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REVIEW

Direct and host-mediated interactions between *Fusarium* pathogens and herbivorous arthropods in cereals

Running title: Arthropods in FHB and FER epidemiology

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Abstract

Fusarium head blight (FHB) and Fusarium ear rot (FER) diseases of cereal crops are significant global problems, which cause yield and grain quality losses and accumulation of harmful mycotoxins. Safety limits have been set by the European Commission for several Fusarium mycotoxins, and mitigating the risk of breaching these limits is of great importance to crop producers as part of an integrated approach to disease management. Here we review current knowledge regarding the role of arthropods in disease epidemiology. In the field, diseased host plants are likely to interact with arthropods which may substantially impact the disease by influencing spread or condition of the shared host. For example, disease progress by *Fusarium graminearum* can be doubled if wheat plants are aphid-infested. Arthropods have been implicated in disease epidemiology in several cases and the evidence ranges from observed correlations between arthropod infestation and increased disease severity and mycotoxin accumulation, to actual experimental evidence for insect infestation causing heightened pathogen prevalence in hosts. *Fusarium* pathogens differ in spore production and impact on host volatile chemistry, which influences their suitability for arthropod dispersal. Herbivores may allow secondary fungal infection after wounding a plant or they may alter host susceptibility by inducing changes in plant defence pathways. Post-harvest, during storage, arthropods may also interact with *Fusarium* pathogens, with instances of fungivory and altered behaviour by arthropods towards volatile chemicals from infected grain. Host-mediated, indirect pathogen-arthropod interactions are discussed alongside comprehensively reviewing the evidence for direct interactions where arthropods act as a vector for inoculum.

Keywords: Fusarium head blight, Fusarium ear rot, host-pathogen-herbivore interactions, disease epidemiology, volatiles, cereals

Introduction

Fusarium head blight (FHB) is a disease that affects small-grained cereals and is caused by a complex of up to 17 *Fusarium* and 2 *Microdochium* fungal species (Parry *et al.*, 1995; Glynn & Edwards, 2010). FHB is a relatively well studied plant disease in terms of virulence (Goswami & Kistler, 2004), management and crop resistance (Bai & Shaner, 2004 , Rudd *et al.*, 2001 , Buerstmayr *et al.*, 2009), mycotoxin accumulation (Logrieco *et al.*, 2003) and other aspects of its biology, ecology and epidemiology, but information on the interaction of FHB with arthropods is lacking. Here we provide a review of what is known about how insects and mites may directly or indirectly facilitate Fusarium diseases. Although scientists are often confined to narrow disciplines focussing on insects, plants or pathogens, this separation does not occur in nature where insects and pathogens are exposed to each other and can influence each other either directly or indirectly by altering the condition of a shared host plant. FHB, and Fusarium ear rot (FER) which affects maize, are significant global problems. By understanding and establishing control over arthropod interactions that exacerbate disease or enhance host susceptibility the impact of FER has been demonstrably reduced, and this could be utilised in FHB control also.

FHB infections result in decreased grain yield, quality and production of mycotoxins in grain by *Fusarium* spp. which are harmful to the health of animal consumers (Marin *et al*., 2013). FHB is present in most cereal growing regions in the world (Parry *et al.*, 1995; Gilbert & Haber, 2013) and no variety of cereals is completely resistant to FHB (Wegulo *et al*., 2015). Visible symptoms of FHB appear as water-soaked lesions on glumes, then pink-orange sporodochia and mycelia and black fruiting bodies may develop, followed by discolouration and premature bleaching of spikelets (Bushnell *et al*., 2003; McMullen *et al*., 2012). Harvested grains may have a reduced protein content (Eggert *et al*., 2011) and can appear chalky-white and shrivelled

63 or without outward symptoms (Goswami & Kistler, 2004). Several different species can
64 contribute to disease on any one host, and different causal species induce a varying severity of
65 visible symptoms, ranging from aggressive disease onset by *F. culmorum* and *F. graminearum*
66 to infrequent symptom development by *F. poae* and mostly cryptic infection by *F. langsethiae*
67 (Imathiu *et al.*, 2013).

68 The largest group of mycotoxins produced by *Fusarium* species are trichothecenes, which act
69 on ribosomes to inhibit protein synthesis and cause direct damage to intestines upon ingestion
70 (D'Mello *et al.*, 1999). Trichothecenes are sesquiterpenoid secondary metabolites (Lattanzio
71 *et al.*, 2009) which can be divided into different classes based on chemistry and mode of action.
72 Type A trichothecenes are the most toxic to animals, including T-2 and HT-2 (Paciolla *et al.*,
73 2004). Type B trichothecenes include nivalenol (NIV) and deoxynivalenol (DON), of which
74 the concentration in grain is limited by European law (Anon, 2006). Reports indicate that while
75 mycotoxin levels do not commonly breach legal limits (Streit *et al.*, 2012; Marin *et al.*, 2013;
76 Belakova *et al.*, 2014), different geographic areas are at a higher risk of approaching these
77 limits than others under favourable weather conditions (Placinta *et al.*, 1999; Cui *et al.*, 2013).
78 Different acetylated forms of DON and NIV, varying in their toxicity, phytotoxicity (Suzuki &
79 Iwahashi, 2014) and geographical distribution (Gale *et al.*, 2011), are produced by different
80 pathogen strains. FER of maize is typically caused by *Fusarium verticillioides* (formerly *F.*
81 *moniliforme*). FER infection leads to the production of fumonisin mycotoxins, which are
82 associated with toxicity syndromes in animals (Yazar & Omurtag, 2008). Control of FHB and
83 FER diseases is important not only to prevent physical degradation of the crop and the
84 associated losses in yield, but to prevent mycotoxins from accumulating in the grain and the
85 economic losses associated with breaching safety limits.

Overview of Fusarium disease epidemiology

The most dominant species to cause FHB across temperate regions is *F. graminearum* sensu stricto which, along with *F. culmorum* and other species from the *F. graminearum* species complex, causes the accumulation of the mycotoxins deoxynivalenol (DON) and nivalenol (NIV) in infected grain. These species prevail in climates with mild to warm summer temperatures, alongside *F. avenaceum* and *F. poae.* Both pink and red ear rot diseases of maize (which will both be referred to as FER in this review), which are primarily caused by *F. verticillioides* and *F. graminearum* respectively, also prevail in warm climates (Munkvold, 2003). Colder maritime climates, such as in Northern Europe, are more greatly affected by the non-toxigenic *Microdochium nivale* and *M. majus* (Xu *et al*., 2008; Nielsen *et al*., 2011), and the T-2 and HT-2 toxin producers *F. sporotrichioides* and *F. langsethiae* (Fredlund *et al*., 2013). *F. poae* and *F. langsethiae* have been described as early season colonisers (Sturz & Johnston, 1985; Parikka *et al*., 2012), that are capable of infecting hosts prior to anthesis (GS59, Zadoks *et al*., 1974), possibly facilitating the later colonisation of cereal heads by other species such as *F. graminearum* and *F. culmorum.* In Asia and southern USA other species from the wider *F. graminearum* species complex, particularly *F. asiaticum,* are of more prevalent (Suga *et al*., 2008; Qu *et al*., 2008; van der Lee *et al*., 2015).

In addition to FHB and FER, *Fusarium* spp. can also cause seedling blight and foot rot as part of the Fusarium disease complex on cereals, with different species prevailing in different geographical locations and on different host crops. Wheat is most susceptible to infections of FHB during mid-anthesis, GS65 (Miller, 1994) via ascospores or conidia conveyed directly onto the heads. *F. graminearum* infects wheat plants via the anthers, through stomata or at the base of the glume, then grows through the caryopsis, floral bracts to the rachis and into neighbouring spikelets (Bushnell *et al*., 2003) with the mycotoxin DON produced early in the

colonisation process (Boenisch & Schaefer, 2011) which acts as a virulence factor, facilitating fungal progression through the spike (Jansen *et al*., 2005). Inoculation experiments using *F. langsethiae* on oats also found that direct panicle-applied conidia produced FHB symptoms (Divon *et al.,* 2012), but it was also found that injection of spores into the boot achieved greater pathogen DNA at harvest than inoculation during flowering (Opoku *et al*., 2013). The latter method of inoculation is unlikely to represent an infection mechanism that can be achieved in the field, although it supports the potential importance of wound sites for example through insect feeding on hosts tissues as a risk factor for increased success of these pathogens. Further support for the role of wound sites in infection of wheat with *F. langsethiae* comes from detached leaf assay experiments which showed that artificial wounds were necessary for *F. langsethiae* to cause lesions on leaf samples (Imathiu *et al.,* 2010). Recently it has been demonstrated that wounding of glumes enhanced infection of wheat by *F. langsethiae* and lead to increased symptom development and pathogen DNA accumulation compared to unwounded controls (Ajigboye *et al*., 2016) Damage caused by arthropod feeding on host plants could potentially provide the wound sites required for colonisation by this otherwise weak pathogen of wheat; however this interaction has not been explored in the current literature.

Upon infection, *Fusarium* pathogens carry out a phase of biotrophy upon their host plants prior to switching to necrotrophy on tissues and crop residues (Goswami & Kistler, 2004) where infected material becomes a potential source of inoculum for the next crop in rotation. Figure 1 shows the cycling processes of fungal inoculum types in small-grain cereals, and how each disease in the Fusarium disease complex provides inoculum for the next. *Fusarium* infected heads produce infected seed and can result in Fusarium seedling blight (FSB), conidia arising from FSB can give rise to Fusarium foot rot, and conidia at the stem bases can be moved via rain splash up to the ears via the canopy layers to initiate FHB. While FHB is considered a monocyclic disease (Fernando *et al*., 1997; Kohl *et al*., 2007; Landschoot *et al.,* 2011) evidence

suggests that multiple, and potentially distant, inoculum sources may contribute to the level of starting inoculum, as the population structure of *Fusarium* spp. in mature ears does not always reflect that in crop residues or soil (Landschoot *et al*., 2011). In some cases in a Belgian survey of wheat fields, the *Fusarium* spp. population structure on ears was more similar to that on weeds, indicating that weeds are likely to be an important source of primary inoculum for some FHB outbreaks. Furthermore, this study also revealed that in one season (2008-9), in 50% of the locations *F. poae* was isolated wheat heads, but not found at the start of the season on any of the primary inoculum sources tested from the site. The increase in species diversity over the growing season indicates that inoculum can arrive throughout the season from distant sources either by wind or perhaps through insect dispersal.

The involvement of arthropods in the dispersal of inoculum in small grained cereal crops (Parry *et al.*, 1995) and maize (Munkvold, 2003) has been proposed previously although the exact role played by arthropods in Fusarium disease epidemiology is not well understood and only studied in a limited number of species-specific situations. For example, there are documented cases where insects and mites have been observed to transmit *Fusarium* inoculum between host plants (Kemp *et al*., 1996; Sobek & Munkvold, 1999), and where the activity of pests is correlated with infection by *Fusarium* spp. (Mongrain *et al.*, 1997; Saladini *et al*., 2008). A recent review (Gagkaeva *et al*., 2014) focussed on the potential positive or negative interferences between arthropods and specific *Fusarium* species, with more aggressive pathogens being described as antagonists to arthropods and weaker pathogens offering potential symbiotic or commensal relationships, however the significance of these interactions on host susceptibility, FHB disease progress and/or mycotoxin accumulation were not explored. There are differences in the size and shape of conidia produced by *Fusarium* species; *F. verticillioides*, *F. poae* and *F. langsethiae* produce small, almost spherical microconidia, whereas *F. graminearum*, *F. culmorum*, *F. avenaceum* among others produce larger boat-

shaped septate macroconidia (Leslie & Summerell, 2006). The reduced size of the conidia produced by *F. verticillioides*, *F. poae* and *F. langsethiae* may make them more compatible for transportation by wind or arthropods to move greater distances between sources and host sites.

This review aims to discuss arthropod interactions in the epidemiology of Fusarium disease in cereal crops, which encompasses both FHB in small grained cereals and FER in maize. The interactions studied include those with insects and mites acting as vectors of inoculum, causing damage and weakening of the host so as to increase the severity and infection opportunities of *Fusarium* species, and as potential feeders on both fungi and grains during post-harvest storage. The chemical ecology that governs host-arthropod interactions has also been studied in a number of these cases, and gives an indication for the role of the pathogen in altering insect-host relationships.

Observational studies show correlation between FHB or FEB and arthropods

The majority of studies that link arthropod activity to FHB or FER diseases have documented observed correlations in their incidence, and those described are summarised in Table 1. The orange wheat blossom midge (OWBM), *Sitodiplosis mosellana*, has been investigated as a putative vector of FHB pathogens. Contaminated wheat crops in Canada were found to have midge infestations (Couture *et al*., 1995), and a more extensive study of 14 field sites of different districts in Quebec showed a positive correlation ($R = 0.67$, $P = 0.001$) between the number of OWBM larvae per wheat spike and per spikelet with infection by *F. graminearum*, but not by other species of *Fusarium* (Mongrain *et al*., 1997). However, the number of *F. graminearum* damaged grain was low, with mean values for each site ranging from 0 – 4%, despite midge incidence in spikes ranging from 2 – 98%. Study of OWBM physiology revealed the presence of structures that could carry conidia on adult females (Mongrain *et al*., 2000).

These are features common to other groups within the Cecidomyiidae which can also feed on fungi (Borkent & Bissett, 1985). Despite the original hypothesis of vector activity by OWBM, there is no formal description in the literature of whether the correlation between OWBM and *Fusarium* spp. is due to transmission of the pathogen by the insects, increased host disease following damage from larval feeding, recruitment of insects to infected hosts so as to feed on the fungal material, or in fact whether there was any causation associated with the correlation at all.

In wheat, FHB severity has been associated with aphid infestation of host crops. Field trials in India measured the effect of insecticides on the incidence and severity of FHB. Insecticides were applied, targeting aphid populations; the number of aphids on treated plots was successfully reduced. In treated plots, FHB incidence and severity were also significantly reduced (Bagga, 2008) showing a correlation between aphid and FHB incidence.

A lot of attention has been paid to the interaction between Lepidoptera such as the European corn borer (ECB), *Ostrinia nubilalis*, and *F. verticillioides* infection of maize. Following chemical treatment of maize with the pyrethroid insecticide lambda-cyhalothrin at 0.02 $kg.ha^{-1}$ at 7 days after peak European corn borer (ECB) flight, a significant reduction of FER severity (29%) was observed. Following early sowing of maize in addition to insecticide treatment, severity was reduced by up to 67% (Blandino *et al*., 2008). The consequence to the host of the association between ECB and *F. verticillioides* has been measured in terms of the mycotoxin levels amassed in grain. Field experiments were conducted over a 7-year period in Italy to test the use of two different pyrethroid insecticides, deltamethrin at 0.013 kg. ha^{-1} and lambda-cyhalothrin at 0.019 kg. ha^{-1}, in their effectiveness for controlling ECB and the effect on FER in maize (Saladini *et al*., 2008). In one season where insecticide treatment failed to reduce ECB damage there was no reduction in FER either, showing that the insecticides have no direct effect on the disease. In the seasons with effective ECB control, the levels of the fumonisins

B_1 and B_2 were reduced on average by 75% through the use of insecticide. The infection process of *F. verticillioides* is greatly assisted by insect activity, and insecticides have been shown to be more effective than fungicides in reducing fumonisin levels (Blandino *et al*., 2009). Furthermore, *Bt*-maize which has lower insect damage has been shown to have lower mycotoxin levels (Bakan *et al.*, 2002; Bowers *et al*., 2014). The mechanism appears to be a reduction in secondary infection when there is less insect damage because insect feeding damage can provide an entry point for disease. *Fusarium* fungi that make toxins such as fumonisin B enter through holes made by caterpillars in the cob or stem in non-GM maize.

The incidence of thrips, *Frankliniella occidentalis*, on maize ears has also been correlated with FER caused by *F. verticillioides*. Increased FER severity and fumonisin B_1 concentrations were found in field samples with increased thrips infestation in several sites in southern USA (Parsons and Munkvold, 2010; 2012). Fumonisin B_1 contamination was more strongly correlated with the number of thrips per ear (R = 0.89) than the amount of Lepidopteran feeding damage (R = 0.34). Visibly mouldy ears were also more strongly correlated with thrips frequency (R = 0.78) than with the frequency of Lepidopteran feeding damage (R = 0.37) (Parsons & Munkvold, 2012). Additionally, thrips have been implicated in the development of silk-cut symptoms in maize, and by doing so facilitated FER infection (Parsons & Munkvold, 2010). These correlative studies of thrips show that Thysanoptera pose a taxonomically diverse threat to increased FER in maize in addition to that of Lepidoptera, and as such supports the argument for the control of insects in maize FER management strategies.

Direct interactions: Arthropods as potential vectors of *Fusarium* inoculum

Direct interactions between arthropods and FHB or FER pathogens potentially involve insects or mites vectoring fungal spores. There are few documented cases of insects or mites acting as vectors of FHB and FER pathogens, and in the cases that have been studied the nature of the

vector activity and the relative importance of the arthropod-pathogen association is far removed from the close-knit associations to insect vectors of pathogens such as viruses and phytoplasmas. In such cases, insect transmission is the primary dispersal mechanism, and the pathogens may benefit from propagative transmission whereby the pathogen replicates inside the vector. Although a number of studies have found increases in FER with insects (Attwater & Busch, 1983; Windels et al., 1976; Farrar & Davis, 1991; Darvas et al. 2011; Dowd 2004) they have not definitively shown that this is due to vector activity and not due to secondary infestation after insect damage or other preconditioning of the shared host plant. Incidences of transmission of *Fusarium* inoculum by arthropods reported thus far are restricted to the carriage of fungal material on the external surfaces of insects or mites, and therefore the carrying capacity of the vectors is determined by the availability of fungal material on the surfaces of host plants and the size and surface type of the arthropod bodies. This implies that the life cycle and timing of the arthropod involvement with the host plant relative to the infection process of the pathogen needs to be aligned for insect or mite transmission to be possible. That said, while control of relevant arthropod activity on high risk crops might offer only partial control of FHB or FER disease, any mitigation of the risk of breaching mycotoxin safety limits ought to be considered in FHB and FER management plans.

The association of ECB with FER in maize is well studied (Munkvold, 2003). ECB larvae are known to burrow into the stalks and ears of maize plants, causing large amounts of damage to the host tissues. The first generation of larvae make initial attacks on host plants, but the second generation are the most relevant in FER epidemiology as they emerge during ear development. Emerging larvae have been described to be able to act as vectors of *Fusarium verticillioides* inoculum, bringing conidia upwards from leaf surfaces to the developing ears and the site of ear infection (Sobek & Munkvold, 1999). However in glasshouse experiments in this study, larvae-free controls still became infected at a low incidence, so it could be argued that the insect

attack on the host merely increased the host susceptibility to the disease, leading to the increased incidence in plants treated with ECB larvae. It has not been suggested that these insects can introduce inoculum from distant sources, and as such ECB is only described as a vector on a local scale. From field trials conducted in the same study, larvae that were artificially coated with a strain of *F. verticillioides* and placed on leaf axils were able to transmit that strain to maize ears, which supports the hypothesis that the external surfaces of larval bodies are able to carry inoculum to susceptible tissues, although the acquisition of the inoculum has not yet been satisfactorily demonstrated.

In addition to ECB, several other insects have also been associated with the epidemiology of *F. verticillioides* in maize. These include western flower thrips, western bean cutworms (Bowers *et al*., 2014), sap beetles and corn rootworm beetles; with sap beetles and rootworm beetles having been described to commonly carry *F. verticillioides* and *F. graminearum* spores (Munkvold, 2003). Furthermore, sap beetles were shown to be attracted to the volatile chemical emissions of maize plants infected with *F. verticillioides* (Bartelt & Wicklow, 1999; Munkvold, 2003), indicating compatibility between potential insect vectors and infected hosts, thus revealing a possible mechanism for the recruitment of insects that may enhance the dispersal of *Fusarium* inoculum. Increased populations of both Lepidopteran stem borers and Coleopteran beetles were observed on maize infected with *F. verticillioides* compared to uninfected plots (Cardwell *et al*., 2000), although the authors here note that this increased level of infestation may not be due to attraction of the insects but rather due to improved survival on the infected hosts.

In small-grained cereals, there are fewer reports of arthropods acting as vectors for *Fusarium* inoculum. One such report is that of *F. poae*, which similarly to *F. verticillioides* produces mostly microconidia (Leslie & Summerell, 2006). Mites, *Siteroptes avenae*, were shown to transmit *F. poae* inoculum. Mites were fed from cultures on agar plates placed in open petri

dishes between rows of wheat plants at ear emergence. Up to 6 symptomatic spikelets per ear were observed after 3 weeks (Kemp *et al*., 1996). Light microscopy also revealed the presence sac-like structures on female mites concluded by the authors to be sporothecae containing *F. poae* microconidia. This study is limited in that the inoculum source was not from an infected host such as infected seedling leaves or a realistic reservoir of inoculum such as crop debris. Rather the inoculum was from a fungal colony, which presumably would be a much denser source of inoculum than on living or decaying plant material as would occur in the field, so does not demonstrate a realistic infection route in nature, although it demonstrates that mites have the carrying capacity to deliver inoculum to new hosts when sufficient inoculum can be acquired. *F. poae* infection of cereals is favoured by warm and dry environmental conditions, for which insect and mite activity is also favoured.

In an attempt to demonstrate the capacity of OWBM to carry *Fusarium* spores, midge samples collected from the field were washed and the washings plated onto antibiotic amended agar (*pers. comms.*, Ray, 2010). *Fusarium* spp. were successfully grown and identified to be *F. oxysporum, F. langsethiae* and *F. poae*. However, the success rate of transmission of this fungal material to new hosts was not examined, and while correlations of OWBM and FHB incidences have been reported (described above), evidence of insect transmission by OWBM is lacking.

Fungivory

The capacity of arthropods to alter the disease impact caused by *Fusarium* spp. after harvest has been investigated in several species-specific studies. Studies report fungivory of *Fusarium* species by insects and mites, for example by psocids, which are able to feed on *Fusarium poae* and *F. sporotrichiodes* (Mills *et al*., 1992). Mites, *Tyrophagus putrescentiae,* are able to feed on several species of *Fusarium* reared on oatmeal agar and *F. poae, F. verticillioides, F. culmorum* and *F. avenaceum* from inoculated barley grain, but two other mite species *Acarus*

siro and *Lepidoglyphus destructor* experienced negative rates of growth on the *Fusarium* feeding substrates (Nesvorna *et al*., 2012). In low abundances, *T. putrescentiae* have been reported to be able to transmit *F. poae* inoculum from fungal cultures to stored barley grain (Hubert *et al*., 2014) as seen by the detection of *F. poae* operational taxonomic units in sampled DNA. When the pest pressure was increased, the fungus was considered to have been too heavily grazed by the mites to achieve inoculum transfer that could be detected by amplified cloning. DON levels were also raised in both pest pressure treatments, and although the authors cite this as evidence for fungal transmission, *F. poae* is not a known producer of DON (Thrane *et al*., 2004) and so this increase is likely to be due to the increased activity of other toxigenic fungi in the grain as the substrate used was not autoclaved. While further work is required to determine if mites would be capable of transmitting the fungus from a more realistic inoculum source, i.e. from infected grain, these studies lend support for the need to control insect populations and grain residues that can act as inoculum reservoirs in grain storage sites, or risk contamination of grain with mycotoxin producing fungi such as *F. poae* and also potentially increasing the mycotoxin output by *Fusarium* spp. that infected grain prior to harvest.

Mycotoxins produced by toxigenic fungi on stored grains have been tested for toxicity on certain insect species (Magan *et al*., 2003). Arthropods that are not harmed by or are able to tolerate the toxins are considered more compatible dispersal agents for the fungi and long-term herbivores of the storage products. DON and T-2 were found not to be toxic to the confused flour beetle, *Tribolium confusum* (Wright *et al*., 1973). Mites *Tyrophagus putrescentiae* were also found to be able to feed on DON without harm (Hubert *et al*., 2014) but previous studies showed them to be sensitive to T-2 and zearalenone (Rodriguez *et al*., 1979). Screening common storage pests for toxin sensitivity may be a useful step in understanding the importance of pest pressures and mycotoxin contamination in stored grain.

Indirect interactions between FHB or FER and arthropods

Indirect interactions of arthropods and FHB or FER pathogens have consequences that are relevant to the disease process in such a way that is mediated by the host plant. In cases where dispersal of the fungus is not enhanced by arthropod activity, the effects of arthropod activity on the host can still increase the susceptibility of the host to the disease (Munkvold, 2003; Drakulic *et al*., 2015). This can include damage allowing secondary fungal infection because wounded plant tissue is easier to enter, changes in volatile emissions from disease plants that alters arthropod behaviour, or pre-conditioning of the host plant by suppression of plant defence pathways.

Host weakening by arthropod activity

Synergy between the insect and fungal host attackers is thought to have a modest impact on *F. graminearum* epidemiology in maize (Munkvold, 2003). *F. graminearum* can infect maize systemically or through the silks and neither of these infection routes rely on insect involvement, but in addition to these routes the pathogen can enter the host through wound sites created by insect activity. The significance of this route in host acquisition of the pathogen varies depending on environmental and agronomic factors, but reduction in DON of up to 59% was recorded in *Bt*-maize hybrids which resist insect feeding, in comparison to non-transgenic hybrid plants (Schaafsma *et al*., 2002; Munkvold, 2003). This shows that insect activity can promote *F. graminearum* infection and accumulation of DON in maize, although the circumstances under which insect involvement is most likely to impact on the disease has not been elucidated for *F. graminearum*. Insect wounding has also been linked to the increased prevalence of *F. verticillioides* in maize, with attention being drawn to *Helicoverpa zea*, the corn earworm (Dowd, 2000; Clements *et al*., 2003) as populations appear to vary greatly in

sensitivity to *Bacillus thuringiensis*, and as such can continue to wound Bt maize hosts, leading to failure to control FER despite adequate ECB control.

The effect of aphid feeding on plant hosts in terms of consequences of disease has also been measured in terms of mycotoxin accumulation in the host. When aphids, *Rhodopsium padi,* were fed on wheat leaves whilst ears were inoculated with *F. graminearum* an increase in DON was observed in infected grain compared to aphid-free controls (Liu *et al*., 2005). This implies that systemic changes to the host biochemistry are induced upon aphid feeding that leaves the host less able to withstand infection by the fungus. Furthermore, recent findings have examined the interaction between English grain aphids, *Sitobion avenae*, and *F. graminearum* on wheat and found that the combined effect of both plant attackers leads to increased disease severity and mycotoxin accumulation (Drakulic *et al*., 2015). The outcome of the interactions between pest and pathogen in this case also differed depending on the specific timing of the interaction, with infestation of aphids in advance of fungal infection of the hosts bringing about a rise in the level of pathogen DNA at maturity compared to when pathogen infection preceded aphid infestation.

As described earlier, several other examples of correlations between insect incidence and FHB or FER severity have also been observed. One possible mechanism to explain the increase in disease severity and mycotoxin contamination in hosts with insect infestation is suppression of plant defence by insects. Basal resistance to FHB is thought to be mediated by the salicylic acid (SA) pathway (Makandar *et al*., 2012). In contrast, if attack by insects on plant hosts upregulates the jasmonic acid (JA) pathway, which has negative crosstalk with SA-pathway (Bostock, 2005; Cipollini *et al.*, 2004) susceptibility to FHB could well be increased. A key factor that determines the outcome of the defence response by the host plant is the nature of the feeding behaviour of the arthropod attacker. ECB larvae are chewing insects that cause visible wounding to the host which upregulate JA- and wound-dependent plant defence

responses, whereas aphids that feed for a prolonged time on phloem sap cause minimal cellular damage and upregulate different defence pathways including SA- and JA/ethylene-dependent processes (Walling, 2000). Furthermore, insect-produced molecules can alter the host-defence response: chewing insects transfer salivary excretions to the host in the form of foregut regurgitants; aphids and related sap-feeding insects secrete both thick gelling sheath saliva and watery saliva around and through the stylet mouthparts (Dixon, 1973). This can introduce potential elicitors to the host that can upregulate plant defences, but also present the opportunity for insect-produced signalling molecules to be injected into the host plant so as to interfere with the host defence response. Aphids and other phloem feeders in particular have been described to produce effector molecules (Bos *et al*., 2010) that deceive the host into disabling defence responses (Thompson & Goggin, 2006; Walling, 2008) leaving the host increasingly susceptible to secondary attack.

Volatile chemical interactions between infected hosts and arthropods

The frequency of host-mediated interactions between pests and pathogens can be influenced by the volatile chemistry of the host plants (Gagkaeva *et al*., 2014). Infected hosts may emit different volatile chemicals into the environment than healthy hosts, and these chemical signals may be perceptible to proximal arthropods (Drakulic *et al*., 2015). As a result of perceiving volatile chemicals, arthropods may alter their behaviour towards infected hosts and as a result alter the course of the disease (Mayer *et al*., 2008). The study of the chemical ecology of species-specific interactions is one way to identify potentially important relationships between insect herbivores and FHB or FER pathogens.

The behaviour of the cereal leaf beetle, *Oulema melanopus*, is influenced by volatile chemical emissions from maize plants inoculated with a mixture of four *Fusarium* species: *F. avenaceum*, *F. culmorum*, *F. graminearum* and *F. oxysporum* (Piesik *et al*., 2011). An array of green leaf volatiles, terpenes and shikimic acid pathway-derived volatiles were identified as

404 being raised in infected maize emissions above that of controls. Four chemicals, ((Z)-3-hexenyl
405 acetate, (Z)-3-hexenal, linalool and β-caryophyllene) were bioassayed individually for cereal
406 leaf beetle behavioural responses, and significant attraction of *O. melanopus* towards all tested
407 chemicals was observed at specific doses. Similar experiments that used wheat and barley
408 instead of maize, a reduced inoculum mix that omitted *F. oxysporum*, and the related cereal
409 beetle, *O. cyanella*, showed that the beetles were attracted to certain volatile chemicals ((Z)-3-
410 hexnyl acetate and (Z)-3-hexenal) at lower doses but repelled by those chemicals and others
411 ((Z)-β-ocimene and linalool) at high doses (Piesik *et al*., 2013). This work is limited in that the
412 nature of the leaf beetle responses to volatiles induced by different pathogens is not compared
413 to the disease development in infected hosts with and without herbivory, and therefore it cannot
414 be concluded as to the impact altered herbivore attraction would have on disease progression.
415 However this work does show that the severity of infection and the corresponding changes in
416 the level of volatile chemical emissions could have different influences over herbivore
417 behaviour.

418 The chemical ecology of the tripartite interactions between *F. graminearum*, wheat and grain
419 aphids *Sitobion avenae* was studied alongside analysis of the impacts of aphid activity on the
420 disease and vice versa (Drakulic *et al*., 2015). It was shown that grain aphids were repelled by
421 the volatile chemical emissions of *F. graminearum* infected wheat ears, and that aphids fed on
422 infected hosts had an elevated rate of mortality. It was concluded that avoidance of volatiles
423 indicative of *F. graminearum* infection was likely to be a behavioural adaptation by aphids to
424 evade an inhospitable environment. Prior aphid colonisation of the host was shown to increase
425 pathogen DNA and mycotoxin accumulation, so this work revealed that insects relevant to
426 disease processes do not necessarily need to be attracted to the infected host to impact upon the
427 disease, as appears to occur in FER (Cardwell *et al*., 2000; Schulthess *et al*., 2002). Moreover,

428 the work of Drakulic *et al*. (2015) demonstrates that timing is critical in determining the
429 outcomes of volatile organic chemical (VOC) interactions with insect pests.

430 The behaviour of the meal beetle *Tenebrio molitor* towards grain infected with different
431 *Fusarium* species has been assessed on wheat grain, in addition to beetle survival when feeding
432 on the infected grain (Guo *et al*., 2014). Beetles were attracted to grain infected with *F.*
433 *culmorum, F. poae* or *F. proliferatum*, but repelled by grain infected with *F. avenaceum*. In
434 accordance, survival rates were similar to controls for *F. proliferatum* or *F. poae*-infected grain,
435 but infection by *F. avenaceum* or *F. culmorum* lead to increased mortality. This study revealed
436 three different relationships between a single insect species and several related fungi. Meal
437 beetles were not harmed by *F. poae* and *F. proliferatum* and were attracted to infected hosts,
438 potentially increasing dispersal of the fungus or increasing the mechanical and biological
439 damage to grains infected with those species, thus facilitating further infection of the hosts or
440 changes in fungal metabolism as a response. Conversely, the beetles avoided grain infected
441 with *F. avenaceum*, so the insects are observed to avoid the damaging environment by
442 interpreting volatile chemical cues produced by the infected grain. Finally, the beetles were
443 attracted to *F. culmorum* infected grains, despite this environment being detrimental to the
444 survivorship of the insects, which could be interpreted as manipulation of the insect by the
445 pathogen: while the pathogen could benefit from the insect activity, feeding on hosts infected
446 with this pathogen would negatively impact on the meal beetle population. Why this relatively
447 aggressive pathogen bucks the trend is not addressed in this work, although as *F. avenaceum*
448 produces beauvericin, whereas *F. culmorum* does not, it could be the case that the different
449 mycotoxin contributions of the pathogen species is one factor that plays a role in differentiating
450 the response of insects to infected host volatiles.

451 Species-specific interactions between *Fusarium* pathogens and the rice weevil, *Sitophilus*
452 *oryzae*, have been observed (Selitskaya *et al*., 2014). Interestingly, weevils responded

differently to the VOC produced by fungal colonies on agar plates versus infected wheat grain in some incidences, implying that there are host-dependent differences in VOC output from pathogens, so that the same pathogen could produce different volatiles on different host species infected. Moreover, this work highlighted different responses of weevils to volatiles of grain infected with species differing in pathogenicity to the host plant. Weaker pathogens *F. poae* and *F. langsethiae* were attractive to the weevils whereas volatiles from grain infected with *F. graminearum* and *F. culmorum* were repellent. A study has identified the VOC produced by *F. poae* on inoculated wheat grain, showing differences in abundances of chemical groups between two and five days after inoculation (Precisse *et al*., 2006). Some chemicals identified were known to be associated with infections caused by other fungal pathogens, including ethyl acetate which has been associated with *F. culmorum* infection, but others, such as 2,4-Dimethylepten, were considered to be indicative specifically of *F. poae* contamination. Further to this, several carbonyl-possessing chemicals were shown to be suppressed from grain emissions following *F. poae* infection, including 2-butanone, 3-methylbutanal and 2-heptanone. This work shows that pathogen-specific changes in VOC emissions from stored grain could potentially be used to identify early infection of grain and to determine which pathogens are likely to be present, and therefore what arthropod activity would be expected to increase risk of mycotoxin contamination.

Conclusions

This review explores how arthropods may interact with the processes of FHB or FER by drawing together and appraising current knowledge of *Fusarium*-arthropod interactions. In doing this we have identified some important knowledge gaps that merit attention in future studies. Firstly, there is the need for more investigation into insect dispersal of inoculum from natural sources instead of colonies. Secondly, there is a need for identification of the molecular

mechanisms that mediate enhanced host susceptibility to FHB or FER disease following arthropod herbivory. Finally, the role of mycotoxins in mediating arthropod behaviour through host volatile chemistry needs to be clarified. The potential for interaction between Fusarium diseases and arthropods has generally received less attention than other aspects of the disease epidemiology, but this means it is an exciting new area of science. Little work has been done to evaluate the potential role of arthropod involvement in Fusarium disease epidemiology while host plants are growing in the vegetative stage. However, upon the production of reproductive organs, and the beginning of the period of host susceptibility to FHB or FER, arthropod activity has been observed to have varying degrees of impact on the disease depending on the combination of species of host, arthropod and pathogen involved.

The most thoroughly studied system is that of maize, FER caused by *F. verticillioides* and the activity of the European corn borer (*Ostrinia nubilalis*), which has been accepted to act as a vector for the fungus, despite somewhat limited direct evidence (Sobek & Munkvold, 1999; Cardwell *et al*., 2000; Darvas *et al*., 2011), and provides wound sites to the host that leads to an increase in disease symptoms and in the levels of the fumonisin mycotoxins (Munkvold 2003; Saladini *et al*., 2008; Blandino *et al*., 2009). Very few studies have investigated the interaction of arthropods on FHB epidemiology in small-grained cereals, although from the limited amount information available it appears that *F. graminearum* infection can be promoted by aphid infestation without acting as a vector for the pathogen (Bagga, 2008; Drakulic *et al*., 2015). The activity of thrips and mites have also been correlated with increased disease severity in a range of hosts and, along with sap and flour beetles, demonstrated to be capable of altering the disease process in cereal plants, with no definitive evidence to suggest their activity as vectors between infected host plants either (Parsons & Munkvold, 2010; Piesik *et al*., 2011). This small amount of research supports the hypothesis that insect and mite activity can impact

the progress of FHB disease in such a way as to increase the host's susceptibility, and therefore to increase yield and grain quality losses and increased mycotoxin accumulation.

The role of host volatiles in mediating the interactions between *Fusarium* pathogens and arthropod herbivores appears to vary between systems. If pathogen species-specific compounds can be identified, screening crops early in the growth season with devices such as electronic noses might provide an early warning to allow timely application of fungicide treatments when needed. Furthermore, gaining knowledge of field and storage arthropod pest species that respond to infected host volatiles would be beneficial for informing pest monitoring and management strategies of the associated risks. Control of FHB and FER may be improved by using combinations of fungicides and insecticides at important time periods in the disease cycle, and the importance of appropriate storage environments for cereal products has been highlighted by the potentially damaging interactions that can occur between toxigenic *Fusarium* fungi and arthropods in stored grain.

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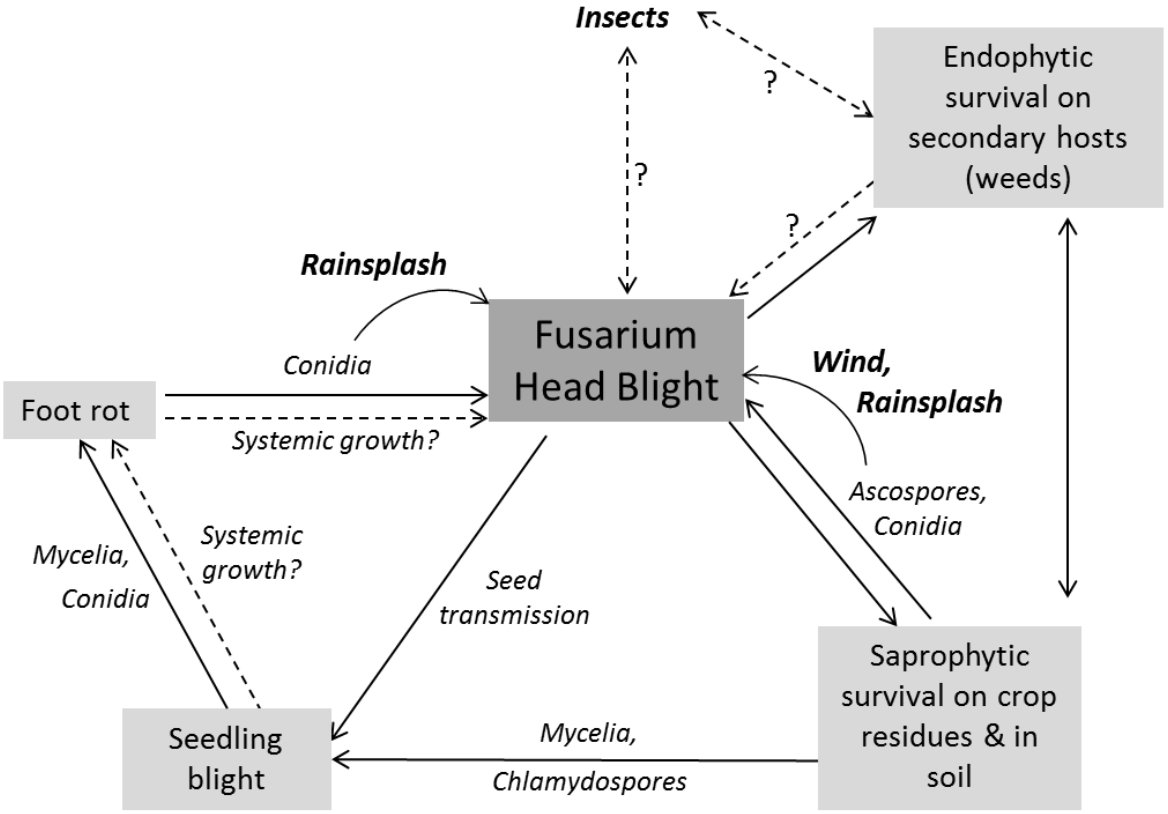
Figure Legends

Fig. 1. Sources of Fusarium head blight inoculum and the factors that promote dispersal of different spore types. Dashed lines indicate unconfirmed processes.



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801 **Fig. 1.** Sources of Fusarium head blight inoculum and the factors that promote dispersal of
802 different spore types. Dashed lines indicate unconfirmed processes.

803 **Table 1:** Summary of studies observing correlations between insect activity and Fusarium disease incidence, severity and mycotoxin levels. FHB
804 – Fusarium head blight; FER – Fusarium ear rot; FB_1 & FB_2 – fumonisin B_1 & B_2

Insect(s)	Crop	Pathogen(s)	Country	Finding	Citation
Sitodiplosis mosellana Orange wheat blossom midge (OWBM)	Wheat	*F. graminearum*	Canada	Number of OWBM larvae per spike and per spikelet positively correlated with *F. graminearum* seed contamination (R = 0.67).	Mongrain *et al*., 1997
Sitobion avenae English grain aphid	Wheat	*F. graminearum*	India	Monocrotophos (0.1%) insecticide at booting and heading or only at heading reduced aphid population by 80% and FHB incidence and severity by 21% and 30% respectively.	Bagga, 2008
Ostrinia nubilalis European corn borer (ECB)	Maize	*F. verticillioides*	Italy	ECB damage 23% greater in late sown maize, and early sowing reduced FHB incidence and severity by 25% and 49%. Early sowing with insecticides (deltamethrin @ 0.012 kg AI ha^{-1}) which reduced ECB reduced $FB_1 + FB_2$ by 79%.	Blandino *et al*., 2008
"	"	"	Italy	Insecticides (deltamethrin @ 0.013 kg AI ha^{-1} or lambda-cyhalothrin @ 0.019 kg AI ha^{-1}) reduced ECB severity and reduced $FB_1 + FB_2$ by 75%, FER incidence by 51% and severity by 68%.	Saladini *et al*., 2008
Ostrinia nubilalis & *Sesamia inferens* Pink stem borer	*Bt*-maize	*F. verticillioides* *F. proliferatum*	France & Spain	*Bt*-maize grain had up to 18 times lower fungal biomass and up to 30 times lower FB_1 than grain from near-isogenic traditional maize hybrids.	Bakan *et al*., 2002
Frankinella occidentalis Western flower thrips	Maize	*F. verticillioides*	USA	Insecticides (lambda cyhalothrin & dimethoate @ 0.035 & 0.56 kg AI ha^{-1}) reduced thrips infestation as well as silk-cut symptoms, FER and FB_1 in field trials. Intra-ear immature thrips were more strongly correlated with FB_1 (R = 0.53) than mature thrips (R = 0.36).	Parsons & Munkvold, 2010
"	"	"	USA	Intra-ear thrips infestation correlated with mould symptoms (R = 0.78) and FB_1 (R = 0.83).	Parsons & Munkvold, 2012

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