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Resistance elements of small grain cereals against Fusarium head blight and contribution of Health Promoting Compounds

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Summary

Fusarium head blight FHB is one of the main cereal disease. FHB is caused by the complex of *Fusarium* pathogens and results in significant yield losses and contamination of grain with mycotoxins. These toxins constitute a substantial risk to human health and a threat for food safety. The most dominant toxin is deoxynivalenol (DON), produced by *F.graminearum.* The use of resistant varieties is the most sustainable way to control disease outbreaks and damages. In all cereals, resistance is a complex character, based on cumulative effects of many elements and so, difficult to select. An accurate assessment of all the impacts of *Fusarium* infection on plants and a perfect knowledge of resistance mechanisms are both necessary to breed resistant varieties. Yet, previous resistance studies of FHB and of resistance elements were mainly focused on wheat. For oats in particular, FHB is an emerging issue, and efficient resistance is now required to face the increasing occurrence of *F.poae* and *F.langsethiae* and their highly noxious toxins. Besides FHB resistance, breeding programs are now focusing more and more on nutritional values and the increase of Health Promoting Compounds HPC in cereal grains. Indeed, wheat grains can contain high amounts of antioxidants including anthocyanin compounds, barley and oat grains are sources of β-glucan. Interestingly, several cereal endogenous HPCs shown inhibitive effects on *Fusarium* growth and toxin synthesis *in vitro* conditions, hence suggesting they could contribute to FHB resistance *in planta*.

The aim of this thesis is to understand how the wheat, barley and oat grains protect themselves against *Fusarium* pathogens. The role of several HPC in resistance was investigated. A first preliminary study allowed us to understand the variability of the aggressiveness of *Fusarium* strains. We demonstrated that strain aggressiveness is related to the chemotype. Aggressiveness is mainly conditioned by the environment and not impacted by the resistance of the host. These observations have been necessary for both optimizing artificial inoculations in our field tests and interpreting the results of subsequent experiments. In a second study, we confirmed that CBS can contribute to FHB resistance *in vivo*. Indeed, we observed that, within a large panel of wheat genotypes, the most resistant varieties contained the highest contents of ferulic acid in the flower tissues. Following these results, the impact of FHB, the resistance elements and the contribution of different HPC in resistance have been successively studied in grains of wheat, barley and oat. For these studies, panels of wheat,

barley and oat varieties have been artificially infected with *Fusarium* pathogens in different field tests across Switzerland. We observed that wheat grain displays the most complete resistance to protect itself against FHB damages DON accumulation and grain deformation. We have detected a novel resistance type that preserves the constituents and the baking quality of the grain. In barley grains, besides significant DON contamination, infections of *F.graminearum* caused damages on grain structure and composition. In particular, infection reduced the concentration of β-glucan in the grain. We demonstrated that the barley grain has distinct resistance elements to protect itself against fungal infection and toxin accumulation. In oat, we observed that both *F.poae* and *F.langsethiae* infections result in severe toxin contaminations, without any other symptoms or visual damages on the plant or the grain. Among the tested varieties, none was able to avoid contaminations. Our results suggest the presence of distinct resistance elements operating against the different *Fusarium* toxins.

The role of HPC in resistance depends on the compound. Our results show, that β-glucans reduce the mycotoxin charge in barley grains, while anthocyanins do not influence the resistance of wheat grains. In oat grain, accumulation of toxin was not modulated by β-glucans, yet increases of β-glucan contents were observed in grains resistant to nivalenol contamination.

To conclude, wheat, barley and oat protect themselves against *Fusarium* pathogens deploying different resistance mechanism. The stability and elevated heritability of resistance highlights the highest genetic gain that can be expected when selecting wheat for FHB resistance than in barley or oat. We demonstrated that if some HPC partially enhance grain resistance, enhancing HPC contents in grains will not drastically limit the threat associated with mycotoxins. However, it allows to develop new varieties that combine elevated HPC content and resistance to toxins.

Key words

Fusarium head blight, cereal grains, resistance types, resilience, anthocyanin compounds, β-glucan

Résumé

La fusariose de l'épi est une des principales maladies des céréales, causée par les pathogènes du genre *Fusarium*. Elle engendre des pertes de rendement conséquentes ainsi que la contamination des grains en mycotoxines. Nocives pour la santé humaine et animale, ces toxines représentent une menace reconnue pour la sécurité alimentaire. L'utilisation de variétés résistantes permet de réduire considérablement les épidémies de fusariose. Dans toutes les céréales, cette résistance est cependant un caractère complexe et difficile à sélectionner. Il faut pour cela disposer à la fois d'une évaluation détaillée de l'impact de l'infection sur la plante, et d'une connaissance parfaite des mécanismes de résistance. Or, l'étude de la fusariose et de la résistance s'est principalement concentrée sur le blé, au détriment des autres céréales. Pour l'avoine particulièrement, la fusariose a été négligée longtemps et l'amélioration de la résistance est devenue une priorité pour lutter contre le nouveau risque sanitaire lié aux toxines de *F.poa*e et *F.langsethiae*. Les travaux de sélection s'intéressent également de près aux composés bénéfiques pour la santé (CBS) présent dans les grains. En effet, les grains de blé contiennent des antioxydants dont les anthocyanes et les grains d'orge et d'avoine sont des sources reconnues de β-glucanes. Or, plusieurs CBS ont montré qu'ils pouvaient inhiber la croissance et la production de toxines des *Fusarium* en conditions *in vitro,* laissant ainsi suggérer qu'ils pourraient contribuer à la résistance contre la fusariose *in planta*.

L'objectif de cette thèse est de comprendre comment les grains de blé, d'orge et d'avoine se protègent contre les *Fusarium*. Le rôle des CBS dans la résistance des grains est étudié. Une première étude préliminaire nous a permis de comprendre les variations d'agressivité des pathogènes *Fusarium* au sein d'une population. Nous avons démontré que l'agressivité est liée au chémotype de la souche, est surtout conditionnée par l'environnement, mais n'est pas impactée par la résistance de l'hôte. Ces observations ont été essentielles pour la mise en place des infections artificielles au champ, ainsi qu'à l'aide à l'interprétation des résultats dans les essais suivants. Une deuxième étude nous a confirmé que les CBS peuvent contribuer *in vivo* à la résistance *in vivo* contre la fusariose. Nous avons en effet observé qu'au sein d'un large panel de variétés de blé, les plus résistantes contenaient, dans leurs épillets, les teneurs les plus élevées en acide férulique, principal antioxydant des céréales. Suite à ces résultats, les impacts de la fusariose, les éléments de résistance et la contribution de différents CBS ont été successivement étudiés dans les grains de blé, d'orge et d'avoine. Nous avons

observé que le grain de blé possédait la résistance la plus complète pour lutter contre tous les dégâts que la fusariose peut causer, de la malformation des grains à l'accumulation de toxine deoxynivalénol (DON) produit par *F.graminearum*. Nous avons démontré qu'il peut également se prémunir contre la dégradation de ses composants, de sa qualité et de ses activités de synthèse. Les infections de *F.graminearum* dans le grain d'orge provoquent en plus d'une contamination en DON, des dégradations de la structure et de la composition du grain, notées entres autres par des réductions des teneurs en β-glucanes. Le grain d'orge possède des éléments de résistance distincts pour lutter indépendamment contre l'infection et contre l'accumulation en toxines. L'infection du grain d'avoine par *F.poae* et *F.langsethiae* a conduit à des contaminations en toxines, parfois sévères, sans causer aucun autre dégât ou symptôme. En comparaison avec le blé et l'orge, l'avoine possède un faible niveau de résistance contre ces contaminations. De plus, nos résultats suggèrent que la résistance d'une variété d'avoine dépend de la toxine en présence.

Le rôle des CBS dans la résistance des grains dépend du composé. Nos résultats montrent que les teneurs élevées en β-glucanes réduisent l'accumulation de toxines dans les grains d'orge, alors que les anthocyanes du grain de blé n'influencent pas la résistance. Dans le grain d'avoine, les contaminations en toxines ne sont pas modulée par les teneurs en β-glucanes, des augmentations de ces teneurs ont cependant été observées dans les grains les moins contaminés en toxine nivalénol.

En conclusion, les grains de blé, d'orge et d'avoine se protègent différemment contre la fusariose. La stabilité et l'héritabilité de la résistance est la plus élevée dans le blé, ce qui laisse présager d'un plus fort gain génétique dans la sélection de la résistance dans cette céréale que dans l'orge ou l'avoine. Par ailleurs nous avons démontré que si certains CBS intervenaient dans la résistance, augmenter leurs teneurs dans les grains ne permettra pas de limiter de façon significative les dégâts causés par la fusariose. Cela permettrait cependant le développement nouvelles variétés à la fois plus bénéfiques pour la santé et résistantes aux toxines.

Mots clés

Fusariose, grain de céréales, type de résistance, résilience, anthocyanines, β-glucanes

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General introduction

Fusarium head blight FHB is a destructive disease of wheat, barley and oat worldwide. FHB is caused by pathogens of the genus *Fusarium* and *Microdochium* spp. First described in wheat in 1884, this disease has become a major threat for wheat and barley crops (Stack 2003). The threat is multifaceted: FHB causes yield losses and contamination of grains with noxious mycotoxins, rendering the production unsuitable for human food or feed (Bai and Shaner 2004; Parry et al. 1995). These mycotoxins jeopardize food safety. For example, ingestion of deoxynivalenol, the prevalent mycotoxin in small grain cereals, can provoke intestinal irritations, nausea, feed refusal in livestock, whereas exposure to other *Fusarium* toxins can lead to fertility issues, immune suppression or carcinoma of internal organs (Eriksen and Pettersson 2004). Moreover, toxins still remain in food and feed after basic treatments, such as milling and baking (Dexter et al. 1996). Many examples of damages caused by *Fusarium* toxins emerge from history. Outbreaks of alimentary toxic aleukia (ATA), a potentially fatal condition caused by long-term exposure to *Fusarium* toxins and characterized by vomiting, hemorrhaging and skin inflammation, have been reported as far back as the 18th century. The most devastating epidemic occurred in Russia between 1942 and 1948 causing the death of an estimated 100,000 people (Mayer 1953; Stack 2003). Severe contaminations of grains with T-2/HT-2 toxins produced by *F. poae* was likely the cause of the epidemic (Yagen and Joffe 1976). As cereal grains constitute a staple food of our diet, it is pertinent that regulations are put into place to minimise the amount of *Fusarium* toxins entering the food chain. The European commission set maximum limits for *Fusarium* toxins in unprocessed cereals for human consumption and established guidance values for feedstuffs, which were adopted in the Swiss legislation. In addition to being a threat for food safety, FHB causes reductions of both yield and grain quality resulting in drastic economic losses (Windels 2000). The most notorious epidemic occurred during the 1990s in North America and the estimated losses reached 3 billion USD (McMullen et al. 1997; Nganje et al. 2002). FHB caused substantial problems for wheat and barley in China between 1950 and 1990, with severe epidemics exceeding 40% of yield losses (Bai et al. 2003).

Worldwide, the introduction of wheat and barley varieties selected for FHB resistance has allowed to significantly reduce severe epidemics. FHB is, however, a new problematic for oat, the disease having been identified on oat panicles and grains less than 20 years ago (McCallum

et al. 1999). As a consequence, study of oat resistance towards FHB causing species is only in its early stages. Despite recent significant progress in assessments of FHB symptoms and impacts on oat, no resistance is yet available to face increasing FHB epidemics caused by *F. poae* and *F. langesthiae* (Bjørnstad and Skinnes, 2008; Tekle et al. 2012).

Besides FHB resistance, cereal breeders are now taking into account the nutritional interest of grains. Cereal grains contain a wide range of bioactive compounds providing benefits for human health. Consumption of these "Health Promoting Compounds" (HPCs) has been shown to prevent various human diseases such as cancer, cardiovascular diseases, type II diabetes, Parkinson disease, while reducing the risk of obesity (Gani et al. 2012; Topping 2007; Ward et al. 2008). Among these HPCs, wheat grains contain numerous antioxidant compounds contributing to the enhancement of the immune system. (Blandino et al. 2013; Nishino et al. 2011). Oat and barley grains are sources of β-glucan, a soluble fiber shown to control glycemic index and to regulate satiety feeling and cholesterol levels (Battilana et al. 2001; Wood 2007). Thus, in order to improve the nutritional balance of consumers, value added cereals with such health-promoting properties are now receiving an increasing interest from customers, food manufacturers and distributors. To date, wheat, barley and oat varieties producing grains with enhanced HPCs contents are already available for the development of health-promoting foods (Cervantes-Martinez et al. 2001; Varga et al. 2013). Interestingly, *Fusarium* growth and toxin synthesis activity can be inhibited by several cereal endogenous HPCs (Bakan et al. 2003; Skadhauge et al. 1997). This statement is, however, limited to *in vitro* conditions. As of yet, it is unclear if the HPC in cereal grains contribute to enhancing resistance against *Fusarium* pathogens *in planta* under field conditions.

This work is a part of the " Healthy and Safe" project (Swiss national research program NRP69) aiming to reduce the risk of contamination by mycotoxins in wheat, barley and oat grains while developing varieties with benefits for health. This thesis examine resistance of the varieties with enhanced HPCs contents and the role of these HPCs in grain resistance. Moreover, the " Healthy and Safe" project will deliver an inventory on the occurrence and the underlying environmental and agronomic factors of *Fusarium* species, and epidemiological investigations on the most prevalent species in oat and barley. This project will result in the development of strategies to reduce the risk of contaminations using combination of suitable cropping factors and resistance improved varieties, while promoting the production of healthy cereals.

This introduction will present the different aspects of *Fusarium* infections, beginning with the typical symptoms of FHB and the impacts on grains, the infection processes and conditions propitious for FHB outbreaks. The current knowledge of resistance elements will then be presented for wheat, barley and oat. Finally, HPCs found in small grain cereals will be described, and our research hypothesis suggesting a contribution of HPCs in resistance against *Fusarium* pathogens will be explained.

1. *Fusarium* infections

1.1. Symptoms and consequences of FHB on wheat, barley and oats

1.1.1. Visual symptoms of FHB

FHB infections cause typical symptoms on wheat and barley spikes. The most easily recognizable symptom is a progressive blighting of the spike after flowering (figure 1.a-b). In wheat spikes, this blighting may be preceded by the apparition of brown, purple to black necrotic lesions on the exterior surface of the florets and glumes. The awns, if present, become deformed and curved downward (figure 1.a). In barley, the infected spikelets show generally a water-soaked or browning appearance before blighting (figure 2a). In both wheat and barley spikes, the presence of pink mycelium, or orange spore masses can be noticed in case of severe infections. Wheat and barley grains heavily infected by *Fusarium* pathogens are easily recognizable by their pinkish discoloration attributed to fungal mycelium or spore masses (figure 1c—2c). Moreover, infected wheat grains are often shrunken or even aborted, and can be identified by their shriveled appearance (figure 1c) (Goswami and Kistler 2004; Jones and Mirocha 1999; Parry et al. 1995). Depending on the severity of the infection and the

environmental conditions, black perithecia can be observed on infected barley grains (figure 2b) (McMullen et al. 1997; Berger et al. 2014).

Figure 1. a. Early symptoms on wheat spike of *Fusarium* infection of the spikelets; b. Progressive blighting of the spike typical of FHB due to the propagation of the pathogen within the spike. c. Wheat grains infected by *Fusarium* pathogens, recognizable by shriveled aspect and in this case presence of pink mycelium typical of severe infections. (Source: MARTIN Agroscope)

Figure 2. a. Early symptoms of FHB on 2 rows barley spikes, recognizable by brown spots on spikelets (Source: SCHONEBERG, Agroscope). **b.** *Fusarium* infected barley grains, recognizable by brown discoloration and presence of black perithecia (Source: Barley Alberta). **c**. Pink discoloration of barley grains due to presence of *Fusarium* spores and mycelium, associated with grain deformation, typical of severe infection (Source: laborundmore.com)

In oat, a lack of typical FHB symptoms on panicles and on grains has been reported by numerous epidemiolocal studies (Bjørnstad and Skinnes 2008; Tekauz et al. 2004; Yan et al. 2011). In certain cases, infected spikelets can be recognized by blighting and orange to brown discoloration, but such symptoms are often restricted to one heavily infected spikelet on the top

of the panicle (figure 3a) (Bjørnstad and Skinnes 2008). Generally, highly infected grains are aborted, and no sign of *Fusarium* infection can be observed in contaminated harvested oats (figure 3b).

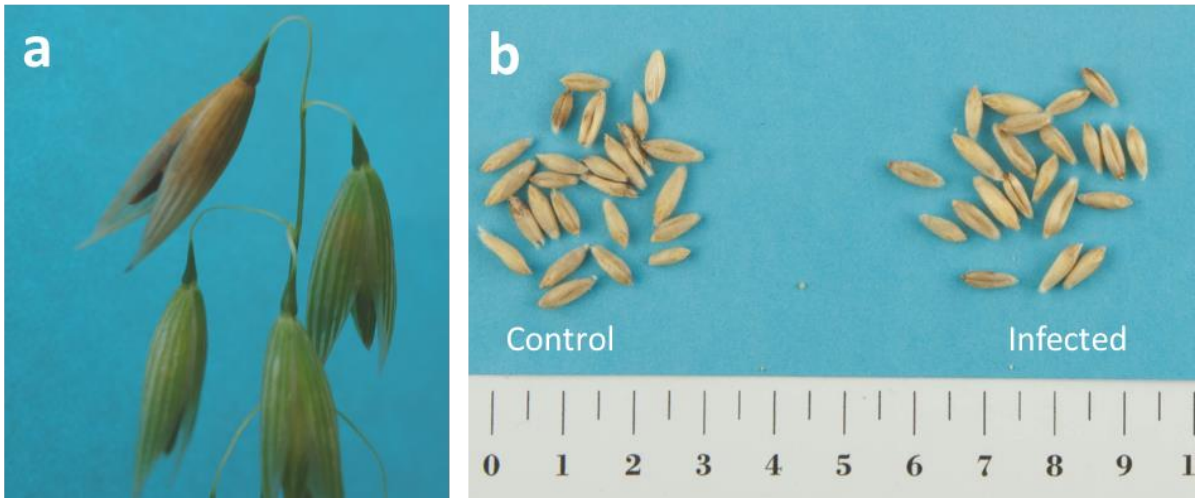

Figure 3 a. FHB symptomatic oat spikelet resulting from natural infection. **b.** Lack of visual symptoms of oat grains artificially infected with *F. graminearum.* (Source: MARTIN Agroscope).

1.1.2. Mycotoxins of *Fusarium* pathogens

Fusarium infected grains can contain a wide range of mycotoxins. The most frequently encountered *Fusarium* mycotoxins in FHB in Europe has proved to be deoxynivalenol (DON) produced by *F. graminearum* and *F. culmorum*, while zearalenone is more common in colder conditions in northern European countries (Bottalico and Perrone 2002). Nivalenol was usually found associated with deoxynivalenol and its derivatives (mono-acetyldeoxynivalenols) (Bottalico and Perrone 2002; Quarta et al. 2005). In Switzerland also, deoxynivalenol is the prevalent mycotoxin in wheat and barley grains (Vogelgsang et al. 2011; Schöneberg et al. 2016). The type B trichothecenes affect human health through acute temporary nausea, vomiting, diarrhea, abdominal pain, headaches, dizziness, and fever (da Rocha et al. 2014). They also lead to feed refusal, and animal health issues, leading to fertility losses. These toxins are produced by the most common pathogens causing FHB on cereals in temperate climate: *F. graminearum* Schwabe (telemorph = *Gibberella zeae* (Schwein)Petch) and *F. culmorum* (W.G Schmidt) (Bottalico and Perrone, 2002; Pasquali et al. 2016). Contaminations with fusarenone-X have been associated with infections with *F. graminearum* and *F. culmorum*. The other widespread *Fusarium* species on small grain cereals are *F. avenaceum*, producing moniliformin and also beauvericin, *F. crookwellensee*,DON producer and *F.poae* mainly causing grain contamination with nivalenol (Bottalico and Peronne 2002) . Furthermore, *Microdochium*

nivale and *M. majus* can lead to FHB symptoms and yield reductions, however, these pathogens do not produce toxins (Glynn et al. 2005; Osborne and Stein 2007; Xu et al. 2005).

Recently, a considerable increase of nivalenol (NIV) and T-2/HT-2 toxin contamination has been observed in oat crops across Europe, especially in Nordic countries (Bernhoft et al. 2012; Edwards et al. 2009; Scudamore et al. 2007; Van der Fels-Klerx and Stratakou 2010). These metabolites are highly toxic (Imathiu et al. 2016). For example, NIV frequently produced by *F. poae* (no teleomorph known), is a vomitoxin ten times more toxic for human health than DON (EFSA 2013; Pitt 2013),. T-2 and HT-2 toxins are produced by *F. langsethiae* (Torp and Nirenberg no teleomorph known) and *F. sporotrichioides* (Nathanail et al. 2015) Toxic effects of T-2/HT-2 include growth retardation, myelotoxicity, hematotoxicity and necrotic lesions on contact sites (Rocha et al. 2005).

Regulations are needed to limit the amount of *Fusarium* toxins entering the food chain and ensuring safety of cereal products. The European Commission Regulation (EC) No 1881/2006 has set 1250µg/kg of DON as the maximum limit in unprocessed wheat and barley grains, and 1750 µg/kg in unprocessed oat grains. The maximum indicative levels of DON content decrease in cereal products for food down to 750 µg/kg. Furthermore, the European Commission Recommendation 2013/165/EU provides indicative levels for the sum of T-2/HT-2 toxins in cereals and cereal products ranging from 15 µg/kg for cereal-based foods for infants and young children up to 2000 µg/kg for oat milling products.

1.1.3. Impact of *Fusarium* Infection on grain properties

Besides contamination with noxious mycotoxins, infection of grains with *Fusarium* pathogens impair grain properties. Reductions of grain weight have been noticed in wheat, barley and oats (Chełkowski et al. 2000; Jones and Mirocha 1999; Martinelli et al. 2014). This finding is due to the reduction of grain filling provoked by the infection. Reduction of seed germination capacity is also a sign of FHB infection (Browne and Cooke 2005; Tekle et al. 2013).

In addition, *Fusarium* infections can alter grain quality. For example, a reduced bread-making quality of wheat has been noticed when using *Fusarium* infected grains (Boyacioğlu and Hettiarachchy 1995; Häller-Gärtner et al. 2008), and beers brewed with *Fusarium* infected

barley grains are prone to gushing (Oliveira et al. 2013). Moreover, reductions of protein and starch contents have been reported in infected wheat and barley grains (Häller Gärtner et al. 2008; Oliveira et al. 2013).

1.2. Infection processes in wheat, barley and oats

1.2.1. Infection routes of *Fusarium* pathogens in wheat, barley and oats

The disease cycle of *Fusarium* spp. on small grain cereals has been extensively studied and is reviewed by Parry et al. (1995). Central to the infection process is the initial source of inoculum from *Fusarium* spp. surviving in the soil, either as mycelium on crop debris or as resting spores (chlamydospores), depending on the *Fusarium* species. It has been recently demonstrated that panicle is also the most propitious access for F.poae and F.langsethiae in oat (Divon et al. 2012). For all cereal species, flowering is the most favourable time for infection (Xue et al. 2015; Divon et al. 2012). With high relative humidity or rainfalls, conidia or ascospores penetrate the floret via the anthers, and germinate on the ovary (Miller et al. 2004). More specifically, in wheat, the hyphae penetrate the rachis and propagate throughout the spike via the vessels (Bushnell et al. 2003). In six-row barley, hyphae often propagate externally from one floret to another close one, hence bypassing the rachis (Jansen et al. 2005). In wheat and barley, infection of developing kernel proceeds through the epicarp, destroying the fruit coat, to finally reach the endosperm (Jansen et al. 2005, Miller et al. 2004). The inflorescence form of oat, with long pedicels between spikelets, limits the spread of the pathogen (Bjørnstad and Skinnes 2008). Yet the pathogen colonise the palea, the lemma and finally the developing kernel (Divon et al. 2012; Tekle et al. 2012).

1.2.2. Ability of *Fusarium* pathogens to infect small grain cereals

From the pathogen side, the infection is a highly complex process, involving a myriad of aggressiveness factors including effectors, cell wall degrading enzymes, such as lipases , proteases, pectinases, cellulases and xylanases, as well as a broad spectrum of proteins involving in plant-pathogen interactions (Kikot et al. 2009; Brown et al. 2010; Yang et al. 2013). All these factors allow the pathogen to face various immune surveillance system and defense mechanisms of the plant.

Fusarium species and strains differ in their ability to infect small grain cereals and to cause FHB (Ward et al. 2002). The toxigenic potential of a strain is considered to be the main

determinant of its aggressiveness. It is, however, unclear if the chemotype of the toxin produced influences the infection and the severity of FHB. To date, higher aggressivess of strains producing 3ADON has been reported in several epidemiological studies (Mesterházy et al. 1999; Carter et al. 2002; Goswami and Kistler 2005). Furthermore, genetic studies have revealed genes associated with aggressiveness. Most of them are included in the *Tri g*ene cluster which are associated with mycotoxin synthesis (Menke et al. 2012; Proctor et al. 1995; Cumagun et al. 2004), others appear to be related to the geographical origin of the strain or to the preferred host (Carter et al. 2002; Carter et al. 2000; Harris et al. 2015).

1.2.3. Factors favouring *Fusarium* infection and toxin contamination

Environmental conditions mainly determine FHB outbreaks (Doohan et al. 2003). Generally, moderate temperatures and elevated relative humidity are necessary for pathogen growth and mycotoxin synthesis. Differences in environmental requirements have been noticed between species. For example, *F. graminearum* caused greater FHB symptoms on spikes at 16 °C than at 20 °C, whereas *F. culmorum* is more pathogenic at 20 °C (Brennan et al. 2005). Under *in vitro* conditions, warm and humid conditions (25–28°C, a_w=0.97) are required for growth and toxin synthesis of both *F. graminearum* and *F .culmorum,* whereas *F. poae* appears to prefer lower temperatures with humid condition (20–25°C, a_w=0.99) (Hope et al. 2005; Kokkonen et al. 2010). Generally, growth of *F. langsethiae* is slow, even at its optimal temperature (25°C) (Torp and Nirenberg, 2004).

FHB outbreaks and mycotoxin contaminations are also heavily influenced by agricultural practices. Using *Fusarium* hosts as the previous crop, *a fortiori* susceptible genotypes, as well as practice of no-tillage increase the risk of FHB caused by *F. graminearum* (Dill-Macky and Jones 1993; Dorn et al. 2009; Schöneberg et al. 2016; Vogelgsang et al. 2011). Moreover, lower occurrence of toxins produced by *F. graminearum* was observed in organically cultivated cereals, and was attributed to cropping techniques such as wider crop rotations, use of plough and lower nitrogen fertilization (Edwards 2004; Schöneberg et al. 2016).

2. Resistance of small grain cereals against FHB

Worldwide, introduction of less susceptible varieties and adaption of agronomic practices have allowed to reduce outbreaks of FHB epidemics and thus to ensure the production of safer

grains. Nowadays, resistance against FHB and trichothecenes is one of the key characteristics when breeding for new varieties of wheat and barley, and resistance against toxin contamination is currently desired in oat breeding programs (Bjørnstad and Skinnes 2008; Gagkaeva et al. 2011; Yan et al. 2010).

Resistance to FHB is a highly complex character: quantitative in nature, horizontal, and based on cumulative effects of many genes. Furthermore, most breeding studies concentrate on resistance towards *F. graminearum* or *F. culmorum*, while only little is known about the resistance mechanisms against other FHB causing species. To date, total immunity to the disease has not been observed. For a long time, the poor understanding of the genetic and molecular basis of resistance as well as the lack of precise phenotyping has hindered the development of FHB resistant varieties. Gene discoveries and identification of resistance mechanisms in wheat has allowed the distinction of different resistance types and the development of marker assisted selection (Bai and Shaner 2004). Yet, knowledge and resistance materials are broadly inadequate to improve FHB resistance of oat and barley.

Generally, the development of FHB resistant genotypes has proven to be a difficult task. The challenges breeders are confronted with are:

(1) the effects of environmental conditions on resistance elements that reduce the stability of resistance in genotypes.

(2) the complexity of the resistance character that include resistance elements of head and grains, against both fungal colonization and toxin accumulation.

(3) the poor agronomic traits of resistant germ-plasms used as a source of resistance in breeding programs. For example, the wheat genotypes Sumaï 3 (exotic germ-plasm from China), Alsen (USA) and Frontana (Brazil) are sources of resistance in numerous wheat breeding programs but all are characterized by poor agronomic traits and yield potential. The Swiss variety Arina is used as a source of resistance in the European winter wheat gene pool, but it is susceptible to brown rust and to lodging. (Ruckenbauer et al. 2001; Fossati, personal communication)

(4) the lack of gene-for-gene resistance that impede the prospect of total immunity.

2.1. Resistance types of wheat

Numerous studies of FHB resistance on wheat have allowed to identify different types of resistance (table 1). These different resistance types can interact with each other to improve the overall resistance.

Table 1. Resistance types of wheat against Fusarium head blight.

Resistance	Description
Type I	Resistance to primary infection (Schroeder & Christensen, 1963)
Type II	Resistance to disease spread (Schroeder & Christensen, 1963)
Type III	Resistance to kernel infection (Mesterházy, 1995)
Type IV	Tolerance against FHB and trichothecenes (Mesterházy, 1995)
Type V	Resistance to trichothecene accumulation (Miller et al. 1985)
- Type V class 1	- by chemical modification of trichothecene (Boutigny et al. 2008)
- Type V class 2	- by inhibition of trichothecenes biosynthesis (Boutigny et al. 2008)

The type I and type II resistance assemble resistance mechanisms of the spike aiming to limit primary infection of flower tissues and then the propagation of *F. graminearum/F. culmorum* throughout the spike. The type III resistance encompasses all resistance mechanisms avoiding the colonisation of developing kernel by hyphae of *F. graninearum/F. culmorum* hyphae. The type IV resistance characterises the stability of production, meaning that yield and quality in terms of low toxin contamination is maintained despite *Fusarium* infection. Finally, the type V resistance is subdivided in two classes. Type V-1 results in plants able to chemically modify trichothecenes, and type V-2 refers to genotypes with the ability to inhibit the trichothecene biosynthesis from *F. graminearum/F. culmorum* pathogens.

2.2. Phenotyping resistance types

Basically, the aim when selecting for resistance in plant breeding programs is to assemble all types of resistance in new germ-plasms. For that, accurate evaluations of all resistance types are required.

2.2.1. Tests for resistance evaluation

Field tests remain the most reliable way to assess the multiple elements of resistance while evaluating agronomic traits of genotypes (Mesterházy 1978). In the field tests devoted to FHB resistance, plants are routinely artificially inoculated by spraying solutions containing *F. graminearum/F. culmorum* conidia on flowering heads. This method has the advantage to control the disease pressure applied in the field. In this way, a higher chance of successful infection on wheat, barley and oat in field resistance tests is obtained (Geddes et al. 2008; McCallum and Tekauz, 2002; Xue et al. 2015).

Additional tests are available for rapid screens of the resistance level in cereal genotypes. For example, inoculations in greenhouses offer the possibility of running phenotypic evaluations throughout the year, but cannot afford reliable screening of grain resistance components across variable conditions. Furthermore, detached leaf assays and germination tests of infected grains allow early screening of the general susceptibility level of genotypes (Browne and Cooke 2005; Diamond and Cooke 1999).

2.2.2. Indicators of resistance

Indicators of resistance are available to characterize the different resistance types of wheat genotypes growing in field tests (table 1). Type I and type II resistances are jointly assessed by comparison of FHB symptoms on spikes (Foroud and Eudes 2009). Distinct evaluations of type I and type II resistance separately is impeded by field conditions, the differentiation of symptomatic spikelets caused by primary infection or by propagation of the pathogen within the plant being impossible. Practically, type I resistance can be approximate by measuring the incidence of symptomatic spikes and type II resistance by observation of FHB severity (Dill-Macky et al. 2003). Evaluation of oat head resistance is hampered by the lack of visual symptoms. Concerning wheat grain resistance, the frequency of symptomatic FHB grains, so-called *Fusarium* damaged kernels,(FDK) is used to measure type III resistance of wheat (Jones and Mirocha 1999). Other methods, such as Seed Health Tests (Vogelgsang et al. 2009). allow to screen type III resistance and the fungal colonisation in wheat grains and can be used for barley and oat studies (Schöneberg et al. 2016; Gagkaeva et al. 2011). Reduction of grain weight caused by FHB can be evaluated by comparing thousand kernels weight (TKW) between infected and non-infected cereal grains (Jones and Mirocha 1999). This allows to estimate the

yield stability despite infection, related to type IV resistance. Finally, the quantification of toxins in grains obviously attests of type V resistance.

2.3. Mechanisms of resistance

Wheat, barley and oat differ in their resistance mechanisms. Wheat is the most FHB susceptible cereal, whereas oat has been considered to be generally less suceptible to *Fusarium* infections (Foroud and Eudes 2009). Indeed, the panicle shape of oat, with long pedicels separating the spikelets and the rachis hinders to a certain extent the propagation of the pathogen in plant tissue, while the presence of several layers of glumes act as a barrier limiting the primary infection and fungal colonisation of grains (Bily et al. 2003; Langevin et al. 2004; Tekle et al. 2012). Concerning barley, the spacing between spikelets confers to two-row varieties a higher FHB resistance compared with six-row varieties (Mesfin et al. 2003).

Cereals possess both constitutive and induced resistance mechanisms against FHB. Together, these resistance mechanisms span all the facets of *Fusarium* infections and operate in the five resistance types of cereals (table 1)

2.3.1. Constitutive resistance of wheat, barley and oat

The constitutive resistance mechanisms encompass all structural and physiological traits non-activated in case of infection. In brief, a short anthesis period, closed spikelets at the same stage, reduced number of flowers with weak anther extrusion, long stems and a thick cuticule are constitutive elements of spike resistance that hamper the pathogen accession to the florets (Mascher et al. 2005; Serrano et al. 2014). These resistance mechanisms, based on visible morphological and physiological traits, can be easily evaluated and selected for breeding purposes. Other constitutive elements of resistance act as barriers limiting the propagation of *Fusarium* pathogens at the scale of the grain, such as the presence of specific compounds in the pericarp, and at the scale of the cell, such as thick cell walls (Bily et al. 2003; Skadhauge et al. 1997). In addition, cereals also express non specific chemical defence that can delay *Fusarium* infection. For example, wheat spikes contain cell-walls polymers affecting the efficiency of the ecll-wall degrading enzymes produced by the pathogen (pectinmethylesterase) (Lionetti et al. 2015). Moreover, a recent study pinpointed a large diversity of plant constitutive resistance-related metabolites including several classes of antioxidant compounds (Gauthier et al. 2015).

2.3.2. Inductible resistance mechanisms

These mechanisms of resistance are activated by the plant upon infections by the pathogen. The plant detects the infection by recognition of *Fusarium* effectors (such as specific fungal peptides), or by the damages caused by the pathogen (oxidative burst, degradations caused by fungal peptidases or other cell-wall degrading enzymes, etc.). In response, the plant activates the expression of resistance genes related to detoxification, antifungal compound production, defense of photosynthesis and of metabolites transport. these changes impair the spread of the pathogen and the accumulation of toxins (Montesano et al. 2003; Ravensdale et al. 2014). Even if the underlying mechanisms are not fully understood, it is known that several proteins play an essential role in induced resistance mechanisms (Zhang et al. 2013). Moreover, a wide range of plant metabolites including phenolic compounds are suggested to contribute to induced chemical resistance of cereals against *Fusarium* pathogens (Gauthier et al. 2015). Finally, the induction of a primed state in which the plant can more rapidly and efficiently activate its immune defense system could contribute to enhancing induced FHB resistance (Ravensdale et al. 2014).

2.3.3. Resistance genes of wheat, barley and oats

The need for FHB resistant germ-plasms motivated the mapping efforts for resistance gene discovery. These QTL represent a pool of genetic sources of resistance that can be used for marker-assisted-selection.

In wheat, numerous QTLs associated with low FHB severity and toxin accumulation were identified on different chromosomes (Shen et al. 2003; Zhou et al. 2002; Paillard et al. 2004; Jayatilake et al. 2011). For example, the Swiss variety Arina owes its spike resistance to eight QTL mapped on the chromosomes 6D, 5B and 4A (Paillard et al. 2004). The best characterized and thus the most widely used source of resistance in wheat is the locus Fhb1 on chromosome 3BS. Fhb1 has been detected in the resistant Chinese variety Sumai 3 and can explain alone up to 60% of the phenotypic variation in DON accumulation and symptoms on spikes (Zhou et al. 2002; Rudd et al. 2001). This locus is associated with induction and regulation of the expression of several proteins, acting in detoxification, defense of photosynthesis processes and against fungal penetration in wheat tissues (Zhang et al. 2013).

In barley, several QTLs associated with resistance of spike and against toxin accumulation were identified on several chromosomes (De la Pena et al. 1999). However, most

of these QTL are associated with morphological traits such as plant height, two-row spike morphology, and so, are not useful for marker-assisted-selection (Choo et al. 2004; Ma et al. 2000). Yet the two major QTL for FHB resistance have been mapped in the chromosome 2Hb8 and 6Hb7, and respectively associated with heading date and activation of defense response (Huang et al. 2016). In barley, the genetic effect of the QTLs associated with resistance is weaker than in wheat, never explaining alone more than 10% of phenotypic variation of FHB severity or toxin accumulation (Capettini et al. 2003).

Mapping of resistance source in oat has lagged far behind, as the plant has long been thought to be free of FHB problem. Only very recently studies revealed presence of several minor QTLs associated with resistance to DON (Bjørnstad et al. 2017).

2.4. Stability of resistance

When measuring symptoms on plants and toxin contents in grains, breeders not only observe resistance *per se*, but also the results of the complex interactions between plant resistance mechanisms, pathogen aggressiveness and environmental conditions. To be considered as efficient, a resistance trait has to be stable across a wide range of environmental conditions and common to various *Fusarium* species and strains.

2.4.1. Common resistance to various *Fusarium* species

When selecting for resistance it first has to be clear if the resistance observed applies only for a given *Fusarium* species or for all *Fusarium* species infecting cereals. In wheat and barley, it was suggested that resistance traits are common for all FHB causing species (Mesterházy et al. 2005; Mesterházy et al. 1999). However, the situation for oat is still unclear. To date, responses of oat genotypes in terms of yield reduction and toxin contamination against numerous *Fusarium* pathogens have been observed, but were never compared (Bjørnstad and Skinnes 2008; Mielniczuk et al. 2004; Tekle et al. 2012). More particularly, it has to be further investigated if the resistance to toxin accumulation is common for all toxigenic *Fusarium* species infecting oats.

Secondly, it has to be evaluated whether resistance observed provides an efficient resistance even in with the presence of highly aggressive strains. To date, the mechanisms of interactions between strain aggressiveness and plant resistance are not fully understood. Only a stable

resistance under various disease pressure levels can result in cereal production free of FHB and toxins. For example, the strong resistance of Sumai 3 was repeatedly observed against a worldwide collection of *F. graminearum* isolates with varying levels of aggressiveness (Bai and Shaner 2004).

2.4.2. Impact of the environment on FHB resistance

In all small grain cereals, the expression of resistance elements are modulated by environmental conditions. Practically, it means that a variety can appear resistant to *Fusarium* infection and toxin accumulation in one location, while being susceptible in other environmental conditions (Miedaner et al 2001; Gagkaeva et al. 2011). Hence, to evaluate the stability of resistance, field tests are routinely implemented in multiple locations. The phenotypic variations of disease symptoms are a result of the expression of resistance traits of the host, environmental conditions and the interaction between the two factors (called Genotype x Environment GxE interactions). For the breeder, it is necessary to determine the heritability of the resistance within its screened population, defined as the share of the phenotypic variability in FHB symptoms. It represents the flexibility of resistance in selection and the gain that can be expected when selecting for FHB resistance in the considered population. Indeed, if the genotypic effect explains the largest part of the phenotypic variation, it reveals the presence of resistance traits highly inheritable that can be selected. On the contrary, if the phenotypic variability observed in FHB symptoms is mainly explained by the environment, it shows that resistance elements cannot control FHB outbreaks. In this case, selecting for FHB resistance in the considered population is useless, since the expected genetic gain in FHB resistance is not sufficient to face the strongest environmental effects. Finally, the presence of GxE interactions are a key element when breeding for FHB resistance. Ideally, these GxE interactions should be weak. Indeed, significant GxE interactions indicate a weak stability of the resistance across different environmental conditions and a moderate heritability of the resistance. These interactions reduce the share of resistance that can be controlled by selection (Capettini et al. 2003; Choo et al. 2004; Massman et al. 2011).

2.5. Need for an improved characterization of FHB resistance in small grain cereals

The impacts of FHB on barley and oat grains were considerably less investigated than on wheat grains. Moreover, for all cereal species, little is known about the consequences of infections by different *Fusarium* species on grain properties and functionalities. The state of knowledge of the impacts of *Fusarium* species infection on wheat, barley and oat are summarized in the figure 4. In particular, it is necessary to obtain knowledge about all potential impacts of *Fusarium* species infection on oat grains, before looking for resistance elements.

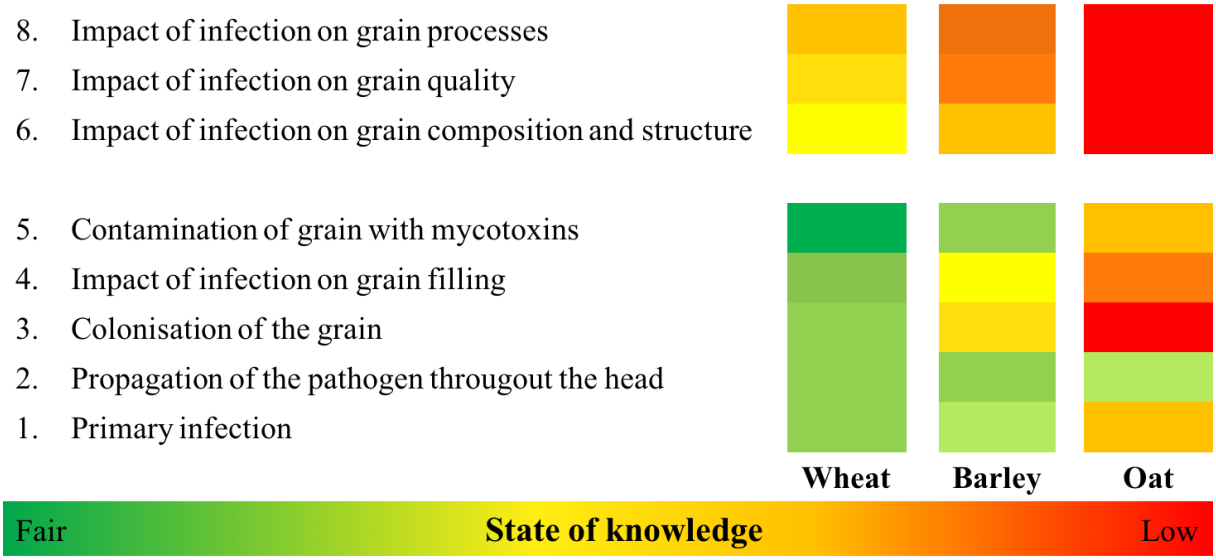

Figure 4. Comparisons of the knowledge about the impacts of *Fusarium* infections on wheat, barley ant oat head (1-2) and grains (3-8). The impacts listed 1 to 5 are considered by the resistance types I to V described in wheat. The presence of resistance traits in cereals against the impacts listed 6-8 have not been investigated yet.

2.5.1. Need for better characterization of resistance types in barley and oat grains

The resistance types I-V, even if they have been described on wheat, are now used to screen resistance of barley and oat genotypes (Bai et al. 2003 ; Gagkaeva et al. 2013; Yan et al. 2010). Yet, wheat, barley and oat differ in their resistance elements and in the infection processes.

Cereal species differ first in their morphological properties and constitutive resistance mechanisms. The panicle shape confers to oat an inherent and strong type II resistance (Bjørnstad and Skinnes 2008; Tekle et al. 2012). Type II resistance is not useful for six-row barley as the fungus does not penetrate the rachis during propagation (Jansen et al. 2005; Maier et al. 2006). The role of trichothecenes during the infection process also differs between the cereal species. In wheat, trichothecenes were suggested to help the fungus entry into the rachis

(Bai et al. 2002). Due to the external propogation in six-row barley, the presence of trichothecenes appears to be redundant. The shape of the oat panicle sems to limit the propagation of the pathogen independently of the presence of trichothecenes (Langevin et al. 2004). In wheat, the severity of symptoms on spike is often correlated with the mycotoxin accumulation in grains (Mesterházy 2002; Schnerr et al. 2002; Snijders and Perkowski. 1990). It is, however, not possible to predict toxin contamination of oat and barley grains by observation of FHB symptoms on the spikes. This example illustrates the need for appropriate resistance indicators and for adapted resistance types specific to oat and barley. Hence, the resistance types previously defined on wheat (table 1) cannot accurately reflect the resistance elements operating in barley and oat.

As of yet, resistance studies in barley and oat aim to evaluate the toxin contamination. DON accumulation seems to be the only criteria available to evaluate resistance of oat genotypes, and absence of hulls was suggested to be the only factor of resistance identified (Yan et al. 2010; Tekauz et al. 2008). Oat resistance against other toxins should be considered and hence, varieties able to avoid NIV and T-2/HT-2 toxin contamination are required.

Besides toxin contamination, potential other impacts of FHB on oat and barley grains received limited interest (Gagkaeva et al. 2011; Nielsen et al. 2014). To date, it is well recognized that FHB in barley results in yield reduction (Chełkowski et al. 2000). Concerning oats, some studies related reduction of grain weight with FHB (Šliková et al. 2010) while others affirmed that *Fusarium* infection does not impair the grain formation and filling (Tekauz et al. 2008)

2.5.2. Impact of *Fusarium* infection on biochemical properties of grains

Previously, reduction of wheat bread making properties and of barley malting and brewing qualities have been reported for grains infected by *F. graminearum and F. culmorum* (Häller-Gärtner et al. 2008; Nielsen et al. 2014; Oliveira et al. 2012). These degradations are evident consequences of the alterations caused by the pathogen on grain structure and constituents, including starch and proteins (Bechtel et 1985; Dexter et al. 1996; Oliveira et al. 2013). These changes can be attributed to the action of fungal enzymes such as protease, α- amylase or other destructive enzymes accompanying the propagation *Fusarium* species in grain tissues (Nightingale et al. 1999; Schwarz et al. 2002; Wang et al. 2005). On the other hand, *Fusarium* infections also reduce the grain filling and transport of metabolites. This could directly impair

grain biochemical processes responsible for the storage of sugars as starch as well as the synthesis of protein and gluten formation (Feillet 2000). Häller Gärtner et al. (2008) observed differences in the reduction of grain bread making quality and protein contents caused by *F. graminearum* between wheat genotypes, but to date, the presence of resistance mechanisms preventing degradation of grain components and structure has not been investigated.

3. Health Promoting Compounds in small grain cereals

Cereal grains contain a wide range of bioactive compounds including several HPCa (Grausgruber et al. 2004; Ward et al. 2008). HPCs have recently received increased attention both in nutrition and agronomic research fields. As a resault, the HPCs present in cereal grains are now identified and the majority of them can be easily quantified. In parallel, *in vitro* studies proved that several HPCs can have an inhibitory effect on *Fusarium* growth and toxin accumulation.

3.1. Health Promoting Compounds in small grain cereals

It is now widely recognized that a regular consumption of wholegrain products provide health benefits, such as prevention of cardiovascular and chronic diseases, obesity, type 2 diabetes and even some forms of cancer (Nishino et al. 2011; Shuren et al. 2008; Tapola et al. 2005). These benefits can be attributed to the presence of high HPC contents in the outer layer of grains (Gani et al. 2012) These health benefits are achieved through multifactorial mechanisms including antioxidant activity and the resulting ability to scavenge free radicals, an enhanced immune system, and the stabilization of glucose and cholesterol levels (Halliwell et al. 2005; Pojer et al. 2013). During the past 10 years, HPCs have been the focus of significant scientific and commercial interests (Delcour et al. 2012; Gani et al. 2012). Consequently, enhanced HPC contents in grains became a new desired character in plant breeding programs (Fossati and Schori, Institute for Plant Breeding Agroscope personal communication). The HPCs considered in the present study are ferulic acid, anthocyanin compounds in wheat grains as well as β-glucan in barley and oat grains.

3.1.1. Antioxidant compounds in wheat

Phenolic compounds are the main antioxidant compounds in cereal tissues and grains. This chemical class encompasses phenolic acid, flavonoids, avenathamides, lignans and

alkylresorcinols. Ferulic acid (FA) (4-hydroxy-3-methoxycinnamic acid) is the dominant phenolic compound and the major contributor to the antioxidant capacity of wheat tissues (Adom and Liu, 2002; Anson et al. 2008; Mpofu et al. 2006) (figure 5). FA is present mainly in cell walls bound with carbohydrates, but also in free form (Klepacka and Fornal 2006). In cereals, FA is concentrated in grains, but also present in other tissues, such as flowers and hulls (McKeehen et al. 1999).

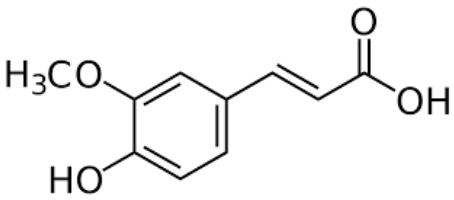

Figure 5. Structure of ferulic acid.

The anthocyanin compounds belong to the flavonoids group of polyphenols, and are responsible for the pigmentation in many plant organs. In wheat, these compounds are mainly concentrated in the aleurone layer and pericarp, bound in cell walls, and provide a blue, purple to dark coloration to the grain (Abdel-Aal et al. 2006; Knievel et al. 2009) (figure 6). These compounds substantially contribute to the significant higher antioxidant activity of colored grains compared with white grains (Hu et al. 2007; Li et al. 2005), and provide recognized benefits for human health (Pojer et al. 2013). Pigmented grains rich in anthocyanins are now desired to produce food associated with a health diet.

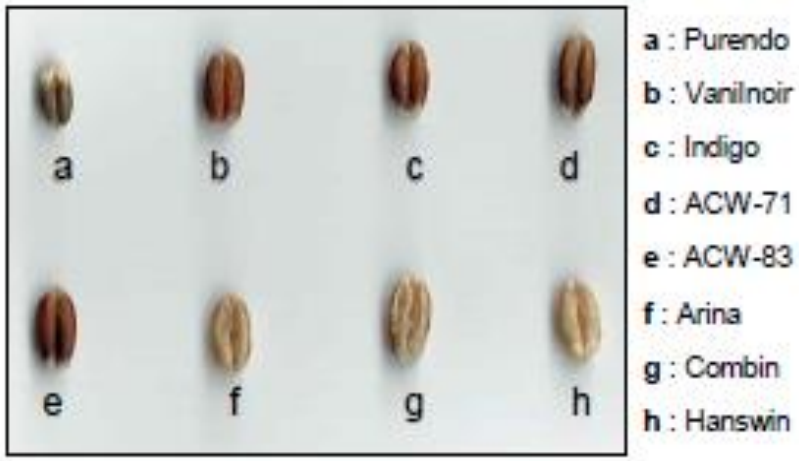

Figure 6. Comparison of grain coloration in eight wheat genotypes due to varied contents in anthocyanin compounds. a. blue endosperm. b-e. dark coloration. f. yellow coloration (high content in lutein). g-h. white grain. (Source: MARTIN Agroscope)

The anthocyanin compounds naturally occur in plants as glycosides, and are composed of an anthocyanidin molecule bound to a sugar group. Chemically, anthocyanidins molecules are polyhydroxy-2-phenylbenzopyrylium of flavylium salts. Few major anthocyanin compounds are responsible of grain coloration, including glycosylated cyanidins, malvinidins and peonidins (Abdel-Aal et al., 2003; Hu et al. 2007) (figure 7). Blue wheat genotypes accumulate delphinidin compounds in endosperm (i.e the wheat variety Purendo) (figure 6). Anthocyanin compounds in wheat grains are synthesised in developing and maturing grains, and synthesis activity grain is directly correlated with the quantity of assimilates in grain tissues (Bustos et al. 2012; Piliarová, 2012). Synthesis activity is also influenced by environmental conditions. (Chalker-Scott 1999; Gordeeva et al. 2013). The impact of biotic stresses such as FHB disease on anthocyanin contents in grains has never been investigated.

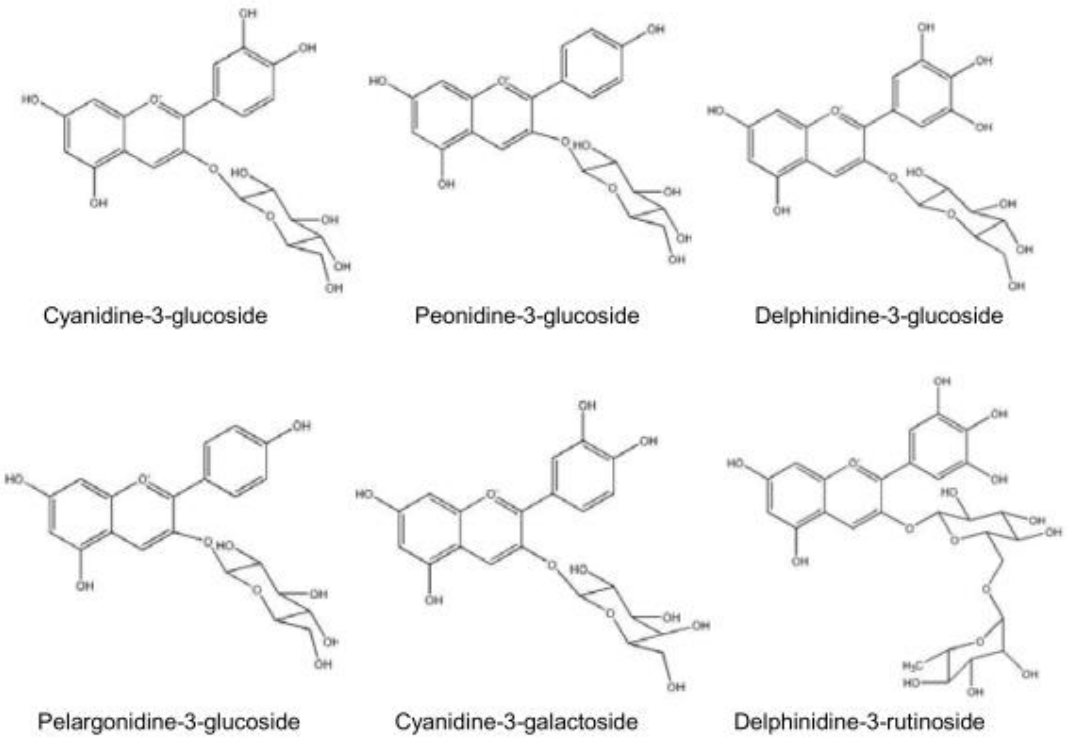

Figure 7. Main anthocyanins of coloured wheat grains (Source: Havrlentová et al. 2014)

The anthocyanin compounds are extractable in ethanol or methanol, and their total concentrations can be measured by analyses of anthocyanins pigmented absorbance extracts, or by chromatographic methods (Abdel-Aal and Hucl 1999) (figure 8). The total amounts of anthocyanins in pigmented wheat grains can reach 160 mg kg^{-1} and up to 460 mg kg^{-1} in flour and bran of blue coloured wheat, respectively (Abdel-Aal and Hucl 1999; Havrlentová et al. 2014).

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Figure 8. Extraction of anthocyanin compounds in acidified methanol, from whole meal flour of the wheat varieties Vanilnoir, Indigo and Purendo (producing coloured grains), and Hanswin (white grains) (Source: MARTINI Agroscope).

3.1.2. β-glucan in barley and oat grains

Oat and barley grains contain high contents of β-glucan, a dietary fiber recognized for its interest in heathy diet by EFSA (European Food Safety Authority, 2011) and FDA (FDA 1997, FDA 2005). To date, more than numerous scientific studies demonstrated the role of β-glucan in stabilization of serum prosprandial glycemic index, reduction of blood LDL cholesterol, increase of satiety filling and in optimization of digestion processes (Wood 2007). Health benefits of β-glucan can be attributed to its high viscosity and antoxidant properties (Anttila et al. 2004; Kofuji et al. 2012). Chemically, β-glucans are mixed-linked (1–3) (1–4)- β-D-glucan (figure 9), organised in ahelical chain.

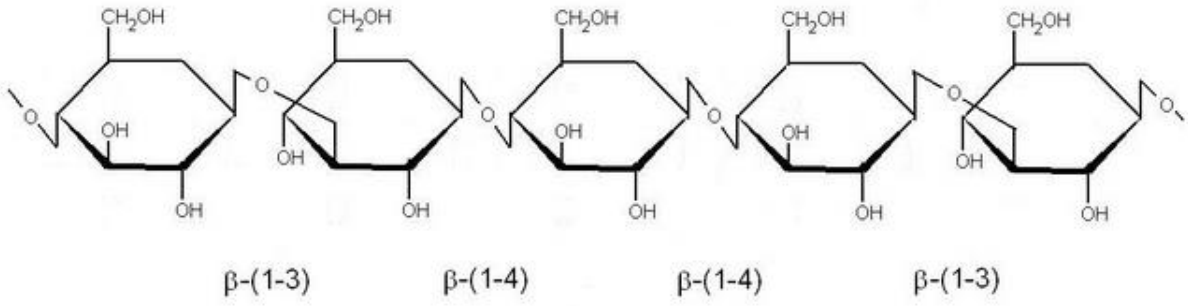

Figure 9. Structure of (1–3) (1–4)-β- D-glucan present in barley and oat grains.

B-glucans are accumulated in the subaleurone layers of oat grains, and are constitutive elements in the endosperm cell walls of barley grains. B-glucan contents range in both species between 2% and 10% of grain dry mass, depending on the variety. The standard concentration is between 4.5 and 5% of β-glucan (Peterson 1991; Zhang et al. 2002). B-glucan are constituted

of glucose molecules and are synthesised during grain maturation. (Becker et al. 1995; Burton et al. 2016; Bustos et al. 2012). The synthesis activity depends on the availability of glucose in the grains and on the cell conditions required for enzymatic synthesis activity. B-glucan content in barley and oat is a polygenic trait influenced by environmental conditions, agronomical practice as well as abiotic stresses (Güler 2011; Redaelli et al. 2013; Saastamoinen et al. 2004).

The suitability of β-glucan in grains depends on their end use: high contents are desired when producing healthy food products, whereas higher contents can impair fermentation and malting processes (Fincher 1975; Le Guennec and Martin, personnal communication). B-glucan contents in flour of cereals and in transformed products can be measured with enzymatic methods or by viscosity analysis (Anttila et al. 2004; Cho and White 1993).

3.2. Health Promoting Compounds in wheat, barley and oat as factors of resistance

Our research hypothesis is that HPCs can contribute to FHB resistance of grains. Several elements support this assumption.

Antixoxidant compounds are already recognized as factors of chemical defense system of plants against various pests and diseases (Hucl et al. 2001; Lattanzio et al. 2006; Treutter 2006) and are involved in both constitutive resistance mechanisms and in induced resistance. Indeed, the presence of antioxidant compounds in plant tissues were suggested to act as a physical barrier against pathogens, while moderating the oxidative stress in plant cells caused by fungal infection and pest injuries (Lattanzio et al. 2006). Moreover, it is well acknowledged that in response to pathogen infection, phenolic acids are released from the cell wall or massively synthesized by the plant to accumulate rapidly at the site of infection (Gauthier et al. 2015).

The ability of HPCs to interact with various *Fusarium* pathogens has been demonstrated under *in vitro* conditions. In artificial media, several natural phenolic compounds proved their inhibitive potential on *Fusarium* growth and mycotoxin production (Boutigny et al. 2010; Ferruz et al. 2016). It has been demonstrated that phenolics can directly interact with *Fusarium* pathogens by repressing the expression of fungal *Tri* genes involved in trichothecene synthesis (Boutigny et al. 2010). Moreover, besides antioxidant properties, β-glucan has the ability to bind a wide range of molecules, including most of the trichothecenes produced by *Fusarium*

pathogens (Yiannikouris et al. 2006) and the possibility to use β-glucan for detoxification of *Fusarium* contaminated products is now currently explored (Avantaggiato et al. 2005)). Yet, evidence of the *in vivo* contribution of antioxidant compounds and β-glucan in resistance to FHB are limited. Positive correlations were reported between phenolic compounds contents and resistance level of maize kernels and palm roots against *Fusarium* pathogens (Bily et al. 2003; El Modafar and El Boustani, 2001). In wheat, significantly higher antioxidant capacity was measured in grains of a variety with low susceptibility than in grains of a highly susceptible variety (Zhou et al. 2007). The contribution of HPCs in cereal grains resistance in planta under field conditions needs to be explored.

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Objectives of the project

The purpose of this thesis is to understand whether HPCs contribute to the resistance of cereal grains against infection through different *Fusarium* species.

Our study considers three cereal species, wheat, barley and oats for which the resistance elements and stability across environments were investigated.

- Chapter I is a preliminary study aiming to understand the variability in the aggressiveness of strains within a *Fusarium* population infecting wheat and maize. The results will provide information necessary both for the implementation of field tests with artificial inoculations and interpretations of the results in the following chapters.
- In chapter II, the role of HPCs in grain resistance was evaluated. For this purpose, the contribution of ferulic acid in wheat florets towards inhibition of *F. graminearum* infection of spikes was investigated.
- In chapter III, the impacts of *F. graminearum* infection on wheat grain components, quality and biochemical processes was characterised. Furthermore, the contribution of anthocyanin compounds in grain resistance was investigated.
- In chapter IV, we aimed to characterize the impacts of FHB on barley, to identify the specific resistance elements of barley grains and to assess the role of β-glucan in grain resistance.
- In chapter V, we investigate the disease response of oat varieties against *F. poae* and *F. langsethiae* as well as the role of β-glucan against *Fusarium* pathogens.

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Chapter I

Chemotype and environment condition the aggressiveness of *Fusarium graminearum* and *F. culmorum* on wheat and maize

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Summary

Fusarium head blight of wheat and ear rot of maize is an economically important disease in all wheat producing areas. In Switzerland, the main pathogens are *Fusarium graminearum* and to a lower extent *F. culmorum*. Different studies have shown that natural *Fusarium* populations can be highly divers in terms species, chemotype and aggressiveness. Moreover, the severity of infections is conditioned by the environment and the resistance reaction of the host. The aim of this study was to better understand the factors determining the aggressiveness of 40 *F. culmorum and F. graminearum* strains, isolated on symptomatic wheat and maize ears in different sites in Switzerland. Species and chemotypes of all 40 strains were determined and 17 strains were selected for aggressiveness experiments on wheat in the greenhouse and in the field. Among these, eight strains were also tested on 2 varieties of maize. Assessments of the severity of symptoms indicate a large spectrum of aggressiveness among the strains. On wheat, NIV-producers were less aggressive than DON-producers. Neither the *Fusarium* species nor the original host determined the aggressiveness. Strains that are highly aggressive on wheat caused also severe symptoms on maize and vice-versa. In a second series of experiments, one highly aggressive and one poorly aggressive *F. culmorum* strains were tested in a multi-local test on wheat. Under favourable conditions for infection, both strains caused equivalent symptoms. In contrast, under unfavourable conditions, only the highly aggressive strain caused symptoms. Our results suggest that (a) within a *Fusarium* population, the aggressiveness is strain specific, (b) the chemotype is an aggressiveness factor, (c) aggressiveness is not related to the original host nor to host resistance, (d) the environment modulates the virulence of the pathogen rather than the resistance of the host. This information is important to understand host resistance assessment and to choose appropriate strains for resistance assessments.

1. Introduction

Several *Fusarium* species can infect the ears of wheat and maize, causing yield reduction and qualitative losses by contamination with various types of mycotoxins (Parry et al. 1995). The main pathogens of both diseases are *Fusarium graminearum* and, to a lesser extent, *F. culmorum* (Oldenburg and Ellner 2015). In wheat, spores of the pathogen penetrate into the spike at anthesis infecting the flower tissue and the rachis (Kang and Buchenauer 2000; Brown et al. 2010). The infection results in Fusarium head blight (FHB), recognizable by a progressive scalding of the spike and the formation of shrivelled and discoloured, pinkish or white kernels (Mc Mullen et al. 1997; Goswami and Kitsler. 2004). In maize, spores infect the ear either through physical injuries caused by pests or via the silks, eventually at the beginning of silking (Munkvold 2003; Sutton 1982). The disease caused by *F. graminearum* and *F. culmorum* is called Gibberella ear rot (GER) (Dorn et al. 2009; Mesterházy et al. 2012; Kebede et al. 2016) and is characterised by partial rotting of the cob and the grains either starting from the tip towards the base, in the case of silk infections, or starting at the site of injury, in the case of insect damage (Vigier et al. 2001; Duncan and Howard 2010; Mesterházy et al. 2012). The source of inoculum is crop debris, in particular from maize (Eckard et al. 2011). For successful infection, the fungus needs high relative humidity (close to 100%) and temperatures above 15°C (Doohan et al. 2003).

Disease severity results from host resistance, pathogen aggressiveness, environmental conditions and the interactions between these factors. Aggressiveness is commonly defined as the ability of a plant pathogen to colonise and cause damages to plant and refers to the quantitative variation of pathogenicity (Pariaud et al. 2009; Holliday 1998). The concept of aggressiveness embraces the capacity of the pathogen to overcome the resistance mechanisms and to invade the host tissue. Infections of the host tissue is a highly complex process involving a myriad of aggressiveness factors including effectors, small molecules that lead the resistance mechanisms, many different cell wall, lipid and cellulose degrading enzymes but also mycotoxins (Kikot et al. 2009; Brown et al. 2010; Yang et al. 2013). Pathogenic fusaria produce type B trichothecene mycotoxins such as nivalenol (NIV) and deoxynivalenol (DON) as well as the acetylated derivatives 3 –acetyldeoxinivalenol (3ADON), 15 –acetyldeoxinivalenol (15ADON) (Ward et al. 2002). The mycotoxins produced determine the chemotype of the pathogenic strain (Lee et al. 2002). In moist and warm continental climates, the most frequently encountered chemotypes associated with wheat FHB and GER produce 3ADON, 15ADON or

NIV (Bottalico and Perrone 2002; Bakan et al. 2001; Eckard et al. 2011). In wheat, there is evidence, that mycotoxins promote the primary infection and favour host tissue invasion by the pathogen in the subsequent disease stages (Bai et al. 2002; Maier et al. 2006; Menke et al. 2012). It is however unclear whether the chemotypes within a defined *Fusarium* population differ in aggressiveness as suggested by other authors (Mesterházy et al. 1999; Carter et al. 2002; Goswami and Kistler 2005). In maize, Harris et al (2005) proved that the DON production by *F. graminearum* strain is an aggressiveness factor, but its role is however not known.

Disease outcome also strongly depends on host resistance and on its capacity to detect the pathogen at an early stage to avoid or at least to delay the primary infection (Pritsch et al. 2000; Bai and Shaner 2004; Ding et al. 2011). In both wheat and maize, resistance is a quantitative trait and no complete resistance is known at present (Mesterházy et al. 2005; Presello et al. 2006). It is known that in such quantitative interactions environmental conditions modulate both the aggressiveness of the pathogen and the resistance of the host. First, temperature and water availability are key factors for pathogen growth and mycotoxin production (Brennan et al. 2005; Kokkonen et al. 2010). Second, interactions between genetics and environmental conditions define the resistance phenotype of the host (Bai and Shaner 2004). More particularly, it has been observed that susceptible varieties are environmentally unstable, whereas the resistant ones are more stable across different environments (Miedaner et al. 2000). In breeding programs, the resistance levels of varieties are assessed by the comparison of symptoms across different environments, after artificial inoculations (Mesterházy et al. 2012). Hence, when observing symptoms on plants, breeders not only observe resistance responses of the plant, but also the results of the interactions between plant resistance mechanisms, pathogen aggressiveness and environmental conditions.

During the course of an infection, complex interactions between host and pathogen take place that are modulated by the environment. The aim of this study was to better understand the factors that determine the aggressiveness of *Fusarium* pathogens causing FHB and GER within on the Swiss population of Fusaria. With the aid of artificial inoculations on wheat and maize in the greenhouse and in the field at different locations, we explore the role of pathogen species, the chemotype and the original host as well as host resistance on the disease development. The results will contribute to complete our present knowledge on the epidemiology of *Fusarium* pathogens infecting wheat and maize, and help to improve artificial inoculation techniques in resistance breeding and variety testing.

2. Materials and methods

2.1. *Fusarium* strains

The *Fusarium* strains used for the present work are stored in Agroscope's fungal collection which is accessible at http://mycoscope.bcis.ch. To find suitable strains, 40 strains of *F. graminearum*, and *F. culmorum* were retrieved from long term storage and cultivated on artificial media. All strains originated from Switzerland and could therefore be used for field inoculations in compliance with the Federal Containment Ordinance. Species were identified by cultivation on Potato Dextrose Agar media (PDA, BD Difco, Le Pont de Claix, France) and on Spezieller Nährstoffarmer Agar media (SNA: 0.5 g $MgSO_4$ $7H_2O$, 1 g KNO_3, 0.2 g sucrose, 0.2 g glucose, 1 g KCl, 1 g KH_2PO_4, all purchased from Merck, Darmstadt, Germany, in 1l distilled water) on 9mm diameter plastic Petri dishes (Sarsterdt, Nümbrecht, Germany). To induce the formation of sporodochia, a 1 cm^2 piece sterilized filter paper (Whatman® qualitative filter paper Grade 1, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) was placed on the SNA medium (Leslie and Summerell, 2006).

Species identification was based on colony colour on PDA and on the morphology of macroconidia produced the sporodochia on SNA, according to Nelson et al. (1993). For 17 strains, chosen for their abundant sporulation, species identification was confirmed by PCR amplification and sequencing of the amplified fragment of the elongation factor EF-1α using the primers EF-1 and EF-2 (O'Donnell et al. 1998) (Microsynth, Balgach, Switzerland). For this, conidia from the cultures on SNA were placed in 1ml sterile nanopure water and DNA released by exposing the suspension for 15 min to 97°C. PCRs were performed in a final volume of 20µL with 14.5µl of extracts containing 0.5 units of *Taq* DNA Polymerase (Qiagen, Hilden, Germany), 1X PCR buffer, 1.25mM $MgCl_2$, 0.2 mM of each dNTP and 0.5 µM of each primer. Standard PCR reactions were carried out using a Tgradient thermocycler (Biometra, Germany) with an initial 5 min denaturation at 94°C and 35 cycles of denaturing at 94°C for 30 s, annealing at 62°C for 45 s and elongation at 72°C for 1 min followed by 7 min at 72°C. PCR products were visualized on agarose gels stained with ethidium bromide, purified on MinElute 96 UF Plates (Qiagen), and diluted in 10µl of nanopure sterile water. These PCR products were sequenced by Fasteris SA (Genève, Switzerland). To determine the species of strains, the obtained sequence data were aligned with reference sequences of translation elongation factor EF-1α retrieved from the database Fusarium-ID (http://isolate.fusariumdb.org ; (Geiser et al.

2004)) using Sequencer 4.9 (Gene Codes Corporation, USA). DNA sequences were deposited in the GenBank database (www.ncbi.nlm.nih.gov) under the accession numbers KR337483 to KR337492.

Chemotypes of the 40 strains were determined by multiplex PCR according to the method described by Quarta et al. (2006), with slight modifications. In this case, no DNA purification was carried out, and extracts containing conidia from SNA cultures were prepared as described above. The PCR amplification required primers Tri3F1325 (specific for 3-ADON chemotype), Tri3F971 (specific for 15-ADON) and Tri3R1679 as antisens, as well as TriF340 and TriR965 which are specific for NIV. The same reagents and concentrations as for the amplification of EF-1α were used. PCR products were separated on 1.7% agarose gels at 100 mV and visualized under UV light after staining with ethidium bromide. Chemotypes of strains were assigned according to the size of the amplification products (Quarta et al. 2006).

2.2. Production of *Fusarium* spores for inoculation

To promote sporulation of the 17 *Fusarium* strains chosen for artificial inoculations, conidia grown on SNA were diluted in 1ml of distilled sterile water and spread on Czapek-V8 agar plates (containing: 18g of Agar (Merck, Darmstadt, Germany), 32g of Czapek Dox Broth (Difco, Le Pont de Claix, France), and 10ml of V8® vegetable juice (Campbells, Camden, USA) in 1l distilled water). After incubation for 10 days at room temperature under near ultra violet light (Philips TL 40W/08), conidia were eluted in 5ml sterile distilled water. The suspension was centrifuged at 4,000 rpm for 10 minutes. The supernatant was discarded, the pellet resuspended in 40 ml sterile distilled water amended with 0.2% Skim Milk (Difco) and stored in 50 ml Falcon tubes at -20°C until further use.

2.3. Artificial inoculations

For greenhouse and field inoculations of wheat plants, suspensions containing 10^6 spores.ml^{-1} were sprayed on the spikes, at anthesis (BBCH65) until runoff. After inoculation, humidity was maintained at approx. 100% for 24 hours using overhead irrigation systems. A second inoculation took place two days later. For maize, inoculations took place 5 days after silk emergence (about BBCH 71), by injecting 1.5 ml of suspension containing 5 x 10^5 spores.ml^{-1} with a syringe into the silk channel.

2.4. Comparison of strain virulence on wheat in the greenhouse

The spring wheat variety Apogee (from Utah Agricultural Experiment Station, USA) was chosen for greenhouse tests due to its high susceptibility to FHB (Garvin et al. 2015). Three seeds were sown per pot and grown under controlled conditions in continuous light at 25°C until anthesis, and then maintained at 17°C. Seventeen *Fusarium* strains were inoculated on plants. One strain was inoculated on three pots.

2.5. Comparison of strain virulence on spring wheat

Three spring wheat varieties were chosen Carasso, Nadro and Toronit, all from Agroscope / DSP. Toronit is recognized for its high resistance level, whereas Carasso is susceptible (Häller Gärtner et al. 2008; Mascher et al. 2013). The field test was conducted in 2013 in Changins (Switzerland; coordinates: 6°14'/46°24', altitude: 455m). The experimental field was divided in 54 microplots (1 m^2); a micro-plot was composed of 5 lines measuring 1m long. The two outer lines were sown with triticale and the three wheat varieties, Carasso, Nadro and Toronit, were sown in the three inner lines in a random order. The same 17 strains previously inoculated in greenhouse were used to inoculate three micro-plots each; non infected micro-plots were used as control.

2.6. Comparison of strain virulence in maize

Two maize varieties were chosen for maize field test, Birko (RAGT) and LG.32.12 (Limagrain). Birko is more resistant to GER, whereas LG 32.12 is more susceptible (Schürch 2016). The test was implanted in Changins in 2013 and split in three plots. In each plot, six lines of app. 25 plants were sown with the variety Birko and six lines with LG 32.12. Eight *Fusarium* strains were inoculated on 10 to 12 plants each side by side on the same line.

2.7. Comparison of strain virulence on winter wheat in a multi-local test

Six winter wheat varieties were chosen for the multi-local test: Arina, Forno, Levcis, Runal, Segor (Agroscope / DSP) and Royssac (RAGT). Among them, Arina is widely recommended for its high resistance (Draeger et al. 2007). Field tests were sown in Changins in 2008 and 2009, and in Cadenazzo (Switzerland, coordinates: 8°56'/46°10', altitude: 203 m) in 2008. The

environmental conditions are described in the table 1. Two *F. culmorum* strains were used: FC808 known as very aggressive, and FC376 as poorly aggressive (Kellenberger S, personal communication). The experimental design was the same at all locations. Subplots ($2m^2$) were composed of five lines 2m long: the two outer lines were sown with triticale and the three inner lines were divided into six 1m long segments sown with the six winter wheat varieties in a randomized order. Each treatment (artificial inoculations with FC808, with FC876, and no inoculation) was applied to the whole subplot.

Table 1. Environmental conditions recorded during the multi-local trials. Data are expressed in mean per day of temperature, humidity and evapotranspiration and sum of precipitations from the 1st June to 31th July, according to www.meteoswiss.ch/idaweb

	Temperature (°C)	Humidity (%)	Evapotranspiration (mm)	Precipitation (mm)
Cadenazzo 2008	20.7	73.6	2.4	458.1
Changins 2008	18.4	68.2	3.2	157.2
Changins 2009	18.8	64.4	3.3	142.0

2.8. Evaluation of disease severity

Symptoms on wheat spikes were registered every three days, starting 10 days after inoculation. Severity of symptoms was scored according to Häller-Gärtner et al. (2008) based on a 1 to 9 scheme with 1 = no symptom and 9 = complete scalding. In the greenhouse experiment, each single spike was scored individually. In the wheat field test, symptoms were observed on five spikes per entry. In all wheat experiments, the area under the disease progress curve (AUDPC) was calculated by integrating symptom severity over time of exposure. The AUDPC values in the multi-local test were divided by the number of days between the last inoculation and the last observation. These relative AUDPCs (AUDPCrel) allowed comparing the severity between different environments (Martin et al., 2017). For the maize experiment, infected ears were harvested manually at maturity. Symptoms on ears were scored according to Reid et al., (1996) on a 1 to 7 scheme with 1 = no symptom and 7 = total rotting of the ear. The means of AUDPC, AUDPCrel or disease rating on maize were calculated for each replicate. Data calculations were conducted on Microsoft® Excel 2013 spreadsheets (Microsoft Inc, Redmond, USA).

2.9. Experimental set-up and data analysis

Four experimental approaches were used to investigate the factors conditioning the aggressiveness of *Fusarium* strains. Out of initial 40 *Fusarium* strains, 17 were chosen to set up a panel of *F. culmorum* and *F. graminearum* strains with all combinations of chemotypes, original host (maize or wheat) and geographical origin. In a first step, aggressiveness was tested on Apogee- wheat in greenhouse experiments and on a set of 3 spring wheat varieties in field tests. Eight out of the 17 strains were selected for inoculation on maize field tests according to their elevated of low aggressiveness on wheat and to represent the two ungal species, the original host and the chemotypes. Finally one highly aggressive and one poorly aggressive *F.culmorum* strains were deployed in a multi-local tests aiming to study the stability of aggressiveness across different environments.

For all experiments three replicates were available. Wheat and maize field experiments followed split-plot designs with three replicates. In the spring wheat field test, in each replicate, the factor "treatment", which was the inoculated strain or non-inoculation, was considered the main plot, and the factor "variety" was the subplot. Inversely for the maize field test, the main plot were the varieties and the factor "treatment" was considered the subplot. The multi-local test followed a split-split plot design considering the environment as the main plot, the treatment as subplot and varieties as sub-subplot.

Statistical analyses were done with the statistical software R (R Core Team, 2015) using the package "agricolae" (de Mendiburu, 2016). Comparison of strain aggressiveness in the greenhouse was performed using one-way ANOVA. Effect of aggressiveness of the strains and of the resistance level of varieties in field tests were investigated using two-way ANOVA on AUDPC (wheat) or means of symptom ratings (maize) according to the experimental split plot designs. To analyze data from the multi-local test, a three-way ANOVA was performed considering as factors the different environments, treatments, varieties as well as their interactions. Tukey range tests were performed as post-hoc analysis. Then nested ANOVAs were separately performed to analyze the effect of strain characteristics on symptoms in the greenhouse and in the spring wheat and maize field tests. For this, the factor "strain" was nested in the factors "species", "chemotype" or "original plant". Association strength between symptoms on spring wheat and on maize was measured with a Pearson correlation analysis.

3. Results

3.1. Description of *Fusarium* strains

The *Fusarium* strains from long-term storage were identified as *F. graminearum* or *F. culmorum*. One strain of *F. crookwellensee*, a NIV producer, was identified (supplementary material). All *F. culmorum* strains were either NIV or 3ADON producers. No *F. culmorum* strain producing 15ADON was found. Among the 25 *F. graminearum* isolates, two were 3ADON producers, one was NIV and the others were 15ADON producers. A general description of all 40 strains can be found in the supplementary material. The 40 strains are described in annex 1. The 17 strains used in the subsequent experiments are described in detail in the table 2.

Table 2. Description of *Fusarium* strains used for artificial inoculations. The accession number is the identifier used in the Mycoscope database (http://mycoscope.bcis.ch). "Host" designates the plant species from which a strain was isolated.

Description of the strains							Inoculations		
Strain ID	Accession number	Species	Chemotype	Host	Origin (Switzerland)	Year of isolation	G	WM	W-ML
FC1073	1073	FC	3-ADON	Maize	Goumoens-la-Ville (VD)	2006	+	+	+
FC1068	1068	FC	NIV	Maize	Goumoens-la-Ville (VD)	2006	+	+	+
FC808	808	FC	3-ADON	Wheat	Changins (VD)	2001	+	+	+ +
FC376	376	FC	NIV	Wheat	Peney-le-Jorat (VD)	1988	+	+	+
FG1153	1153	FG	15-ADON	Maize	Baden (AG)	2006	+	+	+
FG1088	1088	FG	15-ADON	Maize	Goumoens-la-Ville (VD)	2006	+	+	+
FG13	13	FG	15-ADON	Wheat	Fravental (ZG)	1998	+	+	+
FG1149	1149	FG	15-ADON	Wheat	Ependes (VD)	1992	+	+	+
FG1069	1069	FC	NIV	Maize	Goumoens-la-Ville (VD)	2006	+	+	
FC378	378	FC	3-ADON	Maize	Savagnier (NE)	1988	+	+	
FC1879	1879	FC	NIV	Wheat	Nyon (VD)	2010	+	+	
FC254	254	FC	3-ADON	Wheat	Nyon (VD)	1996	+	+	
FC1065	1065	FC	NIV	Maize	Goumoens-la-Ville (VD)	2006	+	+	
FG2113	2113	FG	15-ADON	Wheat	Ellighausen (SH)	2011	+	+	
FG1151	1151	FG	15-ADON	Maize	Baden (AG)	2006	+	+	
FG1145	1145	FG	15-ADON	Wheat	Ependes (VD)	1992	+	+	
FC876	876	FC	NIV	Wheat	Changins (VD)	2007	+	+	+

Abbreviations: G= Greenhouse, W= Wheat field test, M= Maize field test, W-ML= Wheat multi-local field test, FG=*F. graminearum* , FC=*F. culmorum*

3.2. Effect of the strains and varieties on disease severity in wheat

In both greenhouse and field tests, artificial inoculations resulted in FHB symptoms. The comparisons of disease severities revealed significant differences between the strains. In the greenhouse test, the *F. graminearum* strain FG1153 caused the strongest disease severity, followed by *F. culmorum* strain FC808. On the contrary, *F. culmorum* FC876 and *F. graminearum* FG2113 were the least aggressive strains (Table 3).

Table 3. Disease severity on spikes of the wheat variety Apogee following infection by *Fusarium* strains. Different letters indicate means of AUDPC significantly different according to Tukey honest significant difference at the probability level 0.05.

Strain ID	Mean AUDPC on Apogee spikes ± SE
FC1073	24.9 ± 3.7 ab
FC1068	25.3 ± 7.8 ab
FC808	48.1 ± 7.0 ab
FC376	29.7 ± 5.2 ab
FG1153	54.1 ± 3.2 a
FG1088	41.4 ± 4.7 ab
FG13	28.0 ± 8.6 ab
FG1149	15.1 ± 8.3 ab
FC1069	19.1 ± 9.2 ab
FC378	22.5 ± 4.4 ab
FC378	22.5 ± 4.4 ab
FC1879	23.8 ± 7.4 ab
FC254	26.3 ± 2.9 ab
FC1065	13.7 ± 4.4 ab
FG2113	8.6 ± 5.1 b
FG1151	36.2 ± 9.4 ab
FG1145	16.7 ± 7.2 ab
FC876	7.5 ± 4.7 b

In the spring wheat field test, the factor strain had a higher impact on disease severity and explained a higher proportion of the observed variance (45.5%) than the factor variety (10%) (P <0.01). Disease development was significantly weaker in the variety Toronit than on Carasso or Nadro and the strains FC808 and FG1153 were the most aggressive in this field test (P <0.05) (Figure 1). There was no significant interaction between strain aggressiveness and host resistance (P <0.05).

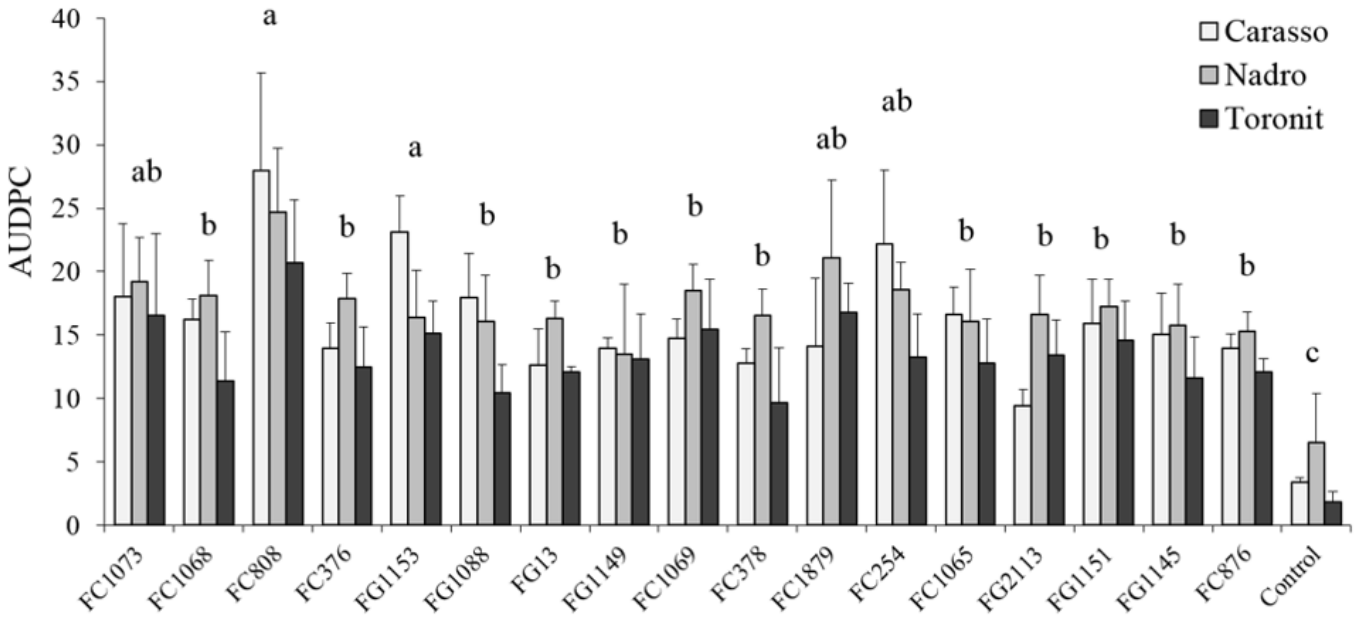

Figure 10. Severity of Fusarium head blight (mean AUDPC +/- standard deviation) recorded in a field trial on three wheat varieties inoculated with 17 *Fusarium* strains. Control bars designate natural infections. Different letters indicate significant differences between strains over all wheat varieties (P<0.05).

3.3. Effect of strain characteristics on disease severity

The impact of strain species, chemotype and the original host on strain aggressiveness were investigated by analysis of variances of AUDPC recorded in greenhouse and field tests. Results show that the factors "species" and "chemotype" significantly impacted the disease severity in the greenhouse and in the field (Table 4). In the greenhouse test, *F. graminearum* strains caused higher FHB severity than *F. culmorum* strains (P <0.05), and the 15ADON chemotype was generally more aggressive than the 3ADON or NIV chemotypes. On the contrary, in the field test, disease severity was higher on spikes infected with *F. culmorum* strains than with *F. graminearum* strains and this for all three varieties (P <0.05). *F. culmorum* strains belonging to the 3ADON chemotype caused higher symptoms than those producing NIV (P <0.001). However in both greenhouse and field tests, even if the strain species and chemotype significantly affected the disease severity, these effects were weak as species and chemotype accounted only for 2.0% and 10% respectively to the AUDPC variances in the greenhouse, and 3.5 % and 9% in the field (Table 4). Strains isolated from maize caused significantly higher symptoms than those isolated from wheat in the greenhouse trial (P <0.001). Whereas, no effect of the original host on disease severity was detected, in the field test (Table 4).

Table 4. Results of the analyses of variance on disease severity in three inoculations experiments. The effect of the strains, chemotypes and species were analysed in a double nested design. A nested design was used to study effect of the plant origin of the strain. (***: significant at P <0.01, **: significant at P <0.01,*: significant at P <0.05)

	Apogee greenhouse test		Spring wheat field test		Maize field test	
	Sum of Square	Mean Square	Sum of Square	Mean Square	Sum of Square	Mean Square
Species	398	398.0**	121.9	121.9*	0.0	0.01
Species : Strain	15356	1023.7***	1022.0	68.1***	12.3	2.0***
Residuals	3984	49.2	2258.7	17.0	11.5	0.3
Chemotype	1908	953.8***	309.2	154.6***	0.3	0.12
Chemotype : Strain	13846	989.0***	834.8	59.6***	12.0	2.4***
Residuals	3984	49.2	2258.7	17.0	11.5	0.3
Plant origin	1091	1091.0***	0.1	0.1	0.3	0.1
Plant origin : Strain	14663	977.5***	1143.9	76.3***	12.0	2.0***
Residuals	3984	49.2	2258.7	17	11.5	0.4

3.4. Aggressiveness on maize and comparison with aggressiveness on wheat

On maize, ANOVA revealed significant differences in disease severity ratings among pathogen strains and among varieties (P <0.001). Differences in resistance between the two varieties explained 8.4% of the variance observed and the variety Birko was more resistant than LG 32.12. Differences in aggressiveness level of the strains explained 13.4% of the symptoms variability. The strains FC808 and FG1153 were significantly more aggressive than FC1073, FC376, and FG1149 (P<0.05) (Figure 2). Moreover, significant interactions were recorded between resistance of the varieties and pathogen aggressiveness (P <0.05), explaining 4.2% of the symptoms variability. Indeed, infection by the two highly aggressive strains and by FC1068 led to similar disease severity on both varieties, whereas less aggressive strains caused considerably more severe symptoms on LG 32.12 than on Birko.

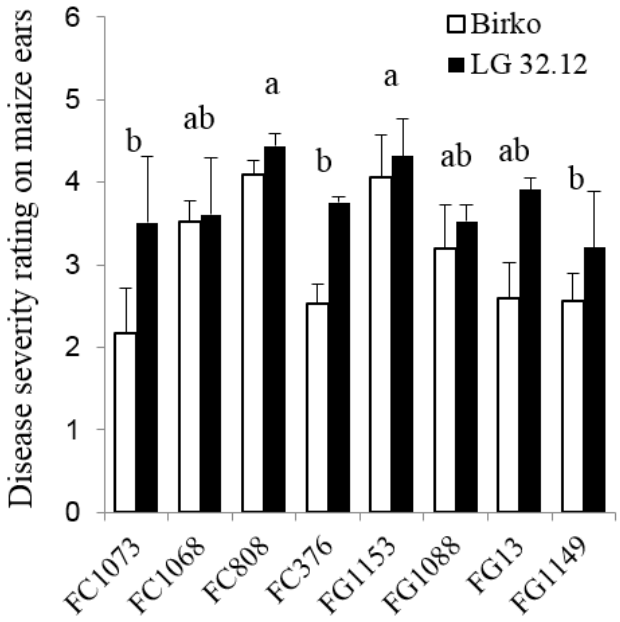

Figure 2. Mean disease severity ratings (+/- standard deviation) on maize ears of two varieties inoculated with eight *Fusarium* strains. Different letters indicate significant differences between strains overall maize varieties.

Comparisons of symptoms on wheat and on maize revealed that strain FC808 was highly aggressive on both hosts. Pearson correlation analysis confirmed a trend between the symptoms caused by the eight strains on wheat and on maize (Figure 3). It has to be noticed that strain FC808 heavily influenced this trend and was opposed to strains FG13, FG1149, FG1088, FC1068 and FC376 that were less aggressive on both maize and wheat. The two strains FG1153 and FC1073 were more remote from the correlation. The aggressiveness of these two strains was markedly different on maize, but similar on wheat. On maize no effect of the strain species, the chemotype or the original host species on aggressiveness was observed.

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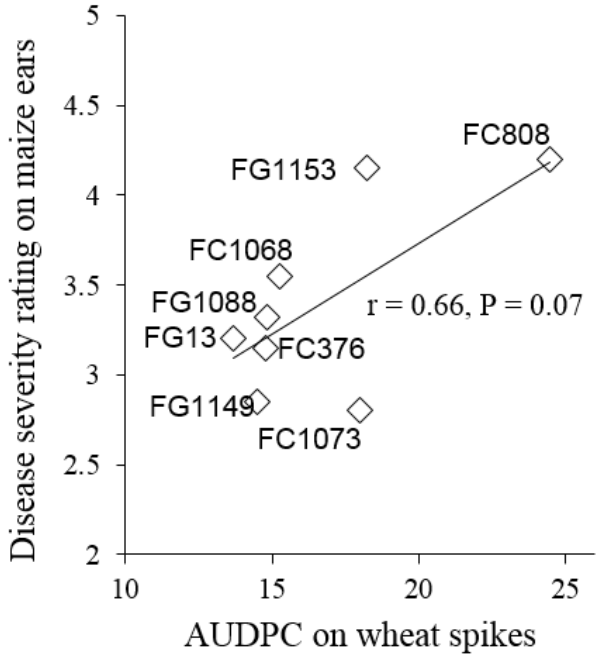

Figure 3. Relationship between disease severity on wheat spikes and on maize ears after inoculation with 8 *Fusarium* strains (Pearson correlation coefficient).

3.5. Comparison of strain aggressiveness under different environmental conditions

The *F. culmorum* strains FC808 and FC876 were respectively among the most aggressive and the least aggressive strains in the previous wheat trials. In a multi-local test we studied the aggressiveness of the two strains on six winter wheat varieties at Changins in 2008 and 2009 and in Cadenazzo in 2008 (Figure 4 and Table 5). As expected, strain FC808 caused the highest symptoms in all environments while FC876 was generally weak and at the same infection level as the non-inoculated control. In Changins in 2008, the severity of symptoms was significantly higher than in the other two environments. Here, symptoms caused by the strain FC808 were more than two time higher compared to strain FC876 and the control. In Changins 2009, the infections were rather weak. Interestingly, the infection caused by the highly aggressive strain FC808 in the low symptoms environment Changins 2009 were equivalent to the symptoms caused by the low aggressive strain in the high symptoms environment Changins 2008 (Figure 4).

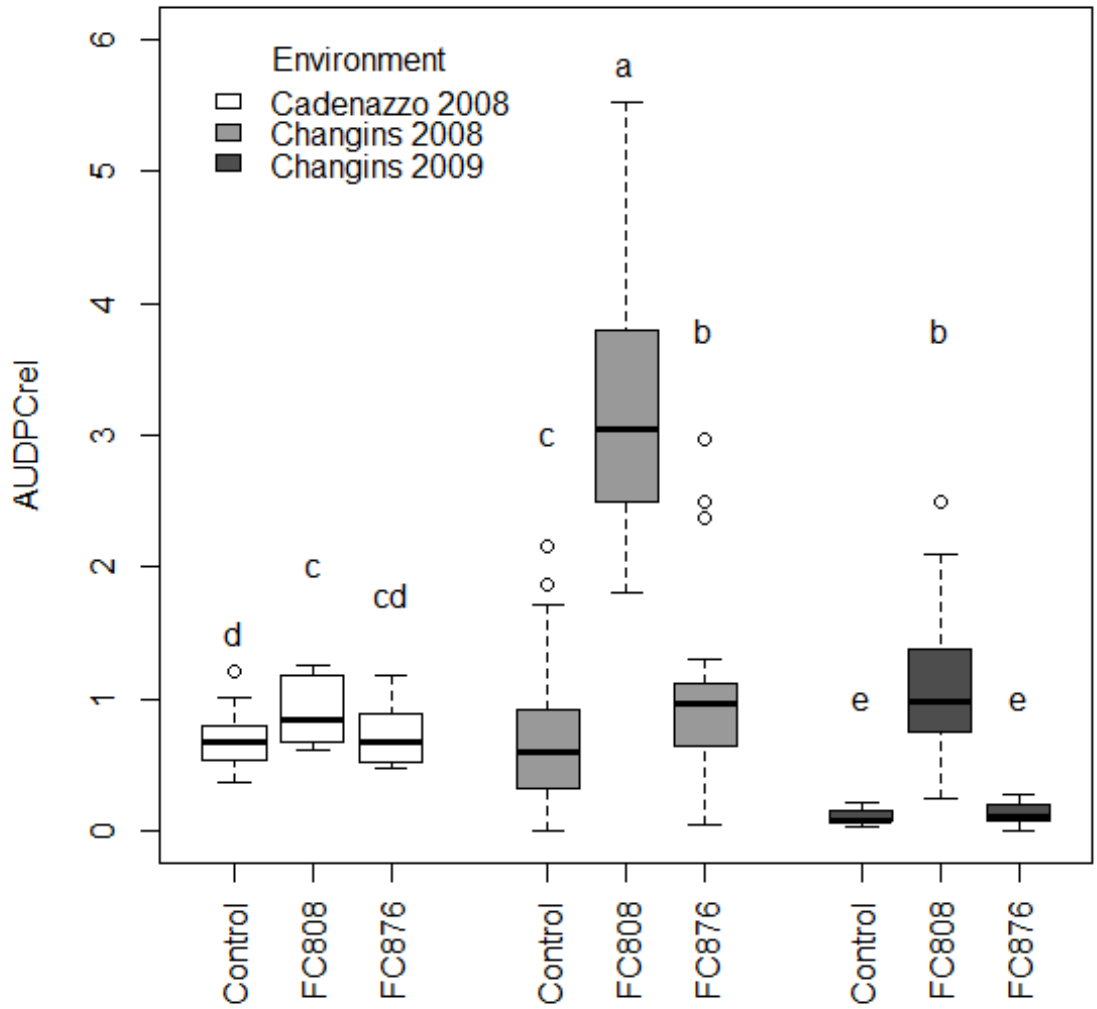

Figure 4. Boxplot AUDPCrel calculated with evaluation of symptoms on wheat spikes caused by isolates FC876, FC808 and natural infection in three different environments. Different letters indicate significant differences between AUDPCrel ($P<0.05$).

The severity of symptoms and the ranking of the 6 varieties were very similar in all environments. Royssac was the most susceptible variety, while Arina and Segor were the most resistant varieties. Yet, infections with strain FC 808 allowed discriminating the resistance level of all varieties in all environments. Strain FC876 was able to discriminate the resistance level of all varieties only in Changins 2008 and the highly susceptible variety Royssac also in Cadenazzo 2008 (Table 5)

Table 5. Means of AUDPCrel ± standard errors caused by FC876 and FC 808 isolates, on six wheat varieties in the three environments Changins 2008, Changins 2009 and Cadenazzo 2008. Control designates natural infections. Different letters indicate significant differences between AUDPCrel within one environment, according to the High Significant Differences (P <0.05).

Cadenazzo 2008						
	FC876		FC808		Control	
Arina	0.51 ± 0.02	de	0.63 ± 0.03	de	0.46 ± 0.06	e
Forno	0.80 ± 0.1	bcde	0.87 ± 0.05	abcd	0.80 ± 0.00	bcde
Levis	0.66 ± 0.1	cde	1.04 ± 0.23	abc	0.58 ± 0.07	de
Royssac	1.07 ± 0.09	ab	1.22 ± 0.03	a	1.02 ± 0.15	abc
Runal	0.59 ± 0.09	de	0.88 ± 0.04	abcd	0.70 ± 0.09	bcde
Segor	0.72 ± 0.21	bcde	0.68 ± 0.05	cde	0.60 ± 0.08	de
Changins 2008						
	FC876		FC808		Control	
Arina	0.17 ± 0.08	g	2.19 ± 0.21	d	0.12 ± 0.12	g
Forno	1.09 ± 0.18	e	3.17 ± 0.22	bc	0.78 ± 0.17	efg
Levis	0.96 ± 0.09	ef	3.79 ± 0.00	b	0.75 ± 0.09	efg
Royssac	2.61 ± 0.26	cd	5.34 ± 0.19	a	1.91 ± 0.18	d
Runal	1.04 ± 0.08	e	3.01 ± 0.07	c	0.60 ± 0.25	efg
Segor	0.59 ± 0.16	efg	2.21 ± 0.39	d	0.32 ± 0.20	g
Changins 2009						
	FC876		FC808		Control	
Arina	0.07 ± 0.01	f	0.51 ± 0.20	cde	0.05 ± 0.02	f
Forno	0.11 ± 0.02	f	0.89 ± 0.13	bc	0.08 ± 0.00	f
Levis	0.13 ± 0.05	ef	1.43 ± 0.09	ab	0.12 ± 0.06	f
Royssac	0.22 ± 0.08	def	2.17 ± 0.24	a	0.14 ± 0.05	ef
Runal	0.20 ± 0.07	ef	0.96 ± 0.15	bc	0.13 ± 0.03	ef
Segor	0.19 ± 0.16	f	0.99 ± 0.22	cd	0.13 ± 0.05	ef

The interaction of the factors variety, treatment and environment are shown in (Table 6). The environment conditions variety and treatment significantly and independently. Here, we remark also statistically significant interactions between the aggressiveness of the two inoculated strains and natural infections and the resistance of the host. Significant interactions between the environmental conditions, the strain aggressiveness and the host resistance lead to an elevated level of disease severity, as observed in Changins 2008 on the variety Royssac infected with FC808.

Table 6. Analysis of the variance of AUDPC observed in three different environment after three different treatments (artificial inoculations with FC808, FC876 or control) on six different winter wheat varieties.

Source of variation	Sum of squares	Mean squares	
Environment	42.1	21.1	***
Error (A)	0.9	0.05	
Treatment	44.8	22.4	***
Treatment : Environment	24.8	6.2	***
Error (B)	0.9	0.08	
Variety	25.4	5.1	***
Environment : Variety	13.3	1.3	***
Treatment : Variety	5.4	0.5	***
Environment : Treatment : Variety	3.6	0.18	***
Error (C)	4.4	0.005	

4. Discussion

All *F. graminearum* and *F. culmorum* strains were isolated on wheat and on maize at different sites across Switzerland. *F. culmorum* strains were mainly 3ADON producers with a minority of NIV producers, while most of the *F. graminearum* strains produced 15ADON. This chemotype pattern is consistent with other studies of European populations (Quarta et al. 2005; Pasquali et al. 2016). A recent study on 1147 *F. graminearum* isolates showed that 83% of the strains were 15ADON producers while the rest were 3ADON and NIV producers, mainly from Eastern European countries (Pasquali et al. 2016). Out of these 40 strains, we chose 17 strains that represent the distribution of pathogenic strains and chemotypes on wheat and maize in Switzerland, to study factors that govern aggressiveness in pathogenic *Fusaria*.

On wheat, the 17 strains showed a large spectrum of aggressiveness in field and greenhouse trials. Among those 17 strains, the NIV producers were significantly less aggressive than the DON producers. By trend, 15ADON producers were slightly more aggressive in the greenhouse while 3ADON producers were superior in the field tests. There was yet no statistically established difference between the two chemotypes. In conclusion, within the 17 strains tested here aggressiveness of the two DON producing chemotypes was equivalent, independent from the *Fusarium* species, while the aggressiveness of NIV producers was weaker. This is consistent with reports on the low aggressiveness of the NIV chemotypes of *Fusarium graminearum* from USA, Europe, and Nepal (Carter et al. 2002; Desjardins 2006), and comparisons of the damages caused on grains by 3ADON, 15ADON and NIV *F. graminearum* producing strains in greenhouse conditions (Foroud et al. 2012). Moreover, NIV producing strains of *F. culmorum* were observed to be less aggressive than DON producers on both wheat and rye (Miedaner and Reinbrecht 2001). Regardless of the species of the strain, NIV was also shown to be less phytotoxic than DON or 3ADON (Eudes et al. 2000; Foroud et al. 2012; Miedaner and Reinbrecht 2001). In epidemiological studies in North America, and Southern China, 3ADON chemotypes of *F. graminearum* and the closely related species *F. asiaticum* were more aggressive than 15ADON chemotypes from the same species. In those populations, 3ADON producers have rapidly emerged in the last years and are becoming the predominant chemotype (Ward et al. 2008; Puri and Zhong 2010; Zhang et al. 2010). This might be due to a better resilience towards the change of environmental conditions (Ward et al. 2008). Arguably, in our collection, no 3ADON chemotype of *F. graminearum* has been detected, but the superior aggressiveness performance of the 3ADON *F. culmorum* in the field test may indicate a similar

evolution. These findings are in accordance with Ward et al. (2008), suggesting a selective advantage and a greater aggressiveness of the 3ADON chemotype that is also independent from the strain species. Even though we have detected a statistically significant difference between *Fusarium* species, this difference is due to the low aggressiveness of the NIV producing *F. culmorum* strains. We measured a 10% impact of the chemotype on the total aggressiveness of the strains indicating that aggressiveness is mainly determined by other factors as reported by Goswami and Kistler (2005).

The original host, wheat or maize, had no impact on the aggressiveness on maize and wheat. The isolates caused a similar range of severity on the plants as found previously (Windels and Kommedahl 1984; Fernando et al. 1997; Carter et al. 2000). The two strains FG1153 and FC1073, both isolated from maize, showed a preference for maize and wheat, respectively. The expression of particular host-preferential genes as suggested by Harris et al. (2015) is conceivable. However, the fact that an isolate from maize shows a preference for wheat confirms also that, in the field, the pathogen is not host specific, at least in the context of the present population. Moreover, both hosts (wheat and maize) reacted in a similar manner to all chemotypes and species as previously observed (Miedaner and Reinbrecht 2001). Small interactions between wheat resistance and aggressiveness have been detected in the multi-local test. Snijders et al. (1990) obtained comparably small but significant wheat genotypes x strains interactions across four different environments and explained their results by the divergence of a very pathogenic strain causing elevated symptoms on all genotypes regardless the resistance level and environmental conditions. This case is comparable with the results we obtained with the strain FC808. However, in the present study, when looking at FHB symptoms in wheat spikes within one single environment, no interaction were detected between pathogen aggressiveness and host resistance, consistent with previous results of Xue et al. (2004). Hence, it can be concluded that wheat resistance does not influence the pathogen aggressiveness. The interactions detected in the multi-local test suggest to be simply the consequences of the impact of environmental conditions on both pathogen aggressiveness and wheat resistance (Snijder et al. 1990; Xue et al. 2004). The reciprocal consequence is that there is no strain-specific resistance in wheat as observed by Xue et al. (2004) and Tòth et al. (2008). Furthermore, Mesterhàzy et al., (2005) concluded that all wheat genotypes exhibit similar resistance reactions to all the members of the *Fusarium* spp. complex infecting wheat. Our results are consistent with this observation, furthermore no chemotype–specific interactions with the host have been detected in our wheat experiments in the field. Interestingly, wheat grains accumulate both

acetylated forms of DON simultaneously (von der Ohe et al., 2010). Also in maize, no differences in aggressiveness was observed neither between species nor chemotype. As demonstrated by Harris et al. (2005), production of DON is an aggressiveness factor for *F. graminearum* strains infecting maize. Our results suggested that 3ADON, 15ADON and NIV all have a similar role in pathogenesis and that other strain characteristics are more important than the chemotype for aggressiveness.

The crucial influence of environmental conditions on growth, mycotoxin production and pathogenicity of *Fusarium* pathogens was demonstrated under controlled conditions (Doohan et al. 2003). More particularly, Hope et al. (2005) observed *in vitro* that the ecological requirements for *F. graminearum* and *F. culmorum* for growth and toxin production differ considerably. Yet, both require abundant humidity and high temperatures for grain colonization. Environmental conditions can also highly influence the expression of resistance traits in cereals, leading in significant genotype x environment interactions (Miedaner et al. 2001). In breeding programs, multi-local field tests are necessary to assess the stability of resistance of the genotype across various environmental conditions. In the present experiment, we followed an original approach by studying pathogen x environment interactions. Arguably, culture management can determine the outcome of FHB on wheat. Yet, in this study, agronomical practices were similar at all three sites, differences observed in disease severity were attributed to different environmental conditions. When comparing the interactions in the triangle host-pathogen-environment, it was revealed that, in terms of disease severity, the environmental conditions affected rather the pathogen than the host. Indeed, only few symptoms were observed on wheat in Changins 2009, while in the year with more precipitation, in Cadenazzo 2008, symptoms caused by artificial inoculations and natural infections were equivalent. In particular, the strain FC808 showed high aggressiveness in all environments, while FC876 showed aggressiveness only under very favourable conditions. Here, the differences between the recorded environmental conditions do not fully explain the differences in symptoms severity. It is likely that environmental conditions and more specifically relative humidity at flowering had a higher impact on symptoms severity than the conditions during the grain maturation period (Xu 2003).

Finally, our results showed that several minor and major factors determine the aggressiveness in pathogenic *F. culmorum* and *F. graminearum* strains. Neither chemotype, nor environmental conditions, nor host preference can completely explain the difference in

aggressiveness within the pathogen population. The major determinant in aggressiveness diversity among strains are intrinsic factors determined by the ability of the strain to degrade host cell membranes (Wanjiru et al. 2002), secretion of extracellular lipases that may be involved in the adherence of the pathogen to the plant cuticle and generation of a triggering signal (Voigt et al. 2005), the toxigenic potential (Langevin et al. 2004; Puri and Zong 2010) and the capacity to withstand oxidative stress during the first steps of infection (Ponts et al. 2009; Montibus et al. 2013).

Results of this study allow defining a "aggressive strain" as a strain able to provoke elevated damage to the plant, whatever its resistance level, and throughout a wide range of environmental conditions. For the use in resistance studies in wheat, we recommend the use of highly aggressive strains such as FC808 that enable to discriminate various levels of resistance regardless the environmental conditions. Since no interaction between pathogen aggressiveness and host resistance was detected, it may be sufficient to deploy only one isolate rather than isolate mixtures for artificial inoculations.

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Supplementary materials

Table S1: Description of the 40 *Fusarium* strains. "Host" designs the original plant of isolation and the accession number refers to the identifier used in the Mycoscope data *(http://mycoscope.bcis.ch).* The accession number followed by a sign "*" indicates the strains chosen for the artificial inoculations.

Accession number	Species	Chemotype	Host	Origin (Switzerland)	Year of isolation
254 *	*F. culmorum*	3ADON	Wheat	Nyon (VD)	1996
302	*F. culmorum*	3ADON	Wheat	Changins (VD)	1977
808 *	*F. culmorum*	3ADON	Wheat	Changins (VD)	2001
378 *	*F. culmorum*	3ADON	Maize	Savagnier (NE)	1988
1071	*F. culmorum*	3ADON	Maize	Goumoens-la-Ville (VD)	2006
1073 *	*F. culmorum*	3ADON	Maize	Goumoens-la-Ville (VD)	2006
376 *	*F. culmorum*	NIV	Wheat	Peney-le-Jorat (VD)	1988
1879 *	*F. culmorum*	NIV	Wheat	Nyon (VD)	2010
876 *	*F. culmorum*	NIV	Wheat	Changins (VD)	2002
876L	*F. culmorum*	NIV	Wheat	Changins (VD)	2002
1065 *	*F. culmorum*	NIV	Maize	Goumoens-la-Ville (VD)	2006
1068 *	*F. culmorum*	NIV	Maize	Goumoens-la-Ville (VD)	2006
1069 *	*F. culmorum*	NIV	Maize	Goumoens-la-Ville (VD)	2006
1070	*F. culmorum*	NIV	Maize	Goumoens-la-Ville (VD)	2006
13 *	*F. graminearum*	15ADON	Wheat	Fravental (ZG)	1998
15	*F. graminearum*	15ADON	Wheat	Ependes (VD)	1992
405	*F. graminearum*	15ADON	Wheat	Ependes (VD)	1992
407	*F. graminearum*	15ADON	Wheat	Ependes (VD)	1992
803	*F. graminearum*	15ADON	Wheat	Chevilly (VD)	2000
1145 *	*F. graminearum*	15ADON	Wheat	Ependes (VD)	1992
1146	*F. graminearum*	15ADON	Wheat	Ependes (VD)	1992
1147	*F. graminearum*	15ADON	Wheat	Ependes (VD)	1992
1148	*F. graminearum*	15ADON	Wheat	Ependes (VD)	1992
1149 *	*F. graminearum*	15ADON	Wheat	Ependes (VD)	1992
1150	*F. graminearum*	15ADON	Wheat	Ependes (VD)	1992
1469	*F. graminearum*	15ADON	Wheat	Changins (VD)	2007
2113 *	*F. graminearum*	15ADON	Wheat	Ellighausen (SH)	2011
1084	*F. graminearum*	15ADON	Maize	Goumoens-la-Ville (VD)	2006
1085	*F. graminearum*	15ADON	Maize	Goumoens-la-Ville (VD)	2006
1088 *	*F. graminearum*	15ADON	Maize	Goumoens-la-Ville (VD)	2006
1151 *	*F. graminearum*	15ADON	Maize	Baden (AG)	2006
1152	*F. graminearum*	15ADON	Maize	Baden (AG)	2006
1153 *	*F. graminearum*	15ADON	Maize	Baden (AG)	2006
1154	*F. graminearum*	15ADON	Maize	Baden (AG)	2006
1155	*F. graminearum*	15ADON	Maize	Baden (AG)	2006
1156	*F. graminearum*	15ADON	Maize	Baden (AG)	2006
395	*F. graminearum*	3ADON	Wheat	Changins (VD)	1988
1086	*F. graminearum*	3ADON	Maize	Goumoens-la-Ville (VD)	2006
1087	*F. graminearum*	NIV	Maize	Goumoens-la-Ville (VD)	2006
97003	*F. croockwellensee*	NIV	Wheat	Changins (VD)	2007

Chapter II

Ferulic acid contributes to the resistance of wheat flowers and developing grains against *Fusarium graminearum*

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Summary

Fusarium head blight (FHB) is one of the most threatening cereal diseases, caused by various *Fusarium* pathogens and results in spike scalding, yield reduction and accumulation of mycotoxins in grains. *Fusarium* pathogens infect the spike during anthesis, via the wheat flowers, and then colonise the developing grains. Wheat spikes dispose of several resistance elements to face pathogen infection and propagation. Ferulic acid (FA) is the main phenolic and antioxidant compound in wheat tissues, and is known to have an inhibitory potential on *Fusarium* growth and toxin synthesis under *in vitro* conditions. However, the contribution of FA in wheat resistance against *Fusarium* pathogens has not been demonstrated *in planta*. The present study aims at quantifying FA in wheat flower tissues and developing grains to then investigate its contribution in resistance of wheat against *Fusarium* pathogens. Subsequent to the confirmed inhibitive effect of FA on the growth of the *F. graminearum* strain FG13 under *in vitro* conditions, this strain was inoculated on 29 wheat genotypes in a field experiment. Resistance levels of the genotypes were determined by analyses of spike symptoms severity, deoxynivalenol accumulation and symptoms on grains. In parallel, FA contents were determined in flower tissues during anthesis, and in developing grains 10 days post anthesis from non-inoculated spikes. These analyses were carried out on single plants, using high-performance-liquid-chromatography HPLC, with three replicates for the two growth stages and the 29 genotypes. Our results revealed a general increase of FA contents between the two growth stages and differences between genotypes. FA contents varied from 0.22µmol.g^{-1} to 0.86 µmol.g^{-1} dry weight in flower tissues and from 0.42 µmol.g^{-1} to 1.25 µmol.g^{-1} dry weight in developing grains. FA concentrations above 0.50 µmol.g^{-1} in flowers and 0.6 µmol.g^{-1} in developing grain tissues resulted in a high FHB resistance level of the genotype, determined by analyses of symptoms on spikes and grains, indicating a contribution of FA in FHB resistance *in planta*. Yet, this contribution appeared to be weak and no significant correlation was observed between symptoms on spikes and FA contents in flower tissues and developing grains. Hence, FA concentrations *in planta* might be insufficient to effectively inhibit *Fusarium* infection. Nevertheless, the genotype showing the lowest spike symptoms also had the highest FA contents in developing grains, which suggest a "dose-effect" of FA in inhibition of *Fusarium* propagation in wheat spikes.

1. Introduction

Fusarium head blight (FHB) is one of the most destructive diseases of cereals, causing yield reduction, grain deformation and the accumulation of mycotoxins in the grains (McMullen et al. 1997). Basically, *Fusarium* pathogens infect the spikes during anthesis, first by penetrating into the florets and then by spreading throughout the spike via the rachis (Kang and Buchenauer, 2000). The principal pathogen causing FHB on wheat in Europe is *Fusarium graminearum* Schwabe (Parry et al. 1995; Botalicco and Peronne 2012). This pathogen produces deoxynivalenol (DON) as secondary metabolite, which was suggested to contribute to its spread in spikes tissues after primary infection (Langevin et al. 2004; Jansen et al. 2005). During infection process, DON accumulates in plant tissues and contaminates the developing kernels. Cunsumption of DON affect human health through acute temporary nausea, vomiting, diarrhea, abdominal pain, headaches, dizziness, and fever (da Rocha et al. 2014). They also lead to feed refusal, and animal health issues, leading to fertility losses.. Regulations were put into place to limit the amounts of DON entering the food chain and ensuring safety of cereal products (European Commission Regulation (EC) No 1881/2006).

Wheat disposes of several mechanisms to avoid *Fusarium* pathogen infection of spikes and grains, and toxin contamination (Bai and Shaner 2004; Gauthier et al. 2015). Among the biochemical defences of wheat, phenolic compounds have already been mentioned as constitutive elements of wheat resistance against *F. graminearum* (Gauthier et al. 2015). These compounds have antioxidant activities and are suggested to be involved in moderation of oxidative stresses caused by fungal infection and insect damage (Abdel-Aal et al. 2001; Lattanzio et al. 2006; Matern and Kneusel 1988). These phenolic compounds mainly occurring bound in cell-walls are believed to provide a physical barrier against invasive insects and microorganisms (Abdel-Aal et al. 2001; Anson et al. 2008; Lattanzio et al. 2006; Serrano et al. 2006). Moreover, several *in vitro* studies demonstrate their inhibitive effect on several *Fusarium* species growth and toxin synthesis (Boutigny et al. 2010; Pagnussatt et al. 2014; Pani et al. 2014; Atanasova-Penichon et al. 2016; Ferruz et al. 2016).

Ferulic acid (FA) (4-hydroxy-3-methoxycinnamic acid) is the main phenolic compound in wheat grains and spikes and the major contributor to the antioxidant activity of wheat cells (Adom and Liu 2002; Zhou et al. 2004; Mpofu et al. 2006; Anson et al. 2008; Kim et al. 2006).

Previous studies demonstrate significant correlations *in planta* between *Fusarium* disease severity and FA contents in maize kernels, and high FA concentrations were measured in date palm roots resistant to *F. oxysporum* infection (Bily et al. 2003; El Modafar and El Boustani 2001). In wheat grains, FA is mainly concentrated in the fruit coat and cell walls of the aleurone layer (Mpofu et al. 2006; Anson et al. 2008; Klepacka and Fornal 2006). Zhou et al. (2004) measured higher antioxidant activities and FA contents in grains from a resistant genotype than in grains from a susceptible one. To date, the role of FA in spike resistance has never been investigated. Yet, wheat flowers are the first tissues that the *Fusarium* pathogen colonizes during the infection process (Miller et al. 2004). As the presence of FA in the flower tissues early after anthesis has been already demonstrated (Ma et al. 2016; McKeehen et al. 1999), it is pertinent to investigate the potential contribution of FA in resistance against primary infection and *Fusarium* spread throughout the spike. Only limited data is available concerning the contribution of phenolic compounds in FHB resistance *in planta*.

The present study aims at measuring and comparing FA content in wheat flower tissues and early developing grains between different wheat varieties, and subsequently to investigate the contribution of FA in constitutive resistance of wheat against *Fusarium* infections. Resistance levels of 29 wheat genotypes were determined in a field experiment after having verified the inhibitory effect of FA on the growth of a chosen *F. graminearum* strain used for field inoculations. FA contents were determined for all genotypes in both wheat flower tissues and in developing grains 10 days post anthesis from spikes harvested in non-inoculated parts of the field.

2. Materials and methods

2.1. FG strain and production of conidia

The strain *F. graminearum* FG13 was used for field inoculations (Martin et al. 2017). The strain was initially isolated from a symptomatic wheat head in Fravental (ZG, Switzerland, 1998). It is a DON producer and was previously identified as a virulent strain (Martin et al, unpublished). The strain was retrieved from long term storage (-80°C conserved in glycerol) and cultivated on potato dextrose agar (PDA) (Difco, Le Pont de Claix, France). After incubation for 10 days at room temperature under near ultra violet light (12h/12h) (Philips TL 40W/08), conidia were eluted in 5ml sterile distilled water. The suspension was centrifuged at 4,000 rpm for 10 minutes. The supernatant was discarded, the pellet resuspended in 40 ml sterile distilled water amended with 0.2% skim milk (Difco) and stored in 50 ml Falcon tubes at -20°C until further use.

2.2. Inhibitory effect of FA on growth in culture media

The inhibitory effect of FA on the growth of strain FG13 was assessed on PDA (39g.L^{-1}) supplemented with increasing concentrations of FA. FA 90% purity (Sigma-Aldrich, Switzerland) was added in media at four different concentrations: 1.0µmol.L^{-1}, 2.0µmol.L^{-1}, 3.0µmol.L^{-1}, 4.0µmol.L^{-1}, 5.0µmol.L^{-1} and no FA added as control. All Petri dishes (∅ 9 cm) were inoculated with 0.1mL of solution containing 10^6 conidia.mL^{-1} collected in fresh PDA culture and placed in the centre of the dish. Inoculated Petri dishes were then incubated for 6 days at 21°C and 12hours light per day. Growth of FG13 was expressed as the area of mycelium produced after 6 days (see Annex 2). Ten Petri dishes were prepared for each FA concentration.

2.3. Plant material

Twenty-nine genotypes of winter wheat (*Triticum aestivum*) were included in this study. The collection was composed of varieties known to contain elevated contents of FA, varieties with coloured grains (yellow, blue, dark), modern varieties and breeding lines from different European breeders as well as Münstertaler, a Swiss landrace from the Gene Bank in Changins. All genotypes are described in Table 1.

Table 1. Description of wheat genotypes used for resistance test and examination of FA content in flower tissues

Genotype	Status	Breeder (Country)	Coloration of the grain
21112014	Breeding line	Agroscope/DSP (CH)	Clear
21113931	Breeding line	Agroscope/DSP (CH)	Clear
08BA243F	Breeding line	Agroscope/DSP (CH)	Dark
08BA243N	Breeding line	Agroscope/DSP (CH)	Blue
08BA249	Breeding line	Agroscope/DSP (CH)	Dark
08BA250C	Breeding line	Agroscope/DSP (CH)	Clear
08BA250F	Breeding line	Agroscope/DSP (CH)	Dark
08BA250N	Breeding line	Agroscope/DSP (CH)	Blue
ACW15083	Breeding line	Agroscope/DSP (CH)	Dark
ACW15271	Breeding line	Agroscope/DSP (CH)	Dark
Apache	Variety	Nickerson SA (FR)	Yellow
Arina	Variety	Agroscope/DSP (CH)	Clear
Caral	Variety	Agroscope/DSP (CH)	Yellow
Combin	Variety	Agroscope/DSP (CH)	Clear
Hereward	Variety	RAGT (UK)	Clear
Indigo	Variety	KWS Ldt (UK)	Blue
Jazzi	Variety	Agroscope/DSP (CH)	Clear
Munstertäler	Swiss Landrace	BND, Swiss gene bank (CH)	Clear
Purendo38	Breeding line	Crop Development Center, Saskatoon (CAN)	Blue endosperm
Renan	Variety	Agri-Obtention (FR)	Clear
Rosso	Variety	Saatzucht-Donau (AT)	Dark
Royssac	Variety	SERASEM GIE (FR)	Clear
Rubisko	Variety	RAGT (FR)	Dark
Runal	Variety	Agroscope/DSP (CH)	Clear
Skorpion	Variety	RICP (CZ)	Blue endosperm
SZD375	Breeding line	Saatzucht-Donau (AT)	Clear
Titlis	Variety	Agroscope/DSP (CH)	Clear
Toronit	Variety	Agroscope/DSP (CH)	Clear
Vanilnoir	Variety	Agroscope/DSP (CH)	Dark

2.4. Field test implementation

The field test was conducted in Changins in Switzerland (Canton Vaud, south-west [46 ° 24’36“/6 ° 14’06”], altitude: 455m). The trial was composed of three artificially inoculated blocks and one untreated block. In each block, all 29 genotypes were sown in a random order in 1m lines with an interspace of 25 cm. The blocks were surrounded by a 1.5 m triticale buffer zone to avoid cross contamination.

Spikes for the quantification of constitutive FA concentrations in flower tissues were collected in the non-inoculated block. Resistance of wheat genotypes was evaluated by scoring disease severity on the head and on the grains in the three infected plots.

2.5. Collection of flower samples

Spikes of the non-inoculated plants were collected at flowering (BBCH65) and 10 days after anthesis (10DP-anthesis). After cutting the peduncle at 1cm below the bases, the spikes were instantly immersed in liquid nitrogen. Samples were kept at -80°C until further handling. Samples were prepared by manually unhusking the frozen spikes. Tissue at the flowering stage collected for further analyses comprised ovary and stigma, the anthers and their filaments as well as the inner palea. Lemma and glumes were discarded. The same tissues were collected in the 10DP-anthesis samples, yet the ovaries had turned into developing grains. Each sample consisted of tissues from one spike. Three replicates for the two growth stages and the 29 genotypes were prepared.

2.6. Analyses of FA content in flower tissues

2.6.1. FA extraction

The flower tissue samples were weighed while frozen and transferred into 50 mL glass tubes containing 10 mL of HPLC grade methanol (Sigma-Aldrich, Switzerland). The tubes were capped and sonicated for 15min, 60°C, 25KHz (TIH10 sonicator, Elma Schmidbauer GmbH, Germany). The solutions were then filtered through folded paper filters 512 ½ (Schleicher and Schuell, Switzerland) into fresh 50 mL clean glass tubes. The filtrate was dried in a centrifugal solvent evaporator for 90 min at 60°C (EZ-2 evaporator, Genevac Ldt, UK). Subsequently, 1mL of methanol was added to the dry extracts in the tubes and then sonicated until complete dissolution of the pellet at 60°C, 25KHz. The 1mL solution was transferred into 1.5 mL Eppendorf tubes (Sigma-Aldrich, Switzerland) and evaporated for 12h to 24h in a concentrator (SpeedVac concentrator, ThermoFischer Scientific, USA) until complete evaporation. These dry extracts were weighted precisely at 0.1mg to obtain the dry weight (representing on average 5% of the wet weight). Finally, the concentration was adjusted at 25 $mg.mL^{-1}$1 of methanol per 25mg of dry extract was added and the solutions filtered through a 0.45 µm filter (0.45 µm x 13mm, BGB, USA) and transferred into 1.8 mL amber screw top vials (Wheaton, USA). Vials were stored at 4°C until analysis.

Extractions of FA were organised in series of 29 samples corresponding to one sample per genotype. Six series of samples were analysed.

2.6.2. Chromatographic conditions

The extracts were run on a Dionex HPLC (P680 Pump System) using a photodiode array detector (PDA-100, Dionex, Switzerland). The separation was done on a LiChroCART column (Merck, 4x 250mm, 5 µm). Mobile phases were A: H_2O+0.1% formic acid and B: CH_3CN+0.1% formic acid. The gradient conditions were as follows: Solvent B: 0min, 20%; 4-6 minutes, 60%; 10-15 minutes, 100%; 16-20 minutes, 20%; 6.5 minutes, 0%. The flow rate was 1mL.min^{-1}. Detection was monitored at 307 nm.

2.6.3. Calibration and quantification

Peak identification of FA as well as FA quantification were performed with Chromeleon Version 6.60. Calibration was established by HPLC analysis of standard solutions containing 1, 0.5, 0.1, 0.05, 0.01 and 0.005µg of FA (R^2 = 0.998). The concentration of FA is expressed in µmol.g^{-1} of dry weight.

2.7. Resistance test

2.7.1. Artificial inoculations

For the mass production of conidia for the artificial inoculations, FG 13 was cultured on Czapek-V8 agar plates (18g of agar (Merck, Darmstadt, Germany), 32g of Czapek Dox Broth (Difco, Le Pont de Claix, France), and 10 mL of V8® vegetable juice (Campbells, Camden, USA) in 1l distilled water) as described previously (Martin et al. 2017). After incubation for 10 days at room temperature under near ultra violet light (Philips TL 40W/08), FG13 conidia were eluted in 5 mL sterile distilled water. The suspension was centrifuged at 4000 rpm for 10 minutes. The supernatant was discarded, the pellet re-suspended in 40 mL sterile distilled water amended with 0.2% Skim Milk (Difco) and stored in 50 mL Falcon tubes at -20°C until further use. For infections, the suspensions were adjusted to 10^6 conidia.mL^{-1} and sprayed on spikes at mid-anthesis (BBCH 65) until runoff. After inoculation, relative humidity was maintained and at approx. 100% for 24 hours using a high pressure, low volume, overhead irrigation system. A second inoculation took place 3 days later.

2.7.2. Harvest and preparation of grain and flour samples

For the assessment of the severity of symptoms of the grain, infected lines were harvested manually at maturity (BBCH 89) and collected in linen bags. The grains were recovered by threshing in a laboratory thresher (Saatmeister, Kurt Pelz) and cleaned with a vertical air flow (Baumann Saatzuchtbedarf). The grain samples were stored at 4°C. Representative subsamples of 50 grams were obtained using a riffle divider (Schieritz und Hauenstein AG, Switzerland). After analyses of grains, sub-samples were milled with a sample mill (1093 Cyclotec Sample Mill, FOSS, Sweden) (1 mm screen) to obtain whole-meal flour. The flour samples were stored at -20°C.

2.7.3. Assessment of resistance

Resistance of the 29 genotypes was investigated by measurement of four resistance indicators of spikes and grains, following artificial inoculations with conidia suspension of FG13, the three blocks inoculated corresponding to three repetitions of the measurements

- Symptoms on spikes

Symptoms on wheat spikes were scored at 10 days after inoculation and then every three days during 20 days. Blighting of spikelets typical of FHB were observed and severity of symptoms was scored according to Häller Gärtner et al. (2008), based on a 1 to 9 scheme with 1 = no symptom and 9 = complete scalding. Disease severity was expressed using the area under the disease progress curve (AUDPC), calculated by integrating the symptom severity over time of exposure.

- Reduction of the thousand kernel weight due to the infection

Thousand kernel weight (TKW) was measured for all grains from inoculated and non-inoculated plots with a MARVIN optical grain counter (Digital Seed Analyser, GTA Sensorik GmbH, Germany) and a balance (Mettler PM2000, include company and country). The reductions of TKW (%) were obtained by comparing TKW of infected grain samples with TKW of untreated grain sample of the same genotype.

- *Fusarium* damaged kernels ratio

The ratio of *Fusarium*-damaged kernels (FDK) in grains was determined by counting the number of shrivelled and deformed grains for one hundred randomly chosen grains (Häller Gartner et al. 2008).

- Contamination with the mycotoxin DON

DON content was determined in whole meal flour using the DON ELISA kit (R-Biopharm AG, Germany), according to the manufacturer's instructions. Samples with high levels of contamination were diluted 10 times in double distilled water before analysis.

2.8. Statistical analyses

The statistical software R (version 3.3.3) was used for all statistical analyses.

2.8.1. Analyses of inhibitory effect of FA *in vitro*

Average values of FG13 growth were determined for each FA concentration. A polynomial regression of FG13 growth in function of FA contents was performed (R^2=0.99). A polynomial regression was used to determine the half maximal inhibitory concentration (IC_{50}) of FA on the growth of FG13.

2.8.2. FA contents in wheat tissues

FA content variations in tissues were analysed by two-way ANOVA using the development stage as main factor and the genotypes as sub-factor. Data were normally distributed.

2.8.3. Analyses of resistance

Determination of plant resistance level was carried out by observations of the four resistance indicators. For all parameters, average values were determined. Resistance of the 29 genotypes was displayed by a principal component analysis (PCA) using the four resistance indicators as factors and the 29 genotypes as individuals. A hierarchical cluster analysis was performed after the PCA to class individuals into distinct groups of resistance level (R packages "FactoMineR", version 1.29, (Le et al. 2016) and "factoextra", version 1.0.4, Kassambara and Mundt, 2017).

2.8.4. Links between FA content and resistance traits

Finally, FA contents within the different clusters were investigated by a two factor ANOVA, with "Group of resistance" as main factors and the different individuals nested in their respective cluster. Pearson correlations were carried out between average of resistance indicators for the 29 genotypes and FA contents in tissues at the two growth stages.

3. Results

3.1. Inhibitory effect of FA on the growth of strain FG13 *in vitro*

The mycelial growth areas of FG13 on PDA medium supplemented with FA in different concentrations ranged from 1.00 µmol.L^{-1} and 5.00 µmol.L^{-1} (Figure 1). Addition of FA to culture media reduced the mycelial growth over the time measured. The growth area continuously decreased with increasing FA concentrations. Calculation of IC_{50} indicate that the growth of FG13 on PDA media was reduced by half at 3.15 µmol.L^{-1} FA.

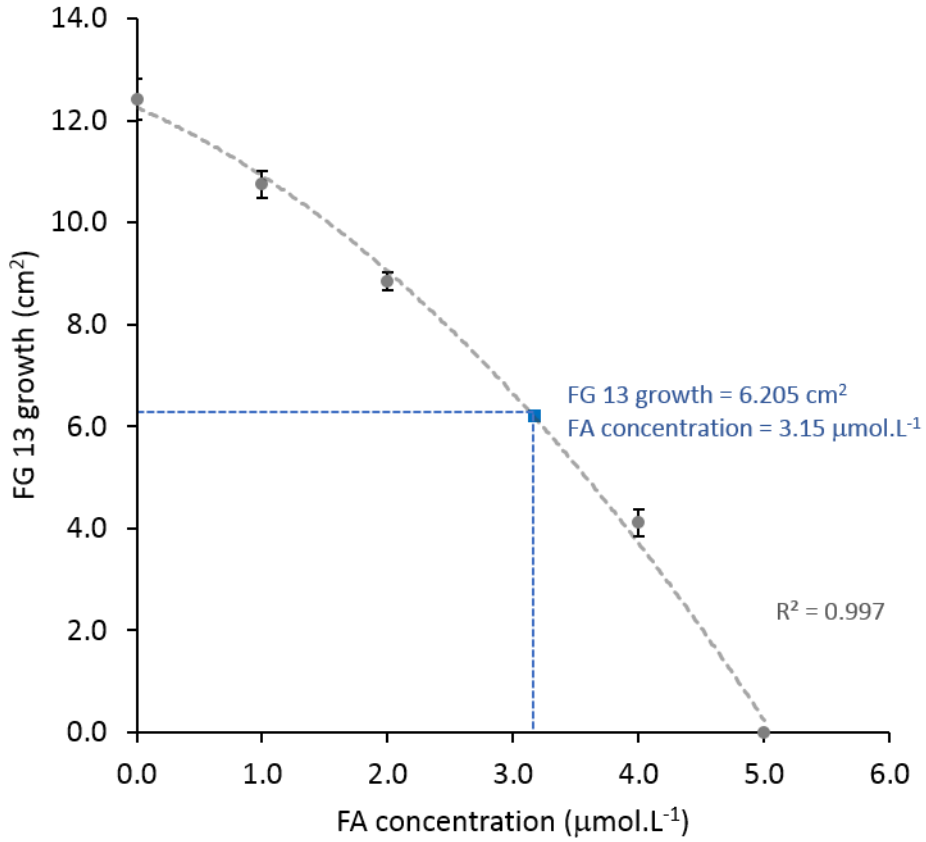

Figure 1. Averages and standard deviations of growth of FG13 on PDA amended with increased concentrations of ferulic acid (FA). The blue square point indicates the calculated IC_{50}.

3.2. Content of FA in flower tissues

The FA content at anthesis varied from 0.22µmol.g^{-1} to 0.86µmol.g^{-1} dry weight. Most of the genotypes contained between 0.30 µmol.g^{-1} to 0.60 µmol.g^{-1} FA (Figure 2a). FA contents in developing grains varied from 0.67 µmol.g^{-1} to 1.20 µmol.g^{-1} dry weight, with most of the

values ranging between 0.30 and 0.75 µmol.g^{-1} (Figure 2b). Over all genotypes, he FA content increased significantly (P<0.05), from average 0.50µmol.g^{-1} in flowering tissues to 0.62 µmol.g^{-1}10 days after anthesis.

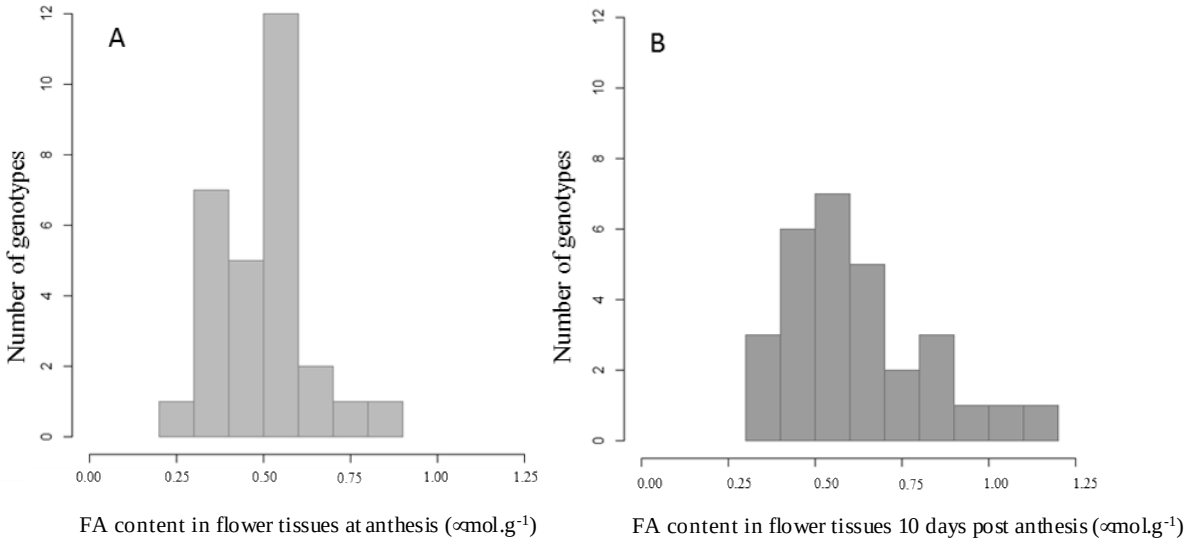

Figure 2. Histograms of ferulic acid (FA) contents in flower tissues (a) and in developing grain tissues 10 days post-anthesis (b) in function of the number of wheat genotypes (n=29).

Significant differences (P<0.05) in FA contents in flowers and developing grains were observed among the genotypes. At anthesis, the lowest FA content was measured in cv. Skorpion, and the highest in the breeding line ACW15083 (Figure 3). In developing grains, the lowest content was measured in the breeding line 211.12014 followed by the genotypes 08BA243F, Rosso, 08BA243N, 211_13931, Runal and Vanilnoir, all containing less than 0.45 µmol.g^{-1} of FA in flower tissues while cv. Munstertäler showed the highest FA content. Significant increases (P<0.05) of FA contents in tissues over time were observed for the genotypes 08BA249, 08BA250, ACW-15271, Munstertäler, Purendo38, Royssac and SZD375. FA contents in flower tissues and developing grains were significantly correlated (Pearson correlation coefficient r=0.45, P<0.05). However, the colour of the grain was not correlated with the FA contents in flowers and developing grains (P>0.05).

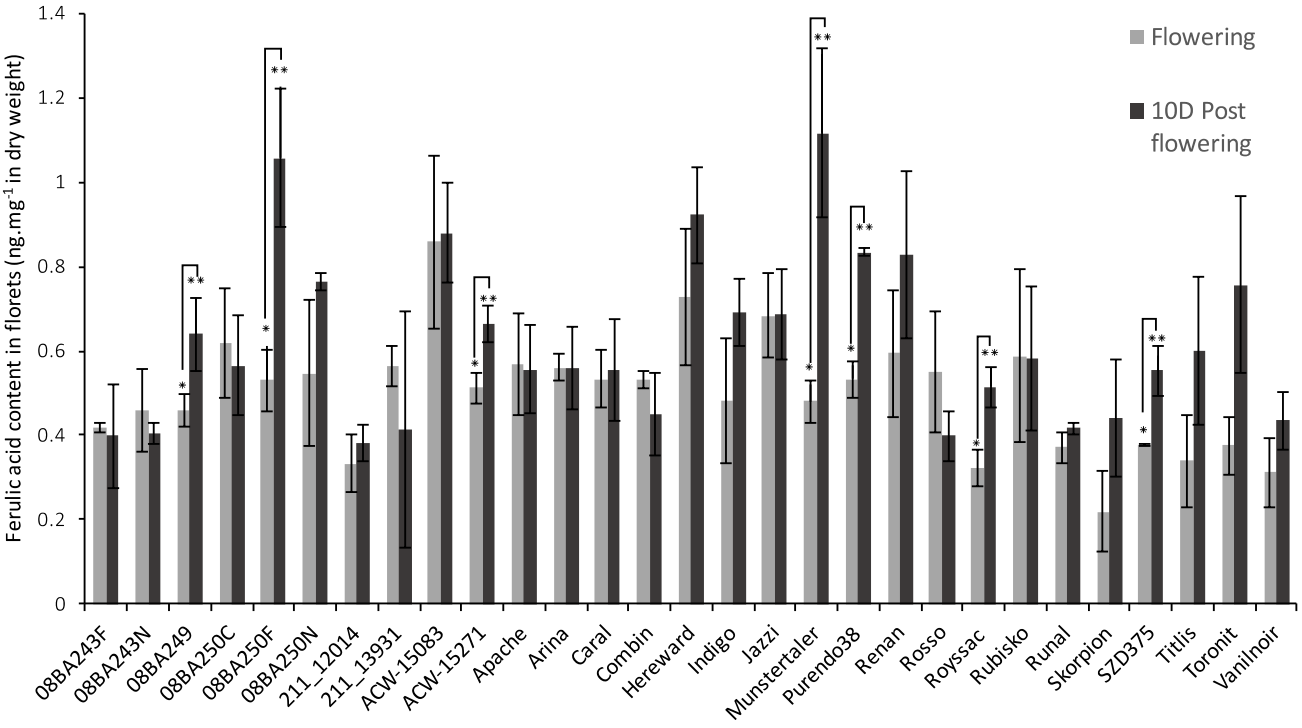

Figure 3. Averages and standard deviations of FA content in flower tissues of 29 wheat genotypes at two stages in wheat flower tissues at anthesis (light grey columns) and in developing grain tissues 10 days after flowering (dark grey columns). The symbol "*" indicates genotypes with a significant increase of FA content observed between the two growth stages (P<0.05).

3.3. Resistance phenotype of the wheat genotype

Based on disease severity assessed on the spikes, Münstertaler, Titlis, Arina and Vanilnoir were the most resistant varieties (Table 2). In comparison, susceptible varieties such as Royssac and Skorpion showed a tenfold higher disease severity. The symptoms on the grains varied largely among the varieties. The proportion of the *Fusarium* damaged kernels (FDK) ranged between 3% (variety Arina) up to 54% (varieties Caral and Royssac), with an average of 21% FDK. Overall, reductions of TKW were observed, indicating a reduction of the grain filling. Taking all varieties into account, an average reduction of 15% of TKW was calculated. Up to 38% of TKW reduction was measured in Caral grains, while Titlis, Rosso and SDZ375 conserved a good grain filling despite *F. graminearum* infection (Table 2). Interestingly, for Titlis and Rosso, a rise in TKW was observed. An average DON concentration of 15.3 mg.kg^{-1} was measured in the grains. In the varities Purendo38, Vanilnoir, Rosso and Runal, DON concentrations were below the detection limit, while up to 39 mg.kg^{-1} were measured in the varieties Royssac and Hereward (Table 2). The links between the resistance indicators were examined with correlation analyses (Table 3). Resistance of spikes was significantly correlated

with resistance of grains. Moderate to strong correlations between the resistance elements (0.44 to 0.71, $P<0.001$) were detected. Regarding grains, indicators revealed moderate but significant correlations between FDK ratio and reduction of TKW, and between FDK and DON content. DON contaminations were not correlated with the reductions of TKW.

Table 2. Averages ± standard deviation of AUDPC, FDK, reductions of TKW and DON content measured for the 29 wheat genotypes after artificial inoculation with *Fusarium graminearum*. "nq" indicates a DON content below the limit of quantification (0.25 $mg.kg^{-1}$).

Genotypes	AUDPC	FDK (%)	Reduction of TKW (%)	DON content ($mg.kg^{-1}$)
21112014	2.5 ± 0.2	19.3 ± 3.1	18.4 ± 10.2	10.3 ± 1.8
21113931	2.0 ± 0.2	31.7 ± 2.1	26.7 ± 7.0	14.9 ± 5.0
08BA243F	1.9 ± 0.5	22.0 ± 3.5	11.4 ± 9.6	24.5 ± 3.5
08BA243N	2.2 ± 0.3	23.0 ± 4.0	6.3 ± 16.7	13.3 ± 4.0
08BA249	2.1 ± 0.6	22.0 ± 5.6	21.1 ± 6.2	17.8 ± 8.7
08BA250C	1.0 ± 0.1	18.7 ± 0.6	14.2 ± 7.0	17.4 ± 3.0
08BA250F	2.1 ± 0.3	18.7 ± 8.0	26.6 ± 9.4	16.2 ± 0.9
08BA250N	1.4 ± 0.6	18.0 ± 2.0	4.7 ± 2.2	26.8 ± 6.1
ACW15083	1.4 ± 0.2	15.7 ± 2.5	22.0 ± 0.4	15.4 ± 4.5
ACW15271	1.1 ± 0.4	14.3 ± 3.5	9.6 ± 2.9	10.4 ± 9.9
Apache	0.7 ± 0.6	11.3 ± 3.5	24.4 ± 4.3	5.6 ± 3.3
Arina	0.5 ± 0.3	3.0 ± 0.0	5.2 ± 2.9	8.6 ± 3.3
Caral	2.0 ± 0.6	53.7 ± 16.3	38.0 ± 13.9	17.5 ± 8.6
Combin	1.6 ± 0.1	21.0 ± 2.0	5.9 ± 10.8	29.1 ± 6.6
Hereward	2.1 ± 0.2	29.3 ± 11.2	3.0 ± 20.1	41.8 ± 11.3
Indigo	1.5 ± 0.5	17.7 ± 5.1	29.1 ± 4.0	8.4 ± 2.0
Jazzi	2.4 ± 0.1	25.3 ± 1.5	24.2 ± 8.3	28.7 ± 1.1
Munstertäler	0.3 ± 0.4	12.3 ± 3.8	10.9 ± 5.5	5.4 ± 2.7
Purendo38	1.5 ± 0.0	22.3 ± 4.7	14.0 ± 9.0	nq
Renan	1.6 ± 0.8	17.3 ± 8.5	18.1 ± 21.3	2.8 ± 1.2
Rosso	0.9 ± 0.2	10.7 ± 2.5	-4.1 ± 6.3	nq
Royssac	3.1 ± 0.2	51.7 ± 15.8	30.5 ± 5.5	38.9 ± 2.9
Rubisko	1.5 ± 0.1	14.0 ± 2.0	11.0 ± 5.9	17.2 ± 7.0
Runal	1.4 ± 0.6	11.0 ± 10.4	6.9 ± 8.0	6.1 ± 8.0
Skorpion	2.9 ± 0.6	41.3 ± 4.0	32.1 ± 2.0	23.6 ± 5.1
SZD375	1.8 ± 0.4	9.0 ± 1.7	0.0 ± 7.7	12.1 ± 1.6
Titlis	0.4 ± 0.3	5.7 ± 2.1	-5.6 ± 4.4	nq
Toronit	1.7 ± 0.0	19.0 ± 6.2	6.7 ± 5.7	26.2 ± 5.6
Vanilnoir	0.4 ± 0.2	8.7 ± 2.5	21.2 ± 4.7	nq
MIN	0.3	3.0	-5.6	<0.25
MAX	3.1	53.7	38.0	41.8

AUDPC : Area Under Disease Progress Curve, FDK: proportion of *Fusarium* damaged kernels, DON: deoxynivalenol toxin

Table 3. Pearson correlation coefficients between observed indicators of resistance of spikes and grains. *** significant at $P<0.001$, ns=not significant.

	AUDPC	FDK	DON
FDK	0.71***		
DON	0.57***	0.60***	
Reductions of TKW	0.44***	0.60***	ns

The resistance elements of the 29 genotypes were visualized with a Principal Component Analysis using the four resistance elements as factors (Figure 4). The 1st dimension (representing 67.3% of the model) discriminated the genotypes according to their general resistance level while the 2nd dimension (20.7%) discriminated genotypes susceptible to reduction of grain filling from those susceptible to DON accumulation. The genotypes were finally grouped into three clusters using a hierarchical clustering of the principal components. Cluster "S" designates the most susceptible genotypes for both spikes and grains. This cluster is essentially composed by the genotypes Caral, Skorpion and Royssac. The cluster "MR" corresponds to the moderately resistant genotype comprising 11 out of the 29 genotypes. Finally, the cluster "R" includes to the most resistant genotypes characterized by low AUDPC, FDK, reduction of TKW and DON content. Fifteen genotypes formed this cluster.

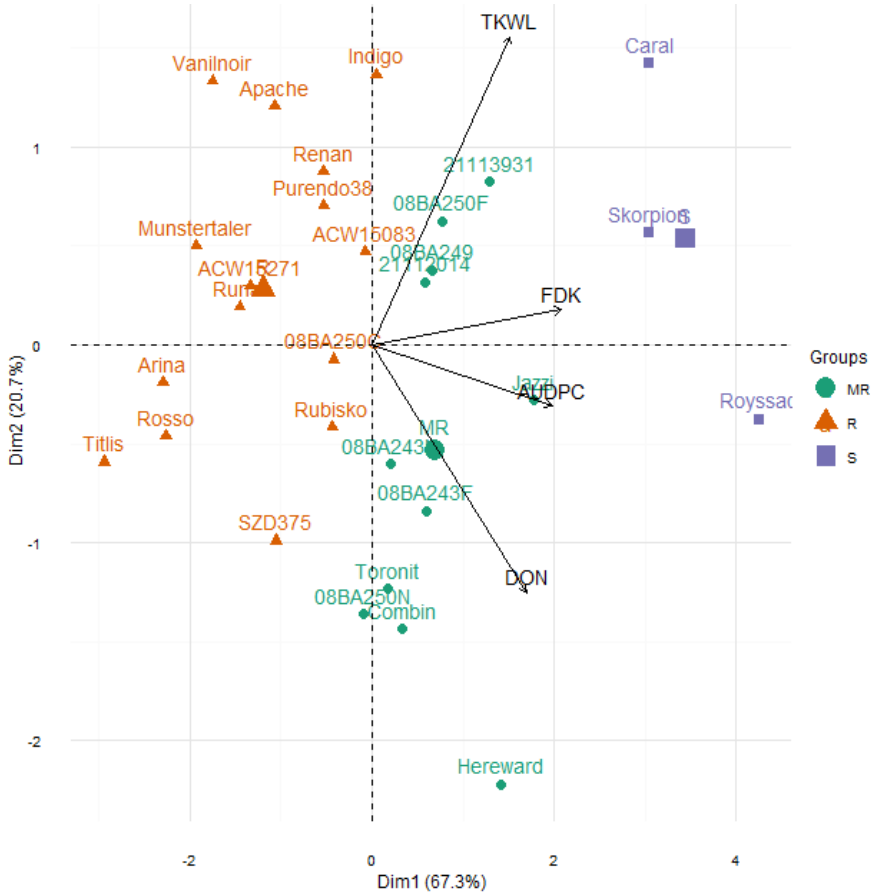

Figure 4. Principal Component Analysis of the resistance of 29 wheat genotypes. The 29 genotypes are gathered in three groups "R": resistant, "MR": moderately resistant, "S": susceptible. Abbreviations: "AUDPC": Area Under Disease Progression Curve, "FDK": ratio of *Fusarium* Damaged Kernels, "TKWL": Losses of TKW due to the infection, "DON": deoxynivalenol content in grains.

3.4. Links between FA contents in wheat tissues and resistance of genotypes

In the previous paragraph, genotypes were gathered in three clusters according to their resistance level. Figure 5 shows significant differences (P<0.05) in FA concentrations between the three clusters of resistance previously defined. The average of FA content in flower tissues of genotypes of cluster "S" was 0.36µmol.g^{-1} in dry weight. In cluster "MR" and "R", 0.51µmol.g^{-1} and 0.52µmol.g^{-1} of FA in dry weight, respectively, were found in the flowers at anthesis (P<0.05). The situation was very similar in the developing grains 10 days after anthesis. The average FA concentration in cluster "S" was significantly lower (0.50 µmol.g^{-1} in dry weight) (P<0.05), than in the cluster of the resistant genotypes "R" (0.65 µmol.g^{-1} in dry

weight). An average of 0.63µmol.g^{-1} of FA in dry weight was obtained in flower tissues in the group "MR".

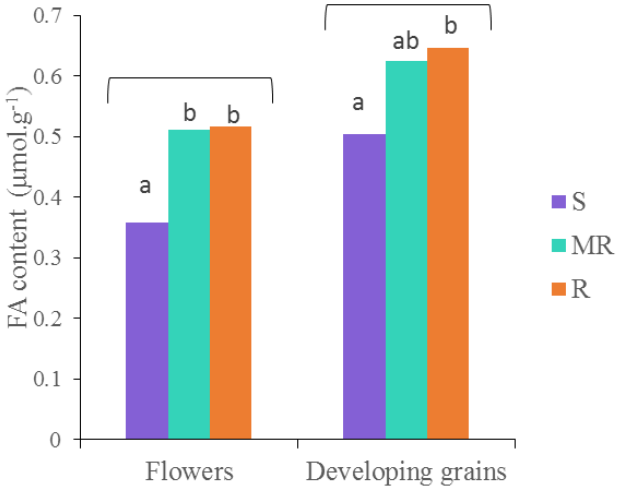

Figure 5. Comparisons of the averages of ferulic acid (FA) contents in flower tissues between the three resistance groups (S: susceptible, MR: moderately resistant, R: resistant) in flower tissues at flowering and in developing grains 10 days after flowering. Different symbols indicate significant differences between average of FA contents between the three groups within one development stage according to Tukey Honest Significant Difference test (α=0.05).

According to the two nested ANOVA on FA content, the factor "Group of resistance" had significant (P<0.05) but weak impacts on variability of FA contents (Table 4). The three distinct resistance groups explained 8.0% and 3.2% of FA content variabilities in flowers and developing grains, respectively. Correlation analyses were performed between FA contents and the average values of the four resistance indicators. However, none were significant (data not shown).

Table 4. Nested analyses of variance of ferulic acid contents in flowers at anthesis and in developing grains 10 days post anthesis. For both, the 29 genotypes were considered as factor nested in their respective resistance groups. "*": significant at P<0.05, "**": significant at P<0.01, "***" significant at P<0.001.

	Flowers			Developing grains		
Source of variance	Sum Square	Mean Square		Sum Square	Mean Square	
Resistance group	7852	3926	**	6039	3019	*
Genotype	54894	2111	***	131483	5057	***
Error	39470	651		50623	904	

4. Discussion

Phenolic acids and their derivatives play an important role in the resistance of plants against fungal pathogens (Nicholson and Hammerschmidt 1992). They can have a toxic or inhibitory effect on microorganisms, contributing to the formation of a constitutive barrier in plant tissues to hinder or to delay infection (Sinaridou et al. 2002; Treutter 2006). In wheat, ferulic acid (FA) is abundant in ear tissues with a putative role in resistance against *F. graminearum* (Engelhardt et al. 2002). The growth of the highly virulent strain FG13, used in the present experiments, was reduced on PDA medium amended with FA in increasing concentrations. We calculated that a FA concentration 3.15µmol.L^{-1} can halve the growth of FG13 on PDA. A similar impact of FA on other pathogenic *F. graminearum* strains and *Fusarium* species has been observed on artificial media in other studies (Assabgui et al. 1993; McKeehen et al. 1999; Ferruz et al, 2016; Ferrochio et al. 2013; Lintz et al. unpublished). In the present field experiment, we compared the concentration of FA in the flowers of 29 wheat genotypes with different resistance levels after infection at the ear and at the kernel stage (respectively at anthesis and 10 post-anthesis). It became evident that the higher the concentration of FA, the higher the resistance parameters. Notably, concentrations above 0.5 µmol.g^{-1} at anthesis and above 0.6 µmol.g^{-1} at 10 days post-anthesis (developing grain tissues) contributed to a higher resistance level. In the genotype with the highest FA concentrations in developing grains (Munstertäler), a dose-effect on the reduction of severity of symptoms on the ear was observed. We therefore conclude that FA inhibits the development of the fungus in the flower and consequently reduces the initial infection. It has been demonstrated under *in vitro* conditions that FA can inhibit the synthesis of different trichothecenes, produced by the genus *Fusarium*, by a direct action on the expression of the *Tri* genes (Boutigny et al. 2009). Deoxynivalenol, a trichothecene mycotoxin of *F. graminearum,* affects the host tissue and has been suggested to act as a major virulence factor in the first infection stages (Kikot et al. 2009; Brown et al. 2010; Yang et al. 2013). Besides a proven effect on the growth of the fungus, FA may contribute to resistance with a direct effect on DON. However, there was no detectable effect of the FA content on the DON content in the grains at harvest.

The increase of FA during the first growth stages of grain development is consistent with previous studies. McKeehen et al. (1999) showed that synthesis and accumulation of FA in the cell walls is initiated at anthesis and continues throughout kernel development. Ma et al. (2016) underlined that the highest FA synthesis activity is reached in developing kernels between the

end of anthesis and 14 days after anthesis. Indeed, in mature grains, Anson et al. (2008) measured 8.00 μmol of FA.g^{-1} in bran, and up to 16.00 μmol of FA.g^{-1} in the aleurone layer. In comparison, we measured on average 1.00 μmol of FA.g^{-1} in the immature grain at 10 days post-anthesis. Other phenolic compounds such as *p*-coumaric acid are also synthesised in flower tissues during the early step of grain development (Mc Keehen et al. 1999). The increasing phenolic compound concentration in grain tissues accompanies the formation of the grain pericarp (McCallum and Walker 1990; McKeehen et al. 1999). We observed that the amounts of FA in flower and developing grain tissues were not linked with the colour of mature grain pericarp, consistent with previous studies (Mpofu et al. 2006; Ma et al. 2016).

Yet, elevated contents of FA in flower tissue did not fully protect the grain from infection and DON accumulation. In addition, the presence of other antioxidant compounds such as anthocyanins in the present study and in previous studies (Martin et al. 2017) or carotenoids (Dougoud, personal communication) did not influence the grain resistance or the accumulation of DON. Overall, our results show that FA contributes significantly but only weakly (about 8%) to resistance of wheat against FHB caused by *F. graminearum*. The almost linear correlations between FA content in the flower and resistance to ear symptoms and DON accumulation as proposed in earlier publications (Engelhardt et al. 2002) could not be confirmed in this study. The weak effect of FA in resistance can be attributed to its poor concentration in flower and developing grain tissues. Here, we measured FA contents between 0.4 μmol.$g^{-1}$and 1.2 μmol.g^{-1} in dry weight of flower and developing grain tissues, which corresponds to concentrations between 0.02 μmol.g^{-1} and 0.06μmol.$g^{-1}$of FA in tissues *in vivo*. These values are far below those of IC_{50} of FA on FG13 growth inhibition observed *in vitro* (3.15 μmol.g^{-1}), and below the effective concentration to inhibit production of trichothecenes *in vitro* (0.5mM) as observed by Boutigny et al. (2009). Hence, it is likely that the FA content in flower and developing grain tissues was not high enough to effectively inhibit the initial infection and growth of *F. graminearum* in the plant tissues.

Based on a large phenotyping database with 29 wheat varieties, we demonstrated that FA contributes statistically significantly but weakly to resistance against ear symptoms. However, resistance in wheat against FHB is quantitative and as of today, no complete resistance is known (Bai and Shaner 2004). Many minor resistance QTLs have been identified, but with a low percentage of explanation to the global resistance (Shen et al. 2003; Zhou et al. 2002; Paillard

et al. 2004; Jayatilake et al. 2011). We assume that FA provides a small contribution to resistance. As FA can also provide resistance to other ear diseases and insect pests and is beneficial for human health, breeding for resistance should consider the FA concentration in the flower tissue (Abdel-Aal et al. 2001; Stadnik and Buchenauer 2000; Ding et al. 2000).

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Chapter III

Factors of wheat grain resistance to Fusarium head blight

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Summary

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is an important wheat disease that affects grain yield and conformation, and contaminates grains with mycotoxins, including the trichothecene deoxynivalenol (DON). The impacts of *Fusarium* infections on grain filling, grain deformation and rheological properties were assessed under different environmental conditions. Genotypes with elevated grain anthocyanin content were used. Resistance of seven wheat varieties and breeding lines was assessed with artificial infections in the field. Grains from infected and control plots were assessed for proportion of *Fusarium* damaged kernels, grain filling (thousand kernel weight) and DON accumulation. Biochemical and rheological properties of harvested grain were also assessed. Grain resistance to *Fusarium* is has several components, including resistance against DON accumulation, deformation and stability of grain filling. These mechanisms are interdependent but act independently. Resistance against DON contamination was highly influenced by environmental conditions, but environment had little effect on the other resistance components. Anthocyanins and protein concentrations were unchanged in infected grains, suggesting that FHB does not affect grain biosynthesis processes but impacts the transport of assimilates caused by changes in grain composition. We suggest that this is the reason for the alterations of rheological properties. The greater the grain resistance, the less was the impact on dough properties. This study suggests that the resilience of rheological properties under FHB infection pressure is an additional component of grain resistance to the disease.

1. Introduction

Fusarium head blight (FHB), caused by different species of *Fusarium*, is one of the most important diseases of wheat. Besides considerable yield losses, infections lead to contamination of grain with mycotoxins and to grain morphological changes, produce unsuitable for consumption and trade (McMullen *et al.*, 2012). In temperate climates, *F. graminearum* Schwabe is the prevalent FHB-causing species, and this fungus produces, among others, the mycotoxin deoxynivalenol (DON) (Parry *et al.*, 1995). At host flowering and with high relative humidity, ascospores or conidia of the fungus reach high numbers, and penetrate at the base of the florets to infect the floral tissues. Subsequently, the fungus spreads throughout the spikes via xylem and phloem causing characteristic symptoms on the spikes and colonising the developing grains (Kang and Buchenauer, 2000).

Management strategies for FHB aim to avoid primary infection from debris of previous crops (in particular maize) (Vogelgsang *et al.*, 2011), and the use of resistant varieties (Mascher *et al.*, 2005). Resistance of wheat against FHB includes a large number of resistance mechanisms (Ravensdale *et al.*, 2014). Schroeder and Christensen (1963) described “type 1 resistance” as resistance to primary infection, and “type 2 resistance” as resistance against spread the pathogen throughout the host inflorescences (spikes). This concept was subsequently extended with types of kernel resistance, yield stability (Mesterhazy 1995) and resistance against mycotoxin accumulation (Miller *et al.*, 1985).

The presence of shrivelled, misshaped and so-called scabby grains reflects *Fusarium* infection of grain. The proportion of healthy looking grains is a measure of grain resistance, termed “type 3 resistance” (Jones and Mirocha 1999). The underlying symptoms can be differentiated, firstly in grains that have been damaged by the infection, so-called *Fusarium* damaged kernels (FDK), and secondly where grains are were poorly filled (Mesterhazy, 1995; Foroud and Eudes 2009). Poor grain filling is quantified by measuring the difference in thousand kernel weight (TKW) of infected and non-infected grains. Reduced TKW is part of type 3 resistance, but is also a component of “type 4 resistance” defined as the conservation of yield despite pathogen presence (Foroud and Eudes 2009; Almeida *et al.*, 2016). Type 5 resistance is considered the capacity of host plants to impede the production of mycotoxins by the pathogen, and to detoxify the mycotoxin (Miller *et al.*, 1985; Boutigny *et al.*, 2008). *Fusarium* infection can also alter the biochemical constituents of grains resulting in

deterioration of rheological and baking quality traits (Dexter *et al.*, 1996; Häller Gärtner *et al.*, 2008). Resistance mechanisms against changes in grain biochemistry have not yet been described.

All of these resistance types are partly interdependent, but are probably based on distinct mechanisms and are likely to be independently inherited. (Mesterházy *et al.*, 1999; Bai *et al.*, 2000). Furthermore, strong genotype-environment interactions have been observed in FHB outcomes and trichothecene accumulation (Miedaner *et al.*, 2001). For these reasons, the evaluation of grain resistances towards FHB remains difficult in a modern breeding context. Usually, only symptoms on the wheat ears, and sometimes mycotoxin accumulation (particularly DON), are taken into account by plant breeders (Foroud and Eudes 2009). However, these few resistance parameters do not span all facets of resistance, and may be insufficient when looking for specific combinations of traits in breeding lines or when phenotyping for resistance in mapping populations.

Between host flowering and maturity, developing grains are supplied with all required nutrients such as sugars, minerals, and amino-acids (Feillet 2000). Subsequently, these nutrients are transformed into starch, storage protein, functional proteins and other constituents. The effects of *Fusarium* pathogen on these biochemical mechanisms have not been studied.

This study aimed to investigate the impacts of *Fusarium* infection on grain filling and modifications in rheology of flour from infected grains. We have tested seven wheat varieties and breeding lines with different resistance levels against FHB in field tests with artificial inoculations at three experimental sites in Switzerland. Four genotypes with coloured grains, due to elevated anthocyanin content (Abdel-Aal *et al.*, 2007), were included in the study. Anthocyanins are synthesised by grain tissue during maturation, starting at the milk-dough stage (Knievel *et al.*, 2009; Žofajová *et al.*, 2012). Biosynthesis of these compounds depends on the availability of photosynthesis assimilates (Bustos *et al.*, 2012), and is conditioned by abiotic stresses (Gordeeva *et al.*, 2013). In the present study, coloured grains were used to measure the impacts of a biotic stress on grain filling and biosynthesis of anthocyanins and proteins.

2. Materials and methods

2.1. Plant material

Seven winter wheat genotypes, including five registered cultivars and two breeding lines, have been studied in this experiment (Table 1). The cultivars 'Arina', 'Combin', 'Hanswin' and 'Vanilnoir' show different FHB resistance levels in the field (Häner *et al.*, 2014; Mascher, unpublished). ACW 083, ACW 271, 'Indigo' and 'Vanilnoir' have coloured grains due to the presence of anthocyanins in the grain pericarps. The cultivar 'Purendo 38' has dark blue grains with anthocyanins in the grain pericarps and endosperms. This additional genotype was employed for comparison of anthocyanin synthesis production in two field tests (at Changins and Vouvry). Due to high susceptibility to yellow rust, this variety could not be included in the resistance evaluation.

Table 1. Description of winter wheat genotypes investigated in this study. + = resistant, - = susceptible, and Ø = non-significant resistant comportment

Genotype	Resistance level	Baking quality class	Coloration of the grains	Breeder	Pedigree
ACW 083	unknown	unknown	dark	Breeding line Agroscope	TABOR/NS1201/6/ZENITH/BRST-KV4666-56//17R4/5/VUKA/4/PROBUS MS×2/ FLINOR/3/BEZOSTAYA 1//CAN3842/ HEINE 7/7/KONINI
ACW 271	unknown	unknown	dark	Breeding line Agroscope	TABOR/NS1201/6/ZENITH/BRST-KV4666-56//17R4/5/VUKA/4/PROBUS MS×2/ FLINOR/3/BEZOSTAYA 1//CAN3842/ HEINE 7/7/KONINI
'Arina'	+	I	clear	Agroscope/DSP (CH)	MOISSON//CANADA3842-3663/Heines-VII
'Combin'	-	I	clear	Agroscope/DSP (CH)	VIRTUE/3/ZENITH/NS611// LICHTI/4×PROBUS/4/W'ST480-73/3/ ZENITH/NS611//LICHTI/4×PROBUS
'Hanswin'	Ø	I	clear	Agroscope/DSP (CH)	OBELISK/TAMARO//PEGASSOS
'Indigo'	unknown	unknown	dark	KWS Ltd (UK)	
'Vanilnoir'	+	Top	dark	Agroscope/DSP (CH)	RUNAL/W'ST479-77//KONINI
'Purendo 38'	unknown	unknown	blue	Crop Development Center, Saskatoon, (CAN)	

2.2. Fungal isolates and production of inoculum

Fusarium graminearum strains used in the current study were the single spore isolates FG 1145[1] (isolated in 2006, Canton Vaud, Switzerland), FG 13[1] (1998, Canton Zug) and FG 0410[2] (2005, Canton Schaffhausen). The three strains were isolated from symptomatic wheat ears, and were chosen to represent the average level of virulence of various *F. graminearum* strains isolated in Switzerland (Martin *et al.*, unpublished). Conidia of the strains were stored in a 1:1 mix of water with glycerol at -80°C. Routinely, the strains were cultured on Potato Dextrose Agar (PDA, BD Difco) for 1 week in the dark at 4°C. For mass production of conidia of each isolate, two discs (5mm diam.) from a well-grown colony were transferred to 200 mL of liquid V8-medium (1:5, V8 juice (Campbell Soup Company): distilled water, + 2g sodium carbonate L^{-1}) in a 1L capacity Erlenmeyer flask. Cultures were incubated for 7 d at 24°C, on a shaker at 200 rpm in the dark. The culture was then filtered through sterile cheesecloth to remove all mycelium, and conidia were collected by centrifugation at 4,500 rpm for 10 min. The resulting pellet was re-suspended in sterile, demineralised water. These preparations were either used immediately or stored at -80°C as described below.

2.3. Field experiments and artificial inoculations

Field experiments were conducted in 2014 at three locations in Switzerland: Changins (VD), Vouvry (VS), and Reckenholz (ZH) (Table 2). All winter wheat genotypes were sown in 1 m^2 microplots on 5 lines with a Seedmatic seeding machine (HegeMaschinen) in autumn 2013.

Table 2. Locations and environmental conditions for three field experiment locations (data from 01/06/2014 to 31/07/2014). (source :http://www.meteoswiss.admin.ch)

	Changins	Vouvry	Reckenholz
Coordinates (North/Est)	46°24′36″/6°14′06″	46°20′16″/6°53′28″	47°16′30’’″/8°26′45″
Altitude (m)	455	387	494
Daily mean temperature (°C)	18.4	18.0	18.2
Total degree-day (°C)	1119.8	1100.9	1112.0
Daily mean rainfall (mm)	4.0	5.6	4.1
Total rainfall (mm)	245.6	344.0	247.5
Daily mean humidity (%)	70.3	77.1	72.3

[1]Strains deposited in the Agroscope data base http://mycoscope.bcis.ch/
[2]Strain deposited at the CBShttp://www.cbs.knaw.nl/fusarium/

Artificial inoculations took place when 50% of the plants inside each microplot were at flowering stage (BBCH 65). Inoculum was prepared just before the inoculations by mixing equal proportions of liquid cultures of each of the three *F. graminearum* strains and adding 0.0125% of TWEEN®20 (Sigma-Aldrich Chemie GmbH). Concentration of conidia and volume of suspension prepared were adjusted to 1.5×10^7 conidia per microplot. Suspensions were applied with a hand sprayer (Spray-matic 1.25P, Birchmeier) at dawn. According to the climatic conditions, the plots were irrigated to maintain humidity on the ears for at least 24 h. A high pressure/low volume overhead spray irrigation system was available at Changins. In Vouvry and Reckenholz, water (600 L ha^{-1}) was sprayed manually with a back-pack sprayer. A second inoculation took place 2 d later.

2.4. Disease assessments on spikes

Disease severity and incidence were recorded on 30 randomly marked spikes in each plot. Disease severity was recorded by counting the number of infected spikelets, and disease incidence was scored by counting the number of infected spikes. The severity was expressed as percentage of infected spikelets relative to the total number of spikelets. The first assessments were carried out 15 d after the last inoculation, and then at 3 d intervals. At least three assessments were carried out for each plot.

2.5. Harvest and milling

All wheat plots were harvested at full maturity (BBCH 89) using the locally available facilities. In Reckenholz, a combine harvester (HEGE 140, Mähdreschwerke GmbH) was used. The airflow on the harvester was reduced to recover a maximum of kernels. In Changins and Vouvry, spikes were harvested by hand. After threshing with a laboratory thresher (Saatmeister, Kurt Pelz), grains were dried to 14% moisture and cleaned using a vertical airflow (Baumann Saatzuchtbedarf) to remove dust and other debris. All grains were stored at 4°C and processed in Changins. Two hundred gram sub-samples were extracted after 3 times homogenisation with a riffle divider (Schieritz & Hauenstein AG). Samples were milled separately with a sample mill (1093 Cyclotec Sample Mill, FOSS) to obtain wholemeal flour. The flours were stored at -20°C until used for further analyses.

2.6. Analyses of grains

2.6.1. Morphological analysis of grains

The thousand kernel weight (TKW) of each grain sample was measured with a MARVIN optical grain counter (Digital Seed Analyser, GTA Sensorik GmbH) and a balance (Mettler PM2000). The ratio of *Fusarium* damaged kernels (FDK) in each sample was determined by counting the number of shrivelled and misshapen grains for one hundred randomly chosen grains.

2.6.2. DON content

Content of deoxynivalenol was determined in wholemeal flour using a DON ELISA kit (Ridascreen® FAST DON, R-Biopharm AG), according to the manufacturer's instructions. Samples with high levels of contamination were diluted 10 times in double distilled water before analysis.

2.6.3. Anthocyanin concentrations

The total anthocyanin concentrations in grains were measured in wholemeal flour as described elsewhere (Eticha *et al.*, 2011) with modifications. Extractions were each carried out on 2.5 g of wholemeal flour with 20.0 mL of methanol/hydrochloric acid solution (85:15, v/v) in a 250 mL capacity flask. The mixture was homogenized for 20 min with a magnetic stripper and stored at 4°C for 20 min. The mixtures were transferred in 50 mL plastic tubes, centrifuged at 4,000 rpm for 5 min, and then stored at 4°C for 20 min. The supernatants were filtered into 50 ml volumetric flasks (S-Pak Filters, 0.45µm diam. 47 mm, on a Swinnex Filter Support 47 mm, Millopore SA). The extraction processes were repeated once on the pellet. Before measuring, 25 mL of each supernatant was mixed and stored on ice cubes in the dark. Measuring was done within 2 h. The absorbance of the extract was measured at 538 nm with a VIS/UV spectrometer (UViLine 9400, SCHOTT Instruments). The anthocyanin concentration was calculated based on a calibration curve obtained with cyanidin chloride as a standard (Sigma-Aldrich Chemie GmbH) according to Abdel-Aal and Hucl (1999). All anthocyanin concentrations were expressed in milligrams of equivalent of cyanidin chloride kg^{-1}. (Abdel-Aal and Hucl 1999).

2.6.4. Protein content

The protein contents (%) of infected and non-infected grains were analysed by near-infrared reflectance spectroscopy (NIRS) using a NIRFlex N-500 (Büchi Labortechnik AG). The protein calibration of the NIRFlex was regularly adjusted with 50-100 wheat samples from different

varieties and origins. Basis analyses were made with the Kjeldahl method, according to ICC standard method No. 105/2. The coefficient of confidentially of the calibration is $R^2 = 0.93$ (Cécile Brabant, pers. comm.). The protein content of infected and non-infected samples fitted into the range of the NIRS calibration.

2.6.5. Rheological properties

Dough stability duration (min), dough softening (Farinograph Units; FUs) and water absorption capacity (%) during kneading were measured using the microdough LAB farinograph (model 2800, Perten Instruments). The measurements were based on the microdough LAB 120 rpm method 02.01 (Perten Instruments Method Description for micro-doughLAB, Perten Instruments). For each sample, wholemeal flour (4 g) was placed into the "120 rpm kneading device". Distilled water was automatically added until the dough reached the consistency of 650 FUs. The volume of added water was recorded. The dough stability duration is the time the dough consistency remains at 650 FUs, while dough softening corresponds to the decrease in FUs after 10 min of kneading.

2.7. Experimental set up and statistical analyses

Crop planting, inoculation methodology and disease assessments were the same at the three field sites. The trials each consisted of three replicates with, and three without, artificial inoculation, planted in a split-plot design.

Data of disease incidence and severity were integrated with the number of observation days (number of days between infection and the last scoring) and divided by the number of the observation day, and were thus expressed as relative area under the disease pressure curve (AUDPCrel). Variations of TKW and differences in rheological properties of grains due to the infections were analyzed for each infected sample by calculating the difference (%) with the average of the three non-infected samples of the same wheat genotype from the same location. All calculations were conducted on Microsoft® Excel 2013.

Statistical analyses were carried out using statistical R software (R Core Team, 2015). Analyses of variances (ANOVA) of resistance indicators were used to compare the effects of genotype and environment on inoculated plants. Data for disease incidence, and DON content

were square root transformed to obtain normal distributions. The effect of an infection on grain properties was investigated using analyses of variance for the three factors presence of inoculation, genotype, or environment. Data of dough stability and anthocyanin content were square root transformed to obtain normal distributions. Comparison of TKW losses and relative differences in rheological properties of dough were carried out with two factor ANOVAs on the factors genotype and environment. When significant, multiple comparisons analyses were based on Tukeys HSD (package "agricolae", de Mendiburu, 2015). Correlations between the parameters were investigated using Pearson correlations on normalized data.

3. Results

3.1. FHB severity and incidence

The assessments of disease severity and disease incidence were carried out in inoculated and in non-inoculated (control) plots. Symptoms were significantly more severe in Changins than in Reckenholz or Vouvry (P <0.05) (figure 1). Genotypes gave different resistance levels to infection, but no genotype × environment interactions were detected. Overall, the cultivars 'Combin' and 'Indigo' showed greater disease scores than 'Vanilnoir' and the breeding lines ACW 083 and ACW 271. 'Arina' and 'Hanswin' showed intermediate incidence and severity. No FHB symptoms were found in the non-inoculated plots.

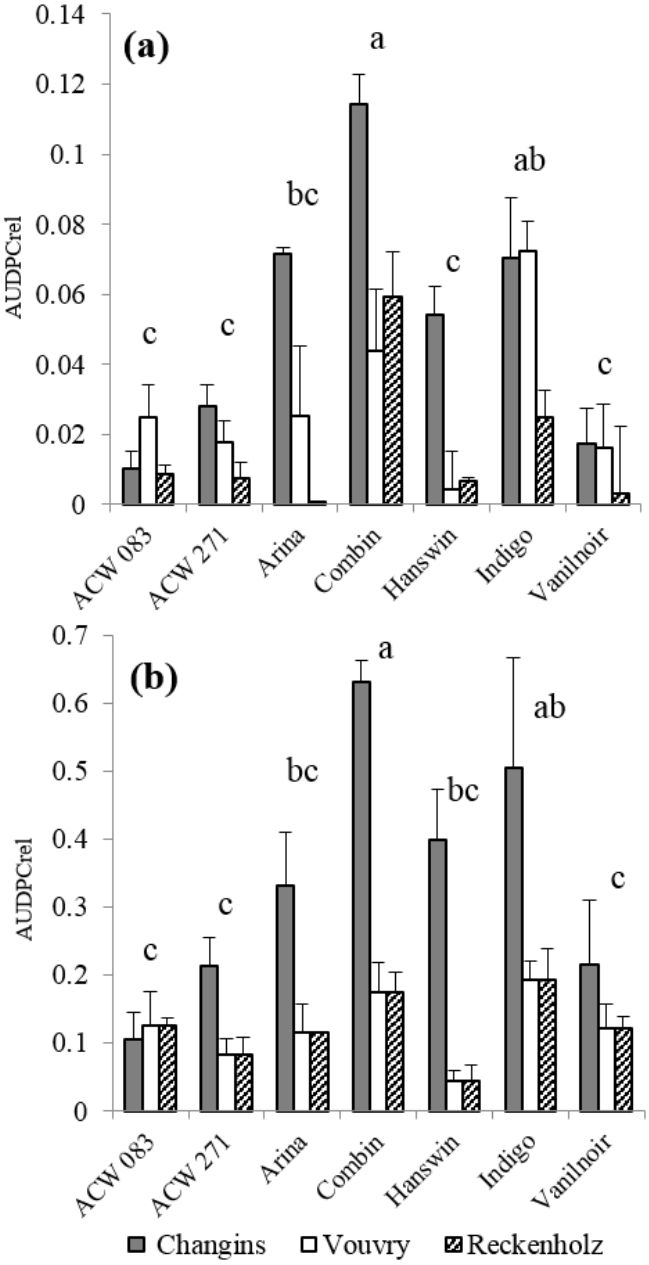

Figure 11. Means of rel. AUDPC for (a) FHB severity and (b) FHB incidence on 7 wheat genotypes in Changins, Vouvry and Reckenholz. Bars accompanied by with the same letter are not statistically different ($P < 0.05$).

3.2. Proportion of *Fusarium* damaged kernels (FDK)

Generally, all samples from inoculated plots, including all varieties in all environments, contained damaged grains in various proportions (figure 2).. The proportion of FDK was significantly less in samples from Vouvry than from the other sites ($P < 0.05$). In non-inoculated plots, 1-2% of the grains showed morphological damage attributed to *Fusarium*. 'Combin' presented the greatest proportion of *Fusarium* damaged kernels in all environments, while the 'Vanilnoir' was the least affected.

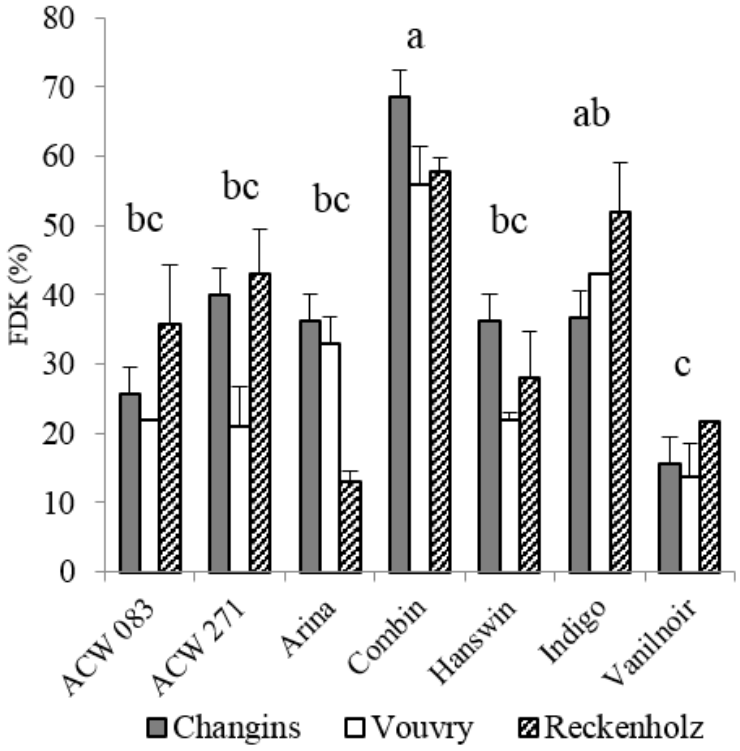

Figure 12. Mean *Fusarium* damaged kernel (FDK) ratios of three replicates, for seven wheat genotypes grown at three different field sites. Bars accompanied by the same letter are not statistically different ($P < 0.05$).

3.3. Thousand kernel weight (TKW)

Grain filling was measured as TKW, and differences in TKW between grains from inoculated and non-inoculated plots revealed the impacts of the infection on grain filling. In Changins and Reckenholz, infection had significant impacts on grain filling ($P < 0.05$) (Table 3). For example, losses in TKW reached 25% for 'Combin' in Reckenholz and Changins. In contrast, TKW was hardly affected at the Vouvry site. Over all three environments, the genotypes with coloured grains (ACW 083, ACW 271, and Vanilnoir) showed the smallest losses in TKW due to inoculation.

Table 3. Means of Thousand Kernel Weights (TKW), losses (%) for all genotypes across at the three field sites (environments). Data followed by the same letter are not significantly different ($P < 0.05$).

Genotype	TKW losses (%)			Mean	
	Changins	Vouvry	Reckenholz		
ACW 083	9.0	8.3	7.0	8.1	c
ACW 271	8.9	6.3	11.3	8.8	c
Arina	23.7	7.1	8.1	13.0	bc
Combin	24.7	16.8	25.8	22.4	a
Hanswin	15.0	-2.9	8.8	11.9	bc
Indigo	19.6	6.6	17.8	14.7	ab
Vanilnoir	8.4	1.7	5.7	5.3	c
Mean	15.6 (a)	7.8 (b)	12.1 (g)	12.0	

3.4. Accumulation of DON

The accumulation of the mycotoxin DON is considered key for the evaluation of the severity of kernel infection by *F. graminearum*. No DON was detected in non-inoculated samples. The content in DON was significantly ($P < 0.05$) conditioned by the environment (Figure 3). In Reckenholz, Changins and Vouvry, the average content in DON in grains was 31.9 $mg.kg^{-1}$ for Reckenholz, 20.7 $mg.kg^{-1}$ for Changins and 12.3 mg kg^{-1} for Vouvry. Over the three environments, the greatest DON contents were measured in grains from 'Combin' and 'Indigo', while grains of 'Arina', 'Hanswin' and 'Vanilnoir' contained significantly less ($P < 0.05$) DON.

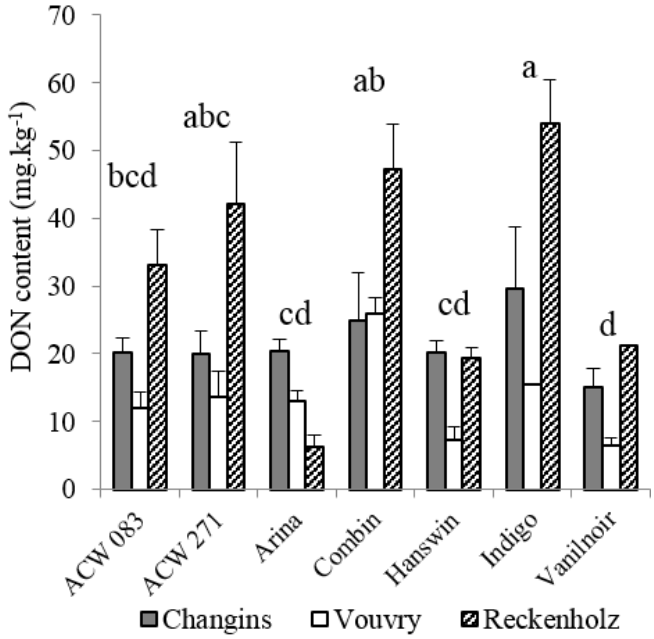

Figure 13. Mean deoxynivalenol (DON) concentrations in grains from seven wheat genotypes grown at three field sites (environments). Bars accompanied by the the same letters are not statistically different ($P < 0.05$).

These analyses allowed the genotypes to be classified according to their different types of resistance (Table 4). 'Vanilnoir' was the most resistant for all types of resistance while 'Combin' was generally the most susceptible. Overall, these types of resistance were affected by environmental conditions but to different extents: impacts of *Fusarium* infection were significantly less at Vouvry regarding FDK ratio and TKW losses while DON accumulation were greater at Reckenholz for all the tested wheat genotypes.

Table 4. Classification of the different types of resistance for the wheat genotypes used in this study. + = resistant, - = susceptible, and Ø = non-significant resistant comportment.

Genotype	Resistance of the spikes	Resistance category		
		DON accumulation	*Fusarium* incidence of grains	Reduction of the grain filling
ACW083	+	Ø	Ø	+
ACW271	+	Ø	Ø	+
'Arina'	Ø	+	Ø	Ø
'Combin'	-	Ø	-	-
'Hanswin'	Ø	+	Ø	Ø
'Indigo'	Ø	-	Ø	Ø
'Vanilnoir'	+	+	+	+

3.5. Impacts of Fusarium infections on grain biochemical and dough rheological properties

Anthocyanin concentrations were measured in wholemeal flour samples from all genotypes, including the cultivar 'Purendo 38', all locations and from inoculated and non-inoculated plots. Anthocyanins were detected in all genotypes. In the non-coloured varieties 'Arina', 'Combin' and 'Hanswin', anthocyanin concentrations were approx. 10 mg kg^{-1}. The contents were between 20 and 35 mg kg^{-1}in the genotypes with coloured grains, and were an average of 85 mg kg^{-1} for the blue genotype 'Purendo 38' (Figure 4). Anthocyanin concentrations in grains were affected by environmental conditions, explaining 9% of anthocyanin concentration variability (Table 5). The concentrations were significantly less in grains from Vouvry irrespective of the genotypes. The *Fusarium* inoculations did not change the anthocyanin concentrations in the grain samples (Table 5).

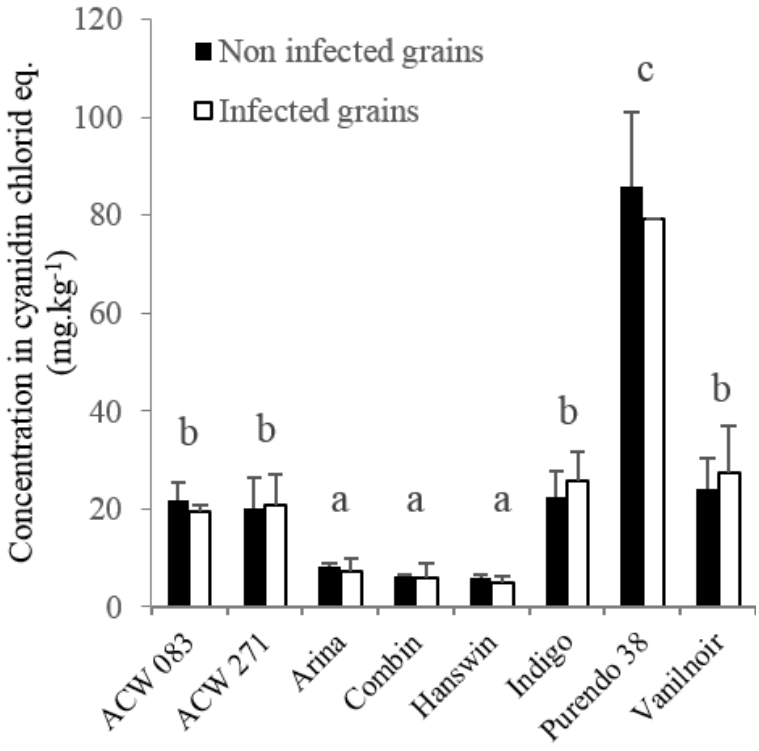

Figure 4. Mean anthocyanin content in grains from seven wheat genotypes overall three environments. Bars accompanied by the the same letters are not statistically different ($P < 0.05$).

The total protein contents ranged between 11% and 16%. Statistical analyses revealed significant differences between genotype, experimental site and inoculation. The variance of protein content depended mainly on the experimental site (21%). Protein contents were on average less in samples from Reckenholz than in those from Changins or Vouvry, while the

impact of the *Fusarium* inoculation accounted for only 7% of the total variance (Table 5). Significant interactions were detected between the genotypes and inoculation, accounting for 7% of the total variance, with the genotypes affected differentially.

Water absorption (%), dough stability duration (min) and dough softening (FU) were measured on flour from infected and non-infected grains. Water absorption showed significant differences between the genotypes (Table 5). The *Fusarium* inoculations (6% of total variance) and differences in environmental conditions (1%) had weak impacts on water absorption (Table 5).

Table 5. Composition of the variances (%) between the different factors (genotype, inoculation, site/environment and interactions) affecting the parameters: anthocyanin concentration and protein content, water absorption, dough stability and dough softening during kneading. Significance levels: *** at P < 0.001, **: at P < 0.01, * at P < 0.05, ns: Not significantly different (P > 0.05).

Factor	Anthocyanin concentration (%)	Total protein content (%)	Water absorption (%)	Dough stability duration (%)	Dough softening during kneading (%)
Genotype	77.1 ***	42.5 ***	53.6 ***	18.8 ***	8.2 ***
Inoculation	ns	6.5 ***	5.9 ***	48.4 ***	68.8 ***
Environment	9.4 *	20.8 ***	0.9 *	3.8 ***	2.8 ***
Interactions					
Genotype×Inoculation	ns	4.1 **	3.7 ***	11.1 ***	4.6 ***
Genotype×Environment	3.2 ***	6.6 **	6.1 ***	6.5 ***	4.1 ***
Inoculation×Environment	0.8 **	ns	13.1 ***	2.9 ***	5.0 ***
Genotype×Inoculation×Environment	2.9 ***	ns	ns	4.1 ***	3.9 ***

The *Fusarium* inoculations gave strong effects on dough stability duration and dough softening during kneading, irrespective of the genotypes and the sites. Analyses of variances revealed that inoculation explained 48% of the stability duration variances and 68.8% and softening variances (Table 5). The effects of the different genotypes on the two parameters (respectively, 18.8 and 8.2% of the total variance) were weaker than the effect of the inoculations. . The impacts of environmental conditions on stability duration and softening variances (respectively 4 and 2.8% of the total variances) were also weak. The significant interactions between genotype and inoculation indicated that rheological properties of grains were not affected to the same extent in all the genotypes. The relative differences in dough stability duration and dough softening caused by the disease were calculated to reflect the

differences in FHB impacts between genotype (Table 6). Overall, *Fusarium* inoculations decreased the duration of dough stability and increased dough softening, and the genotypes were affected differentially. For dough stability duration, 'Hanswin' and 'Indigo' were more affected compared to the other genotypes, with mean reductions across all environments of 59% in 'Hanswin' and 64% in 'Indigo' (Table 6). Differences in dough stability duration were less in ACW 083, ACW 271 and 'Vanilnoir'. The greatest impact of *Fusarium* inoculations on dough softening was measured for 'Hanswin' with more than a three-fold increase for samples from inoculated plots compared with uninoculated plots (Table 6). Impacts of inoculation on dough softening were less for ACW 083 and 'Vanilnoir with decreases, respectively of 79 and 75%. Environmental conditions also affected the impacts of inoculation, as overall differences in dough stability duration and in dough softening were greatest in samples from Reckenholz (Table 6).

Table 6. Relative differences (%) in dough stability duration and dough softening during kneading resulting from *Fusarium* inoculation. Differences in dough stability duration are the means of relative reductions due to inoculation. Differences in dough softening during kneading are the means of relative increases due to inoculation. Means followed by the same letter are not significantly different (P < 0.05)

Genotype	Differences in dough stability duration (%)					Differences in softening during kneading (%)				
	Changins	Vouvry	Reckenholz	Mean		Changins	Vouvry	Reckenholz	Mean	
ACW083	20.2	32.3	45.1	32.5	c	65.8	59.9	111.6	79.1	d
ACW271	29.1	19.5	38.5	29.0	c	34.6	110.3	173.4	97.7	cd
'Arina'	44.1	46.2	42.5	44.3	b	129.3	163.3	81.0	124.5	c
'Combin'	37.3	42.1	72.3	49.4	b	161.2	59.6	462.2	97.7	b
'Hanswin'	59.3	33.6	76.7	59.4	a	245.9	90.5	626.3	349.7	a
'Indigo'	54.7	58.8	74.6	63.8	a	137	216	313	224	b
'Vanilnoir'	35.2	5.0	33.8	24.6	c	105	27	95	75	d
Mean	40.0 (a)	33.9 (a)	54.8 (b)	43.3		135 (a)	79 (b)	260 (g)	150	

3.6. Links between the components of resistance and rheological properties

The relationships between the different FHB resistance components have been examined with Pearson's correlation analysis (Table 7). Disease severity and incidence on the spikes were only weakly correlated with the symptoms on grains. The different components of grain resistance (FDK ratio, TKW loss and DON accumulation) were linked, yet the correlations were moderately strong (0.57 to 0.61; $P < 0.001$) but not complete. Differences caused by infections in dough stability duration and softening indicate significant and positive correlations with FDK

ratio, DON content and TKW losses. This suggests that varieties that accumulate more DON and show reduced grain filling were also more impacted by changes in their rheological properties than less affected varieties. No correlations were detected between differences in rheological properties and the symptoms scored on the spikes in the field.

Table 2. Pearson correlation coefficients between observed indicators of resistance[3] and relative differences in grain rheological properties caused by Fusarium inoculations. *** significant at P = 0.001, ns = not significant (P > 0.05).

Indicators of resistance	FHB severity on spike	FHB incidence on spike	FDK (%)	DON content (mg.kg^{-1})	TKW losses (%)	Differences in dough stability duration (%)
FHB incidence on spike	0.92***					
FDK (%)	0.61***	0.51***				
DON content (mg.kg^{-1})	0.47***	0.34***	0.59***			
TKW losses (%)	0.72***	0.64***	0.61***	0.57***		
Differences in dough stability duration (%)	ns	ns	0.46***	0.55***	0.41***	
Differences in softening during kneading (%)	ns	ns	0.47***	0.54***	0.36***	0.88***

Abbreviations: FDK = *Fusarium* damaged kernel ratio; DON = deoxynivalenol; TKW = thousand kernel weight.

[3]Abbreviations : FDK = *Fusarium* damaged kernel ratio ; DON = deoxynivalenol ; TKW = thousand kernel weight

4. Discussion

In this study, seven wheat genotypes were assessed for spike and grain resistance to FHB caused by *F. graminearum*, in multi-locality field experiments with artificial inoculations. Theses differing environmental conditions have challenged the resistance reactions and allowed examination of resistance and genotype × environment interactions under different infection conditions.

The genotypes showed different levels of disease severity and incidence. While the overall intensity of the infections was different at each of the three sites, the ranking of the varieties was the same across all sites and no genotype × environment (G × E) interactions were detected. Intensity of infection was the least at Vouvry, intermediate at Reckenholz and greatest at Changins. This was likely to be due to the climatic conditions, as Vouvry was characterized by low temperatures and high relative humidity, and at Changins the infections were favoured by the permanent irrigation facility.

The mycotoxin DON was detected in all inoculated samples across all sites. Even at Vouvry, with very low disease severity and incidence, elevated concentrations of DON were detected in the grain samples. Therefore, DON accumulation in grains was not directly linked to type 1 or type 2 resistance of the host spikes, confirming findings in other environments and with other wheat genotypes (Liu et al. 1997; Mesterhàzy et al. 1999; Mesterhàzy 2002). The accumulation of DON was also affected by the wheat genotype and by statistically significant G × E interactions. These results emphasise the important role of grain resistance for wheat breeding, in order to enhance food safety. Besides the accumulation of DON, resistance to FHB effects is characterised by ability to withstand grain deformations, detected as FDK, and to maintain grain volumes, measured as TKW losses or test weight (hectolitre weight). Our observations indicate that the *Fusarium* infections cause deformations in varying percentages and reduce the TKW of all genotypes across all environments depending on the severity strength of infection and the degree of varietal resistance. As for symptoms on spikes, the ranking of resistance of the varieties, in terms of FDK and TKW, was the same in all environments, thus excluding G × E interactions.

These results indicate that grain resistance to FHB is made of several resistance components. The correlation coefficients between these factors were medium, ranging from 0.41 and 0.61, and indicating only moderate links. This was illustrated with the cultivar ‘Hanswin’, which accumulated only low concentrations of DON despite developing average amounts of FHB on the spikes and considerable numbers of *Fusarium* damaged kernels.

'Hanswin' mechanisms that impede DON accumulation, or that favour DON degradation (Miller *et al.*, 1985; Boutigny *et al.*, 2008; Boutigny *et al.*, 2010). Overall, these observations suggest that all kernel resistance types are interacting and may be interdependent, and that they can act individually, confirming other reports (Mesterházy *et al.*, 1999; Mesterházy 2002; Langevin *et al.*, 2004; Snijders 2004).

This study also focused on the impacts of FHB on grain protein content and the dough rheology. Since *Fusarium* infections impede translocation of assimilates to grain, we have used different genotypes with coloured grains to provide knowledge of grain filling, and the impacts of FHB on biochemical processes in the grain. Anthocyanins and proteins are synthesised in the grains (Knievel *et al.*, 2009; Feillet 2000; Bustos *et al.*, 2012; Žofajová *et al.*, 2012), and changes in anthocyanin biosynthesis in grains is part of abiotic stress responses (Gordeeva *et al.*, 2013). In the present study, for all genotypes and across all environments, the concentration of anthocyanins has not changed due to infection. This also held true for the cultivar 'Purendo 38', which contains anthocyanins in the grain endosperms and the pericarps. Although the concentration of anthocyanins in the grains depended on the environment (Chalker-Scott, 1999), we detected only very weak G × E interactions (3.2% difference) and no genotype × infection interactions. The total protein content of the grains was hardly affected by the infection, and was overshadowed by the dominant genotype and environment factors. Similar studies on the impacts of infection on grain protein content have showed either slight increases (Boyacioğlu and Hettiarachchy, 1995) or slight decreases (Häller Gärtner *et al.*, 2008). However, these previous studies were conducted in single environments. The present study demonstrated the interactions between genotype, environment and infection, and revealed negligible impact of the *Fusarium* infection on the grain protein content. Similarly, Wang *et al.* (2005) concluded that the total protein content was not impacted by *Fusarium* infection, but they showed that seriously infected grains had lower glutenin contents. However, high disease pressure does not influence sulphur speciation of glutenins that are responsible for the functionality of the gluten network and the resulting baking quality (Andrews and Skerritt 1996; Birzele *et al.*, 2003; Prange *et al.*, 2005). DON is mainly found in the grain pericarps and diffuses gradually into the core of the grains (Häller Gärtner *et al.*, 2005) Therefore, flour is generally less contaminated with DON than the bran (Cheli *et al.*, 2013), with the exception being for heavily infected grains (Bechtel *et al.*, 1985). It is therefore likely that the pathogen hardly penetrates through the outer grain layers. The reduced glutenin content described by Wang *et al.* (2005) may be due to the degradation activity of *Fusarium* proteases, once in

contact with the gluten and activated after addition of water during dough preparation (Nightingale *et al.*, 1999; Wang *et al.*, 2005). In the present study, the measures with the automatic kneading device did not exceed 10 min. per sample. Hence, it is unlikely that the reduced dough performance in flour from infected grains was due to the fungal enzymatic activity.

The assessment of dough stability duration and softening by kneading revealed degradation of dough properties by *Fusarium* infections in all the wheat genotypes assessed, with important differences between the genotypes. This is in accordance with previous observations of the rheological and baking quality performance in spring wheat varieties (Häller Gärtner *et al.,* 2008). Wang *et al.* (2005) associated alteration of bread-making quality with damage on starch granules and storage proteins caused by *Fusarium* pathogens. Overall, the greater the grain resistance to FHB effects, the lower was the impact on dough properties. The concentration of DON was the best indicator for the reductions in dough properties.

Our results have shown that *Fusarium* infections do not affect the biosynthesis of proteins and anthocyanins nor physiological processes in wheat grains. However, the spread of the pathogen in the spikes associated with the reduction of grain filling demonstrated that the infection modulates the transport of assimilates and consequently changes grain composition. These observations confirm that the evaluation of resistance of grain only with measurements of DON content, as often made in plant breeding programs, is not sufficient to fully characterise grain resistance.

Grain resistance is not a simple trait but is composed of several components, including resistance to DON accumulation, morphological changes and transport of photosynthesis products. This list must be completed with aspects of resilience of rheological properties. Future studies should focus on the impacts of infection on starch and other baking-quality determinants such as hemicelluloses and non-gluten proteins. Important aspects also include plant factors that modulate fungal pathogenesis factors, such as protease inhibitors (Pekkarinen *et al.*, 2000), DON accumulation (Boutigny *et al.*, 2010) and their roles in grain resistance. In this study, some coloured wheat genotypes displayed increased grain resistance to FHB effects. This may be due to the antioxidant activity of anthocyanins, known to enhance resistance in plants (Zhou *et al.*, 2007, Pani *et al.*, 2014). Additional studies are required to elucidate the potential roles of anthocyanins in resistance to FHB.

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Chapter IV

Resistance against *Fusarium graminearum* and the relationship to β-glucan in barley grains

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Abbreviation: β-glucan: 1,3:1,4-β-D-glucan

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Summary

Fusarium head blight (FHB) caused by *Fusarium graminearum* (FG) is a destructive disease impacting barley worldwide. The disease reduces the grain yield and contaminates grains with mycotoxins, such as the trichothecene deoxynivalenol (DON). Although the infection affects mainly the grain yield, only little is known about its impact on grain structural and biochemical properties. Yet, such information is instrumental to characterize the facets of resistance in the grains. After artificial inoculation of six barley cultivars with FG in a 2 years field test, different levels of symptoms on spikes, of colonisation of grains and of DON content were observed. The infections caused a reduction in grain weight and an average decrease by 10% of the β-glucan content in grains, indicating alterations of grain filling, composition and structure. According to our results, we postulate the presence of two distinct resistance mechanisms in the grain, tolerance to grain filling despite infection as well as the inhibition of mycotoxin accumulation. Differently to wheat, in barley, type IV resistance (tolerance of the grain to infection) is directly linked with type III resistance (resistance to kernel infection). The resistance against toxin accumulation (named type V resistance in wheat) appeared to be independent to all other resistance types. Generally, the resistance was significantly influenced by the environment and by genotype x environment interactions explaining the generally weak stability of resistance in barley. Interestingly, a significant and inverse relationship between DON contamination and β-glucan content in grains suggests that high β-glucan content in grains contributes to type V resistance.

1. Introduction

Fusarium head blight (FHB) of small grain cereals is caused by different species of the genus *Fusarium*. The disease is known in all cereal producing areas of the world. Besides yield losses, infections lead to accumulation of different mycotoxins in the grains, grain deformation, resulting in so called tombstones and reduced process quality (Häller Gärtner et al. 2008; Martin et al. 2017). FHB infections can cause significant economic losses along the entire value chain (McMullen et al. 1997). In temperate climates, *Fusarium graminearum* (FG) is the prevalent species causing FHB on barley (Schöneberg et al. 2016; Nielsen et al. 2014; Parry et al. 1995). Primary inoculum originates from crop residues, particularly maize debris, that favour production of asco- and conidiospores (Parry et al. 1995, Schöneberg et al. 2016, Xue et al. 2004). Transported by wind and rain, the spores reach the spikelets. The primary infection takes place at anthesis and under high humidity conditions and temperatures between 16°C-20°C (Xu et al. 2003; Brennan et al. 2005; Musa, et al. 2007). Once established in the ear (primary infection), the infection progresses throughout the spike (secondary infection), interfering with grain development and altering the maturation of grains (Bai and Shaner 2004).

During the infection, *F. graminearum* produces the mycotoxin deoxinivalenol (DON) that accumulates in grains. With other FG toxins, such as zearalenone (ZEA), DON can cause acute nausea, vomiting, diarrhea, abdominal pain, headaches, dizziness, and fever. In pigs, mycotoxins can cause feed refusal, and fertility disorders (Dersjant-li et al. 2003). In Europe, contaminations with DON and ZEA in cereal products are subject of strict regulations to guarantee food and feed safety (European Commission Regulation (EC) No 1881/2006).

The cultivation of resistant varieties is the most sustainable and cost effective way to control yield losses and contamintions with mycotoxins (Mascher et al. 2005). In wheat, FHB resistance involves a multitude of resistance mechanisms (Bai and Shaner 2004; Ravensdale et al. 2014). Schroeder and Christensen (1963) have first observed the resistance against primary infection (called type I resistance) and the resistance impeding the spreading of the infection throughout the spike (called type II resistance). Resistance types of the grain include resistance against kernel infection type III), tolerance to yield loss (type IV), resistance against the accumulation of trichothecenes mycotoxins in the grain (type V) and also the resistance against the alteration of grain constituents (type VI) (Mesterházy et al. 1999; Boutigny et al. 2008; Martin et al. 2017). In wheat, all these resistance types are interdependent, but independently inherited (Bai et al. 2000).

The wealth of knowledge on FHB in wheat can only be partially applied to barley (Berger et al. 2014). In wheat, the mycotoxin DON is considered to be an essential virulence factor allowing the pathogen to invade the rachis and to overcome type II resistance (Maier et al. 2006). In barley, the fungus was observed to grow externally from one spikelet to another, without penetrating the rachis (Jansen et al. 2005). Thus, DON appears to be redundant for the spread throughout the spike (Langevin et al. 2004). In barley, resistance evaluation should focus on type I resistance, since type II resistance appears to be strong. Concerning grains, differences in resistance to FHB and DON accumulation between spring barley genotypes have been reported (Buerstmayr et al 2004). Moreover, significant effects of the genotype on yield loss and yield component were described by Chełkowski et al. (2000). Yet, the knowledge about the impact of FHB in barley grain structure, composition and functioning are far lower those in wheat grains (Foroud and Eudes 2009). Without this information, the underlying mechanisms of type III, IV, V and VI resistance cannot be accurately characterize.

Barley grains contain β-glucan, a soluble fibre accumulated in the cell walls of the endosperm during maturation. β-glucan represents between 2 to 10% of the dry mass of the grain (Fincher 1975; Izydorczyk et al. 2000; Zhang et al. 2002; Wilson et al. 2012). High β-glucan grains are recommended for a healthy diet and benefit of health claims by EFSA (European Food Safety Authority, 2011) and FDA (FDA 1997, FDA 2005, Wood et al. 2007). β-glucan biosynthesis depends on the availability of polysaccharides, in particular sucrose, and on the proper functioning of the cells (Becker et al. 1995). Hence, tracing the content in β-glucan of the grain after infection helps to understand the impact of the infection on the grains' metabolism. β-glucan also possess also antioxidant activity (Kofuji et al. 2012). Antioxidant compounds are recognized factor of chemical plant defence system to cope with biotic aggressors (Lattanzio et al. 2006; Zhou et al. 2007). The role of β-glucan against *Fusarium* pathogens remains to be investigate.

The aim of the current study was to investigate resistance elements of the barley grain against *F. graminearum* and the accumulation of deoxynivalenol toxin. For this, we precisely characterized the outcome of *Fusarium* infections on developing grains of barley. The experimental approach was based on the study of six winter barley varieties with and without artificial infection at four experimental field sites with distinct climatic conditions. In the field,

disease incidence and severity were scored and after harvest, grains were examined for DON content, fungal infection and thousand kernel weight (TKW). The β-glucan content was determined to study the impact of FG infection on grain composition, structure and functioning. By this, the contribution of β-glucan in resistance of barley grains was also investigated.

2. Materials and methods

2.1. Plant material

Six winter barley genotypes, all registered varieties from different European breeders, have been studied in this experiment (Table 1). The set includes the 2 row variety "Cassia", the hybrid variety "Hobbit". The variety "Waxyma", was specially bred for an elevated β-glucan content and recommended for human consumption. The other varieties are popular in Switzerland and figure on the national recommended list (Courvoisier et al. 2017). Besides "Waxyma", all varieties are generally used as animal feed.

Table 1. Description of winter barley genotypes used in the experiments.

Genotypes	Row type	Breeder	Year of registration	Country
Cassia	2	KWS	2010	United Kingdom
Fridericus	6	KWS	2006	Germany
Hobbit	6	Syngenta	2009	United Kingdom
Landi	6	Saatzucht Schmidt	2002	Germany
Semper	6	KWS	2009	Germany
Waxyma	6	Dieckmann Seeds	2008	Germany

2.2. Fungal isolates and production of inoculum

Infections were carried out with a mix of three *Fusarium graminearum* single conidia isolates from symptomatic barley spikes: FG 13170 (isolated in 2013, canton Fribourg), FG 13192 (2013, Basel-Land) and FG 13269 (2013, Graubünden). Strains were stored in a 1:1 water - glycerol mix at -80°C. For mass production, strains were retrieved from deep freezing and cultured on Potato Dextrose Agar (PDA, BD Difco, Le Pont de Claix, France) for 1 week at 18°C with 12/12h UV light and dark. Subsequently, two mycelial discs (5mm diam.) from a

well-grown colony were transferred to 100 mL of liquid V8-medium in a 250ml Erlenmeyer flask. The V8-medium consisted of a 1:5 mix of V8 juice (Campbell Soup Company, Camden, USA) and distilled water, amended with 2g sodium carbonate per litre as a pH corrector (pH_{media}=8.5). Cultures were incubated for 7 days (d) at 24°C on a shaker at 200 rpm in the dark, subsequently filtered through sterile cheesecloth and centrifuged at 4'500 rpm for 10 min. The generated pellet was re-suspended in sterile distilled water and either directly used or stored at -20 °C.

2.3. Field experiments and artificial inoculations

Field experiments were conducted at 4 different locations across Switzerland: Changins (46°24'36''/6°14'06''), Vouvry (46°20'16''/6°53'28''), Reckenholz (47°16'30''/8°26'45''), and Cadenazzo (46°09'00''/8°57'00''). Field tests were carried out in 2014 in Changins and Vouvry and in 2014 and 2015 in Reckenholz and Cadenazzo. The sites and the particular climatic conditions are described in the table 2.

Table 2. Average temperature, relative humidity, evapotranspiration and sum of precipitation at the 6 field sites between 15.05. (flowering stage) and 15.07. (grain maturity) in 2014 and between 15.05.and 15.07. in 2015. Source: meteoswiss.ch/idaweb

	Temperature (°C)	Precipitation (mm)	Relative humidity (%)	Evapotranspiration (mm)
Changins (VD) 2014	17.1	201	68	3.1
Vouvry (VS) 2014	16.9	221	73	2.2
Reckenholz (ZH) 2014	16.8	208	70	2.9
Reckenholz (ZH) 2015	18.1	161	69	3.2

Cantons: VD = Vaud, VS = Valais, ZH = Zurich

The varieties were planted during the month of October in 1 m^2 plots with 5 rows and 15 cm interline using a Seedmatic seeding machine (Hege Maschinen, Eging am See, Germany). According to the local habits, seed density was 350 seeds m^{-2} for all varieties. All trials at all sites passed winter without significant losses. At flowering, rows were well established, dense and without gaps.

Artificial inoculations took place when 50 % of the plants of a plot were at mid anthesis (BBCH 65). Inoculum suspension was prepared from fresh cultures adjusted to $2x10^5$ conidia.ml^{-1}. Immediately before inoculation, equal proportions of liquid cultures from each of

the three FG strains were mixed and 0.0125% of Tween® were added. According to the climatic conditions, the inoculated and control plots were irrigated to maintain humidity on the ears for at least 24 h to promote primary infection. A high pressure/low volume overhead spray irrigation system was used at Changins. In Vouvry, Cadenazzo and Reckenholz, water (600 L ha^{-1}) was sprayed manually with a back-pack sprayer. The additional water supply did not increase the water balance significantly at all field sites (table 2). To ensure that the plants received an adequate quantity of conidia, a second inoculation took place 2 d later.

2.4. Disease assessments on spikes

In inoculated and control plots, disease severity and disease incidence on spikeswere determined. For this, 30 spikes were randomly chosen and labelled, in each plot. Disease severity was recorded by counting the number of infected spikelets on each of the 30 spike and expressed as percentage of infected spikelets. Disease incidence was scored by counting the number infected spikes among the 30 spikes. The first assessments were carried out 7 to 10 d after the last inoculation. Subsequent scorings were done at 3 d intervals. At least three assessments were carried out in each plot.

2.5. Harvest and preparation of grain and flour samples

In Changins, Vouvry and Reckenholz field tests plots were harvested at full maturity (BBCH 89). The trials at Cadenazzo could not be harvested, in both years. In Reckenholz, a plot combine harvester (HEGE 140, Mähdreschwerke GmbH, Germany) was used. The airflow on the harvester was reduced to recover a maximum of kernels. In the other locations, all spikes from each plot were harvested by hand and collected in linen bags. The spikes were threshed with a laboratory thresher (Saatmeister, Kurt Pelz, Germany) and grains were cleaned using a vertical airflow (Baumann Saatzuchtbedarf, Germany) to remove dust and other debris. Grains were dried to 14% moisture and stored at 4°C.

The following assessments on the grains were done on 200g sub-samples extracted after mixing the entire samples 3 times with a riffle divider (Schieritz & Hauenstein AG, Arlesheim, Switzerland).

2.6. Analyses of grains

The proportion of grains colonised by the inoculated FG strains and other *Fusarium* spp pathogens was determined for all samples from artificially inoculated plots using to the seed health test procedure described by Vogelgsang et al. (2008). For this, one hundred grains from each sample were surface sterilized and placed on PDA. After one week, the *Fusarium* species were identified according to the laboratory manual by Leslie and Summerell (2006).

The presence of naturally occurring Fusarium species was tested on three samples from non-inoculated plots from each environment.

The thousand kernel weight (TKW) (g) was measured with a MARVIN optical grain counter (Digital Seed Analyser, GTA Sensorik GmbH, Neubrandenburg, Germany) and a balance (Mettler PM2000, Mettler-Toledo, Greifensee, Switzerland). TKW was determined for all grain samples.

The DON content in whole meal flour from inoculated plots was determined with the DON ELISA kit (Ridascreen® FAST DON, R-Biopharm AG, Darmstadt, Germany), according to the manufactures' instructions. Samples with high contaminations were diluted 10 times in double distilled water. Whole meal flour was obtained by milling 10g of sample with a sample mill (1093 Cyclotec Sample Mill, FOSS, Sweden), using a 1mm screen. Samples were stored at -20°C until further use. The DON content was measured in samples from inoculated plots and one non-inoculated sample from each environment served as a control.

The β -glucan content was also determined in whole meal flour (see above). For quantification, the Mixed-linkage β-glucan kit (Megazyme International Ireland Ltd., Wicklow, Ireland) was used. The streamlined method of mixed-linkage β-glucan in barley flour — (ICC Standard Method No.166) was adapted to the laboratory facilities. As a negative control, the β-glucan content was measured in 0.1g of dry FG mycelium from the strain FG13170. β -glucan content was measured in both infected and non-infected grain samples.

2.7. Experimental set-up and statistical analysis

Field tests conducted in a comparable way at all locations and included inoculated and non-inoculated treatments with 3 replicates arranged in a split–plot design. The treatments were the main plot whereas the different barley varieties were the sub-plots.

Data of disease incidence and severity were expressed as AUDPCrel (relative area under the disease pressure curve) by integrating the single scores with the number of days between infection and the scoring. After this, the integrated score was divided by the total number of the observation days. This standardized average daily disease progress value enables the comparison of disease incidence and severity between the trial sites. The infection with *Fusarium* caused a reduction of the thousand kernel weight TKW. This difference in TKW was calculated for each single infected replicate as the difference with the average TKW of the three non-infected samples. All calculations were conducted on Microsoft® Excel 2013.

Statistical analyses were carried out using the statistical software R (R Core Team, 2015). Results of AUDPCrel of disease severity in the six environments were illustrated with a biplot (R package "GGEBiplotGUI", version 1.0-9, Frutos Bernal et al. 2016). The data of DON content were squared-root transformed to obtain normal distribution. The proportion of grains infected by FG (%), the DON content ($mg.kg^{-1}$) and the reduction of TKW (%) were analysed with two-factors analyses of variances (ANOVA), with the trial site as main factor and the varieties as sub-factor. Pearson correlations were carried out to test the relation between disease incidence, disease severity, the proportion of infected grains, the reduction of TKW and the DON content. To better understand kernel resistance and the role of β-glucan therein, a principal component analysis (R packages "FactoMineR", version 1.29, (Le et al. 2016)) was performed with the proportion of grains infected by FG, DON content and reduction of TKW as "factors" and one variety in one environment as "individuals". A hierarchical cluster analysis was carried out after the PCA to class individuals into distinct clusters (R packages "factoextra", version 1.0.4, Kassambara and Mundt, 2017). Variation of β-glucan content (%) in the dataset was analysed with a three-factors ANOVA, using, according to the experimental design, the "environment" as a main factor, the "treatment" indicating the presence of inoculation as sub-factor and the different varieties as smallest source of variation. Then β-glucan content (%) in grains from inoculated plots were compared between the three groups defined by the clustering

on the PCA. All along the study, multiple comparisons were used based on Tukeys HSD (package “agricolae”, de Mendiburu, 2015).

3. Results

3.1. Symptoms on spikes

Symptoms on spikes were scored in all six field tests. The results of disease severity are shown in the figure 1. Symptoms were significantly more severe in Changins and Reckenholz in 2014 than in all other environments ($P<0.05$). Only very weak symptoms were observed in Reckenholz 2015, and in Cadenazzo 2014. Symptoms in Cadenazzo 2015 and Vouvry 2014 were moderate. Pooled over all environments, the significantly highest ($P<0.05$) disease severities on spikes were measured on “Hobbit” and the lowest on “Cassia” followed by “Waxyma” and “Semper” (Figure 1). Results indicate that the varieties performed differently in the different environments. While "Hobbit" showed the highest disease severity in Changins 2014 and Cadenazzo 2015, it was not the most affected genotype in Reckenholz in 2014. The varieties "Fridericus" and "Waxyma" showed more symptoms in Vouvry and Reckenholz in 2014 in comparison with other environments. Disease severity observed on “Semper” was very similar across the different environments. Disease incidence and disease severity were strongly correlated (Pearson coefficient 0.80, $P<0.001$), therefore only disease severity is displayed here. All data of disease severity and incidence are presented in supplementary materials. In all field tests, none or very low symptoms were observed on non-inoculated plots.

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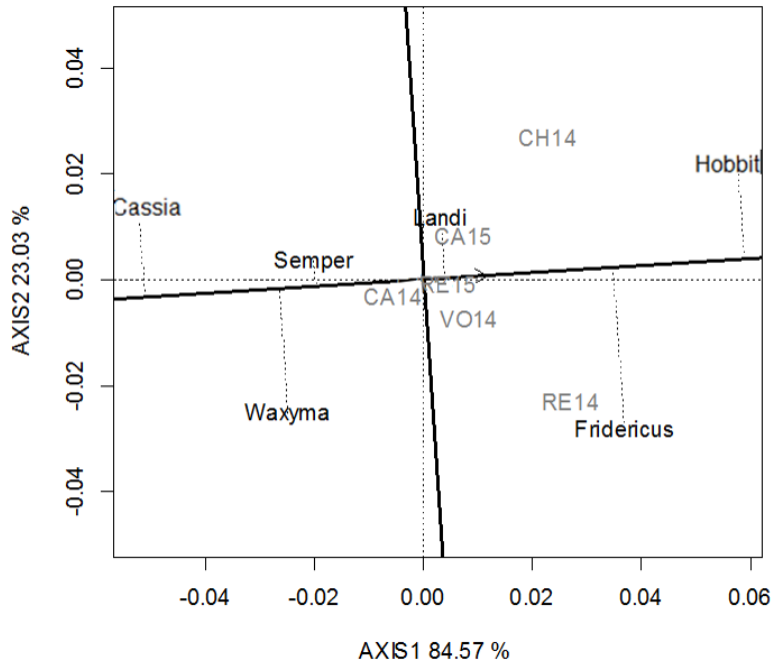

Figure 1. Genotype main effect plus Genotype x Environment (GGE) biplot showing the average disease severity and its stability of the six barley genotypes over all six experimental sites. The plot has been obtained with R backage GGEBiplotGUI with parameters "scalling=0" and "centering =2" to illustrate both the 6 barley genotypes and the genotype x environment interactions. The parameter "singular value portioning" was set to 1 (SVP=1) scaling by the the visualising the average points (variety / site) and the stability of the genotype. Position of the varieties along the horizontal axis indicates the average disease severity over all environments, with "Cassia" displaying weakest symptoms and "Hobbit" with strongest symptoms. The distance between variety and horizontal axis indicates the stability of disease severity. Short distances indicate high stability. CH14= Changins in 3014, in 2014, RE14= Reckenholz in 2014, VO14= Vouvry in 2014, CA15=Cadenazzo in 2015, RE15= Reckenholz in 2015.

3.2. Resistance of grains

Analyses of grains and of the β-glucan content were carried out in samples from Changins 2014, Reckenholz 2014, Vouvry 2014 and Reckenholz 2015. These four environments represent the largest array of disease severity ranging from Changins 2014 and Reckenholz 2014 with severe infections, Reckenholz 2015 with low infections and Vouvry 2014 with an intermediate infection pressure.

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3.2.1. Proportion of grains colonised by *Fusarium* species

FG was found in all grain samples from inoculated plots. Less than 3% of the grains from inoculated plots presented *Microdochium nivale* and less than 1% were colonised by *F.poae* or *F.culmorum*. In non-inoculated plots, less than 5% of grains were colonized by *M.nivale* and *F.graminearum*, and sporadically by *F.poae* and *F.culmorum*.

The proportion of grains colonized by FG with artificial inoculation on the 6 barley varieties in the 4 locations is displayed in figure 2A. The highest colonization was found in Reckenholz in 2015 (75% of grains colonised) followed by Changins 2014, Reckenholz 2014 and Vouvry 2014 (22%). Pooled over all environments, grains from "Fridericus" were significantly more colonised ($P< 0.05$) than grains of "Cassia" (60% and 38%, respectively) (Figure 2). Within the same environment, the proportion of colonised grains between varieties was rather similar, except for Changins in 2014 where "Waxyma" was only weakly colonised while the 5 other varieties, were strongly colonised (Figure 2). The impact of the environment on FG colonisation prevailed over the impact of the genotype (Table 3).

3.2.2. Thousand kernel weight reductions

A reduction of grain filling due to FG infection, measured by TKW comparisons was observed for all barley varieties in all environments, and ranged between 2% and 20% (Figure 2c). The lowest reduction was measured in grains from "Cassia" with an average of 4% over all environments, whereas for the five other varieties, TKW reductions ranged between 8% and 11%. The reduction of the TKW was also significantly influenced by the GxE interactions ($P<0.01$) (Table 3). In particular, in Changins 2014, FG infection caused only weak reduction of TKW in grains from "Cassia" and "Fridericus" (respectively 2.5% and 6.9% of reduction), but elevated reductions of up to 14.5% in grains of "Hobbit". In contrast, in Reckenholz 2015, no differences between varieties were observed (Figure 2c).

3.2.3. Accumulation of deoxynivalenol

The mycotoxin DON was detected in all samples from inoculated plots. Contents varied from 5.2 to 49.4 $mg.kg^{-1}$. The contamination was significantly lower in Reckenholz 2015 (on average 9.4 $mg.kg^{-1}$) than in all other environments (on average 18.1 $mg.kg^{-1}$) (Figure 2C). Over the four environments, the highest average content was detected in grains from "Hobbit" (24.3 $mg.kg^{-1}$), while grains from "Waxyma" were significantly ($P<0.05$) less contaminated (on average 7.9 $mg.kg^{-1}$) than all other varieties (figure 2b). The DON content in grains was

mainly influenced by the environment and to a lesser extent by the variety (Table 3). Nevertheless, not all varieties performed similarly in all environments, as indicated by significant GxE interactions (P<0.05) (table 3). Indeed, higher DON contaminations in "Cassia" and in "Fridericus" were observed in grains from Reckenholz 2014 than from Changins 2014. The opposite was observed for "Semper" with highest contamination measured in Changins 2014. Overall, the genotype and the GxE interactions determined in the same measure the DON content (Table 3).

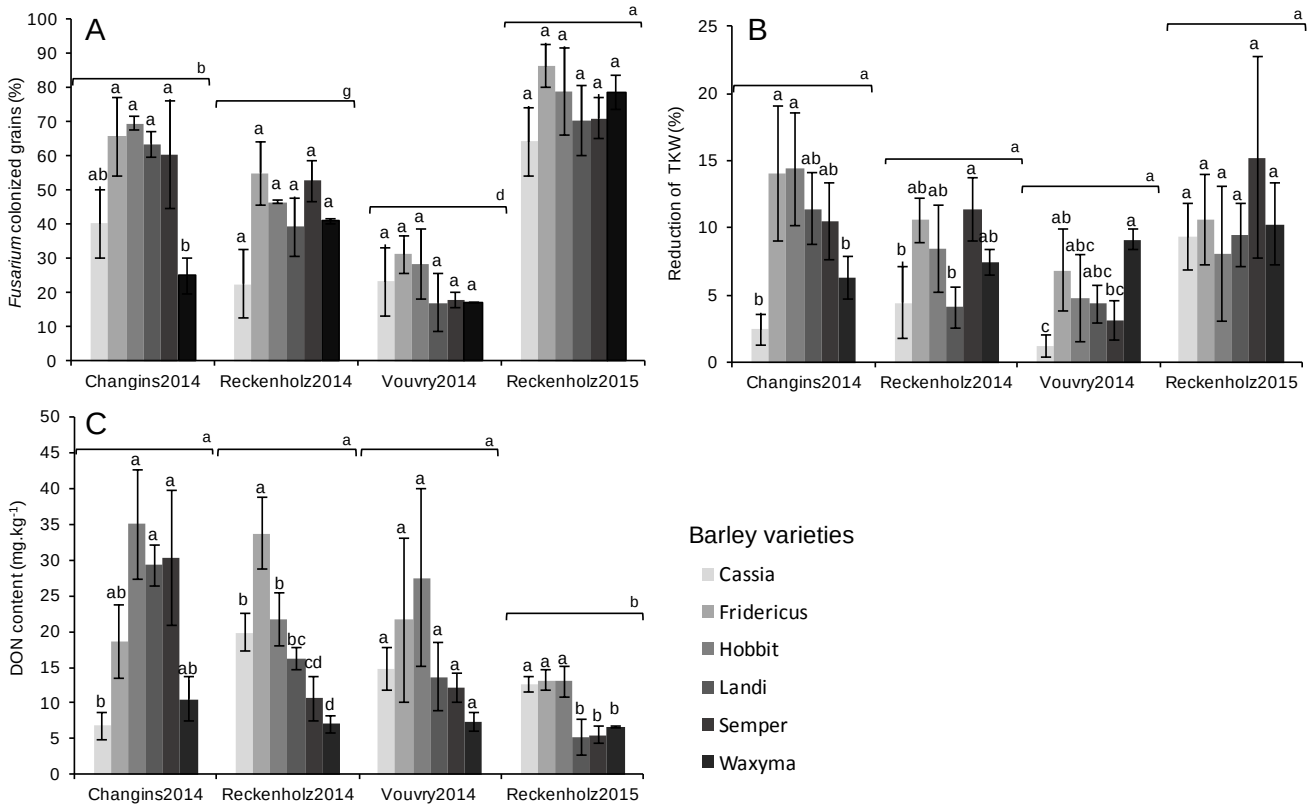

Figure 2. Proportion of *Fusarium* colonised grains (A), reduction of thousand kernel weight (TKW) (B) and content of deoxynivalenol (DON) (C) and for the 6 barley varieties in 6 environments. Error bars represent the standard error of the means. Different symbols indicate significant differences in averages between the four environments (Changins2014, Revkenholz2014, Vouvry2014, Reckenholz2015) and different letters indicate significant differences between varieties in the same environment according to a Tukey-Test with α= 0.05.

Table 2. Compositions of the variances of proportion of colonised grains, deoxynivalenol (DON) content and reduction of thousand kernel weight (TKW) in function of the factors environment, genotype, and genotype x environment interaction . Significance level: ***: $P<0.001$, **: $P<0.01$, *: $P<0.05$.

	Proportion of *Fusarium* colonized grains (%)			DON content (mg kg^{-1})			Reductions of TKW (%)		
Source of variation	Sum of Square	Mean Square		Sum of Square	Mean Square		Sum of Square	Mean Square	
Environment	26920	122.9	***	21.4	7.2	*	335.9	112.0	
Error (A)	916	153.0		4.8	0.8		307.5	51.2	
Genotype	4100	819.9	**	31.1	6.2	***	287.1	57.4	***
Genotype x Environment	4284	285.6	*	30.4	2.0	***	399.8	26.7	**
Error (B)	4939	123.5		19.1	0.5		337.3	8.43	

3.3. Interactions between resistance traits

Disease incidence and disease severity were highly correlated ($P<0.001$) (Table 4). A significant ($P<0.001$) correlation was also found between the proportion of grains colonised by FG and the reduction of TKW due to artificial inoculation. However, DON content in grains was neither related with colonisation nor with the reduction of TKW. The severity and the incidence of symptoms on the spikes were neither correlated with TKW reduction nor with the proportion of FG colonised grains but with the DON content.

Table 4. Pearson correlation coefficients between observed of spikes and grains at 4 field tests. Significant level: ***: $P<0.001$, ns: not significant.

	Disease incidence[a]	Disease severity[b])	Proportion of *Fusarium graminearum* colonised grains	DON[c] content in grains
Disease severity (% spikelets with symptoms)	0.85***			
Propotion of *Fusarium graminearum* colonised grains	ns	ns		
DON content in grains	0.48***	0.42***	ns	
Reduction of TKW[d]	ns	ns	0.62***	ns

[a] Number of spikes with symptoms; [b] Percent spikelets with symptoms; [c] DON = deoxynivalenol; [d]TKW = thousand kernel weight

The interactions between grain resistance factors were studied with a Principal Component Analysis (PCA) (Figure 3). Here again, the PCA showed the positive link between the colonisation of grains and the reduction of TKW, whereas the DON content in the grains was clearly not linked with colonisation and TKW reductions. The hierarchical cluster analysis identified three distinct clusters: cluster A includes low DON content, low TKW reductions and low proportion of grains colonised by FG. Cluster B is composed of individuals affected by the reduction of TKW and with a high proportion of FG colonised grains but low DON contaminations. Cluster C gathers individuals highly contaminated with DON, along with a wide range of TKW reduction and proportion of FG colonised grains. Overall, cluster A consisted mainly of "Waxyma" and "Cassia", regardless of the environment. Cluster B gathered most of the individuals from Reckenholz 2015, and cluster C contained all other remaining variety x environment combinations (Figure 3).

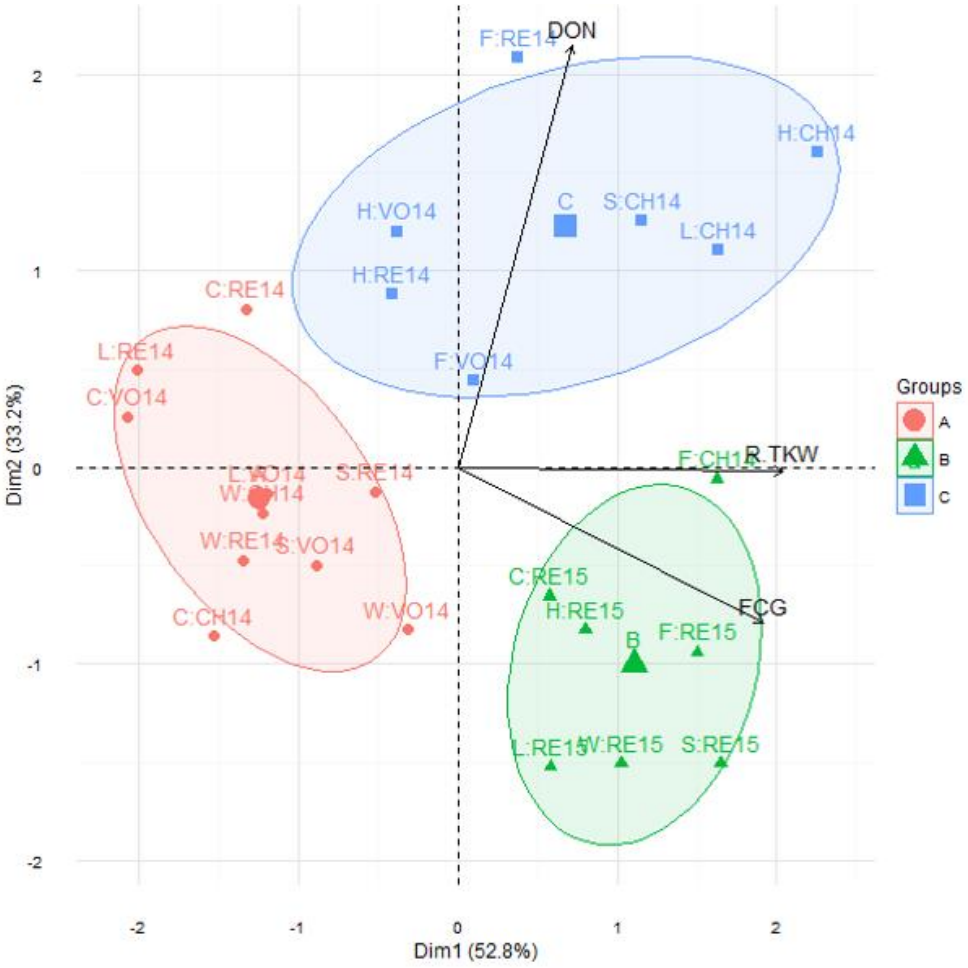

Figure 3. Biplot representation of a Principal Component Analysis (PCA) of grain resistance against accumulation of DON (deoxynivalenol), grain colonisation (FCG) and reduction of thousand kernel weight (TKW) for 6 varieties in 4 environments. Each pair "Genotype:Environment" represents one individual point in the PCA. Individuals have been attributed in three groups (A-C) using a hierarchical cluster analysis. C: Cassia, F: Fridericus, H: Hobbit, L: Landi, S: Semper, W: Waxyma; CH14: Changins 2014, RE14: Reckenholz 2014, RE15: Reckenholz 2015, VO14: Vouvry 2014.

3.4. Impact of the *Fusarium* infection on the β-glucan content in grains

Grains in our data set contained between 3.1% to 6.8% of β-glucan in dry weight. The highest contents were measured in "Waxyma" grains (on average 6.5%), and the lowest contents in "Landi" grains (4.0%). Generally, higher β-glucan contents were measured in grains from Reckenholz in 2014 and 2015 (4.9% and 4.8%, respectively) compared with grains from Vouvry 2014 (4.5%). Contents in grains from "Cassia" varied between the different environments from 4.4% in Changins 2014 to 5.1% in Reckenholz 2014; all other varieties showed stable contents across environments (data not shown). It resulted in weak but significant GxE interactions (table 5).

In grains from inoculated plants, the β-glucan content was generally lower by 10% compared to non-inoculated grains (P<0.01) (Figure 4, Table 5). This decrease was the same in all varieties and in all environments. The main source of variation for β-glucan content were the genotype and the inoculation. The influence of the environment, interactions between the environment and the genotype or the environment and inoculation were not significant (Table 5).

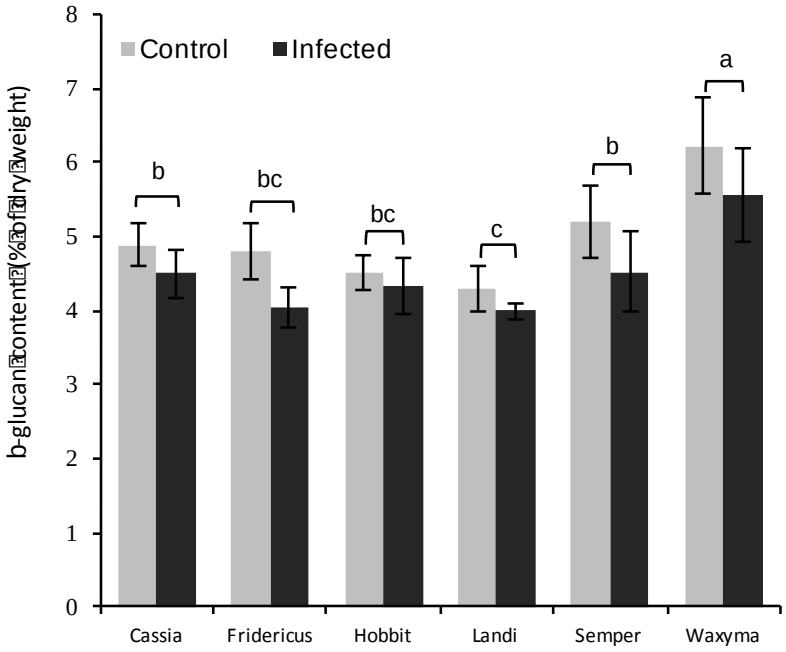

Figure 4. Comparison of β-glucan content in barley grains from inoculated and control plots, pooled over 4 environments. Black and grey bars represent average of β-glucan content in dry weight of grains. Error bars represent the stand error of the means. Different letters indicate significant differences between varieties over all environments in inoculated and control samples (P<0.05).

Table 5. Analysis of variance of β-glucan content in grains of 6 barley varieties, from 4 different field sites, inoculated or not with *Fusarium graminearum.* Significance: ***: *P*<0.001, **: *P*<0.01, *: *P*<0.05.

	β-glucan content (%)		
Source of variation	Sum of Square	Mean Square	
Environment	3.2	1.1	*
Error (a)	0.9	0.2	
Inoculation	7.6	7.6	**
Environment x Inoculation	2.3	0.8	
Error (b)	3.0	0.4	
Genotype	48.8	9.8	***
Genotype x Environment	6.2	0.4	*
Genotype x Inoculation	1.0	0.2	
Genotype x Environment x Inoculation	4.0	0.3	
Error (c)	16.3	0.2	

3.5. Link between β-glucan content and grain resistance traits

The comparison of the β-glucan content in infected grains between the three groups of grain resistance previously defined by hierarchical clustering revealed significant differences (*P*<0.05). While grains in cluster A (resistance to FG infection and DON contamination) contained on average 4.8% β-glucan, grains from cluster C (grains accumulating DON) contained significantly less β-glucan (average of 4.2%) (Figure 5). The grains of cluster B (elevated proportion of colonized grains and reduced TKW) had an intermediate content (4.5%). A correlation analysis (Pearson correlation coefficient: -0.29, *P*<0.01) confirmed the significant inverse relationship between DON accumulation and β-glucan content. The concentration of β-glucan was not linked with any other grain resistance trait (data not shown).

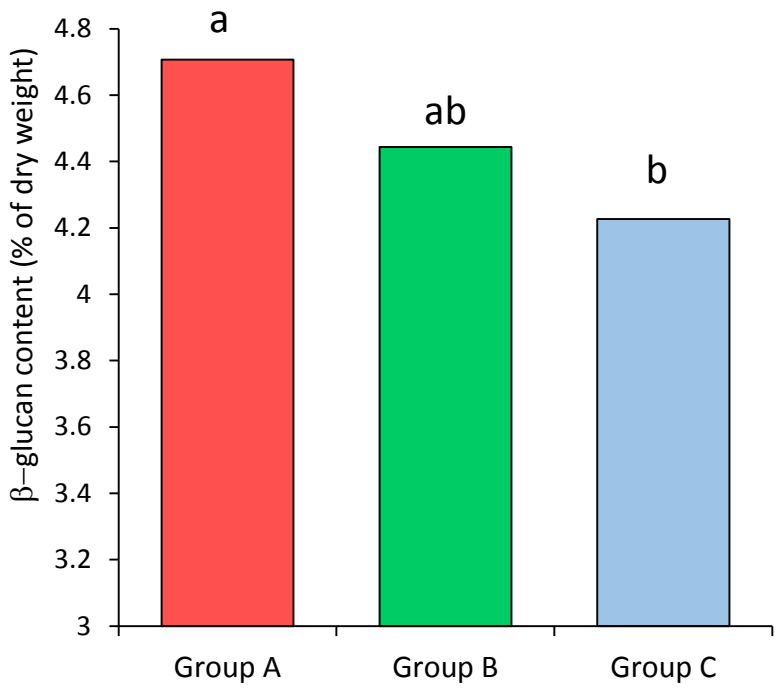

Figure 5. The average β-glucan content in barley grains within the three resistance groups. Group A = grains with general high resistance level, Group B = grains susceptible to Fusarium colonization and reduction of grain filling, Group C= grains generally susceptible, with high a DON accumulation. Different letters indicate significant differences according to a Tukey test with α=0.05.

4. Discussion

In the current study, the resistance of 6 barley varieties against infection with FG was investigated under different field conditions. Besides the symptoms on the spikes, a particular focus was the symptoms of the grains, the accumulation of the mycotoxin deoxynivalol and variations of the β-glucan content. The severity of symptoms on the spike differed between the varieties and between the environments. Generally, varieties with a higher spike resistance showed reduced accumulation of the mycotoxin DON. Positive correlations between spike resistance and toxin accumulation have also been reported by other authors (e.g. Tekauz et al. 2000; Choo et al. 2004, Berger et al. 2014; He et al. 2015). Yet, the present results show that DON content is not linked with the colonization of the grain by FG. High DON contamination but a weak grain colonisation was found in all samples from Vouvry 2014, where conditions after anthesis were quite humid. In contrast, in Reckenholz in 2015, where elevated temperatures and low precipitations were registered, we found a high proportion of colonised grains but only low symptoms on the spikes and low accumulation of DON in the grains. In Vouvry in 2014, characterized by elevated rainfalls and high humidity between flowering and grain maturity, elevated DON content was found despite a moderate symptom level on the spike. This supports the important role of environmental conditions on symptoms development and DON accumulation in barley and confirms findings of other authors (Tekauz et al. 2000; Bai and Shaner 2004; Berhoft et al. 2012). In practical terms, similar to wheat and triticale, the absence of symptoms on the spike is not a reliable indicator of low mycotoxin accumulation in the grain (Arseniuk et al. 1999; Mesterházy. 2002). Arguably, the different genetic basis for spike resistance and for DON accumulation in the grain leads to the differential reaction between spike and grain (Massman et al. 2011). In the following we aim to accurately phenotype the infection induced symptoms on the grains of the 6 barley varieties.

Therefore, we expected to observe typical symptoms of *F.graminearum* on the grains such as the presence of scabby grains, pinkish discolouration, or grain deformation as described by Mc Mullen et al. (1997) and He et al. (2015). None of these symptoms were found in our samples, besides the presence of black perithecia, attributed to *Fusarium*. Yet, the colonization of the FG inoculum on the grain proves the presence of the pathogen on the grain. It is conceivable, that the elevated disease pressure and conducive meteorological conditions allowed the development of the typical symptoms on the grains in these studies. Indeed, Berger et al. (2014) and He et al. (2015) rated pinkish discolouration in grains from intensively irrigated fields and inoculated with a highly aggressive FG isolate.

The FG infection caused significant reduction of the TKW indicating a disruption in grain filling that impacts grain morphology and shape. The quantification of β-glucan in the grains revealed a general decrease by 10% in infected grains compared to non-infected grains, regardless the variety and the environment. The lower of β-glucan content in infected grains might be attributed on the one hand to the activity of fungal β-glucanases and other cell-wall degrading enzymes of *Fusarium graminearum* (Schwarz et al. 2002, Wang et al. 2005; Oliviera et al. 2012a, Oliviera et al. 2012.b). On the other hand, to a lack of sugars and other nutrients can lead to a reduced synthesis of β-glucan in the developing grain (Fincher. 1975; Becker et al. 1994; Wilson et al. 2012). In conclusion, our analyses demonstrated FG colonises barley grains, synthesizing mycotoxins and causing the reduction of the grain size and shape as well as reducing the content in β-glucan.

The present trials show significant differences of the symptoms of spike and grain between barley genotypes and environmental conditions. Strong interactions between the genotypes and the environments indicate a weak stability of barley resistances traits of spike and grains previously mentioned by genetic studies (Capettini et al. 2003; De la Pena et al. 1999). In our field analyses, we observed that the interactions of the genotype with the environment determine to the same extent the phenotypic variation of symptoms on the spike, the DON content and the reduction of grain filling. Heritability of resistance against FG infection is therefore lower that in wheat, confirming finding of other authors (Bai et al. 2000; Urrea et al. 2002; Capettini et al. 2003). Consequently, in practical breeding, the expected genetic of FHB resistance is only rather weak.

Nevertheless, among the tested varieties, we found differences in resistance against all facets of FHB. The two-row variety “Cassia” showed the highest resistance with respect to spike symptoms, FG grain colonisation and TKW reduction. “Waxyma” was the most resistant variety with respect to DON accumulation and second to “Cassia” in all other categories. In contrast to six-row varieties, two-row spikes may impede the external propagation of the pathogen from one spikelet to the other (Choo et al. 2004; Langevin et al. 2004). Even though in “Cassia” and “Waxyma” both spike and grain resistances are present, results of the less resistant genotypes show that the two resistance types are acting independently. The degree of colonisation of the grain was linked to the reduction of grain filling but not linked with

accumulation of DON. It is known for barley, that the colonisation of the grain is independent from the accumulation of trichothecenes mycotoxins (Langevin et al. 2004, Maier et al. 2006). Yet, the degree of abundance of the FG pathogen may impair the grain filling processes. No resistance was found in any of the 6 barley varieties to prevent β-glucan degradations caused by the infection. Overall, our resistance analyses suggest the existence of two distinct resistance mechanisms in the grain, namely (i) the continuity of grain filling and reduced colonization and (ii) the inhibition of mycotoxin accumulation. Type IV resistance (tolerance of grains to FHB) seems to be directly linked to type III resistance (resistance to kernel infection). Meanwhile, type V resistance (resistance against trichothecenes accumulation) appears to be independent from all other resistance types.

Interestingly, varieties with the highest β-glucan content (Fig. 3, cluster A) displayed the lowest DON content. The negative correlation between DON contamination and the β-glucan content suggests that β-glucan might intervene in resistance against DON accumulation thus contributing to type V resistance. It is likely that the antioxidant activity of β-glucan (Kofiji et al. 2012) contributes *in planta* to resistance against different *Fusarium* spp. Indeed, several studies demonstrated the inhibitive potential of natural antioxidant compounds on the production of mycotoxins (Boutigny et al. 2010; Pani et al. 2014; Zhou et al. 2007). Moreover, it has been demonstrated that β-glucan is able to bind several *Fusarium* toxins *in vitro* conditions (Yiannikouris et al. 2004, 2006). Previous researchs demonstrated its interest as a potential detoxifiant of food products contaminated with *Fusarium* toxins (Meca et al. 2012; El-Naggar & Thabbit 2014). Hence, cultivating barley varieties with high β-glucan contents can be recommended to serve two purposes at once: to reduce the risk of mycotoxin contaminated grains and to further promote the production of health promoting food (Ames and Rhymer 2008).

Acknowledgments

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Supplementary materials

Supplementary table S1: Averages ± standard deviations of incidence and severity of FHB disease on barley spikes for six varieties in six environments

	Incidence of disease on spike			Severity of disease on spike		
Changins 2014						
Cassia	0.295	±	0.064	0.024	±	0.008
Fridericus	0.309	±	0.057	0.046	±	0.016
Hobbit	0.593	±	0.079	0.097	±	0.016
Landi	0.352	±	0.067	0.047	±	0.011
Semper	0.302	±	0.086	0.031	±	0.016
Waxyma	0.160	±	0.076	0.013	±	0.006
Vouvry 2014						
Cassia	0.348	±	0.087	0.121	±	0.022
Fridericus	0.565	±	0.119	0.104	±	0.050
Hobbit	0.595	±	0.048	0.089	±	0.015
Landi	0.295	±	0.096	0.061	±	0.027
Semper	0.269	±	0.052	0.043	±	0.017
Waxyma	0.461	±	0.055	0.060	±	0.012
Reckenholz 2014						
Cassia	0.161	±	0.023	0.009	±	0.003
Fridericus	0.445	±	0.057	0.037	±	0.008
Hobbit	0.291	±	0.202	0.032	±	0.014
Landi	0.146	±	0.000	0.007	±	0.003
Semper	0.216	±	0.121	0.024	±	0.005
Waxyma	0.333	±	0.013	0.017	±	0.006
Reckenholz 2015						
Cassia	0.174	±	0.077	0.021	±	0.013
Fridericus	0.210	±	0.036	0.029	±	0.011
Hobbit	0.205	±	0.011	0.033	±	0.011
Landi	0.169	±	0.063	0.018	±	0.007
Semper	0.146	±	0.037	0.015	±	0.003
Waxyma	0.217	±	0.090	0.023	±	0.014
Cadenazzo 2014						
Cassia	0.000	±	0.000	0.000	±	0.000
Fridericus	0.027	±	0.010	0.001	±	0.000
Hobbit	0.044	±	0.034	0.000	±	0.000
Landi	0.000	±	0.000	0.000	±	0.000
Semper	0.000	±	0.000	0.000	±	0.000
Waxyma	0.019	±	0.006	0.000	±	0.000
Cadenazzo 2015						
Cassia	0.123	±	0.102	0.013	±	0.013
Fridericus	0.519	±	0.044	0.027	±	0.012
Hobbit	0.361	±	0.176	0.035	±	0.012
Landi	0.422	±	0.131	0.027	±	0.013
Semper	0.406	±	0.083	0.031	±	0.004
Waxyma	0.077	±	0.021	0.004	±	0.001

Chapter V

Oat grain responses to *F. poae* and *F.langsethiae* infections and related resistance elements

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Under review in Toxins

Summary

Recent increases of Fusarium head blight (FHB) disease caused by infections with *F. poae* (FP) and *F. langsethiae* (FL), have been observed in oats. These pathogens are producers of nivalenol NIV and T-2/HT-2, respectively, two highly toxic compounds accumulated in oat grains and now considered as major issues for cereal food and feed safety. To date, the impacts of FP and FL on oat grains are not identified, and consequently, little is known about oat resistance elements against these pathogens. In the present study, the impacts of FL and FP on oat grain properties were assessed under different environmental conditions in field experiments with artificial inoculation. The severity of FP and FL infection of grains were compared between three environmental conditions (field sites) and the resistance against NIV and T-2/HT2 accumulation were assessed on seven oat genotypes. In addition, grain weight, contents in β−glucan and protein were compared between infected and non-infected grains. For all genotypes, no visible symptoms of infection were observed on panicles nor on grains. Analyses of the proportion of grains colonised by FP and FL and quantification of fungal DNA showed that FL was able to cause infection on grain only in the field site with the highest relative humidity whereas FP infected grains in all field sites. FP infection of grains resulted in NIV contamination (between 30 and 500 $\mu g.kg^{-1}$) not conditioned by environmental conditions. FL provoked contamination of grains with T-2/HT-2 (between 15 and 132 $\mu g.kg^{-1}$). None of the genotypes was able to fully avoid toxin accumulation. The general resistance of oat grains against toxins was weak and resistance against NIV was strongly impacted by genotype x environment interactions. Only the genotype with hull-less grains showed partial resistance to both NIV and T-2/HT-2 contamination. FP and FL infections increased β-glucan contents in grains, depending on genotypes and environmental conditions. Up to 63.5% β-glucan content increase was measured in hull-less grains infected with FL. FP and FL did not have a significant impact on TKW and protein contents. Hence, resistance against toxin accumulation remains the only indicator of FHB resistance in oat. Our results highlight the need of new oat genotypes with high resistance against both NIV and T-2/HT-2 to insure food and feed safety.

1. Introduction

Fusarium head blight FHB is recognized as a major threat for oat production (Clear et al. 1996; McCallum et al. 1999; Yan et al. 2010). The disease is caused by different species belonging to the genus *Fusarium* and leads to accumulation of mycotoxins in grains that are toxic for humans and animals (Müller et al. 1998; Šliková et al. 2010). In oats from Northern Europe, the most dominant FHB causing species is *F. graminearum* producing deoxynivalenol (DON) (Paavanen-Huhtala et al. 2007; Bjørnstad and Skinnes 2008; Fredlund et al. 2013). However, in other European areas, a considerable increase of *F. poae* (FP) and *F. langsethiae* (FL) infections have been observed in oats (Scudamore et al. 2007; Edwards et al. 2009; Van der Fels-Klerx and Stratakou 2010; Bernhoft et al. 2012; Schöneberg et al. 2018). These pathogens produce highly noxious toxins, putting food and feed safety in jeopardy. FP is the main producer of nivalenol (NIV), a vomitoxin more then ten times more toxic than DON (EFSA 2013; Pitt 2013). FL has been associated with the recent increases of oat grain contaminations with hazardous T-2 and HT-2 toxins (Scudamore et al. 2007; Schöneberg et al. 2018). In fact, T-2/HT-2 contaminations caused an outbreak of alimentary toxic aleukia that was responsible for the death of thousand people in Russia during the 1940s (Yagen and Joffe 1976). Legislative limits have been put in place to limit the amount of T-2/HT-2 entering the food chain (European Commission 2013/165/EC), however, limits for NIV are not yet in place. FP and FL are emerging pathogens, and in contrast to *F. graminearum*, very little is known about the life cycle, the epidemiology, and environmental requirements for infection and toxin production. Without such information, it remains difficult to forecast FL and FP infection and thus to avoid toxin contaminations (Schöneberg et al. 2018).

Oat has long been regarded as more resistant to FHB than wheat or barley. This may be attributed to the lack of visual symptoms on infected panicles, and the presence of long pedicels between spikelets that usually prevent the spread of fungal mycelia throughout the panicle (Bjørnstad and Skinnes 2008; Tekauz et al. 2008). Yet, oat grains can accumulate considerable mycotoxin amounts. The use of resistant oat varieties would be the most sustainable way to overcome such contaminations (Langevin et al. 2004 ; Tekauz et al. 2004). However, the selection of FHB resistant oat varieties experienced a strongly delay due to the lack of knowledge about the FP and FL infection processes and their impact on oat tissues. Nevertheless, it has been demonstrated that oats, like other small grain cereals, are susceptible to primary infection during anthesis (Xue et al. 2015; Kibler 2016). Fungal hyphae enter via

the tips of the glumes, quickly colonise the anthers, reach the developing kernels which are frequently aborted (Divon et al. 2012; Tekle et al. 2012). Besides toxin contamination, the consequences of *Fusarium* infections on oats are not yet clearly identified. Some epidemiological studies relate significant reduction of grain weight and germination ability (Mielniczuk et al. 2004; Tekle et al. 2012), whereas others report minimal consequences on crop yields and grain properties (Bjørnstad and Skinnes 2008; Tekauz et al. 2004). Moreover, the vast majority of studies aiming to describe FHB symptoms only focus on plants infected with *F. graminearum* or *F. culmorum* (Bjørnstad and Skinnes 2008; Šliková et al. 2010; Tekle et al. 2012; etc.). As of yet, it is not known how oat grains react to the infection with FP and FL.

As in other small grain cereals, oat resistance against FHB is a complex, quantitative character and based on the cumulative effect of several genes (He et al. 2013). Up to now, all resistance studies in oats aimed to limit toxin contamination in grains, defined as type V resistance in wheat (Miller et al. 1985). It has been observed that naked grains accumulate lower amounts of DON than hulled grains (Gagkaeva et al. 2013; Yan et al. 2010). Recently, several minor QTLs associated with resistance to DON accumulation have been identified in oats (Bjørnstad et al. 2017; He et al. 2013). It is not clear if resistance against DON contamination also provides resistance against NIV and T-2/HT-2 toxins. Besides resistance type V, other resistance types have not been described in oat. The resistance type II, described in wheat as the resistance against disease propagation in head tissue (Mesterházy 1995; Schroeder and Christensen 1963), is inherent to the panicle shape (Bjørnstad and Skinnes 2008). Due to the limited knowledge of oat grain responses to FHB, it was so far not possible to examine the resistance against kernel infection (type III) and the tolerance of grain in terms of yield (type IV).

The aim of this study is to investigate the responses of oat grains to infection by FP and FL. Seven oat genotypes were artificially inoculated at three different field sites. Each experiment was composed of plots inoculated with FP, FL or non-inoculated. The success of infections was determined by the observation of grains infected by the pathogens and measurements of fungal DNA in grains of three varieties. The contents of NIV and T-2/HT-2 toxins were measured in all grains from inoculated plots. The impacts of FP and FL on grain properties were assessed by comparison of grain weight, β-glucan and protein contents between inoculated and non-inoculated grains.

2. Materials and methods

2.1. Plant material

Seven oat varieties were used for this study. The varieties differ in their morphological properties, favoured environmental conditions for cultivation and resistance to DON accumulation (Stefan Beuch, personal communication). Plant height, panicle and grain characteristics are presented in table 1 (IBPGR 1985). Descriptions of these characteristics were carried out in the field at Changins in 2015. Ten plants of each variety from non-inoculated plots were randomly chosen and the averages of plant height and number of seeds in a panicle were calculated.

Table 1. Description of the 7 oat genotypes investigated in the current study.

Variety	Breeder	Plant height (cm)	Panicle shape	Panicle erectness	Number of seeds in a panicle	Kernel covering	Lemma colour	Hairiness of lemma	Hairiness at basal part of the primary grain
Canyon	NORDSAAT Saatzucht (DE)	110	Equilateral	Drooping	71	Covered	Yellow	Glabrous	Slightly pubescent
Expander	SZ Edelhof (DE)	105	Equilateral	Drooping	96	Covered	Yellow	Glabrous	Highly pubescent
Husky	NORDSAAT Saatzucht (DE)	110	Equilateral	Drooping	90	Covered	Yellow	Slightly pubescent	Moderately pubescent
Melody	NORDSAAT Saatzucht (DE)	110	Unilateral	Drooping	69	Covered	White	Slightly pubescent	Moderately pubescent
Poseïdon	NORDSAAT Saatzucht (DE)	115	Equilateral	Drooping	85	Covered	Yellow	Slightly pubescent	Moderately pubescent
Samuel	Nufarm Deutschland GmbH (DE)	120	Equilateral	Drooping	65	Naked	/	/	Slightly pubescent
Triton	NORDSAAT Saatzucht (DE)	105	Equilateral	Semi-erected	62	Covered	White	Highly pubescent	Moderately pubescent

2.2. Fungal material

Artificial inoculations were carried out with three strains of FL and three strains of FP, all isolated from infected oat grains (Table 2).

Table 2. Description of *Fusarium* strains used for the study. All strains are deposited at the CBS http://www.cbs.knaw.nl/fusarium/.

Species	Strain ID	Origin (Swiss canton)	Year	Toxin produced
F. poae	13013	Luzern	2013	NIV
F. poae	13045	Thurgau	2013	NIV
F. poae	13059	Vaud	2013	NIV
F. langsethiae	13014	Jura	2013	T-2/HT-2
F. langsethiae	13005	Schaffhausen	2013	T-2/HT-2
F. langsethiae	14001	Vaud	2014	T-2/HT-2

Strains were retrieved from long term storage in a 1:1 mix of water with glycerol at -80 °C. For mass production of each isolate, strains were cultured on Potato Dextrose Agar (PDA, BD Difco, Le Pont de Claix, France) for 1 week at 18 °C with 12h/12h UV light/darkness. Subsequently, two discs (5 mm diameter) from the outer margin of a well-grown colony were transferred to 200 mL of liquid V8-medium in a 1L capacity Erlenmeyer flask. V8-medium consisted of a 1:5 mix of V8 juice (Campbell Soup Company, Camden, USA) and distilled water with 2g sodium carbonate per litre. Cultures were incubated on a shaker at 200 rpm for 7 days at 24 °C in the dark. Cultures were then filtered through sterile cheesecloth to remove all mycelia. Finally, the culture medium was removed by centrifugation at 4,500 rpm for 10 min and the pellet was re-suspended in sterile distilled water. These preparations were either used immediately or stored at -20 °C.

2.3. Field tests and artificial inoculations

Field experiments were conducted in 2015 at three locations in Switzerland: Changins (Canton Vaud, south-west [46 ° 24’36“/6 ° 14’06”]), Reckenholz (Canton Zürich, north-east [47 ° 16’30”/8 ° 26’45”]), Cadenazzo (Canton Ticino, south of the Alps [46 ° 09’00”/8 ° 57’00”]). In Changins and Reckenholz, oat grains were sown in March 2015, in Cadenazzo, oat grains were sown in November 2014. At all field sites, planting and

management was conducted with the same experimental design and protocol. The varieties were sown with a Seedmatic seeding machine (HegeMaschinen) in $1m^2$ micro-plots with 5 rows and 15 cm space between rows . Artificial inoculations took place when 50% of the plants within each micro-plot were at mid-anthesis (BBCH65). Inoculum was prepared immediately before the inoculation by mixing equal proportion of the liquid culture of each of the three strains of FP or FL and adding 0.0125% of Tween®20 (Sigma-Aldrich Chemie GmbH). Concentration of conidia and volume of suspension prepared were adjusted to 7.5×10^6 conidia per microplot. Suspensions were applied with a hand-held sprayer (Spray-Matic 1.25P, Birchmeier) at dawn. If required and according to weather conditions during inoculations, the plots were irrigated to maintain humidity on the panicles at least 24h with a maximum of 600 l of water per hectare. A high pressure/low volume overhead spray irrigation system was available in Changins while in Cadenazzo, an equivalent of 300L / ha of water was sprayed manually with a backpack sprayer. Three artificial inoculations were carried out at three days' intervals. The climatic conditions at anthesis at the three sites are described in table 3.

Table 3. Average temperature, relative humidity, evapotranspiration and total rainfall at the three-field tests recorded during flowering period (from 15.06.2015 to 30.06.2015) and from flowering to harvest (15.06.2015 to 10.08.2015). (Source: http://www.meteoswiss.admin.ch).

	Temperature (°C)	Rainfalls (mm)	Relative humidity (%)	Evapotranspiration (mm/day)
from 15.06.2015 to 30.06.2015				
Cadenazzo	20.6	32.1	66.8	3.5
Changins	19.1	21.4	58.9	4.4
Reckenholz	17.0	61.4	71.8	2.8
from 15.06.2015 to 10.08.2015				
Cadenazzo	23.4	129.7	67.8	3.1
Changins	22.1	95.4	56.3	4.8
Reckenholz	20.9	114.7	65.5	3.7

2.4. Harvest and samples collection

All oat plots were harvested at full maturity (BBCH 89) during the first week of August 2015 at the three field sites. A combine harvester (HEGE 140, Mähdreschwerke GmbH, Germany) was used, with a reduced airflow to recover a maximum of kernels. Grains were dried to a maximum of 14% moisture and cleaned using a vertical airflow (Baumann Saatzuchtbedarf, Germany) to remove dust and other debris. At this stage, clean grains with husks were obtained. Two hundred gram grain samples were extracted after three times

homogenisation with a rifle divider (Schieritz & Hauenstein AG, Switzerland). Fifty grams were extracted from these subsamples and milled separately with a sample mill using a 0.25 mm screen (1093 Cyclotec Sample Mill, FOSS, Sweden) to obtain whole meal flour samples. All grains and flour samples were stored at -20 °C until further analyses.

2.5. Analyses of grains

Presence of FP and FL on oat grains confirmed the success of the artificial inoculations. Infection of grains was assessed by the incidence of grains infected by FP and FL (%), and by the quantification of fungal DNA in grains.

2.5.1. Proportion of grains infected by *F.poae* and *F.langsethaie*

The frequency of *Fusarium* colonized grains was determined using the Seed Health Tests method described in Vogelgsang et al. (2008) and used in Schöneberg et al. (2016) and Martin et al. submitted. One hundred randomly chosen grains per sample were examined. Samples of Canyon, Husky and Triton from both inoculated and non-inoculated plots were analysed to determine the spontaneous colonization of the grains under experimental conditions. The different *Fusarium* species were identified according to the *Fusarium* laboratory manual by Leslie and Summerell (2006).

2.5.2. Quantification of fungal DNA

The severity of FP and FL infection was measured by quantitative PCR (qPCR) of fungal DNA in the grains. Protocols for DNA extraction, total DNA quantification and quantitative PCR reactions are described in Schöneberg et al. (2018). In every performed assay, all standards as well as the negative control (double distilled water) were run as triplicates.

Briefly, for FP qPCR, the primer pairs ACL1-F160 and ACL1-R330 and TaqMan-Probe (ACL1_poae1_probe) (Qiagen AG, Hombrechtikon, Switzerland) were used. For FL qPCR, the protocol specifications and thermocycling parameters were employed as described in Edwards et al. (2012) and adapted to the available reaction mixes and laboratory devices as detailed in Schöneberg et al. (2018). The amplification mix consisted of the primer pairs FlangF3 and LanspoR1 (Wilson et al. 2004) and IQ SYBR® Green Supermix (Bio-Rad, Cressier, Switzerland).

2.5.3. Mycotoxin analysis

NIV contents were examined in all samples from plots inoculated with FP. Identification and quantification of NIV was done using LC-MS/MS. Sample preparation and LC-MS/MS measurements followed the protocols detailed in Schöneberg et al. (2018).

Contents in T-2/HT-2 toxins in grains were measured in all samples from plots inoculated with FL. Contents were determined in flour usng an enzyme immunoassay kit for quantitative screening of toxins (Ridascreen® T-2/HT-2 Toxin, R-biopharm AG Switzerland), according to the manufacturer's instructions. The protocol specific for extraction of T-2/HT-2 in oat flour was followed.

2.6. Analyses of grain properties

2.6.1. Thousand kernel weight analysis

Grain weights were measured for all grain samples using the Thousand Kernel Weight (TKW) indicator. TKW were measured with the MARVIN optical grain counter (Digital Seed Analyser, GTA Sensorik GmbH, Neubrandenburg, Germany) and a balance (Mettler PM2000, Mettler-Toledo, Greifensee, Switzerland).

2.6.2. B-glucan content of grains

Content of β-glucan was determined in all samples (inoculated and non-inoculated plots) with the mixed-linkage β-glucan kit (Megazyme International Ireland Ldt, Wicklow, Ireland). The assay procedure of mixed-linkage β-glucan in oat flour—streamlined method—(ICC Standard Method No.166) was followed and adapted to the laboratory facilities. The β-glucan content was measured in 0.1g of dry *F. graminearum* mycelium (FG13170) as control and was negative.

2.6.3. Protein content analysis

The protein contents (%) were examined in all samples of hulled oat grains using near-infrared reflectance spectroscopy (NIRS) with a NIRFlex N-500 (Büchi Labortechnik AG, Switzerland). The protein calibration of NIRFlex was regularly adjusted and basis analyses were made with the Kjedahl method according to ICC standard method No.105/2 (include a reference). The coefficient of confidentially of the calibration was R^2=0.61 (Cécile Brabant and Carine Oberson, personal communication).

2.7. Experimental design and statistical analyses

Field design, inoculation methodology, harvest and samples preparations were similar in the three field sites. Each field site consisted of three replicates with FP inoculations, three with FL inoculations and three without artificial inoculations, planted in a split-plot design. Proportion of FP and FL infected grains and measurements of fungal DNA were carried out on grains from inoculated plots of the three field sites for three selected oat genotypes (Canyon, Husky and Triton). Mycotoxin measurements were performed on grains from inoculated plots in the three field sites for all seven oat genotypes. TKW, β-glucan and protein contents were measured in all grain samples.

Statistical analyses were carried out using the statistical software R (R core Team, 2015). Effect of FP and FL infection on grains were separately analysed. Analyses of variance ANOVA were performed on proportion of grains infected by FP or FL, fungal DNA, and mycotoxin contents, using the environment (field site) as the main factor and the genotypes as the sub-factor. Data were normally distributed. The impact of FP or FL infections on TKW, β-glucan and protein contents were analysed by ANOVA using the environment as the main factor, the presence of artificial inoculation as the sub-plot and the genotypes as the sub-sub-plots. After ANOVA, a multiple comparison was performed on significant factors using Tukey's test with $\alpha = 0.05$ (package "agricolae", De Mendiburu, 2015). Pearson correlations were used to verify correlations between toxin contents, fungal DNA concentration and proportion of *Fusarium* infected grains.

3. Results

3.1. Outcomes of the FP and FL inoculations

The success of artificial inoculation on panicles to cause infection on grains was assessed by recovering FP and FL colonies from surface sterilised grains. The outcome of inoculation from the three field sites was assessed on three selected oat genotypes, Canyon, Husky and Triton. The percentage of *Fusarium* infected grains and the amounts of fungal DNA were determined in grains from FP and FL inoculated plots using a Seed Health Tests (SHT) (Vogelgsang et al. 2008) and quantitative PCR (Schöneberg et al. 2018), respectively.

The presence of grains infected by FP as well as fungal DNA in all grains (figure 1a.c) confirmed the success of artificial inoculations at the three field sites. Analyses of grains from FL inoculated plots revealed that FL inoculations were successful in Reckenholz. However, FL was not detected in grains from Changins and Cadenazzo, by both SHT and PCR methods (figure 1b.d). FP and FL were not detected in grains from non-inoculated plots. The SHT analyses revealed weak natural infections: in Changins and Cadenazzo, less than 5% of grains from non-inoculated plots were infected by *F. graminearum* while in Reckenholz the non-toxigenic *Microdochium nivale/M. majus* was the main member of the FHB complex. Sporadic presence of *F. graminearum* and *Microdochium nivale/M. majus* was observed in grains from FP and FL inoculated plots (less than 5%).

Based on these results, grains from the three sites were used for analyses of oat grain response to FP. Grains from Reckenholz were used for subsequent assessments of FL impacts on oat grains.

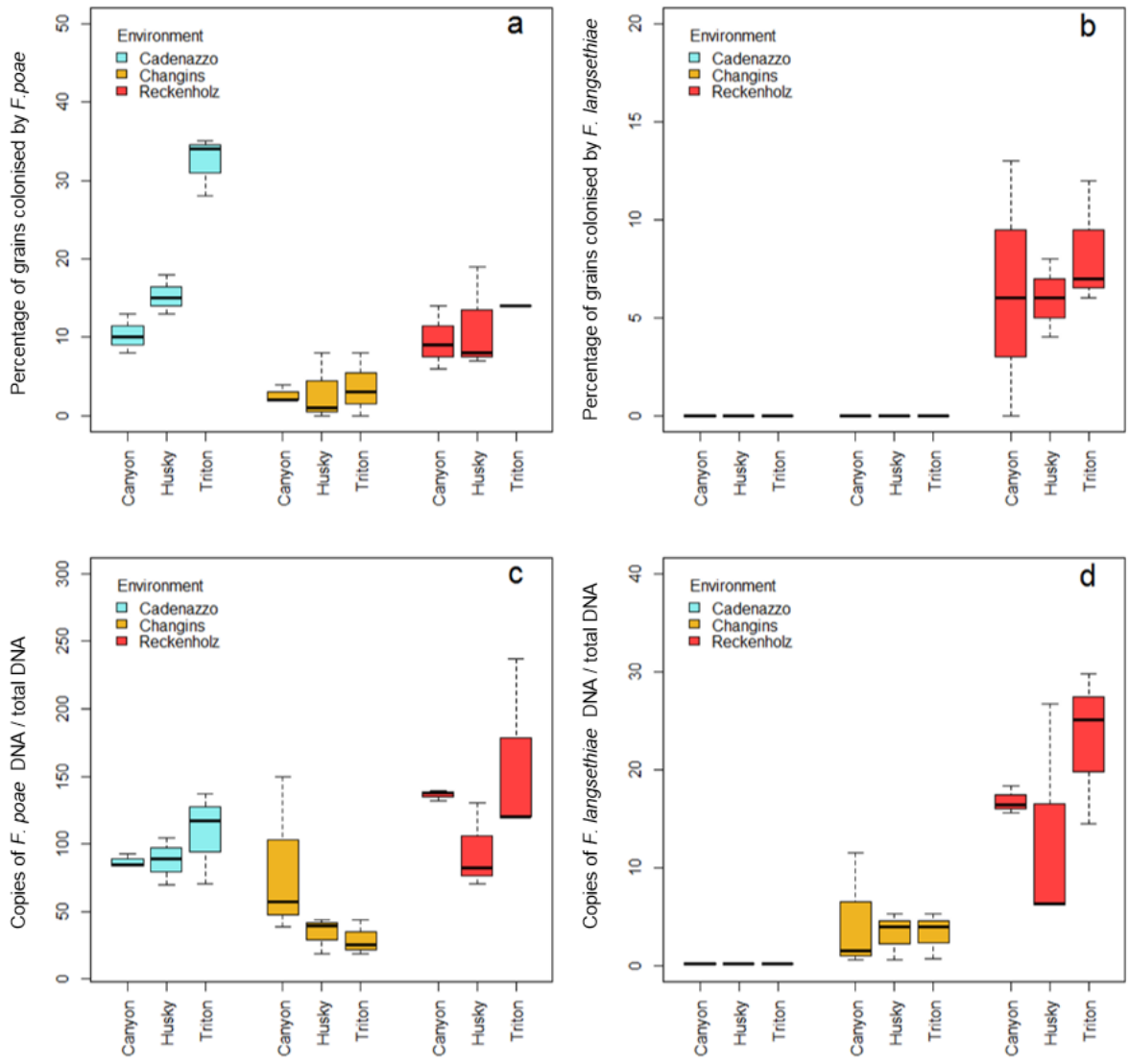

Figure 1. Percentage of grains by colonised *F. poae* (a) and *F. langsethiae* (b), and fungal DNA of *F. poae* (c) and *F. langsethiae* (d) for three oat genotypes from three environments (field sites). Cadenazzo: canton Ticino, Changins: canton Vaud, Reckenholz: canton Zurich.

3.2. Incidence and severity of grain infection with FP and FL

The percentage of *Fusarium* colonised grains and the amounts of fungal DNA were used to determine the incidence and the severity of grain infection, respectively, caused by FP and FL. The incidence and severity were compared between environments and between genotypes.

The proportion of grains colonised by FP ranged from 2 to 35% (figure 1a). Over all genotypes, the highest proportion of FP colonised grains was observed in grains from Cadenazzo (20%), whereas FP was found on 12% of grains from Reckenholz and 3% of grains from Changins. Yet, the FP DNA abundance in grains showed higher quantities in grains from

Reckenholz than in grains from Changins and Cadenazzo (P<0.05) (figure 1c). The SHT revealed higher FP incidence (P<0.05) in Cadenazzo for grains from Triton (33%) compared to grains from Canyon and Husky grains (11% and 16%, respectively), but no differences in FP DNA quantities were detected between the three genotypes. No significant difference in severity of grain infection was observed between the genotypes in Changins and Reckenholz. Over all environments, the percentage of FP infected grains and quantity of FP DNA in grains were significantly correlated (Pearson correlation coefficient: 0.44, P<0.05).

With respect to FL inoculations, the SHT and the DNA quantifications revealed no infection in grains from Cadenazzo and Changins. Even in Reckenholz, FL caused less severe grain infections than FP. Indeed, on average only 9% of grains were infected by FL after artificial inoculation (figure 1.b), and quantities of fungal DNA were 10 times lower than those detected in FP inoculated grains. No significant differences were observed between genotypes considering the percentage of infected grains, but significantly higher quantities (P<0.05) of FL DNA were measured in Triton compared with grains from Canyon and Husky (figure 1d). Nevertheless, the proportion of FL colonised grains and the quantity of DNA in grains were significantly correlated (Pearson correlation coefficient: 0.71, P<0.001).

3.3. Mycotoxin contamination

Mycotoxins contents were measured in grains of the 7 genotypes. NIV contents were measured in grains from FP inoculated plots and T-2/HT-2 were measured in grains from FL inoculated plots.

3.3.1. NIV contamination caused by FP

NIV was detected in all grain samples infected with FP and ranged between 30 to 420 $\mu g.kg^{-1}$ (figure 2), with an average of 172 $\mu g.kg^{-1}$. NIV accumulation was not influenced by the environment (field site), however, significant differences (P<0.05) were observed in NIV between oat genotypes (table 1). Over all environments, grains of Melody accumulated significantly higher (P<0.05) NIV amounts (281 $\mu g.kg^{-1}$) than grains from Husky (143 $\mu g.kg^{-1}$), Samuel (139 $\mu g.kg^{-1}$), Expander (125 $\mu g.kg^{-1}$) and Triton (117 $\mu g.kg^{-1}$) (figure 2). NIV accumulation in grains from Poseïdon and Canyon was intermediate (respectively 218 $\mu g.kg^{-1}$ and 170 $\mu g.kg^{-1}$) and not significantly different from the other genotypes (P>0.05). The accumulation of NIV in oat genotypes was affected by the environmental conditions. Indeed,

in Changins and Reckenholz, Melody grains accumulated the highest NIV quantities, whereas in Cadenazzo, the highest content was measured in grains from Poseïdon (P<0.05). In Cadenazzo, grains from Canyon were among the less affected by NIV contamination, but yet accumulated considerable NIV amounts in Changins. These interactions between genotypes and environments (GxE interactions) explained 46% of the NIV accumulation variability in the present experiment (table 4). No significant correlation was observed between fungal DNA and NIV contents in FP infected grains.

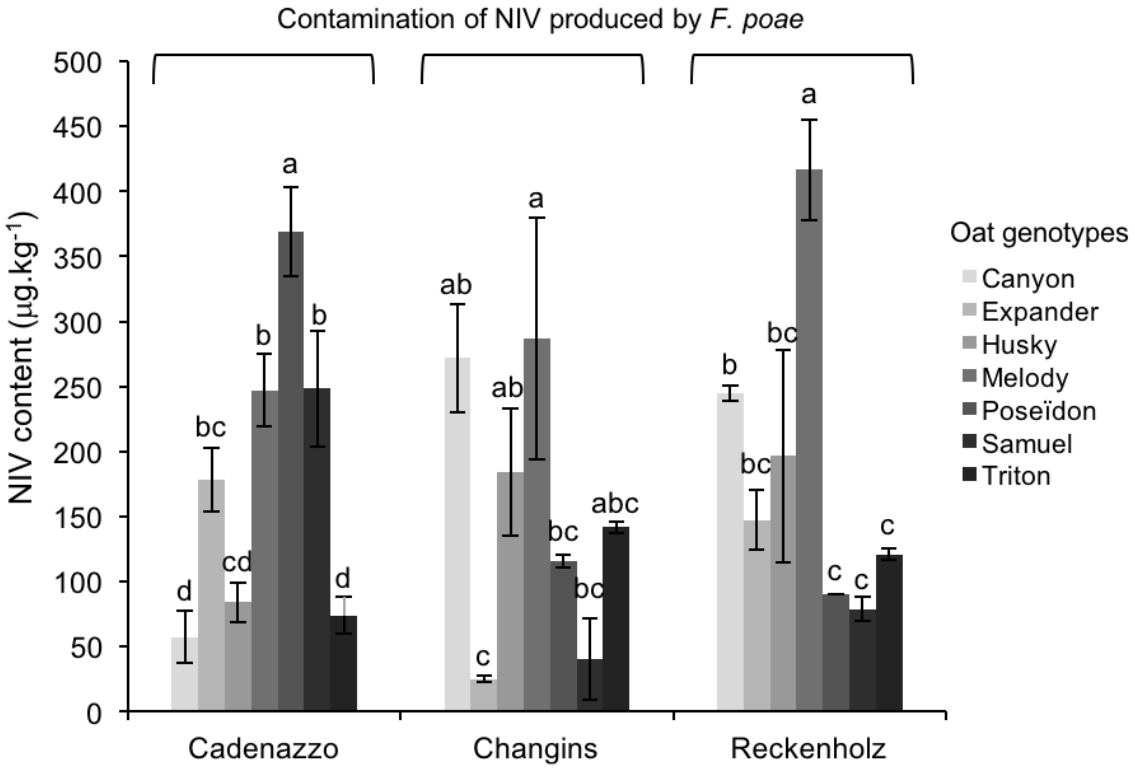

Figure 2. Average ± standard deviation of NIV content measured in seven oat genotypes from three environments (field sites) after artificial inoculation with *F. poae*. Different letters indicate significant differences within one environment according to Tukey HSD (α=0.05).

Table 4.. Variance analysis of NIV contents in oat grains infected with *F. poae* from three environments and seven varieties. ***: significant at P<0.001.

Source of variation	Sum Square	Mean Square	
Environment	13410	6705	
Error (a)	2137	534	
Genotype	188457	31409	***
Environment x Genotype	344011	28668	***
Error (b)	225612	6267	

3.3.2. T-2/HT-2 contamination caused by FL

In Reckenholz, all grains from FL inoculated plots contained T-2/HT-2 toxins. The highest T-2/HT-2 contents were measured in grains from Husky (132 $\mu g.kg^{-1}$) and the lowest in grains from Expander and Samuel (26 $\mu g.kg^{-1}$ and 15 $\mu g.kg^{-1}$). No significant correlation was observed between fungal DNA and both NIV and T-2/HT-2 contents in FL infected grains.

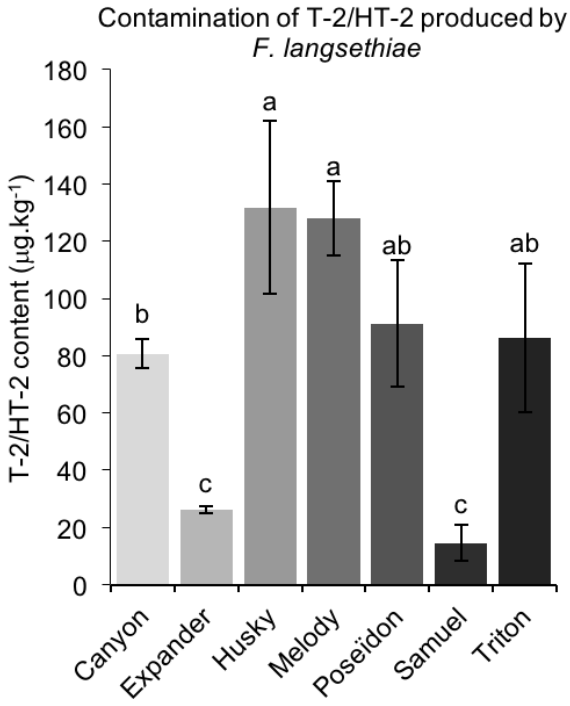

Figure 3. Averages ± standard deviations of T-2/HT-2 contents measured in grains of seven oat genotypes grown in Reckenholz and after artificial inoculation with *F. langsethiae*. Different letters indicate significant differences between genotypes variety according to Tukey HSD (α=0.05).

3.4. Impact of the inoculations on grain properties

Impacts of FP and FL infections on grain properties were assessed by analyses of grain weight, β-glucan and protein contents. These properties were compared between grains from inoculated and non-inoculated plots. In our data set, TKW varied from 38.5 and 23.6g, β-glucan content in grains ranged between 4.8 and 2.8% in dry weight, and protein contents ranged between 11.7 and 12.9%.

3.4.1. Effect of FP inoculation on grain properties

FP infection resulted in minor changes of TKW. Only a slight increase in the TKW of Triton was observed over all environments (from on average 34.0 g for non-inoculated grains to 36.4 g for inoculated grains) (figure 4a). Within each environment, FP inoculation had a different impact on the TKW of oat genotypes. In Changins, infection led to significant TKW increases (P<0.05) in grains from Expander and Melody (31.2 g to 36.3 g and 32.6 g to 35.7 g, respectively). In Cadenazzo and Reckenholz however, FP inoculation reduced the TKW of grains from Melody grains (43.4 g to 34.9 g) and of grains from Canyon (40.0 g to 35.5 g), respectively. Over all environments, FP inoculations reduced β-glucan contents in grains from Melody (-17%), while increasing contents in grains from Samuel and Triton (47.7% and 22.0% increases, respectively) (figure 4b). Moreover, FP inoculations led to a general increase of β-glucan contents in grains from Changins over all genotypes (on average from 3.2% to 3.8% of β-glucan). In addition, FP inoculations resulted in a weak but significant increase of protein content in grains from Triton over all environments (from 12.0% in grains from non-inoculated plots to 13% in grains from FP inoculated) (figure 4c). Within each environment, significant differences in protein contents were observed between infected and not infected grains. In Reckenholz, lower protein contents were measured in FP infected grains over all genotypes (P<0.05) (on average 12.2% of protein in non-infected grains and 11.9% in FP infected grains). In Cadenazzo, FP infection caused significant increases (P<0.05) of protein content in grains from Canyon, Poseïdon and Samuel. In Changins, FP infection resulted in significant (P<0.05) increases of protein content in grains from Triton and Samuel, whereas a reduction was observed in grains from Expander (see Supplementary Material 1).

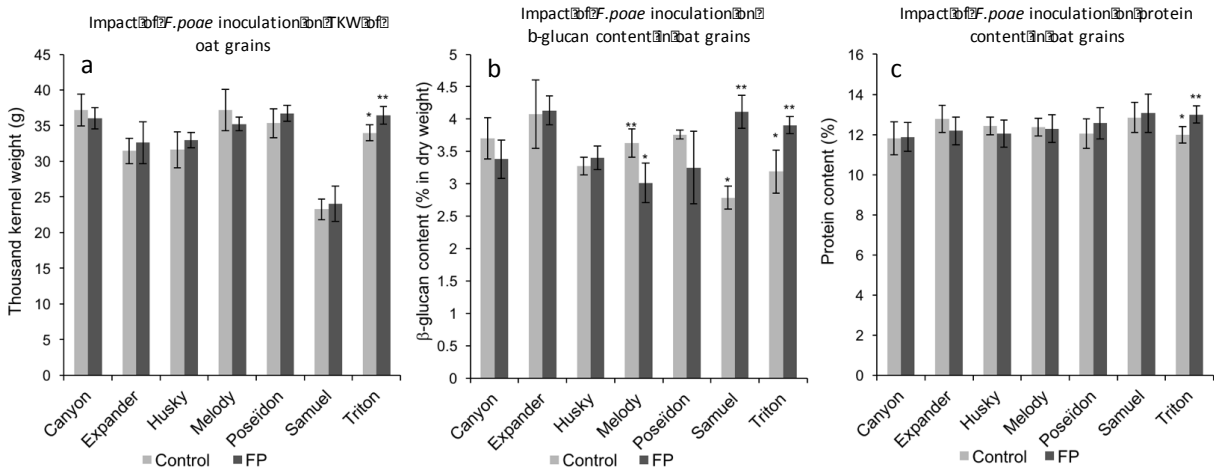

Figure 4. Average and standard deviations of (a) TKW, (b) β-glucan contents and (c) protein contents in grains from plots without (Control) or with *F. poae* (FP) inoculation, for seven oat genotypes and over all three environments (field sites). Different symbols indicate significant differences between infected and non-infected grains of a given genotype according to Tukey HSD (α=0.05).

The variance analyses of TKW, β-glucan and protein contents in oat grains considered as factors the different environmental conditions in the three field sites, the presence of FP artificial inoculations, and the different genotypes. These analyses revealed that FP inoculations did not systematically impact TKW, β-glucan and protein contents of oat grains. Changes of TKW in FP inoculated grains was influenced by environmental conditions and genotypes (ExG, IxG and ExIxG interactions) (table 5). Variability of TKW in our data set was mainly explained by the differences between genotypes, and can be attributed to the hull-less genotype Samuel. Changes in β-glucan contents in infected grains were mainly influenced by the genotypes (table 5) and by the environmental conditions. These IxG and ExI interactions explained a larger proportion of β-glucan content variability than TKW or protein content variabilities. Moreover, no significant ExIxG interactions on β-glucan content were observed, indicating a stability of the impact of FP on β-glucan content across the different environments for each oat genotype. Basically, the variability of β-glucan content was partially attributed to the differences between genotypes as well as to significant ExG interactions. Similarly, changes in protein content in FP infected grains were also influenced by the environmental conditions and the genotypes (ExG, IxG and ExIxG interactions). Over all genotypes, lower protein contents were measured in grains from Reckenholz (12.0%) than in grains from Changins and Cadenazzo (12.5 and 12.6%, respectively).

Table 5. Analyses of variance of Thousand Kernel Wweight (TKW), β-glucan and protein contents in grains between the different factors investigated in this study (Environment, Presence of inoculation with *F. poae* and Genotype) as well as their interactions. Significance levels: *** at $P<0.001$, ** at $P<0.01$, * at $P<0.05$.

	TKW			β-glucan content			Protein content		
Source of variation	Sum Square	Mean Square		Sum Square	Mean Square		Sum Square	Mean Square	
Environment E	4.6	2.3		0.5	0.3		6.4	3.2	*
Error (a)	25.6	6.4		0.2	0.04		1.9	0.4	
Inoculation I	0.5	0.5		0.4	0.4		0.3	0.3	
E x I	97.8	48.9	***	2.3	1.4	***	3.6	1.8	*
Error (b)	9.7	1.6		1.5	0.25		1.5	0.2	
Genotype G	2339.3	390	***	7.6	1.3	***	12.1	2.0	***
E x G	248.4	20.7	***	6.9	0.6	**	20.0	1.7	***
I x G	45.5	7.6	**	13.2	2.2	***	7.5	1.3	***
E x I x G	95.7	8.0	***	2.7	0.2		10.3	0.9	***
Error (c)	166.3	2.3		16.9	0.2		9.3	0.1	

3.4.2. Effect of FL inoculation on grain properties

The impact of FL infections on grain properties was solely investigated in grains from Reckenholz. For all tested genotypes, TKW and protein content were not affected by FL inoculations (figure 5). However, FL inoculation changed β-glucan content in grains depending on the genotype ($P<0.001$). FL inoculations resulted in a decrease of the β-glucan contents ($P<0.05$) in grains of Canyon and Poseïdon (from 3.9% in grains from non-inoculated plots to 2.9% in grains from inoculated plots and from 3.8% to 2.8%, respectively). In contrast, FL inoculation caused a 63.5% increase of β-glucan in grains from Samuel (from 2.6% to 4.1%) (figure 5).

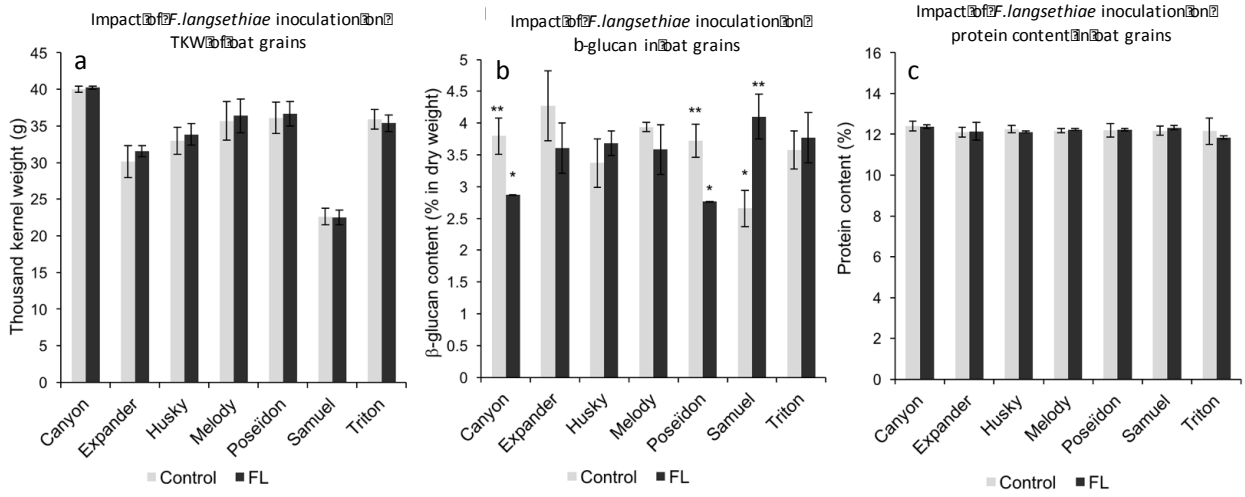

Figure 5. Average and standard deviation of (a)TKW, (b) β-glucan contents and (c) protein contents in grains from plots without (Control) or with *F. langsethiae* (FL) inoculation for 7 oat genotypes in Reckenholz. Different symbols indicate significant differences between infected and non-infected grains of a given genotype genotype according to Tukey HSD (α=0.05).

4. Discussion

The presence of different types of mycotoxins in oat grains is alarming. The mycotoxins found in the current study are produced by several *Fusarium* species. In particular, *F. poae* (FP) and *F. langsethiae* (FL) caused contamination of grains with highly toxic NIV and T-2/HT-2 (Torp and Nirenberg, 2004; Imathiu et al. 2012; Schöneberg et al. 2018), respectively. In some geographic areas, *F. graminearum* and its mycotoxin deoxynivalenol (DON) and zearalenone are also found in oat grains (Bjørnstad and Skinnes 2008; Tekle et al. 2012). This study aims to better understand the response of oat grains in the presence of FP, FL, NIV and T-2/HT-2 toxins. Using artificial inoculations, we intended to trigger the response of oat grains and to challenge resistance of seven oat genotypes. Despite artificial inoculations, no symptoms were detected on the panicles in all experiments. This finding is in agreement with other studies on oats (McCallum et al. 1999; Tekauz et al. 2008; Gagkaeva et al. 2011). Severe symptoms such as blighted spikelets, discoloration of glumes, floral abortions and browning of rachis attributed to *Fusarium* infections are rare and mainly observed in Northern Europe (Bjørnstad and Skinnes 2008; Tekle et al. 2013). Divon et al. (2012) observed these symptoms on oat panicle strongly infected with FL under controlled conditions. Presence of such FHB symptoms on

panicles is probably due to long exposure to high humidity (Hope et al. 2005; Tekle et al. 2012; Martin et al. 2016). In the present study, without visible signs of FHB outbreak, the success of infections was assessed by recovering the inoculum on the grains using a Seed Health Test (SHT) (Vogelgsang et al. 2008) and quantitative PCR for the amount of fungal DNA. Infections with FP were successful at all three field sites, whereas FL infections could only be tracked in Reckenholz. The average temperature of about 20°C was rather similar at the three field sites, but the climatic conditions in Reckenholz, with high relative humidity and a long rainy period during and after anthesis, might have favoured the infection by FL. Indeed, *in vitro* studies showed that FL requires longer periods of high humidity, compared with FP and other *Fusarium* species (Medina and Magan 2010).

Once the grains are infected, both FP and FL produce mycotoxins. Infection of FP caused accumulation of NIV, in similar contents across all field sites. In more contrasting environments across the UK and Canada, Edwards et al. (2009) and Tekauz et al. (2008) ,respectively, noticed the strong influence of environmental conditions on toxin accumulation in grains. In the present study, it was not possible to investigate the impact of environmental conditions on T-2/HT-2 accumulation. T-2/HT-2 were detected in considerable amounts in all FL infected grains from Reckenholz, with up to 180 ppb. Yet, the results of the SHT and the quantification of FL DNA revealed a low degree of kernel colonisation by FL, whereas FP caused more severe grain infections. This result suggests a high toxigenicity of FL and indicate that FL infections on oat crops, although less dominant than FP, nevertheless constitute a real threat for the safety of oat products.

Besides the accumulation of toxins, FP and FL altered the β-glucan content in grains. Depending on the genotypes and environmental conditions, the β-glucan contents increased or decreased subsequent to FP and FL infections. In particular, β-glucan content in grains of the naked variety Samuel substantially increased following infections by both FP and FL (+48% and +64%, respectively), whereas the changes in β-glucan content caused by FHB were limited for the other genotypes (approximately +20%). Hence, with the current dataset, no clear tendency in β-glucan content variation could be identified. In a very similar study on barley grains, 10% reduction of β-glucan content was observed in six barley varieties after inoculation with *F. graminearum* (Martin et al. submitted). On one hand, the reduction of β-glucan content might be attributed to the β-glucanase activity observed from *Fusarium* pathogens (Schwarz et

al. 2002). On the other hand, since β-glucans are mainly concentrated in the outer layers of oat grains (Miller et al. 1995), such variations in β-glucan content might be attributed to changes in grain morphology caused by the infection. With respect to the TKW of grains, FP and FL infections showed only a weak impact in the present study. Over all the three environments, only a weak increase of TKW of grains from Triton was measured when infected with FP. This indicates that both FP and FL had very little to no impact on grain filling, suggesting a minimal impact of FHB on oat yield. However, these observations are likely conditioned by the prevailing environmental conditions in Switzerland. In Brazil, with temperatures above 20°C and relative humidity frequently exceeding 90%, Martinelli et al. (2014) observed significant reduction of oat yield, up to 25%, due to FHB caused by *F. graminearum*. Furthermore, Mielniczuk et al. (2004) detected TKW reductions of 32% in oats after field inoculations with *F. crookwellense* in Poland. We also assume that reduction of grain filling and yield is specific to the particular *Fusarium* species causing FHB. In the present study, we did not observe a significant impact of FP and FL on protein content in oat grains. Finally, our grain analyses showed that even if FP and FL have an impact on some of the grain properties, these alterations are not reliable and useful symptoms of FHB in breeding for resistant varieties.

For the understanding of resistance against FHB in oats, we compared the types of resistance with those for wheat, where type I and type II comprise the resistance against the primary infection and the spread of the infection throughout the spike, respectively (Mesterházy 1995; Schroeder and Christensen 1963). In the present experiments with oats, we could not distinguish these types of resistance. Types III to VI describe the resistance of the kernel, where type III is the resistance against colonisation, type IV is the tolerance against FHB in terms of yield and type V is the resistance against accumulation of trichothecenes (Mesterházy 1995; Miller et al. 1985). The three cultivars Canyon, Husky and Triton displayed a similar degree of fungal incidence and hence, a type III resistance was not detected. Nevertheless, Gagkaeva et al. (2011) noticed significant differences in *Fusarium sporotrichioides* DNA in oat grains among 105 genotypes, indicating the presence of type III resistance elements that remains to be investigated. In the present study, there was no significant weight loss or changes in TKW between the varieties and therefore, no detectable type IV resistance. Among the panel of oat cultivars, none of them was able to completely avoid contaminations with NIV or T-2/HT-2 (type V resistance), nevertheless, we observed significant differences in toxin accumulation between the genotypes. This result is consistent with Yan et al. (2010), previously suggesting that, to date, oat FHB resistance can only be reflected by type V resistance. However, the

authors detected only small differences in mycotoxin accumulation between the genotypes, and none displayed a high type V resistance. The general susceptibility of oat to toxin contamination was also reported in several previous studies on both modern oat varieties and oat landraces (Gagkaeva et al. 2011; Gagkaeva et al. 2013; Gavrilova et al. 2008; Tekauz et al. 2008; Loskutov et al. 2017). In the current study, we observed that resistance of oat genotypes to toxin contamination was strongly impacted by environmental conditions, with strong GxE interactions explaining 46% of NIV accumulation in grains infected with FP. This finding suggests a weak stability of resistance in oat genotypes which could impede the breeding for FHB resistance. Moreover, when comparing T-2/HT-2 and NIV content in FL and FP infected grains from Reckenholz, we noticed that the variety Triton did not accumulate NIV, whereas considerable amounts of T-2/HT-2 toxins (90 ppb) were detected. In fact, Edwards et al (2009) found no correlation between HT-2 and DON content in oat grains. Hence, it is likely that type V resistance depends on the nature of the toxin, and the QTLs associated with low DON accumulation (Bjørnstad et al. 2017; He et al. 2013) will not provide enhanced resistance to other mycotoxins. In our study, only the variety Samuel was resistant to both T-2/HT-2 and NIV across all field sites. This variety produces naked grains, suggesting a role of hulls in mycotoxin accumulation (Gagkaeva et al. 2011; Gavrilova et al. 2008; Tekauz et al. 2008). Interestingly, in grains from Samuel, we also measured high increases of β-glucan contents. We suggest that this reaction of the grain also contributes to the low accumulation of toxins.

Overall, the results presented here show that oats at the panicle level, are constitutively resistant to FHB. However, the pathogen colonises the glumes and grains depositing mycotoxins in the developing grain. Only a weak resistance against mycotoxin accumulation was observed, probably due to the absence of glumes (naked grains) in the variety Samuel. Improving resistance against mycotoxin accumulation may be achieved by the use of resistant wild oats (*Avena sterilis*) and other germplasms as new source of resistance (Bjørnstad and Skinnes 2008; Loskutov et al. 2017).

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Supplementary materials

Supplementary table: Average ± standard deviation of thousand kernel weight, β-glucan content and protein content for seven oat genotypes in three environments (Cadenazzo, Changins and Reckenholz), and presence of three treatments, FP: artificial inoculation with *F. poae*, FL: artificial inoculation with *F. langsethiae*, and absence of artificial inoculations (control).

Genotype	Treatment	Thousand kernel weight (g)	β-glucan content (%)	Protein content (%)
Cadenazzo				
Canyon	Control	36.80 ± 0.83	4.04 ± 0.53	11.37 ± 0.31
Expander	Control	32.57 ± 0.63	4.61 ± 0.37	12.67 ± 0.29
Husky	Control	32.53 ± 0.87	3.37 ± 0.39	13.03 ± 0.37
Melody	Control	43.37 ± 1.94	3.49 ± 0.50	12.47 ± 0.17
Poseidon	Control	35.43 ± 2.09	3.70 ± 0.25	11.70 ± 0.14
Samuel	Control	24.20 ± 1.69	2.66 ± 0.28	13.37 ± 0.38
Triton	Control	31.70 ± 0.96	3.56 ± 0.08	11.40 ± 0.22
Canyon	FP	37.37 ± 0.63	3.65 ± 0.47	12.53 ± 0.30
Expander	FP	31.83 ± 1.11	3.84 ± 0.41	12.99 ± 0.02
Husky	FP	33.43 ± 0.09	3.22 ± 0.12	12.87 ± 0.08
Melody	FP	34.90 ± 1.56	2.74 ± 0.55	12.82 ± 0.25
Poseidon	FP	36.63 ± 0.66	2.88 ± 0.39	13.10 ± 0.25
Samuel	FP	23.70 ± 0.45	3.97 ± 0.30	12.95 ± 0.04
Triton	FP	30.87 ± 1.03	3.72 ± 0.28	12.46 ± 0.35
Changins				
Canyon	Control	34.71 ± 0.35	3.28 ± 0.07	11.67 ± 0.27
Expander	Control	31.21 ± 1.06	3.36 ± 0.23	13.56 ± 0.36
Husky	Control	29.33 ± 2.60	3.08 ± 0.16	12.02 ± 0.43
Melody	Control	32.60 ± 0.22	3.46 ± 0.21	12.46 ± 0.19
Poseidon	Control	34.53 ± 1.52	3.85 ± 0.59	12.25 ± 0.71
Samuel	Control	23.00 ± 0.79	3.03 ± 0.79	13.01 ± 0.34
Triton	Control	35.20 ± 1.07	2.44 ± 0.22	12.52 ± 0.24
Canyon	FP	35.23 ± 1.76	2.97 ± 0.32	11.30 ± 0.72
Expander	FP	36.27 ± 0.39	4.40 ± 0.17	11.79 ± 0.19
Husky	FP	33.50 ± 1.06	3.34 ± 0.47	11.34 ± 0.44
Melody	FP	35.67 ± 0.41	2.85 ± 0.22	12.53 ± 0.41
Poseidon	FP	37.77 ± 1.24	4.05 ± 0.98	12.36 ± 0.69
Samuel	FP	24.23 ± 0.60	4.47 ± 0.37	14.22 ± 0.03
Triton	FP	36.77 ± 1.21	3.97 ± 0.17	14.34 ± 0.04
Reckenholz				
Canyon	Control	40.00 ± 0.41	3.79 ± 0.28	12.40 ± 0.24
Expander	Control	30.13 ± 2.20	4.27 ± 0.55	12.11 ± 0.24
Husky	Control	33.00 ± 1.85	3.37 ± 0.38	12.26 ± 0.17
Melody	Control	35.70 ± 2.62	3.94 ± 0.07	12.18 ± 0.09
Poseidon	Control	36.10 ± 2.12	3.72 ± 0.26	12.19 ± 0.34
Samuel	Control	22.63 ± 1.16	2.66 ± 0.28	12.18 ± 0.22
Triton	Control	35.90 ± 1.34	3.57 ± 0.30	12.15 ± 0.65
Canyon	FP	35.50 ± 0.78	3.52 ± 0.43	11.81 ± 0.29
Expander	FP	29.77 ± 1.60	4.16 ± 0.49	11.77 ± 0.16
Husky	FP	32.00 ± 0.99	3.65 ± 0.07	11.93 ± 0.29
Melody	FP	35.20 ± 0.00	3.45 ± 0.19	11.50 ± 0.40

Poseidon	FP	35.80	±	0.00	2.83	±	0.00	12.24	±	0.00
Samuel	FP	22.00	±	0.08	3.89	±	0.23	12.04	±	0.03
Triton	FP	35.37	±	1.55	4.03	±	0.54	12.10	±	0.37
Canyon	FL	40.25	±	0.20	2.87	±	0.00	12.37	±	0.09
Expander	FL	31.53	±	0.74	3.61	±	0.39	12.14	±	0.43
Husky	FL	33.83	±	1.47	3.68	±	0.19	12.11	±	0.05
Melody	FL	36.40	±	2.30	3.58	±	0.39	12.24	±	0.05
Poseidon	FL	36.67	±	1.70	2.76	±	0.00	12.23	±	0.07
Samuel	FL	22.50	±	1.02	4.10	±	0.36	12.32	±	0.10
Triton	FL	35.37	±	1.14	3.77	±	0.39	11.84	±	0.07

Conclusion and outlooks

Fusarium head blight (FHB) is one of the most noxious diseases in small grain cereals. Besides substantial yield losses, infectins by FHB causing *Fusarium* species seriously damage and contaminate the grains with mycotoxins. The purpose of this thesis was to understand whether health promoting compounds (HPC) might contribute the resistance of wheat, barley and oat varieties against *Fusarium* infections. The identification of resistance elements in grains required to precisely assess all potential impacts of FHB on grains, including fungal incidence, toxin content, impairment in grain filling and alterations of particular biochemical processes in the grain.

In the first chapter of this thesis, we investigated possible factors influencing the virulence of *F. graminearum* and *F. culmorum* strains within a population infecting wheat and maize. The understanding of strain virulence across different environmental conditions and the role of host resistance was subsequently used to adapt the artificial inoculations and to interprete the FHB symptoms in our field resistance tests of the following experiments. We observed that the environment has an impact on the virulence, with each strain reacting differently to environmental conditions. Consequently, we chose to use a mixture of strains, including virulence ones, for artificial inoculations in our multi-local experiments. That way, the effect of environmental conditions on individual strain virulence was taken into account, while mimicking a representative set of FHB causing *Fusarium* strains. We could also point out that strain virulence is independent from host resistance. This observation had two important consequences on the interpretation of the data from the following field experiments: (a) the resistance of a genotype is stable against all strains and (b) phenotypic variations of FHB symptoms are exclusively due to the resistance level of the genotype, the environmental conditions and the interactions between these two factors.

Various cereals contain phenolic acids, β-glucans and other health promoting compounds (HPCs). In the present thesis, the potential contribution of HPCs to increased FHB resistance in small grain cereals was investigated. This hypothesis is supported by recent studies demonstrating that several cereal endogenous HPCs can inhibit the growth and toxin synthesis of *Fusarium* species under *in vitro* conditions. We confirmed the relevance to investigate the

contribution of HPCs in resistance *in planta* under field conditions. In fact, we could show that ferulic acid (FA), which is present in wheat flower tissue during anthesis, but in variable amounts between genotypes, contributes to resistance. Resistance was significantly improved with FA contents of 0.5 $\mu mol.g^{-1}$ at flowering and 0.6 $\mu mol.g^{-1}$ at 10 days after flowering. We assume that FA in flower tissue, depending on the concentration, inhibits primary infection and propagation. However, even with the best genotypes, FA contributed less than 10% to the total resistance against FHB. Arguably, concentrations of FA in plant tissue are well below those inhibiting *Fusarium* growth under *in vitro* conditions. Nevertheless, as it is known that HPCs are mainly concentrated in grains, further investigations on the role of HPCs in grain resistance against *Fusarium* pathogens are required.

The resistance experiments were also used to elucidate the consequences of FHB on deoxynivalenol (DON) accumulation and yield reduction of wheat grains. By analyzing the alterations of the grain composition and the rheological properties after infection, we could propose a model of grain functions under *F. graminearum* infection. We suggest that *Fusarium* infections substantially disturb biochemical processes in grains including protein synthesis, starch and gluten formation, probably through a reduced supply with nutrients, sugars and other assimilates. In contrast to wheat, the accumulation of DON in barley grains was not directly linked to infection with *F. graminearum*. Yet, the presence of the pathogen in the grain seems to directly impair its filling. Similar to what we observed in wheat grains, FHB results in a degradation of the grain structure and composition, in particular by significant reductions of the β-glucan content. In oats, infections by the FHB species *F. poae* and *F. langsethiae* resulted in grain contaminations with T-2/HT-2 and nivalenol (NIV) although no impact on visual symptoms or grain quality was observed. The improvements provide thanks to this project in characterization of FHB impact on small grain cereals are summarize in the figure 1. The remaining gaps at the end of the project concern first the impact of the disease on the quality of barley grains, and on other components than β-glucan. The knowledge of the impact of the disease on oat grain properties (morphology, quality and processes) still have to be improved.

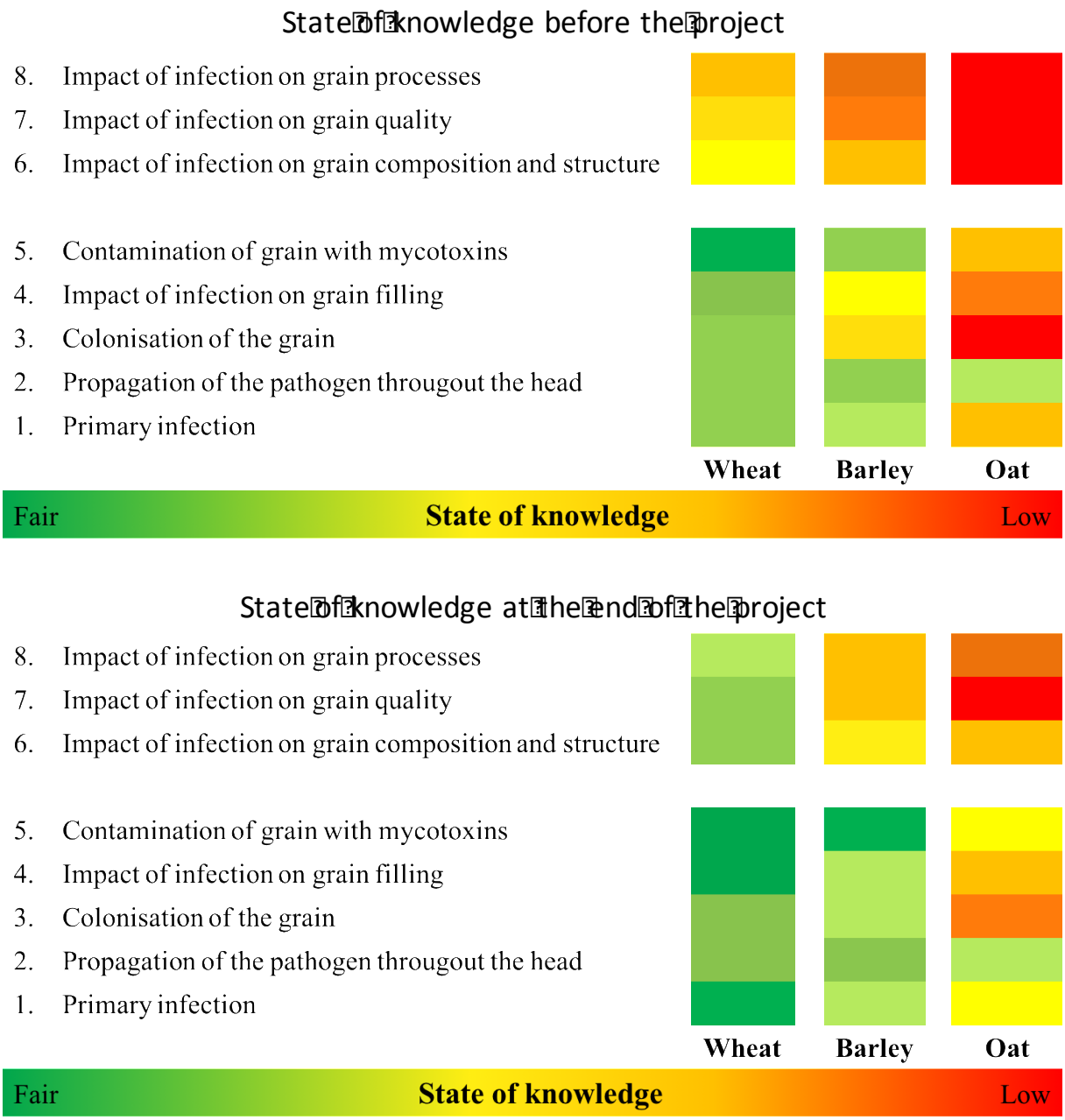


Figure 1. Comparison of the state of knowledge on the impact of *Fusarium* infection on wheat, barley and oat spikes (1–2) and grains (3–8) at the end of the project.

Based on the assessments of FHB impact on grains, the specific resistance elements of wheat, barley and oat grains were characterized and can now be compared. We demonstrated that wheat grain disposes of several mechanisms to protect itself against FHB infections including the resistance against alterations of grain constituents and functionalities. We identified an additional resistance type (named type VI), conferring resilience to changes of rheological and bread-making properties despite infection. Furthermore, we added new information about the functioning of resistance elements of barley grains. Type III resistance

(against kernel infection) and type IV resistance (against the reduction of grain filling) appeared to be strongly intermingled and are very likely based on common mechanisms. Type V resistance in barley was not related with any other resistance type. Thus, certain barley varieties can be highly contaminated by toxins while being only weakly infected with *Fusarium* pathogens. This differs fundamentally from wheat where the resistance against toxin accumulation is moderately correlated with the visible *Fusarium* damages on the grains. This has to be considered when breeding for barley varieties resistant to both FHB infection and toxin accumulation. Finally, and again in contrast with wheat, barley grain seems not to be able to protect its structure and composition, since the β-glucan content in all *Fusarium* infected grains was reduced. We revealed that oat grains dispose only of partial resistance against mycotoxin accumulation (type V resistance). In fact, all oat genotypes accumulated both NIV and T-2/HT-2 toxins, and none was able to completely avoid toxin contamination. As of now it is not clear which mechanisms are responsible for different mycotoxin contamination in different genotypes. This question has to be addressed when selecting oats for efficient toxin resistance. The resistance types known for wheat have been completed and reconsidered for barley and oats (table 1), providing an overview of the available resistance elements, ensuring protection against all the facets of FHB disease.

Table 1. Resistance types against Fusarium head blight in wheat, barley and oat observed in this project.

Resistance mechanisms	Resistance types	Wheat	Barley	Oat
Resistance against primary infection	**I**	✓	✓	✓
Resistance against disease spread throughout the spike	**II**	✓		
Resistance against kernel infection	**III**	✓	✓	
Tolerance to kernel damage despite infection (grain filling)	**IV**	✓		
Resistance against mycotoxin accumulation	**V**	✓	✓	✓
Resilience of grain quality towards infection	**VI**	✓		

Since FA improves FHB resistance in wheat spikes, it is pertinent to further study the role of phenolic compounds with antioxidant activity, and other HPCs in disease resistance. Our results showed that β-glucans reduce DON in barley grains, while anthocyanins did not

have an influence on the resistance of wheat grains. Elevated β-glucan content protected barley grains against accumulation of DON and may be a constitutive element of type V resistance (resistance against accumulation of mycotoxins). Yet, accumulation of toxins in oat grains was not influenced by the β-glucan content.

All wheat, barley and oat field experiments followed the same experimental design and most of them were planted side by side. This experimental setting allowed us to compare the efficiency, stability and heritability of resistance elements between these different cereal types. Generally, wheat was the most susceptible cereal to FHB with strong symptoms on the spike, severe damages on the grains and high toxin contents. However, in wheat we found also the largest spectrum of resistance elements. The resistance was characterized by a high diversity among the chosen wheat genotypes and a relative stability across a wide range of environmental conditions. Arguably, barley appeared to be more resistant to FHB than wheat, with less symptoms and lower toxin contents. The available resistances in the grain were limited to toxin accumulation and fungal incidence in grains. The barley genotypes displayed less variation in resistance and, in contrast to wheat, was also more influenced by environmental conditions. The chosen oat genotypes showed less variability in terms of *Fusarium* resistance. Besides the accumulation of mycotoxins in oat grains and minor variations in β-glucan content, no other alterations have been detected on the plant or in the grains. A significant lower mycotoxin content was detected in grains from naked oats, suggesting an absence of efficient resistances against mycotoxin accumulation in the grain. The heritability of resistance evaluates the share of genetic variation in the expression of a measurable phenotypic character of resistance. For wheat, the heritability represents the capacity of a wheat population to react to selection. We were able to estimate and compare the heritability of resistance within our panels of wheat, barley and oat varieties. The phenotypic variations in the toxin content (DON for barley and wheat, NIV for oats) and in grain weight reduction are described in tables 2 and 3, respectively. The estimated heritability of resistance against mycotoxin accumulation and reduction of grain weight was highest in wheat where genetic factors controlled 45% and 55% respectively. The heritability of resistance traits in barley was lower. Yet genetic effects still explained 30% for both traits of the phenotypic variation. In oats, the heritability of resistance against the mycotoxin NIV was weak and was largely masked by GxE interactions (table 2).

Table 2. Comparisons of the share of environmental and genetic factors and their interactions, in determining the phenotypic resistance against accumulation of toxins between wheat (DON), barley (DON) and oats (NIV). The share explained by genetic factors estimates the heritability of the trait within our panel of chosen varieties.

Source of phenotypic variation in toxin contamination	**Wheat**	**Barley**	**Oat**
Environmental factors	26%	21%	2%
Genetic factors	44%	30%	25%
G x E interactions	22%	30%	45%
Residuals	8%	19%	28%

Table 3. Comparisons of the share of environmental and genetic factors and their interactions, in determining the phenotypic resistance against reduction of grain weight between wheat and barley. No effect on grain weight was observed in oat varieties. The share explained by genetic factors estimates the heritability of the trait within the reduced population.

Source of phenotypic variation in grain weight reduction	**Wheat**	**Barley**
Environmental factors	15%	25%
Genetic factors	55%	21%
G x E interactions	16%	29%
Residuals	14%	25%

Heritability indicates also the interest to select for resistance. The higher the heritability is, the higher the expected genetic gain will be. The GxE interactions are thus the part of resistance that cannot be selected. Hence our last results allow us to prioritize objectives of selection for FHB resistance in the different species. Overall, wheat was the most susceptible cereal to *Fusarium* infection and toxin accumulation. However, the elevated heritability of resistance highlights the high genetic gain that can be expected when selecting wheat for FHB resistance. In barley, the genetic gain was partially masked by strong GxE interactions. Still, breeders can obtain increased resistance when selecting tolerance of breeding lines under differing environmental conditions. By this, it is possible to exclude the GxE interactions and to focus on the heritable part of resistance variability. In modern and ancient oat varieties, we detected only little variability to the accumulation of mycotoxins. Heritability was therefore weak and masked by GxE interactions. Hence, for oats, we recommend to use wild oats (*Avena sterilis*) to introduce new sources of resistances.

Throughout this thesis we demonstrated that wheat, barley and oat grains possess several elements to protect themselves against *Fusarium* infections. Some HPCs in grains partially contribute to resistance, however, enhancing HPC contents in grains will not drastically limit *Fusarium* infections. However, it allows to develop new varieties that combine elevated HPC content and higher resistance to toxins.

List of presentations and posters

List of presentations

- « Comparing impact of host species and environment on virulence in *Fusarium graminearum* and *F. culmorum*» *Zurich Mycology Symposium 2015*

- « Resistance against Fusarium Head Blight in wheat varieties with colored grains » *European Fusarium Seminar 13th, 2015, Martina Franca Italy*

- « Etude de la résistance contre la fusariose dans le blé et l'orge » *Pflanzenschutztagung Feldbau 2015 Murten*

- «*Fusarium* resistance screening in oat, barley and wheat varieties; identification of resistance factors » *NRP 69 Site visit Reckenholz, 2016*

- « Les antioxydants du blé: les couleurs de la résistance *» Colloque Scientifique de Changins 2015*

- « Phenotyping Fusarium Head Blight resistance of oat by analysis of morphological and biochemical properties of grains *» International Oat Congress 2016, St Petersburg, Russia*

- « Phenotyping Fusarium Head Blight resistance of oat by analysis of morphological and biochemical properties of grains *» Journée annuelle Société Suisse d'Agronomie 2016, Zolikofen*

- « Le β-glucan dans les ressources génétiques suisses de l'orge et de l'avoine » *Journée de la recherche agronomique AGRIDEA*

List of posters

- « Des céréales bénéfiques pour la santé, des céréales sûres et sécures ? » *Grandes cultures : Dernières nouvelles de la recherche à Changins 2014*

- « La résistance de type II contre la fusariose de l'épi dans la population de mappage Toronit x 211.12014 » *Journée de la société Suisse de Phytiatrie 2014*

- « Effect of lutein on resistance against Fusarium Head Blight in the Toronit x 211.12014 mapping population » *Journée de la société Suisse de Phytiatrie 2015*

- «Health promoting compounds promote Fusarium head blight resistance in cereals » IFCN 2015

- «Health promoting compounds promote Fusarium head blight resistance in cereals» *PHD symposium ETH Zurich 2016*

- « Impact of *Fusarium* infections on ß- glucans in barley grains » *EUCARPIA 2016 Zurich*

- « Phenotyping Fusarium Head Blight resistance of oat by analysis of morphological and biochemical properties of grains » *EUCARPIA 2016 Zurich*

- « Impact of *Fusarium* infections on ß- glucans in barley grains » *Journée annuelle Société Suisse d'Agronomie 2016, Zolikofen*

- « Impact of Fusarium infections on ß- glucan in barley grains » *PHD Symposium Plant Science 2016, ETH Zurich*

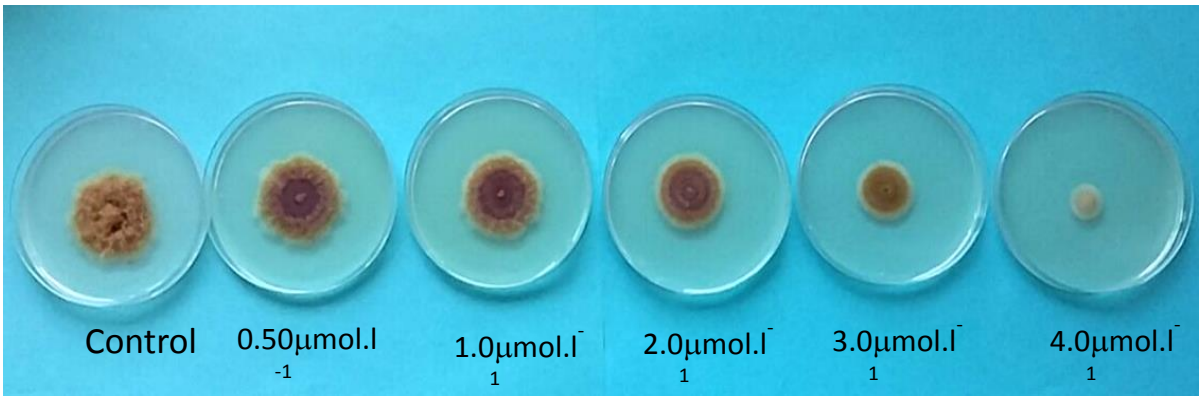
- « Impact de *F. langsethiae* et *F. poae* sur les grains d'avoine » *Journée Bio Grandes Cultures, Cournillens, 2017*

- « Role of ferulic acid in Fusarium head blight resistance of wheat spike » *Future IPM 3.0, Riva del Garda, Italy,2017*

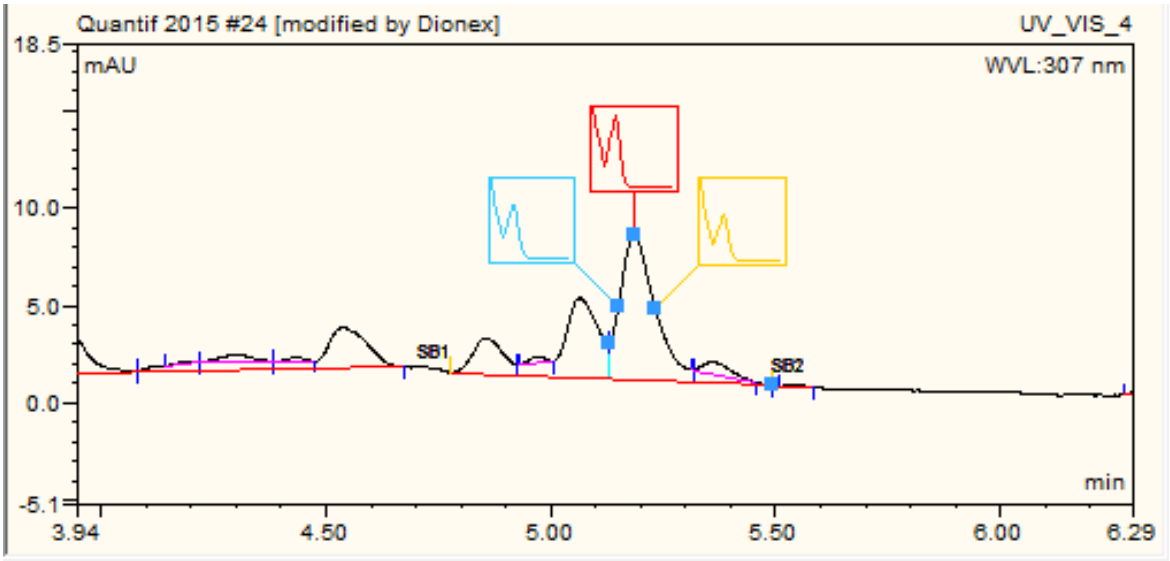
Annexes

Table of annexes

Annex 1. Visualisation of inhibitive effect of ferulic acid on FG13 growth in PDA media, 6 days post inoculation. The FA concentration added in the media are indicated below the dishes.



Annex 2. Example of chromatogram for ferulic acid detection and quantification in wheat flower tissues, monitored at 307 nm, retention time 5.185min.



Annex 3. Beer-Lambert equation for cyanidin chloride quantification via spectrophotometry.

$$C = \left(\frac{A}{\varepsilon}\right) * \left(\frac{vol}{1000}\right) * MW * \left(\frac{l}{sample\ WT}\right) * 10^6$$

With

C= Cyanidin chloride concentration ($mg.kg^{-1}$)

A = Absorbance monitored

ε = Molar absorption coefficient

vol = Total volume of solution of extraction

MW = Molar weight

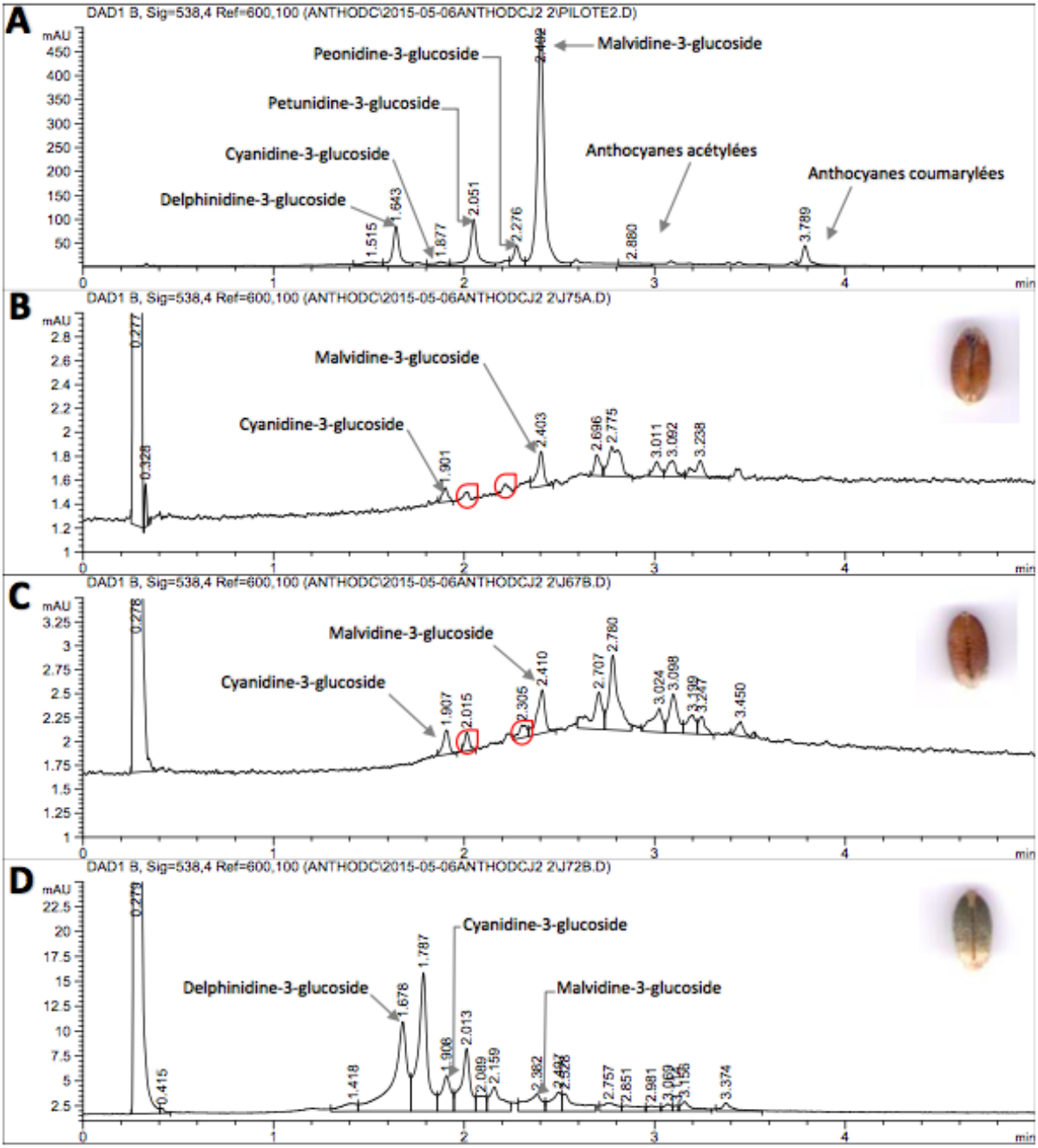
l = width of the cuvette (= 1 cm)

sample WT = flour quantity (g)

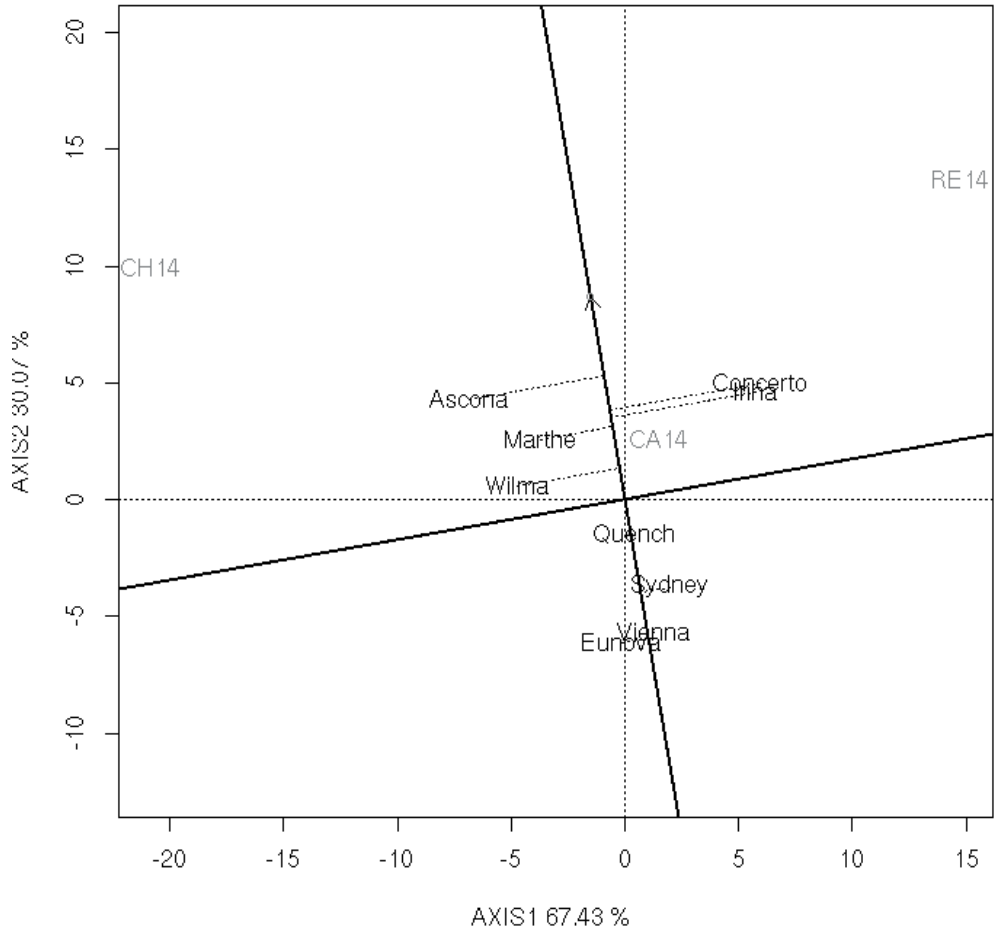
And

	λ_{max} (nm)	ε ($cm^{-1}.M^{-1}$)	MW ($g.mol^{-1}$)
Cyanidin chlorid	538 nm	34500	322.7

Annex 4. Chromatograms of wine (A), extracts of whole meal flour of Vanoilnoir (B), Indigo (C) and Purendo (D), for anthocyanin compounds identification, monitored at 538nm (HPLC-DAD). The anthocyanin compounds are identified by comparisons of anthocyanin chromatograms in wine.



Annex 5. Relationships between DON contents of 9 spring barley varieties after artificial inoculations with *F.graminearum* in 3 environments. The GGE biplot is based on environment centered (centering=2), no scaling, and singular value partitioning environment focused (SVP=2)

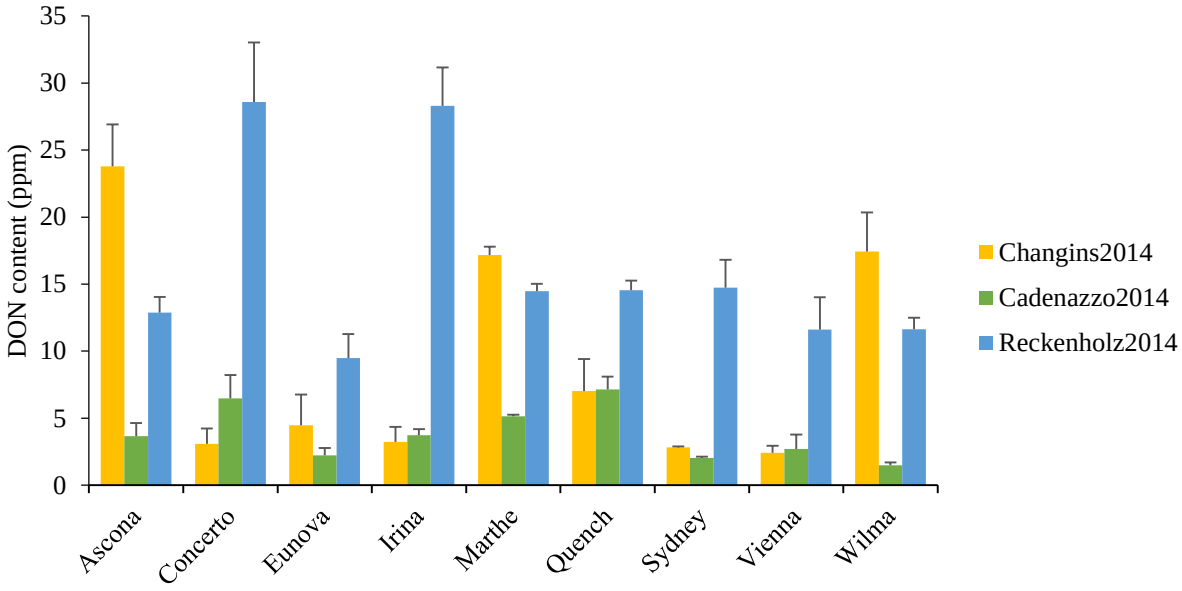


Abbreviations for environments : CA14= Cadenazzo 2014, CH14= Changins 2014, RE14= Reckenholz 2014.

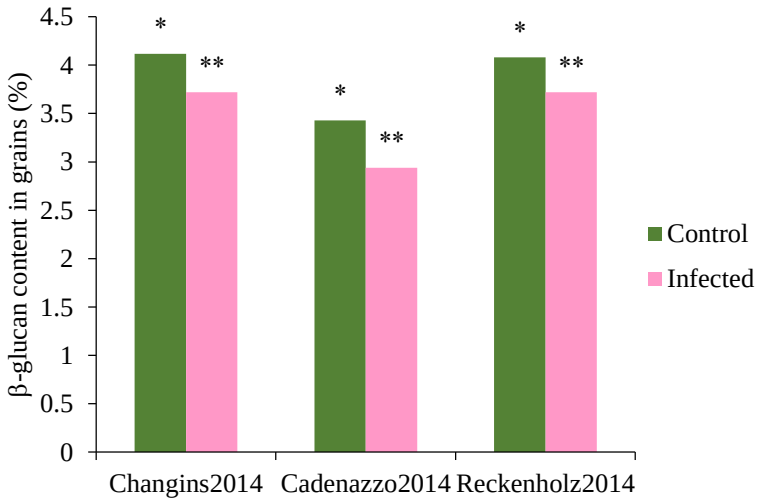
Annex 6. Two-way analysis of variance of DON content in spring barley grains of 9 varieties in three environments after artificial inoculations of *F.graminearum*. The statistical design follows a split-plot design according to the experimental design. Signif: "***": P<0.001.

Source of variation	Sum Square	Mean Square	
Environment	57.85	28.9	***
Error (a)	0.27	0.067	
Variety	18.13	2.26	***
Variety x Environment	44.63	2.78	***
Error (b)	5.83	0.12	

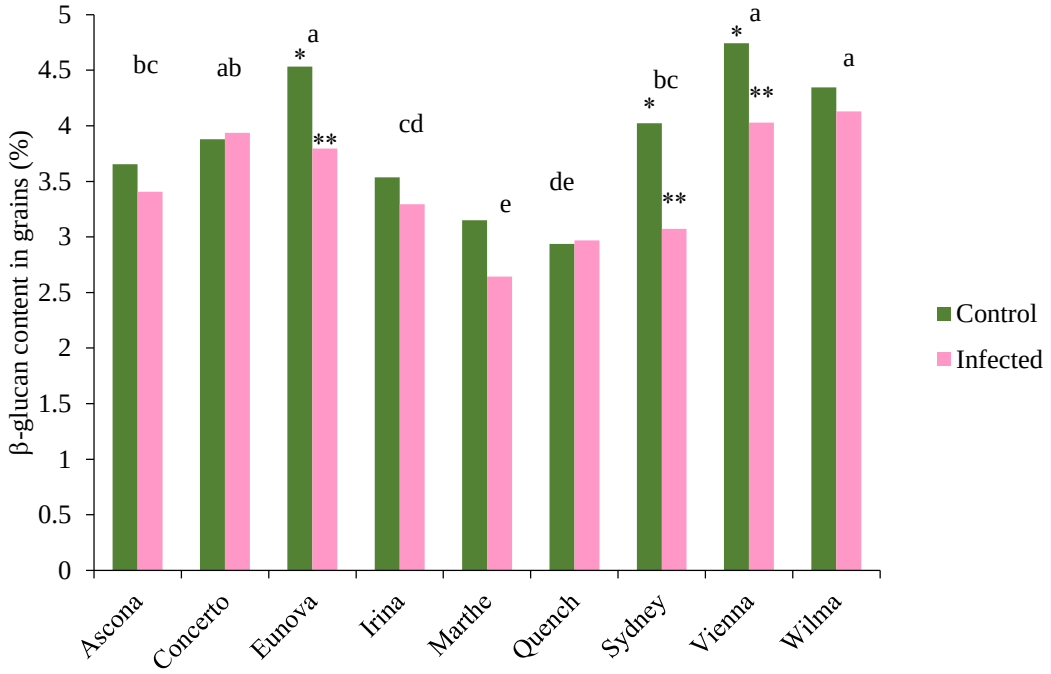
Annex 7. Averages of DON contents in 9 spring barley varieties after artificial inoculations with *F.graminearum* in 3 environements.



Annex 8. Comparisons of β-glucan contents in *F.graminearum* infected and non-infected grains of spring barley in three environments. The bars indicate the averages overall 7 spring barley varieties. Different numbers of "*" indicates significant differences in the β-glucan contents between infected and non-infected grains within one environments, according to Tukey Honest Significant Difference (α=0.05).



Annex 9. Comparisons of β-glucan contents in *F.graminearum* infected and non-infected grains of spring barley of 9 varieties. The bars indicate the averages overall 3 environments. Different numbers of "*" indicates significant differences in the β-glucan contents between infected and non-infected grains within one variety, different letters indicate significant differences in b-glucan content between varieties overall environments and presence of infection, according to Tukey Honest Significant Difference (α=0.05).

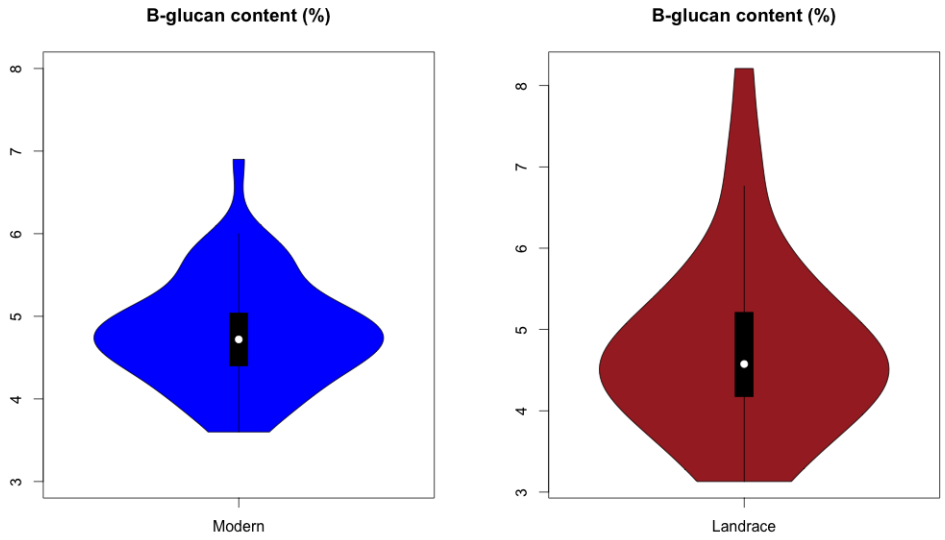


Annex 10. Three ways analysis of variance of β-glucan contents in spring barley grains of 9 varieties, growing in 3 environments, with presence or not of artificial infections with *F.graminearum*. The statistical design follows a split split plot according to the experimental design. Signif: "***": P<0.001, "**": P<0.01.

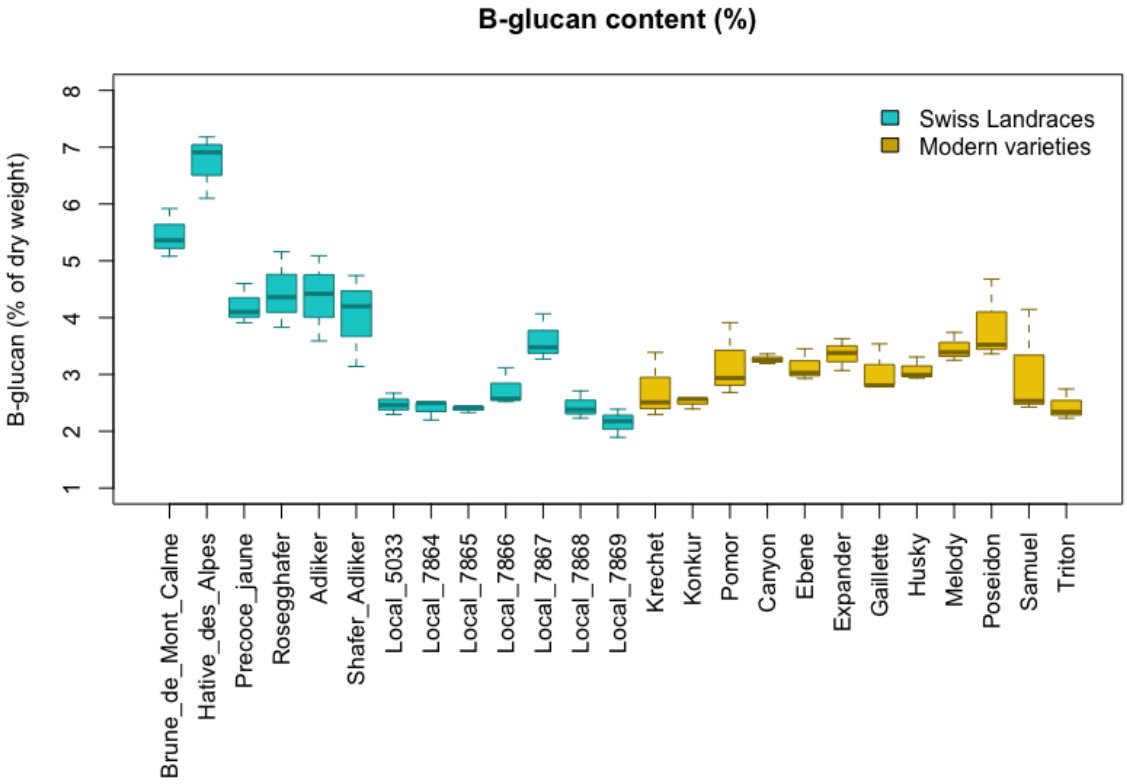
Source of variation	Sum of Square	Mean Square	
Environment	18.8	9.4	**
Error(a)	0.74	0.19	
Inoculation	6.9	6.9	**
Environment x Inoculation	0.1	0.05	
Error(b)	1.813	0.3	
Variety	39.7	5	***
Environment x Variety	13.5	0.85	***
Inoculation x Variety	5	0.63	**
Environment x Inoculation x Variety	10.9	0.68	***

Error (c)	20.6	0.21

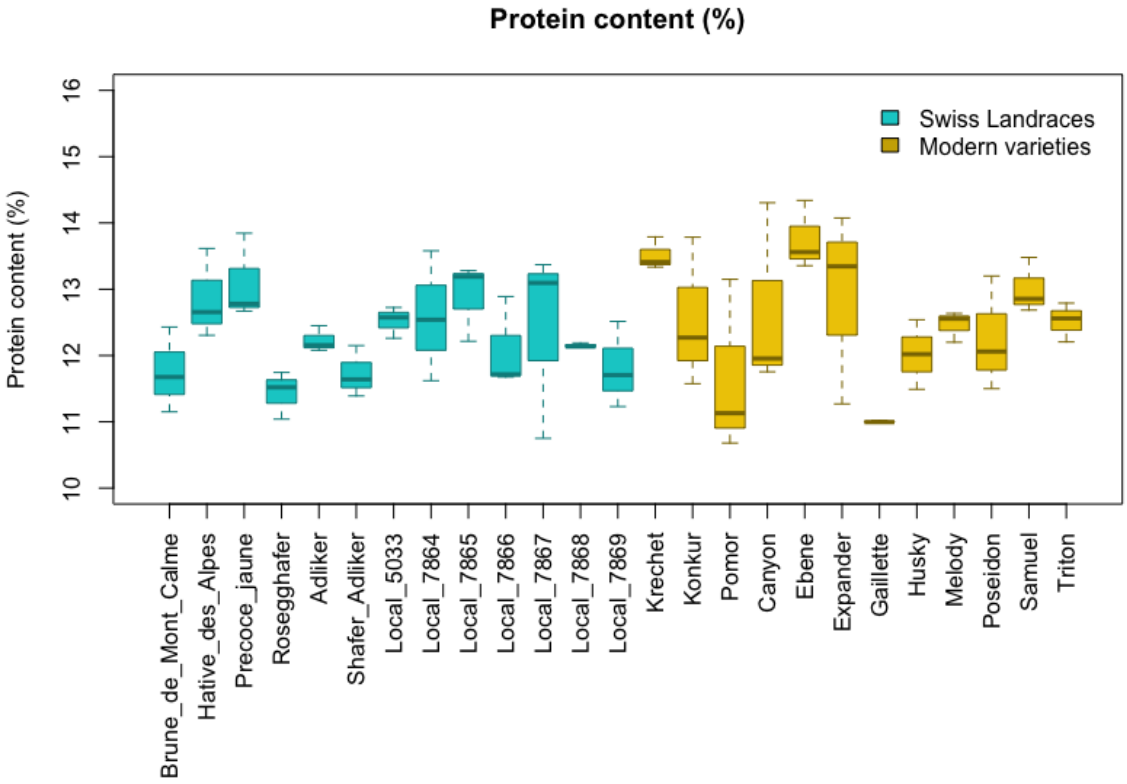
Annex 11. Comparison of β-glucan content in oat grains between a panel of 9 modern and European oat varieties and 6 Swiss landraces collected before 1940. Three repetitions were available for all varieties, and grains were harvested in field in Changins 2015. These violplots represent the higher variability in β-glucan in grains of Swiss landraces compared to modern varieties.



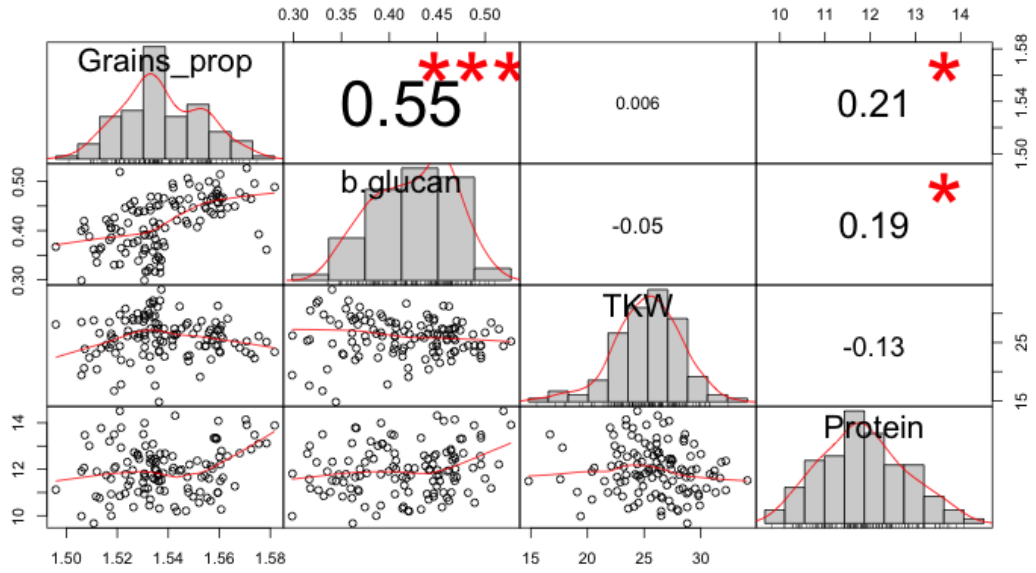
Annex 12. Boxplots of β-glucan contents in oat grains of Swiss Landraces (13 genotypes) and Modern varieties (12 genotypes). Grains were all harvested in Chanigns 2015 and 3 repetitions were available.



Annex 13. Boxplots of protein contents in oat grains of Swiss Landraces (13 genotypes) and Modern varieties (12 genotypes). Grains were all harvested in Chanigns 2015 and 3 repetitions were available.

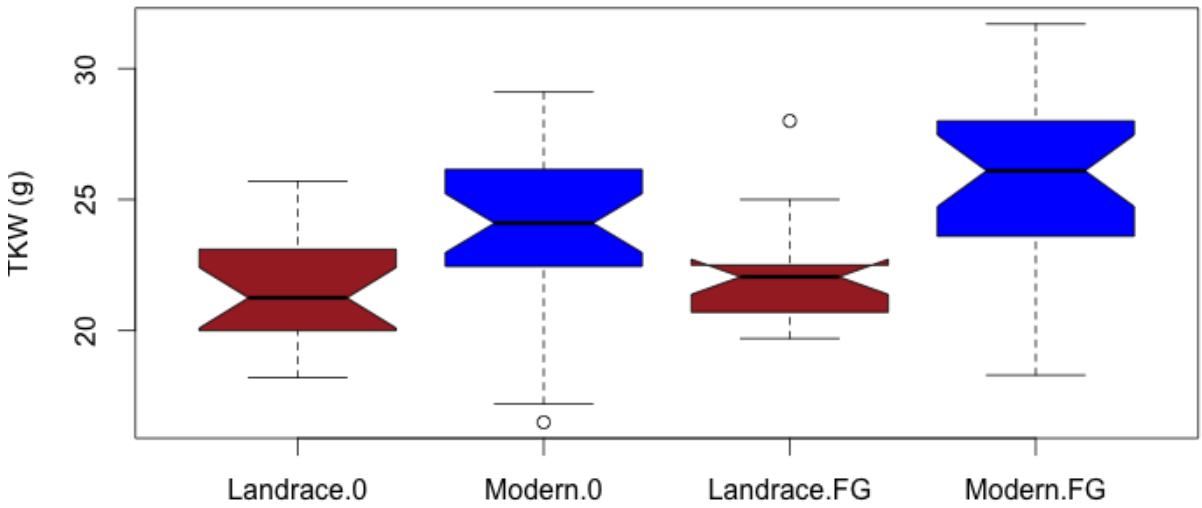


Annex 14. Pearson coefficient correlations (in the upper right part of the figure) and distributions of data (lower left part) between four descriptors of oat grains: proportion of grains compared to proportion of hulls (Grains_prop), b-glucan (b-glucan) and protein contents, Thousand Kernel Weight (TKW). The panel of grains described are constituted of 15 oat varieties (9 modern and 6 Swiss Landraces), growing in Changins in 2015. Three repetitions were available for all varieties.

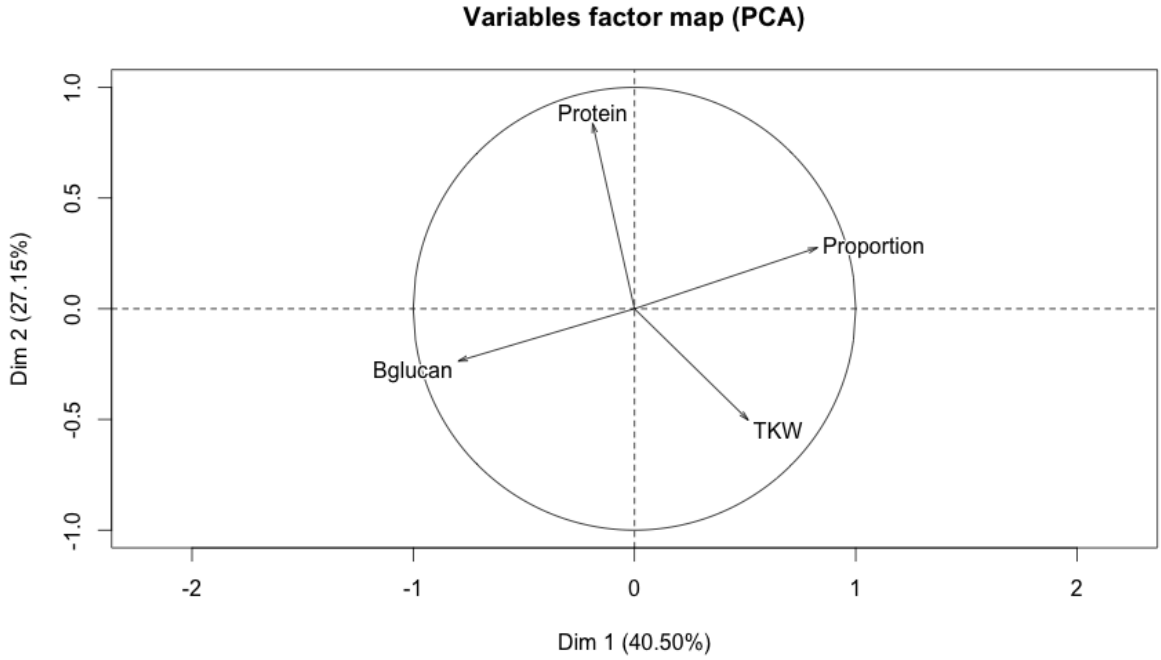


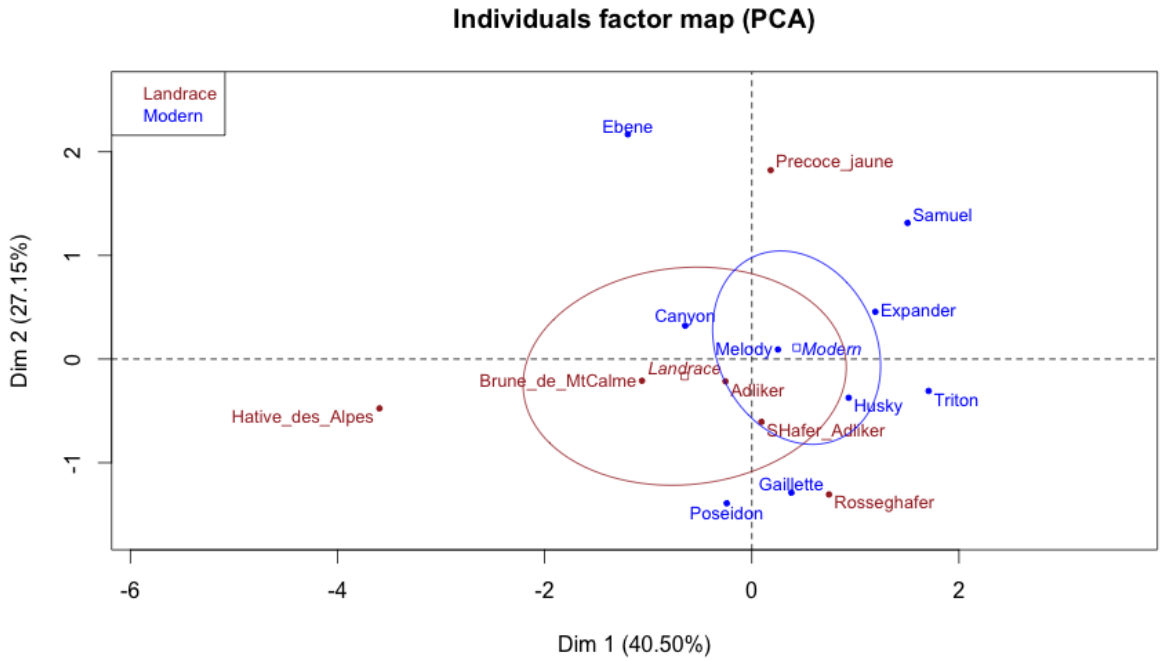
Annex 15. Absence of visual symptoms on oat grain of Husky (modern variety), and Rosseghafer (Swiss Landrace) after field artificial inoculations with *F.graminearum* strains in Changins 2015.

Annex 16. Comparison of Thousand Kernel Weight of oat grains infected or not with F.graminearum strains (FG) and between Swiss landraces (brown) and modern varieties (blue). Basically. Modern varieties had higher TKW than landraces. A slight increase of modern varieties TKW was noticed in case of infection.

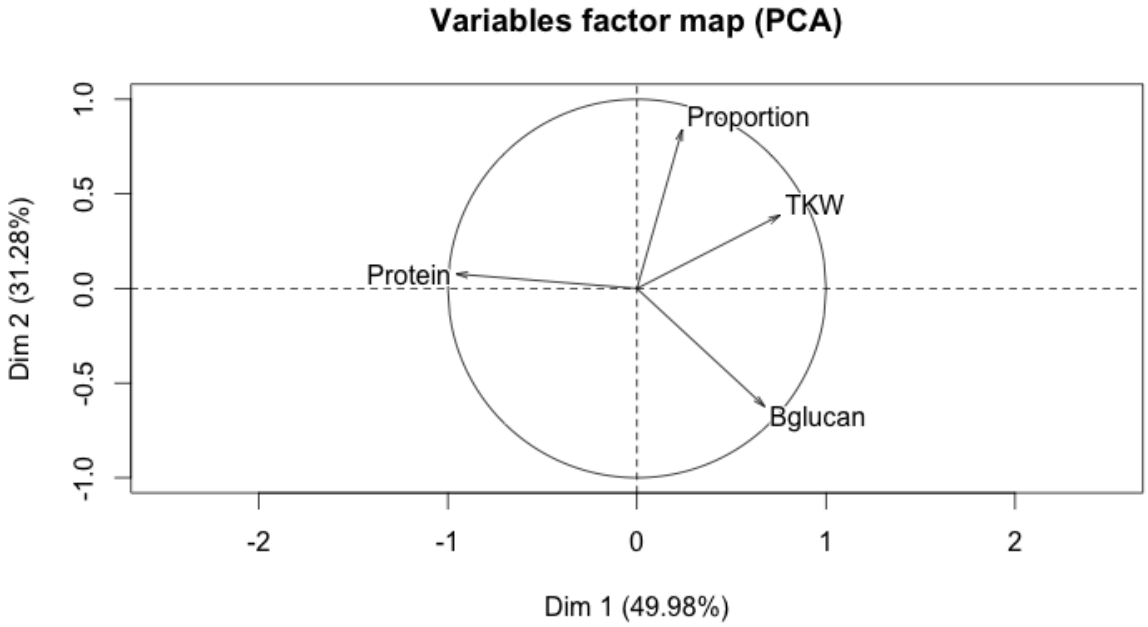


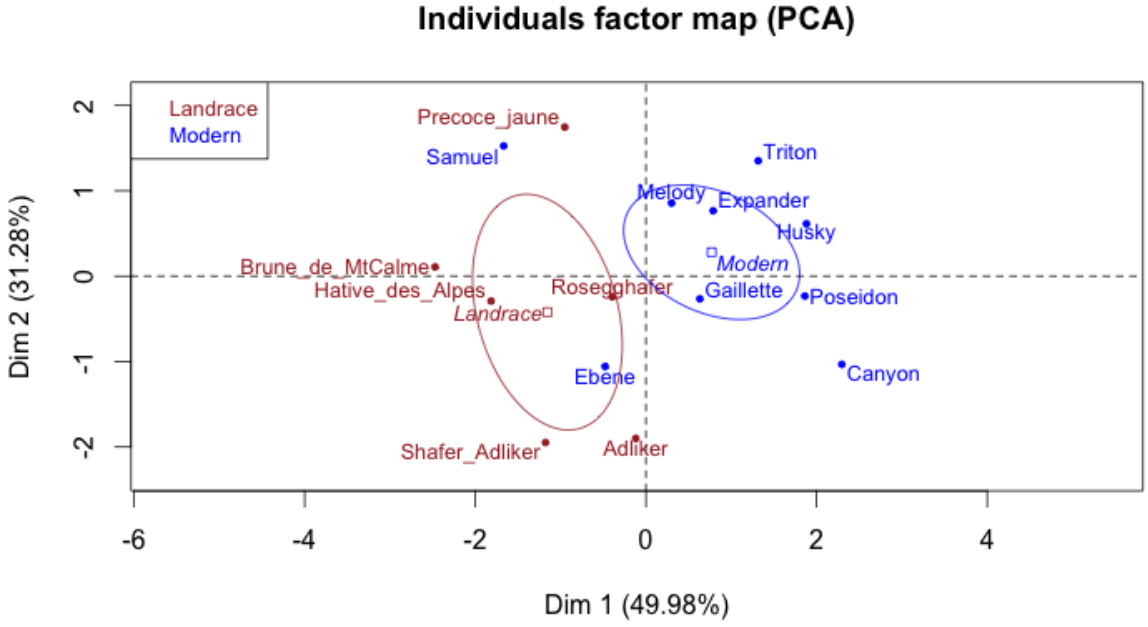
Annex 17. Principal component analysis of 15 oat varieties (9 modern European varieties and 6 Swiss landraces), characterized in the variable factor map by their protein and b-glucan contents, Thousand Kernels Weight (TKW), and proportion of grain compared to the proportion of hulls (Proportion). Data are the averages of three repetitions available for all varieties. The varieties are represented in the Individual factor maps, together with the concentration ellipses for both modern and Swiss landraces varieties. All grains were harvested in Changins 2015.



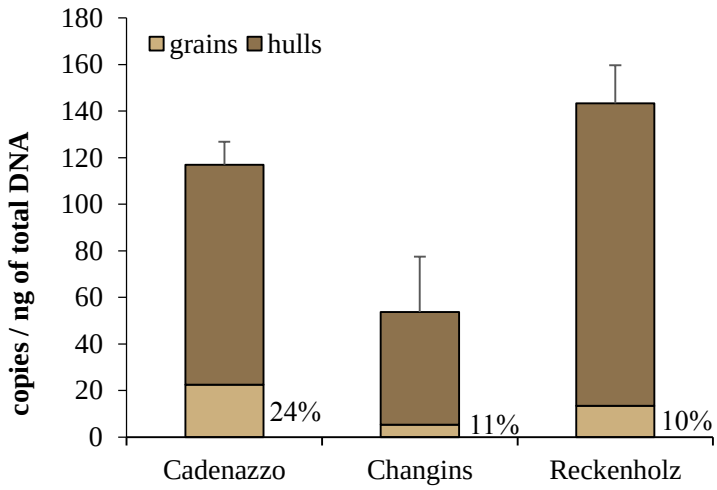


Annex 18. Principal component analysis of 15 oat varieties (9 modern European varieties and 6 Swiss landraces) artificially inoculated with F.graminearum strains, characterized in the variable factor map by their protein and b-glucan contents, Thousand Kernels Weight (TKW), and proportion of grain compared to the proportion of hulls (Proportion). The varieties are represented in the Individual factor maps, together with the concentration ellipses for both modern and Swiss landraces varieties. All grains were harvested in Changins 2015. By comparison with **Annex 18,** these figures illustrate the different responses of modern varieties and Swiss landraces toward Fusarium infection. Infected grains of landraces were characterized by increases of protein contents and reductions of both TKW and proportion of grains. On the contrary, infected modern varieties maintained TKW and proportion of grains in case of infection.

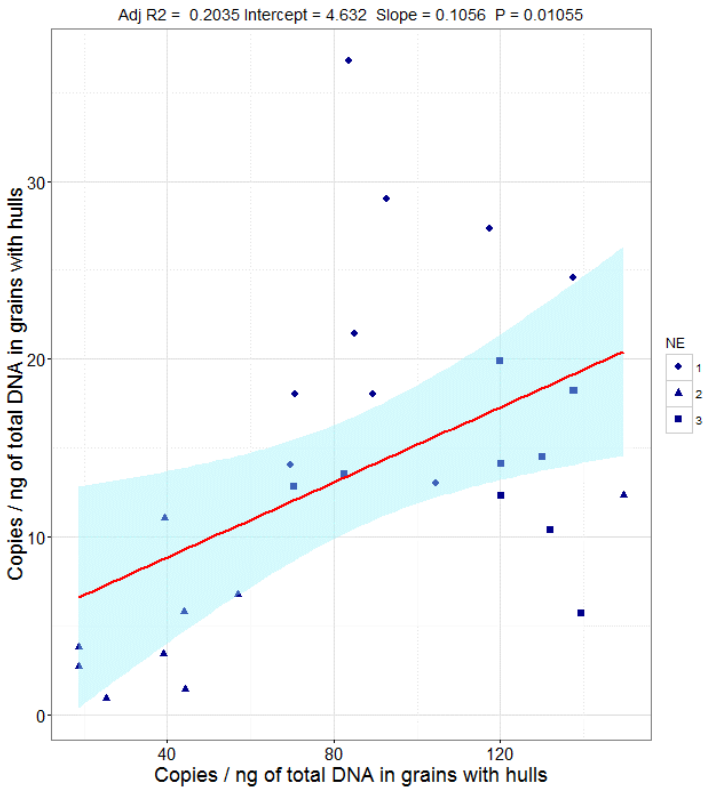




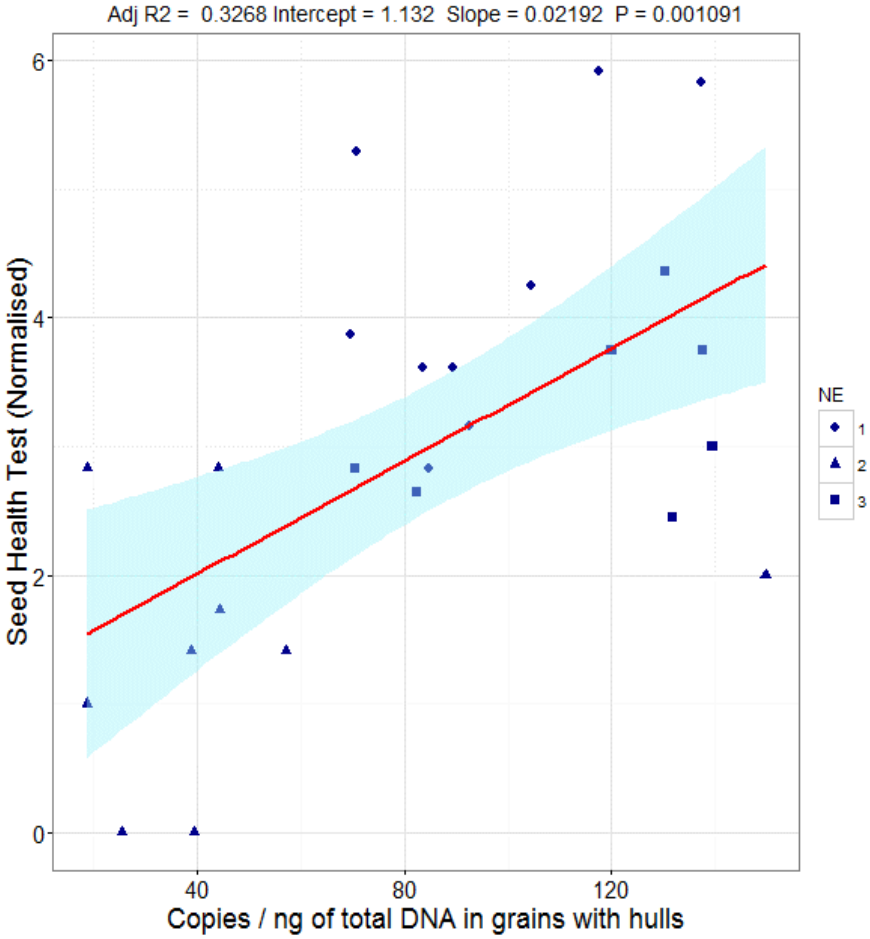
Annex 19. Analysis of presence of F.poae DNA in oat grains and hulls in three environments after artificial inoculations as presented in part V of the thesis. The averages overall three varieties (Canyon, Husky, Triton) are presented. The percentages indicates represent the part of fungal DNA measured in grains. Basically, Fusarium pathogen is mainly present in hulls and only weakly colonises oat grains.



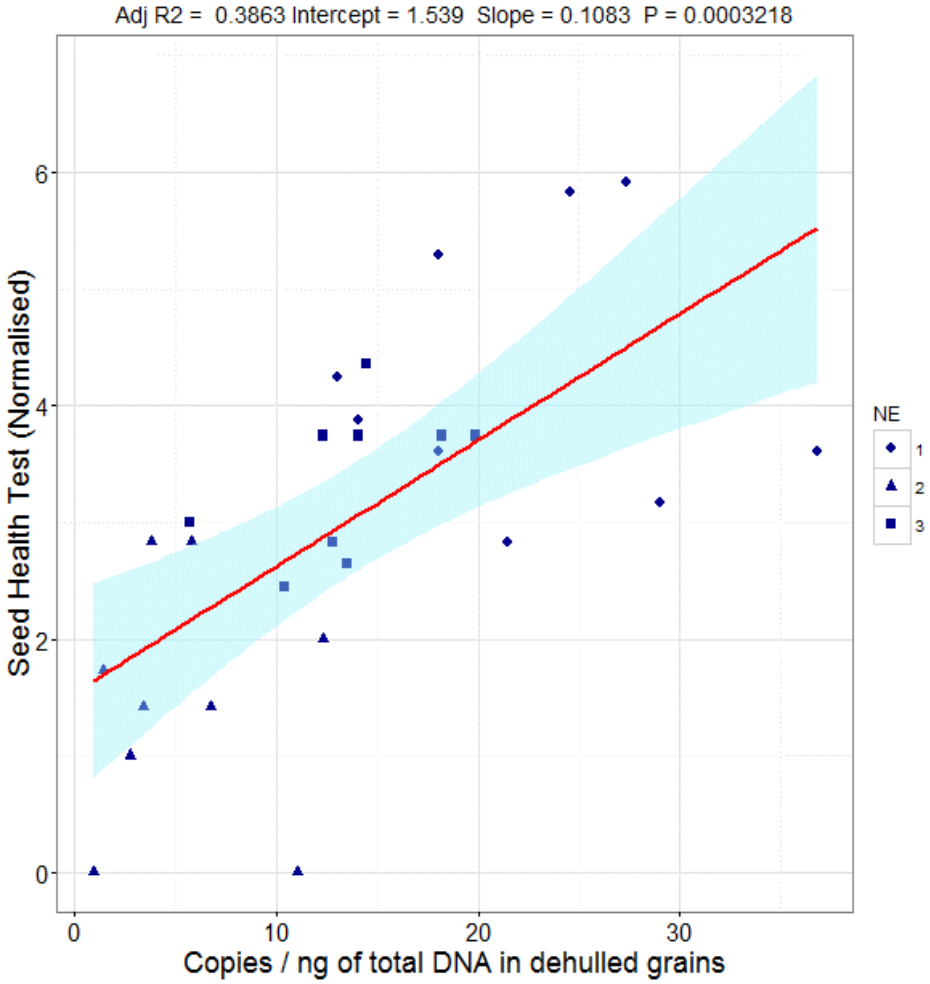
Annex 20. Linean model between F.poae DNA quantities in oat grains with hulls and dehulled grains. The analysed samples are oat grains of three varieties (Canyon, Husky, Triton), from three environments and artificially inoculated as presented in the part V of this thesis. Three repetitions were available for all varieties in all environments. The different environments were illustrated by different point shapes. NE: 1= Cadenazzo, 2=Changins, 3=Reckenholz.



Annex 21. Linear model between proportion of grains colonised by F.poae (data from Seed Health Test square root transformed), and fungal DNA quantity in oat grains with hulls. The analysed samples were oat grains of three varieties (Canyon, Husky, Triton) growing in three environments (Cadenazzo, Changins and Reckenholz 2015) and artificially inoculated as presented in part V of this thesis. Three repetitions were available for all varieties in all environments. The different environments were illustrated by different point shapes. NE: 1= Cadenazzo, 2=Changins, 3=Reckenholz.



Annex 22. Linear model between proportion of grains colonised by F.poae (data from Seed Health Test square root transformed), and fungal DNA quantity in dehulled oat The analysed samples were oat grains of three varieties (Canyon, Husky, Triton) growing in three environments (Cadenazzo, Changins and Reckenholz 2015) and artificially inoculated as presented in part V of this thesis. Three repetitions were available for all varieties in all environments. The different environments were illustrated by different point shapes. NE: 1= Cadenazzo, 2=Changins, 3=Reckenholz.



Annex 23. Type II resistance of double haploid lines from the mapping population « Toronit x 211.12014 » respectively FHB resistant and susceptible parents, evaluated by averages ofAUDPC overall six repetitions. The repartitions of AUDPC indicates that type II resistance is horizontal and based on cumulative effects of several genes partly inherited. Lines with higher type II resistance than Toronit and can be noticed.