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# *Fusarium* infection and trichothecenes in barley and its comparison with wheat

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**REVIEW ARTICLE**

## Abstract

Barley is a small-grain cereal that can be infected by *Fusarium* spp. resulting in reduced quality and safety of harvested barley (products). Barley and other small-grain cereals are commonly studied together for *Fusarium* infection and related mycotoxin contamination, since the infection and its influencing factors are assumed to be the same for all small-grain cereals. Using relevant literature, this study reviewed *Fusarium* spp. infection and mycotoxin contamination, mainly T-2/HT-2 toxin and deoxynivalenol (DON), in barley specifically. For the first time, review results provide an extensive overview of the influencing factors for *Fusarium* infection and mycotoxin production in barley, such as weather, agricultural management and processing factors, and includes the comparison of these mechanisms in wheat. Results showed that *Fusarium* infection in barley is difficult to recognise in the field and mycotoxin levels cannot be estimated based on the symptoms. These factors make it difficult to establish the real severity of *Fusarium* infection in barley. In addition, most pre-harvest measures to mitigate initial *Fusarium* infection, such as cultivar use and soil cultivation, are the same for barley and wheat, but due to anatomical differences, some pre-harvest measures have a different effect on *Fusarium* infection in barley. For example, the effective moment (days after anthesis) of fungicide application in barley and wheat is different. Also, in wheat, there is an additional effect of multiple fungicide applications in reducing *Fusarium* Head Blight and DON concentrations, whereas in barley, no additional effect of multiple application is seen. Hence, care should be taken to use data from one small-grain cereal to draw conclusions on other small-grain cereals.

**Keywords:** small-grain cereals, *Fusarium* Head Blight, deoxynivalenol, T-2 toxin, HT-2 toxin

## 1. Introduction

Barley is the fourth most produced cereal crop worldwide, and grown in temperate climate regions including North-West Europe and Canada. Around 140 million tonnes per year are produced globally, which are mainly used as feed (70%) and for beer production (27%) (FAO, 2004, 2016).

Infection with *Fusarium,* a fungus, can lead to the crop disease *Fusarium* Head Blight (FHB) and *Fusarium* damaged kernels (FDK), resulting in reduced yield, quality of the kernels and the percentage of seed germination (Tekauz *et al.*, 2000). In addition, the presence of *Fusarium* spp. in barley kernels is related to gushing (Sarlin *et al.*, 2005), the eruptive overfoaming of beer upon opening (Christian *et al.*, 2011).

Some *Fusarium* species produce mycotoxins, secondary metabolites that can cause adverse health effects in humans and animals upon consumption (Placinta *et al.*, 1999). *Fusarium* mycotoxins include type A trichothecenes, such as T-2 toxin (T-2) and HT-2 toxin (HT-2), and type B trichothecenes, such as deoxynivalenol (DON). T-2/HT-2 toxins are the most potent trichothecenes and exert immunotoxic, genotoxic and neurotoxic effects (EFSA, 2011). DON is the most studied *Fusarium* mycotoxin in small-grain cereals. It can cause acute and chronic adverse effects on the gastro-intestinal tract, the nervous system, and the immune system in animals and humans (Maresca, 2013). Mycotoxins are chemically stable contaminants; they survive many processing steps and are found in multiple end-products, like flour, feed and beer (EFSA, 2013; Varga *et al.*, 2013). Human chronic dietary exposure to T-2/HT-2 (EFSA, 2017a) and DON (EFSA, 2017b) may exceed their

respective tolerable daily intakes in some sub-populations, in particular young population groups.

The rate of infection and production of mycotoxins by *Fusarium* spp. in small-grain cereals can be influenced by pre-harvest agronomic measures and other factors, like weather and post-harvest processing (EC, 2006c). Although some review papers on infection and these influence factors are available for wheat or small-grain cereals in general (Bai and Shaner, 2004; Dweba *et al.*, 2017; Kabak *et al.*, 2006; Kazan *et al.*, 2012; Liu and Ogbonnaya, 2015; Parry *et al.*, 1995; Van der Fels-Klerx and Stratakou, 2010; Wegulo, 2012; Wegulo *et al.*, 2015), no complete overview exists for barley. In addition, several cited reviews draw conclusions for small-grain cereals based on wheat data. It is generally assumed that *Fusarium* infection and the effect of influence factors on this infection and mycotoxin formation are the same for all small-grain cereals. This literature study aimed to investigate *Fusarium* infection, its related trichothecene contamination (T-2/HT-2 and DON) and the effect of influence factors, like weather, agronomic management and processing in barley specifically, and identify possible differences and similarities with wheat.

## 2. Material and methods

An extensive literature review was conducted including scientific papers published up to July 2017. The keywords (*Fusarium* OR FHB OR mycotoxins OR trichothecenes OR deoxynivalenol OR T-2 OR HT-2) AND (barley OR small-grain cereals) AND (management OR measures) were used to search SCOPUS and PubMed.

The search results were screened for their relevance to the study objectives based on their titles and abstracts. Papers of the relevant records were retrieved, and checked based on their full contents. The reference lists of all relevant studies were checked for additional relevant papers (snowballing effect) of which the abstracts were again checked for their relevance to the study objectives.

## 3. Results

### Anatomy of barley

Barley (*Hordeum vulgare L.*) belongs to the family of grasses and has anatomical similarities and differences with other small-grain cereals, such as wheat. Due to anatomical differences the susceptibility between small-grain cereal types can differ (see next section on Infection).

In small-grain cereal plants, the grain kernels develop in the spike, also called head or ear. This spike consists of multiple spikelets that are connected by a node on the rachis, the main stem. A spikelet consists of one or more florets that can develop to kernels, the actual edible grains. The arrangement of the florets differs between barley types. In barley, three spikelets are connected on a rachis node on alternating sites of the rachis, and each spikelet contains one floret. In six-rowed barley, all three florets are fertile and will develop into kernels. In two-rowed barley, only the middle floret will develop into a kernel (Forster *et al.*, 2007). When viewed from above, six-rowed barley has a ring of six kernels around the rachis whereas two-rowed barley has two kernels on opposite sides of the rachis. During the flowering stage (anthesis) of the plant, anthers extrude from the floret. Barley can be either chasmogamous (open-flowering) or cleistogamous (closed-flowering). Chasmogamous barley has full anther extension whereas cleistogamous barley has no or a limited anther extension (Heta and Hiura, 1963; Vivar *et al.*, 1997). In closed-flowering barley, only self-fertilisation occurs (Briggs, 1978).

### Infection

*Fusarium* spores can survive in the soil, crop residues or grain seeds, and reach the spike via wind or water from rain or irrigation (Osborne and Stein, 2007; Parry *et al.*, 1995). During warm and wet conditions the spores germinate and the fungus infects the plant. Mesterházy (1995) summarized the five types of plant resistance to *Fusarium* infection: (1) resistance to initial infection; (2) resistance to spread of pathogen; (3) resistance to kernel infection; (4) tolerance; and (5) resistance to toxins (Miller *et al.*, 1985; Schroeder and Christensen, 1963; Snijders, 1988). Both type 1 and 2 resistance are found in barley, with type 2 as the predominant type (Bai and Shaner, 2004).

The fungus can penetrate the rachis and spreads via direct floret-floret contamination. Further contamination via direct floret-floret contact occurs mainly in six-rowed barley, because the florets are closer together compared to two-rowed barley (Langevin *et al.*, 2004). In barley, it is possible that only three florets in a spikelet are infected, whereas the neighbouring spikelets are free from infection (Tekauz *et al.*, 2000). Infection is sometimes restricted to these initially infected florets and does not spread to the adjacent florets (Boddu *et al.*, 2007). Chasmogamous barley is most susceptible to *Fusarium* infection during anthesis (Oliveira *et al.*, 2012; Yoshida *et al.*, 2007, 2012), possibly due to production of fungal growth stimulants (Strange and Smith, 1971), whereas cleistogamous barley is most susceptible ten days after anthesis (Yoshida *et al.*, 2007). Although anthesis mainly occurs while the head is still protected from infection (McCallum and Tekauz, 2002), it is observed that barley heads can extrude already fully infected (Osborne and Stein, 2007).

## Symptoms

*Fusarium* infection can be determined in different ways. On the field, FHB can be determined by visual inspection of the percentage of infected florets (Yoshida *et al.*, 2007), percentage of infected spikelets (Ban and Suenaga, 2000; Bérubé *et al.*, 2012; Buerstmayr *et al.*, 2004; Chrpová *et al.*, 2011; Nesvadba *et al.*, 2006; Xue *et al.*, 2006), and percentage of infected kernels in a spikelet (Urrea *et al.*, 2002) or ear (Vančo *et al.*, 2007). These percentages can be used to determine an FHB index (% incidence * % severity) (Tekauz *et al.*, 2000). After harvest, FHB can be determined by the percentage of FDK as described by the visual symptom score of the kernels, the presence of fungi or the weight of the kernels.

In infected barley, symptoms are not distinctive, can be hidden, or may be confused with other diseases. Infected barley can be recognised by necrotic patches and bleaching of the florets (Boddu *et al.*, 2007) and discoloured kernels (tan, orange, brown, pink or red) scattered throughout the head. When the bottom of the head is infected, the stem may turn dark brown (Tekauz *et al.*, 2000). Sometimes, fungal mycelium, (orange-pink) spore masses or black spots are visible on the kernels (Canadian Grain Commission, 2016). A pink-red colour of the kernels can be caused by production of naphthoquinone pigments by *Fusarium* species (Oliveira *et al.*, 2012). Under extreme stressful conditions for the fungus, it can biosynthesise these pigments (Medentsev *et al.*, 2005). In addition to discoloration of the barley kernels, FDK can also decrease in weight by 20% compared to healthy kernels (Tekauz *et al.*, 2000). In hulled barley, FDK cannot be distinguished from healthy kernels, because the hull can conceal the damage (Abramson *et al.*, 2004). In addition, symptoms can be confused with those caused by other pathogens (Bérubé *et al.*, 2012); for example, discoloration at the basal end of the kernel can also be caused by *Helminthosporium sativum* and *Alternaria alternata* (Clear *et al.*, 1996). Overall, these factors make it difficult to establish the real severity of *Fusarium* infection in barley.

## Mycotoxins

Most *Fusarium* species are able to produce mycotoxins. It is suggested that these toxins may act as virulence factors and increase the aggressiveness of the fungus in small-grain cereals (Bai and Shaner, 2004; Jansen *et al.*, 2005; Langevin *et al.*, 2004). Boddu *et al.* (2007) showed that a *Fusarium* strain that produces trichothecenes (DON) and a non-trichothecene producing mutant strain, were both able to infect barley florets without spreading to neighbouring florets. However, the non-trichothecene producing strain resulted in lower disease severity based on the percentage of diseased florets and smaller necrotic patches, less bleaching and lower amount of biomass as compared to the trichothecene producing strain. These results indicate that trichothecene (DON) production is a factor in the pathogenicity and severity of *Fusarium* infection in barley. However, Langevin *et al.* (2004) only found differences in pathogenicity of a non- and trichothecene producing strain (DON) in one of the four barley cultivars studied. Jansen *et al.* (2005) showed that spreading was inhibited by the plant regardless of the presence of DON.

*Fusarium* infection can activate the plant defence system (Hofer et al., 2016b) and mycotoxins might play a role in this activation. When DON was applied to one barley floret, it spread to other florets, diluting its concentration (Gardiner *et al.*, 2010). Upon infection with a trichothecene producing strain, transcription of plant defence genes increased compared to infection with a non-trichothecene producing strain. One of the plant defence mechanisms is detoxification by glucosylation. Glucosylation of mycotoxins by the plant is thought to be the mechanism behind the presence of so called 'masked' or 'modified' mycotoxins. The masked mycotoxin deoxynivalenol-3-glucoside (DON-3G), a plant conjugate of DON, was found when barley was inoculated with DON (Gardiner *et al.*, 2010; Meng-Reiterer *et al.*, 2015). Also, conjugated forms of T-2 and HT-2 were found in barley (Meng-Reiterer *et al.*, 2015). In end-products, high concentrations of DON-3G were found in beer (Varga *et al.*, 2013; Zachariasova *et al.*, 2012).

In Europe between 15 and 55% of the barley (products) is contaminated with DON (EFSA, 2017b) and between 2 and 50% with T-2/HT-2 (EFSA, 2011; 2017a). Mean DON concentrations are around 484 µg/kg in unprocessed barley, 152 µg/kg in barley grains for human consumption, 8.4-11.3 µg/kg in beer, and 187 µg/kg in feed (EFSA, 2013, 2017b; Varga *et al.*, 2013). Mean T-2/HT-2 concentrations are between 22.8 µg/kg in unprocessed barley, 10-13 µg/kg in barley for human consumption and 0.82-3.3 µg/l in beer (EFSA, 2011, 2017a). In the EU, Commission Regulation 2006/1881/EC sets maximum levels for DON at 1,250 µg/kg in unprocessed cereals and 200-750 µg/kg in cereal (products) for direct human consumption (EC, 2006a). Commission Recommendations state maximum levels for DON is 8 mg/kg in cereals and cereal products intended for animal feed (2006/576/EC; EC, 2006b) and maximum levels of T-2/HT-2 at 250-500 µg/kg in barley products for feed and compound feed, 200 µg/kg in processed barley (including malting barley), 50 µg/kg in barley for direct human consumption and 15-100 µg/kg in barley products for human consumption (2013/165/EU; EC, 2013). Regarding exposure to mycotoxins due to barley consumption, barley is a minor contributor to dietary T-2/HT-2 exposure, and its contribution is mainly due to beer consumption by adults (EFSA, 2011, 2017a). In contrast, barley is not a high contributor to DON exposure (EFSA, 2013, 2017b).

### Correlation between symptoms of *Fusarium* infection and mycotoxins

For barley, results for the correlation between disease severity, mycotoxin levels and other symptoms are not consistent. In some studies, a correlation was found between disease severity and the presence of *Fusarium* species (Salas *et al.*, 1999), visually infected kernels (Berger *et al.*, 2014; Legzdina and Buerstmayr, 2004), or a reduction in grain weight (Fernandez *et al.*, 2007a). However, other studies could not find such a correlation between disease severity and presence of *Fusarium* species (Nesvadba *et al.*, 2006; Tekauz *et al.*, 2000) or visually infected kernels (Tekauz *et al.*, 2000). In some studies, presence of DON was correlated to the disease severity (Berger *et al.*, 2014; Buerstmayr *et al.*, 2004; Chrpová *et al.*, 2011; Legzdina and Buerstmayr, 2004; Salas *et al.*, 1999; Thin *et al.*, 2004), visually infected kernels (Berger *et al.*, 2014; Legzdina and Buerstmayr, 2004), a decrease in kernel weight (Chrpová *et al.*, 2011) or presence of *Fusarium* species (Bérubé *et al.*, 2012; Salas *et al.*, 1999; Schöneberg *et al.*, 2016; Tekauz *et al.*, 2000). However, in other studies, no correlation between presence of DON and disease severity (Nesvadba *et al.*, 2006), visually infected kernels (Tekauz *et al.*, 2000), a decrease in kernel weight (Tekauz *et al.*, 2000) or presence of *Fusarium* species (Abramson *et al.*, 1998; Xue *et al.*, 2006) was found. In addition, no significant correlation was found between the presence of *Fusarium avenaceum*, *Fusarium equiseti*, *Fusarium graminearum*, *Fusarium poae*, or *Fusarium sporotrichioides,* and DON content in barley (Abramson *et al.*, 1998; Xue, 2013; Xue *et al.*, 2006).

## 4. Influence factors on *Fusarium* infection and mycotoxin levels

### Weather

Weather is one of the most influencing factors on *Fusarium* infection and the production of mycotoxins in barley (Berger *et al.*, 2014; Bernhoft *et al.*, 2012; Bondalapati *et al.*, 2012; Linkmeyer *et al.*, 2016). Weather conditions determine germination, growth of fungi and selection of species (Doohan *et al.*, 2003). Germination of the fungus normally occurs with warm and moist weather, depending on the type of *Fusarium* species. Presence of these species differ per region and climate conditions. For example, *F. graminearum* is the predominant *Fusarium* species in warmer regions, whereas in cooler regions *Fusarium culmorum* and *F. avenaceum* are predominant (Champeil *et al.*, 2004). Since not all *Fusarium* species produce the same mycotoxins, the type of mycotoxin present is also climate and weather dependent.

Based on a model with barley samples from North-West Europe collected between 1989-2009, presence of DON in barley was positively correlated with temperature and precipitation in April, probably around ear formation (Van der Fels-Klerx *et al.*, 2012). Also, T-2/HT-2 production by *F. sporotrichioides* was associated with wet field conditions in summer, probably during ripening, in Canada in 1993 (Abramson *et al.*, 2004). In the Czech Republic, a high incidence of T-2/HT-2 was associated with relatively low mean temperatures in May and July in 2008 during barley anthesis, which are conditions favourable mainly for type A trichothecene producers such as *F. sporotrichioides* and *F. poae* (Malachova *et al.*, 2010).

### Barley variety

Choosing a resistant barley cultivar can be effective to mitigate *Fusarium* infection and mycotoxin accumulation. Barley cultivars have different susceptibility to *Fusarium* infection and mycotoxin accumulation (Bérubé *et al.*, 2012; Chrpová *et al.*, 2011; Langevin *et al.*, 2004; Xue, 2013; Xue *et al.*, 2006). Susceptible characteristics include six-rowed barley, and open-flowering types and hulled varieties.

A Japanese study with forty-six cultivars, observed higher FHB severity in chasmogamous and six-rowed barley compared to cleistogamous and two-rowed barley from 2001 to 2002 (Yoshida *et al.*, 2005). Also, the number of infected spikelets was higher in wheat than in six-rowed barley (Langevin *et al.*, 2004).

The presence of a hull is another characteristic determining a difference in susceptibility. Most barley cultivars have a hard inedible hull around the kernel (hulled or covered barley), but in some cultivars this hull is loosely attached (hulless barley) and generally falls off during harvest. In the edible parts of both hulled and hulless Korean barley, the highest total mycotoxin content was found in the bran (Hong *et al.*, 2014). Although hulled and hulless barley did not differ in FHB incidence in 18 cultivars in Northern America and 174 cultivars in Austria (Berger *et al.*, 2014; Legzdina and Buerstmayr, 2004), the presence of a hull might be related to the extent of trichothecene contamination in barley. DON, 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol concentrations were higher in hulled barley compared to the hulless variant (Legzdina and Buerstmayr, 2004), whereas T-2/HT-2 can be up to twice as high in hulless compared to hulled cultivars based on data from the Czech Republic in 2005 (Malachova *et al.*, 2010); however, not all studies could find a difference for DON (Berger *et al.*, 2014).

### Sowing date

Barley can be sown in spring (spring barley) or the previous autumn/winter (winter barley), and harvested in summer or autumn. Winter barley cultivars need vernalisation and spring barley cultivars are not always resistant to frost. Spring and winter barley differ in sowing time

and susceptibility to *Fusarium* infection. In 2010, the predominant *Fusarium* species was *F. graminearum* in winter barley (cv. Campanile and Fridericus) and *Fusarium langsethiae* in spring barley (cv. Quench) from Germany (Linkmeyer *et al.*, 2016). In Switzerland, *F. graminearum* incidence and DON content were higher in winter barley (fodder) compared to spring barley (malting) from 2013 and 2014 (Schöneberg *et al.*, 2016). In France, DON levels in malting barley were lower in spring barley compared to winter barley in 2006, but higher in 2007 and 2008 (Orlando *et al.*, 2010). T-2/HT-2 levels in France were higher in spring barley compared to winter barley from 2006-2008 (Fournier, 2009). Another French study reported higher levels of T-2/HT-2 in winter barley from 2006-2007 (Barrier-Guillot, 2008). The levels of T-2/HT-2 in spring barley were reported to be up to four times higher than those in winter barley in France between 2006 and 2008 (Orlando *et al.*, 2010). A study on European malting barley showed no difference between T-2/HT-2 levels in 2007, but reported higher T-2/HT-2 in spring barley compared to winter barley in 2008 (Slaiding, 2008, 2009). In addition, spring barley sown in autumn was less contaminated with T-2/HT-2 compared to spring barley sown in spring. Two potential reasons for these differences are a difference in cultivar susceptibility and difference in co-occurrence of the susceptible time of barley and the infectious time of the *Fusarium* species (Orlando *et al.*, 2010).

### Fertilisation

Fertilisation with nitrogen, applied during sowing or tillage did have a positive effect on growth and yield of barley and wheat grown in Uruguay between 1989-1991 (Baethgen *et al.*, 1995). However, fertilisation can also influence *Fusarium* infection and trichothecene production. When barley was grown on high nitrogen soil, the percentage of FDK, presence of *F. graminearum* and DON levels were higher compared to plants grown on low nitrogen soil in greenhouses (Hofer *et al.*, 2016a; Yang *et al.*, 2010). In contrast, Pageau *et al.* (2008) found that nitrogen fertilisation had no significant effect on DON content in barley in Canada from 2002-2005. No studies could be found on the effect of fertilisation on T-2/HT-2 levels.

### Lodging

Lodging, the bending of the stalk or the entire plant, is mainly influenced by plant characteristics and environmental conditions, such as soil type, high nitrogen fertilisation, high sowing density, drought and strong winds with heavy rain (Nakajima *et al.*, 2008). Two-rowed barley (cv. CI9831) was more resistant to lodging than six-rowed barley (Léger), in Canada and China from 2001-2002 (Thin *et al.*, 2004). Lodging of barley leads to a reduction of the grain yield and quality (Baethgen *et al.*, 1995; Caierão, 2006). In addition, lodging increases the moisture content of the plant and can increase *Fusarium* infection and mycotoxin concentration (Nakajima *et al.*, 2008). In barley, resistance to lodging is associated with lower FHB incidence (Thin *et al.*, 2004). Higher DON concentrations were found after artificial lodging Norwegian barley samples (Tore and Pemilla) from 1991-1993 (Langseth and Stabbetorp, 1996) and natural lodging in Japan from 2002-2006 (Nakajima *et al.*, 2008). No studies could be found on the effect of lodging on T-2/HT-2 levels in barley.

### Fungicide use

Fungicides can be used to decrease *Fusarium* infection during cultivation. However, the evidence of effectiveness of fungicide use to reduce *Fusarium* infection in barley is conflicting. In addition to type and dose of a fungicide, the timing of fungicide application is crucial, because barley is only susceptible during a short period of time.

May *et al.* (2010) concluded that barley seeds (cv. Excel and Westeck) treated with fungicides improved yield in Canada between 2004-2005. Application of fungicides or herbicides during the vegetation state showed either no effect or an increase of the presence of *Fusarium* species in Norway in 1996 (Henriksen and Elen, 2005). This increase might be the result of inhibitory effects of the fungicide on competitor microorganisms. In addition, during the vegetation state, no effects of fungicide application on DON and T-2/HT-2 concentrations were observed in the Czech Republic between 2005-2008 (Malachova *et al.*, 2010). In some years, the combination of fungicides and barley cultivar resulted in higher DON concentrations or lower T-2/HT-2 concentrations. In Japan, between 2005-2006, applying fungicides on two-rowed cleistogamous barley (cv. Nishinochikara) in different development stages (before anthesis and up to 30 days after anthesis), showed that application at the beginning of spent anther extrusion (11-12 days after anthesis) was most effective in reducing FHB incidence, FHB severity, and percentage of discoloured kernels, compared to other fungicide application times (Yoshida *et al.*, 2008a). Spraying fungicides on six-rowed chasmogamous barley (cv. Shunrai) three days after anthesis was more effective compared to later spraying dates. Spraying twice gave no additional effect on FHB and DON concentration comparing to spraying once three days after anthesis in Japan in 2011 (Tateishi *et al.*, 2014).

### Biological control

Biological control, i.e. the application of other microorganisms to suppress fungal growth or infection, is not well examined in barley. Piriformospora indica used as a biological control agent in barley increased grain weight and decreased root rot (Achatz *et al.*, 2010; Deshmukh and Kogel, 2007; Harrach *et al.*, 2013). However, the effect of *P. indica* on FHB or mycotoxin content in barley

is not known. In wheat, *P. indica* reduced FHB and DON concentration, and increased grain weight (Rabiey and Shaw, 2016). No studies could be found on the effect of biological control on T-2/HT-2 levels in barley.

### Soil cultivation

*Fusarium* present on plant debris can survive and contaminate the next planted crop. Tillage and ploughing bring the contaminated plant debris deeper into the soil which can avoid contamination of the next crop. In contrast, with minimum tillage and direct drilling, plant residues are not buried and are associated with higher infection of cereals compared to deep ploughing (Imathiu *et al.*, 2013). In Canada between 1999-2002, incidence of FDK was lower under conventional tillage (seven or more tillage operations) or no tillage compared to minimum tillage (one to six operations) in more than six cultivars tested (Fernandez et al., 2007b). However, the effectiveness of tillage type on FHB differed between susceptible and more resistant cultivars. For example, lowest disease levels were reached under conventional tillage for susceptible cultivars and under zero tillage under more resistant cultivars (Fernandez et al., 2007b).

Incidence of *F. graminearum* and DON content in barley was higher under minimum tillage compared to ploughing, regardless of previous crop in Switzerland between 2013 and 2014 (Schöneberg *et al.*, 2016). DON contamination in spring barley did not differ significantly between tillage, chisel or direct drilling in the Czech Republic between 2007-2014 (Matušinsky *et al.*, 2016). Orlando *et al.* (2010) found no effect of tillage (ploughing/non-ploughing) on T-2/HT-2 levels in France in 2006-2008. Although tillage can reduce barley infection, Bérubé *et al.* (2012) concluded that tillage (mouldboard plough, spring tillage or direct drilling) had minor influence on disease incidence and DON content in three barley cultivars compared to weather and crop rotation in Canada between 2007-2008.

### Crop rotation

With crop rotation, different types of crops will succeed each other in the field, to limit recontamination of crops. For example, sowing *Fusarium* prone crops after each other increases the chance of recontamination from the soil. In barley, incidence of *F. graminearum* and DON content were higher when barley succeeded maize compared to cereal or pasture in Switzerland between 2013 and 2014 (Schöneberg *et al.*, 2016). DON levels in barley were significantly higher when the previous crop was barley compared with dry pea, soybean, or red clover in Canada from 2002-2005 (Pageau *et al.*, 2008). In barley succeeding barley or wheat, T-2/HT-2 levels were higher compared to barley succeeding maize, beet or other crops in France in 2006-2008 (Orlando *et al.*, 2010). Although Fernandez *et al.* (2007a) did not find a difference between FHB in barley succeeding a cereal crop, oilseed, pulse or summer fallow, the percentage of FDK was lower when the previous crop was summer fallow compared to the other crops tested, in Canadian barley between 1999-2002.

### Harvesting

Although harvest date is difficult to influence due to weather conditions, a delayed harvest should be avoided. In three barley cultivars (AC Vision, Brucefield, and OAC Baxter), a delayed harvest by two weeks was correlated with the increase in the incidence of total *Fusarium* and *F. sporotrichioides* in Canada between 2004-2005 (Xue *et al.*, 2008). Harvesting two weeks before the expected harvest significantly lowered the presence of total *Fusarium* and *F. sporotrichioides.* A change in harvest date could not be statistically correlated to presence of other *Fusarium* species or DON (Xue *et al.*, 2008).

Infected kernels are difficult to be separated from healthy kernels because infected kernels might not have distinguishable symptoms and infected kernels weigh on average only 20% less than healthy kernels, based on a Canadian study with six-rowed malting barley (cv. Excel, Foster, Robust and Stander) (Tekauz *et al.*, 2000). Techniques based on weight to separate FDK at harvest (Salgado *et al.*, 2011) might therefore not be effective in barley compared to wheat where the infected kernel weight decreases up to 50% (Tekauz *et al.*, 2000). In Canadian barley harvested in 1994, DON accumulated in the outer part of the kernel. Up to 50% of the initial DON concentration can be lost in hulless barley, because the hull is easily removed at harvest (Clear *et al.*, 1997). In addition, commercial dehulling strategies can remove the outer hull as well (Trenholm *et al.*, 1991). No studies could be found on the effect of harvesting on T-2/HT-2 levels in barley.

### Processing

Although mycotoxins can hardly be removed during processing, mycotoxin concentrations can be diluted or accumulated during certain processing steps. Presence of *Fusarium* fungi and the use of infected kernels during processing can result in a decrease of the quality of the end-product. After harvest, several processing steps like rolling, extruding, cooking and flaking can be applied for feed production (EFSA, 2011). For food consumption, malting and brewing are the most common processing steps. Hong *et al.* (2014) report that washing or boiling of barley can decrease the DON content by 80%. Although very few other studies are available on the effects of barley processing, several studies have assessed the quality and safety regarding *Fusarium* infection during the malting and brewing process (see also the recent review by Schwarz, 2017).

Barley kernels that are smaller or coloured red are suggested to be related to gushing. These red kernels are an indication of *Fusarium* infection (Oliveira *et al.*, 2012). Other studies also report a relationship between *Fusarium* infected kernels and a decrease of malt quality (Nielsen *et al.*, 2014; Oliveira *et al.*, 2013), or a negative relation between *Fusarium* resistance and malt quality (Urrea *et al.*, 2005). The probability of gushing is reduced by eliminating the red kernels from the batch; however, gushing can still occur as some infected kernels show no symptoms (Christian *et al.*, 2011). Primary gushing is caused by elements in the raw materials and malt, whereas secondary gushing is caused by factors during the production process. Two type of proteins have an influence on the extent of gushing and both are the result of fungal infection. Hydrophobins are excreted by fungi, and non-specific lipid transfer proteins (ns-LTPs) are produced by the plant upon fungal infection (Christian *et al.*, 2011). Barley samples inoculated with F. *graminearum* and *F. poae* had increased proteinase, β-glucanase and endoxylanase levels compared to the control samples. In malt prepared from infected grain, levels of free amino nitrogen were elevated and wort β-glucans levels were reduced. The quality of the malt and wort is negatively affected by these enzymes (Schwarz *et al.*, 2002). DON levels slowly increased during the early stages of malting and were also elevated during the kilning process when the temperature was increased, causing a stress response in the fungi (Oliveira *et al.*, 2012).

When barley is contaminated with mycotoxins, the contamination can also be seen in the beer produced from the barley. For example, when barley is initially contaminated with DON, an increase of DON concentration is seen during malting followed by a slight decrease during brewing. Hazel and Patel (2004) suggest that adding certain products to the brewing process (e.g. corn grits, syrups, wheat) may contribute to the mycotoxin content in the beer. Several studies showed an increase of DON-3G during brewing (Kostelanska *et al.*, 2011; Lancova *et al.*, 2008; Zachariasova *et al.*, 2012). Levels of HT-2 decreased from barley to malt and brewing itself had a minor effect on the HT-2 levels (Lancova *et al.*, 2008). Mycotoxins were transferred to the beer or the germ bud, which is used in the feed industry (Lancova *et al.*, 2008). The technological process of beer brewing might affect the mycotoxin concentration. For example, a positive correlation between the mycotoxin concentration and the alcohol content was reported (Kostelanska *et al.*, 2009; Papadopoulou-Bouraoui *et al.*, 2004) with non-alcoholic beers showing the lowest contamination (Varga *et al.*, 2013).

## 5. Comparison of *Fusarium* infection and mycotoxins in barley and wheat

Barley and wheat are both small-grain cereals used for animal and human consumption, and *Fusarium* infection results in both a quality and safety loss of these cereals. Similarities and differences of *Fusarium* infection and mycotoxins in barley and wheat are summarised in Table 1.

The main similarities between barley and wheat are: (1) the influence factors on *Fusarium* infection in the pre-harvest stage, such as cultivar use, fungicide use and soil cultivation; and (2) the contribution of T-2/HT-2 to human exposure, i.e. both barley and wheat contribute to exposure, and current intake levels are above the tolerable daily intake in some (sub-)populations.

The main differences between barley and wheat in terms of *Fusarium* infection and mycotoxin accumulation are summarised as following:

1. Barley and wheat are anatomically different, which results in differences in susceptibility of the plants to *Fusarium* infection and disease severity. Barley is more resistant to the spread of the fungal infection within the plant, whereas in wheat, a fast spread of the infection occurs. Therefore, avoidance of initial infection is more important in wheat than in barley.
2. Determination of infection by visual symptoms is different for barley and wheat. In barley, *Fusarium* infection hardly shows any symptoms or they can be confused with other diseases, whereas in wheat *Fusarium* infection can be apparent in both the field (FHB) and in loose kernels (FDK). This leads to misestimating the presence of *Fusarium* spp. in barley. Therefore, the use of visual inspection to decide to take additional measures to prevent further spread of the disease, as is done in practice by wheat farmers, cannot be done by barley farmers. Also, techniques to separate FDK at harvest might not be as effective in barley as in wheat, because in barley FDK are more difficult to distinguish.
3. The effective moment (days after anthesis) of fungicide application in barley and wheat is different. Also, in wheat, there is an additional effect of multiple fungicide applications in reducing FHB and DON concentration, whereas in barley, no additional effect of multiple application is seen. In this regards, data on effective fungicide application in wheat, cannot be extrapolated to barley.
4. Barley and wheat are used for different end-products, which results into differences in mitigation targets and their timing: limiting fungal presence and growth in barley during post-harvest processing to improve product quality, and minimising mycotoxin contamination in wheat during pre-harvest to ensure the product safety.

**Table 1. Comparison of *Fusarium* infection and mycotoxins between barley and wheat.[1]**

| Section | Barley | Wheat | Reference |
|---|---|---|---|
| Anatomy | Chasmogamous (open-flowering) or cleistogamous (closed-flowering) | Chasmogamous (open-flowering) | Briggs, 1978; Heta and Hiura, 1963; Thomason and Griffey, 2009; Vivar *et al.*,1997 |
| | Florets close together (six-rowed) or apart (two-rowed) | Florets close together | Langevin *et al.*, 2004 |
| *Fusarium* infection | Type I resistance more important | Type II resistance more important | Bai and Shaner, 2004; Jansen *et al.*, 2005 |
| | Direct floret-floret (six-rowed) contamination or limited floret-floret (two-rowed) | Direct floret-floret contamination | Langevin *et al.*, 2004 |
| | Most susceptible at anthesis (chasmogamous) or 10 days after anthesis (cleistogamous) | Most susceptible at anthesis | Oliveira *et al.*, 2012; Yoshida *et al.*, 2007, 2012 |
| | Neighbouring spikelets are free from infection | Neighbouring spikelets are often all infected | Boddu *et al.*, 2007; Tekauz *et al.*, 2000 |
| Symptoms | Affected kernels are scattered throughout the head | Entire spikelet and neighbouring spikelets are affected | Goswami and Kistler, 2004; Tekauz *et al.*, 2000 |
| | Discoloured kernels (tan, orange, brown, pink or red) | FDK smaller, red or white and shrivelled | Boddu *et al.*, 2006; Canadian Grain Commission, 2016; Goswami and Kistler, 2004; Tekauz *et al.*, 1997 |
| | FDK weight decrease 20% | FDK weight decrease 50% | Tekauz *et al.*, 2000 |
| | Hull can cover infection symptoms | No hull | Abramson *et al.*, 2004 |
| | Symptoms can be confused with other diseases | Clear symptoms for *Fusarium* infection | Bérubé *et al.*, 2012; Clear *et al.*, 1996 |
| Mycotoxins | Contradicting results if trichothecenes act as virulence factor | Trichothecenes act as virulence factor | Bai and Shaner, 2004; Boddu *et al.* 2007; Jansen *et al.*, 2005; Langevin *et al.* 2004; Maier *et al.*, 2006; Shah *et al.*, 2017 |
| | Activation of plant defence system | Idem | Berthiller *et al.*, 2013; Gardiner *et al.*, 2010 |
| | Plant defence regardless of mycotoxins | Plant defence inhibited by mycotoxins | Jansen *et al.*, 2005 |
| | Modification of mycotoxins by barley | Idem | Berthiller *et al.*, 2013; Gardiner *et al.*, 2010; Meng-Reiterer *et al.*, 2015 |
| | Occurrence levels DON lower | Occurrence levels DON higher | EFSA, 2013; 2017b; Varga *et al.*, 2013 |
| | Occurrence levels T-2/HT-2 higher | Occurrence levels T-2/HT-2 lower | EFSA, 2011; 2017a |
| | Contribution to DON exposure limited | High contribution to DON exposure | EFSA, 2013; 2017b |
| | Contribution to T-2/HT-2 exposure minor | Contribution to T-2/HT-2 exposure | EFSA, 2011; 2017a |
| Correlation between symptoms | Contradicting results on correlation between symptoms | (Limited) correlation between symptoms | Abramson *et al.*, 1998; Berger *et al.*, 2014; Bérubé *et al.*, 2012; Chrpová *et al.*, 2011; Fernandez *et al.*, 2007a; Legzdina and Buerstmayr, 2004; Nesvadba *et al.*, 2006; Paul *et al.*, 2005; 2006; Salas *et al.*, 1999; Schöneberg *et al.*, 2016; Tekauz *et al.*, 2000; Xue *et al.*, 2006 |
| | Contradicting results on correlation between disease severity and presence of *Fusarium* spp. | - | Salas *et al.*, 1999 |
| | Contradicting results on correlation between presence of DON and disease severity | Significant positive correlation between presence of DON and disease severity | Berger *et al.*, 2014; Buerstmayr *et al.*, 2004; Chrpová *et al.*, 2011; Legzdina and Buerstmayr, 2004; Nesvadba *et al.*, 2006; Paul *et al.*, 2006; Salas *et al.*, 1999; Thin *et al.*, 2004 |

**Table 1. Continued.**

| Section | Barley | Wheat | Reference |
|---|---|---|---|
| Agronomy and management | Presence of DON in barley was positively correlated with temperature and precipitation in April | Presence of DON correlated to temperature in April, May, June and September, rainy days during June, and relative humidity during May and June | Van der Fels-Klerx *et al.*, 2012 |
| | Susceptibility differences between barley varieties | Idem | Bai and Shaner, 2004; Berger *et al.*, 2014; Bérubé *et al.*, 2012; Chrpová *et al.*, 2011; Langevin *et al.*, 2004; Legzdina and Buerstmayr, 2004; Malachova *et al.*, 2010; Xue, 2013; Xue *et al.*, 2006 |
| | Spring and winter barley differ in sowing time, cultivar used, and susceptibility to *Fusarium* infection | - | Barrier-Guillot, 2008; Fournier, 2009; Orlando *et al.*, 2010; Schöneberg *et al.*, 2016; Slaiding, 2008; 2009 |
| | Possible increase of FDK, fungal presence and DON concentration by high nitrogen fertilisation | Inconsistent effects of fertilisation on FHB and mycotoxin levels | Hofer *et al.*, 2016a; Pageau *et al.*, 2008; Yang *et al.*, 2010; Yoshida *et al.*, 2008b |
| | Lodging increases *Fusarium* infection and mycotoxin concentration | Idem | Baethgen *et al.*, 1995; Caierão, 2006; Nakajima *et al.*, 2008; Thin *et al.*, 2004 |
| | Application of fungicides was most effective in reducing FHB incidence, FHB severity and percentage of discoloured kernels 3 days (chasmogamous barley) or 11-12 (cleistogamous barley) days after anthesi | Application of fungicides 4 days after anthesis was most effective to reduce FHB | Tateishi *et al.*, 2014; Yoshida *et al.*, 2008a |
| | Spraying fungicides twice gave no additional effect on FHB and DON concentration | Spraying twice had an additional effect on reduction of FHB and DON concentrations in wheat | Tateishi *et al.*, 2014 |
| | Tillage can reduce *Fusarium* infection in barley | Idem | Bérubé *et al.*, 2012; Fernandez *et al.*, 2007b; Matušinsky *et al.*, 2016; Orlando *et al.*, 2010; Schöneberg *et al.*, 2016; Wegulo, 2012; Wegulo *et al.*, 2015 |
| | Crop rotation can reduce *Fusarium* infection in barley | Idem | Wegulo, 2012; Wegulo *et al.*, 2015 |
| | Biological control leads to increase in grain weight, effect on FHB and mycotoxins unknown | Biological control leads to increase in grain weight and decrease FHB and mycotoxins | Achatz *et al.*, 2010; Deshmukh and Kogel, 2007; Harrach *et al.*, 2013; Rabiey and Shaw, 2016 |
| Harvesting | Delayed harvest increases presence fungus, not DON | Idem | Xue *et al.*, 2004, 2008 |
| | Separation of FDK based on weight probably not effective | Separation of FDK based on weight at harvest is effective | Tekauz *et al.*, 2000 |
| | Loss of DON due to loss of hull in hulless barley; effective commercial dehulling | Effective commercial dehulling | Clear *et al.*, 1997; Trenholm *et al.*, 1991 |
| Processing | Main food processes are malting and brewing | Main food processes are milling and baking | - |
| | Quality issues due to presence fungus | Presence fungus not an issue | Nielsen *et al.*, 2014; Oliveira *et al.*, 2013 |
| | Quality issues due to infected kernels | Contradicting results on quality issues due to infected kernels | Dexter *et al.*, 1996; Horvat *et al.*, 2015; Kreuzberger *et al.*, 2015; Nielsen *et al.*, 2014; Oliveira *et al.*, 2012, 2013; Prange *et al.*, 2005 |
| | Transfer of mycotoxins through processing steps | Idem | Kaushik, 2015; Nielsen *et al.*, 2014; Oliveira *et al.*, 2013; Urrea *et al.*, 2005 |

[1] DON = deoxynivalenol; FDK = *Fusarium* damaged kernels; FHB = *Fusarium* Head Blight; HT-2 = HT-2 toxin; T-2 = T-2 toxin.

## 6. Conclusions

This is the first study providing an extensive literature review on the influence factors for *Fusarium* infection and mycotoxin formation in barley, including weather, pre-harvest and post-harvest factors. It has also comprehensively compared these factors and their underlying mechanisms between barley and wheat.

The unique anatomy of barley leads to differences regarding its susceptibility and susceptible infection time among cultivars. *Fusarium* infection in barley is difficult to recognise in the field and mycotoxin levels cannot be estimated based on the symptoms. Overall, these factors make it difficult to establish the real severity of *Fusarium* infection in barley. Weather influences *Fusarium* infection and mycotoxin production. Reduction of *Fusarium* infection and mycotoxin contamination in barley can be achieved by several pre-harvest measures. Although DON concentrations in barley do not contribute much to exposure of human by consumption of barley related food products, barley in beer can be a contributor to T-2/HT-2 exposure. In addition, the presence of *Fusarium* spp. leads to serious quality issues in beer.

Most pre-harvest measures to mitigate initial *Fusarium* infection are the same for barley and wheat, but due to anatomical differences, some measures (e.g. fungicide application) have a different effect on *Fusarium* infection. Therefore, in future research (e.g. on biological control) care should be taken to use data of wheat to draw conclusions for barley.

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