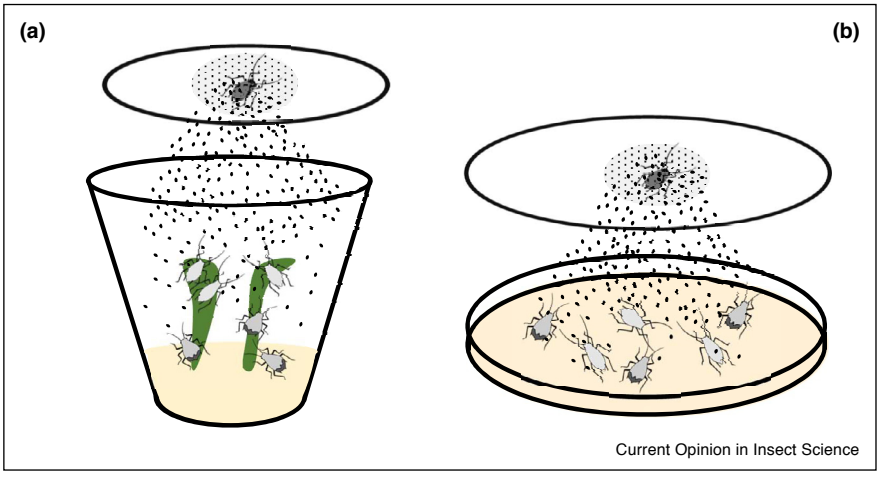
Role of symbionts

Aphids harbor many bacterial species, including endosymbionts [42,43•], and we may speculate if they assist in protecting aphids from fungal infections. The obligate symbiont *Buchnera aphidicola* was reported from aphids, including *S. avenae* and *R. padi* [43•,44]. A few facultative endosymbionts have been reported from *S. avenae*, namely *Hamiltonella defensa*, *Regiella insecticola* and *Serratia symbiotica* [43•,45–47] while no facultative endosymbionts to our knowledge are reported from *R. padi*. Five species of facultative endosymbionts (*R. insecticola*, *Spiroplasma*, *Rickettsia*, *Rickettsiella* and X-type) have been identified as conferring significant protection to *A. pisum* against *P. neoaphidis* [48,49]. They can reduce the mortality of *A. pisum* when infected and decrease *P. neoaphidis* sporulation from the killed aphids [48]. In a study discriminating between intrinsic (genetic) resistance and extrinsic (symbiont conferred) resistance to *P. neoaphidis* across host, it was shown that *A. pisum* biotypes with a higher probability of carrying protective endosymbionts also have a higher intrinsic resistance [50••]. Authors therefore did not find evidence that aphid carrying protective endosymbionts lose (or 'chose to outsource') their own genetic resistance.

Methodological challenges to compare aphid susceptibility

Pandora neoaphidis and *E. planchoniana* conidia can be produced from dead aphid cadavers, but due to their sticky nature [22,24] they cannot be mixed with water to obtain a predefined concentration. Therefore, infection bioassays to study pathogenicity and virulence have to use methods mirroring their biology by allowing one or more dead infectious aphid cadaver(s) to discharge infective conidia to a cohort of aphids [51,52]. Since a predefined conidia concentration cannot be obtained, various

Figure 2



Bioassay procedure to document pathogenicity and virulence of entomophthoralean fungi to grain aphid *Sitobion avenae* and bird cherry-oat aphid *Rhopalosiphum padi*. Infective conidia are discharged from one or more aphid cadavers (conspecific or heterospecific host) onto exposed aphids.

(a) Aphids are allowed to position themselves on their host plant. Aphids which prefer to position themselves in lower parts of plants (*R. padi*, with dark posterior) may to some extent benefit from this position in comparison with aphids that normally position themselves with more exposure to fungal conidia (*S. avenae*, without dark posterior).

(b) Aphids do not have access to their host plant during exposure, so host behavioral effects are avoided.

methods to count conidia on cover slips before and after exposure or during exposure have been applied [52]. In studies comparing virulence toward two aphid species, the challenge is to apply the same dose to each aphid species. Such bioassays may in addition take aphid host behavior into account. An example of a bio-assay, where *S. avenae* and *R. padi* are allowed to position themselves on a host plant according to their biological preferences is depicted in Figure 2a; *R. padi* will position themselves lower than *S. avenae*. Results suggest that a conspecific host *S. avenae* is more susceptible than the heterospecific host *R. padi* [53] (Table 2). In that study, aphid behavior is taken into account and therefore results can more readily be extrapolated to real field conditions. Another set up is to leave out the position behavior of aphids by placing *S. avenae* and *R. padi* in the same Petri dish without a host plant during exposure (Figure 2b), and in that case both aphid species will receive the same dose. A study using this design [37•] proved that also is this case the conspecific host was more susceptible than the heterospecific host. It seems therefore that the different susceptibilities between the two aphid species may include an immune component and a behavior component. Further studies testing the susceptibility of conspecific versus heterospecific aphid hosts may elucidate more in depth the importance of the different components.

Conclusion

In this cereal host–pathogen system, aphid behavior, aphid morph and fungal isolate are important factors governing host susceptibility and resistance. Hosts' immune responses seem weak in *S. avenae* and of limited importance, but further studies are needed to confirm if this also is the case in *R. padi*. When comparing susceptibility of fungal isolates from the conspecific host *S. avenae*, *S. avenae* was more susceptible than the heterospecific host *R. padi* but fungi conspecific to *R. padi* and heterospecific to *S. avenae* are still left to be tested. Alate *S. avenae* and *R. padi* seem to be more susceptible to *P. neoaphidis* than apterae fitting with the hypothesis of energy limitation (cost spend in nymphal stages on the development of wings versus costs spend to ensure a high level of immune response) that we see for resistant but less fecund *S. avenae*. Symbionts may play an important role in aphid resistance to fungal pathogens and other natural enemies, but comparative studies on the influence of symbionts on the resistance to specialist fungal pathogens in *S. avenae* and *R. padi* are lacking.

Conflict of interest statement

Nothing declared.

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