Fusarium infection of bread wheat and subsequent mycotoxin contamination of milling products: Impact on quality parameters and composition of flour

Doctoral Dissertation

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by

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Abbreviations

15-ADON	15-acetyldeoxynivalenol
3-ADON	3-acetyldeoxynivalenol
AU	absorption units
BEA	beauvericin
BMEVL	Bundesministerium für Ernährung, Verbraucherschutz, Landwirtschaft
BSA	Bundessortenamt (Federal Office of Plant Varieties)
BV	baking volume
bw	bodyweight
CIMMYT	International Maize and Wheat Improvement Center
cv.	cultivar
DAS	diacetoxyscirpenol
DDT	dough development time
DNA	deoxyribonucleic acid
DON	deoxynivalenol
DON_{LOG}	logarithmically transformed DON content
DON_{ORG}	original DON content
DS	dough softening
DST	dough stability
DTR	*Drechselera tritici-repentis*
ELISA	Enzyme linked immunosorbent assay
EU	European Union
EU_{Max}	EU maximum levels
EU_{MaxCF}	EU_{Max} for cereal flour
EU_{MaxUP}	EU_{Max} for unprocessed cereals
EXT	extensibility
FAEN	Forschungsverbund Agrar- und Ernährungswissenschaften Niedersachsen
FAO	Food and Agriculture Organization of the United Nations
FB_1	fumonisin B_1
FDK	*Fusarium* damaged kernels
FHB	*Fusarium* head blight
FN	falling number
FPU	*Fusarium* soluble protein units
FUP	fusaproliferin
FUSX	fusarenone X

GC	gas chromatograph
GMF	Vereinigung Getreide-, Markt-, und Ernährungsforschung
GY	grain yield
HMW	high molecular weight
HMW-GS	high molecular weight glutenin subunits
HPLC-MS/MS	high performance liquid chromatography with tandem mass spectrometry
ICC	International Association for Cereal Science and Technology
LH	loaf height
LL	loaf length
LMW	low molecular weight
LMW-GS	low molecular weight glutenin subunits
LOD	limit of detection
LOQ	limit of quantification
LW	loaf width
MON	moniliformin
MV	mean value
MW	molecular weight
N	nitrogen
n	number of observations
NIV	nivalenol
PA	protease activity
PAGE	polyacrylamide gel electrophoresis
PC	protein content
PCR	polymerase chain reaction
$PC_{Type550}$	protein content of flour Type 550
PC_{WGF}	protein content of whole grain flour
QTL	quantitative trait loci
RH	relative humidity
R_{MAX}	resistance to extension
RP-HPLC	reversed-phase high performance liquid chromatography
S	sulfur
SBA	Statistisches Bundesamt
SD	Standard diviation
SDS	sodium dodecylsulfate
SE	standard error
SE-HPLC	size-exclusion high performance liquid chromatography

SoE	severity of effect
SV	sedimentation value
T2	T-2 toxin
TA	Texture Analyzer
TDI	tolerable daily intake
TDI	tolerable daily intake
TKW	thousand-kernel weight
T_{Max}	maximum daily temperature
T_{Mean}	average daily temperature
T_{Min}	minimum daily temperature
WA	water absorption
WG	wet gluten
WGF	whole grain flour
WSG	Wetterstation Göttingen
ZEA	zearalenone

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Preface

This study was conducted within the subproject 2 “Influence of fungicide treatment, pre-crop, cultivar susceptibility and *Fusarium* spp. infection on nutritional and processing quality of bread wheat” of the cooperation project Forschungsverbund Agrar- und Ernährungswissenschaften Niedersachsen (FAEN) and financed by the Ministry of Science and Culture of Lower Saxony, Germany. The influence of the agronomical factors such as cultivar, crop rotation, and fungicide treatment on *Fusarium* infection level and DON accumulation in wheat grain was mainly investigated by the cooperation partners from the subproject 1 “Agronomic factors influencing the mycotoxin accumulation in *Fusarium* head blight infected wheat in different crop rotation regimes”, Phytopathology and Plant Protection, Department of Crop Sciences, Georg-August-University Göttingen. The data and results of this project`s work have been published so far as PhD thesis by GÖDECKE 2010. Since there seems to be some interference between the objectives of this study and from GÖDECKE 2010, it has to be emphasized that even though samples came from the same field trial sampling (composite sample vs. individual sample) and sample preparation (cleaning vs. no cleaning) was different and in accordance with the respective focus of the projects. Additionally, mycotoxin determination was performed with different analytical methods (HPLC-MS/MS vs. ELISA).

1 Introduction

1.1 Importance of wheat in human consumption

Cereals and cereal products are the most important staple foods worldwide. According to the FAO rice, maize, and wheat are staple foods for 4 bn people and make up about 60 % of the world`s food energy intake. In Germany, cereals contribute 20 to 30 % to the average dietary energy supply (FAO 2011). While total protein content of wheat is comparatively low (only 8-15%) when compared to other crops, particularly in developing countries it serves as major source of protein intake (SHEWRY 2009). Winter wheat is the most important cereal and crop in Germany. In 2010, 55 % of Germany`s arable land was used for cereal production, 46 % of this area (3.26 m ha) was utilized for cultivation of winter wheat which made up 53.5 % of the total cereal harvest (44.3 m t) (SBA & BMELV 2010). About 16 m t of soft wheat were domestically utilized in 2007/8, 54.5 % for feed, 35.0 % for consumption, 7.1 % for industrial use (alcohol, malt, technological starch, energy), and 3.4 % for seed. Mills produced about 6.2 m t of bread flour (wheat, rye) in 2008/09 whereas the major proportion (5.4 m t) was milled from wheat. 67 % of wheat flour was produced as flour type 550, 11 % as type 405 (BMELV 2009). In 2008/9 consumption of flours from bread cereals accounted for 72.7 kg per capita per year. Wheat flour made up the major proportion with 63.3 kg per capita per year (SBA & BMELV DIV.). 84.6 kg bread and bakery products per capita were consumed in 2007/08 (BMELV 2009). In Germany, the consumer has the choice out of a worldwide unique diversity of bread and bakery products. The GMF estimated that over 300 kinds of bread and over 1200 types of bakery products are available in Germany (GMF 2004). Due to its great significance in human diet, the production of cereals, including wheat, that is free of contaminants which might pose a threat to human health, is of major interest.

1.2 *Fusarium* head blight in wheat

1.2.1 Causal organisms and global distribution

Already in the field as well as immediately post harvesting and during storage several genera of toxigenic fungi –dominating are *Aspergillus*, *Fusarium*, and *Penicillium*- are able to infest wheat (SWEENEY & DOBSON 1998). In the field, species of the genus *Fusarium* are of major relevance. In Europe, a complex consisting mainly of *Fusarium graminearum* (*F. graminearum*) Schwabe [teleomorph: *Giberella zeae* (Schwein.) Petch], *Fusarium culmorum* (*F. culmorum*) (W.G. Smith) Sacc., *Fusarium avenaceum* (Fr.) Sacc. (teleomorph: *Giberella avenacea* R.J. Cook), and *Fusarium poae* (Peck) Wollenw., and several other less predominant *Fusarium* spp., is associated with the disease in wheat which is known as *Fusarium* head blight (FHB) or scab (PARRY *ET AL.* 1995; BOTTALICO & PERRONE 2002). FHB and *F. graminearum* as its predominant etiologic agent have been described as a destructive disease that affects wheat and barley as well as other small-grain

cereals in the United States, Canada, Europe, Asia and South America (PARRY *ET AL*. 1995; MCMULLEN *ET AL*. 1997; STACK 2000; OSBORNE & STEIN 2007). The CIMMYT has declared FHB a major factor limiting wheat production worldwide (STACK 2000).

1.2.2 Economic relevance

The economic importance of FHB is immense. In the United States, during the 1990s, an estimated loss of 3 bn $ was caused by FHB in wheat and barley and the subsequent yield losses and reduced grain and seed quality. The economic damage partly had a serious social impact on farms and rural communities in FHB stricken regions (MCMULLEN *ET AL*. 1997; WINDELS 2000). Total economic impacts (direct and secondary) from FHB of 2.7 bn $ were calculated for wheat and barley in the Northern Great Plains and the Central United States from 1998-2000 (NGANJE *ET AL*. 2004).

Yield loss

The threat of FHB epidemics is multifaceted. PARRY *ET AL*. 1995 revised studies that quantified the loss of yield in wheat in the field under natural occurrence of the disease from 15-70 % in different countries of the world. Controlled inoculation trials with different *Fusarium* spp. caused losses from 3-60 %. In China, wheat yield losses ranged from 20-40 % in severe epidemical years, in Canada, losses from 30-70 % were mentioned for spring wheat (BAI & SHANER 1994).

SNIJDERS 1990 showed a significant positive relationship between yield reduction and FHB in wheat as well as that yield reduction mainly resulted from the loss of kernel weight and reduction of kernel amount that was caused by FHB. Several studies have demonstrated that FHB damaged grain obtains a lower test weight (e.g. SHOTWELL *ET AL*. 1985; TUITE *ET AL*. 1990; JONES & MIROCHA 1999) and generally shows a reduced thousand-kernel weight compared to healthy grain (e.g. SEITZ & BECHTEL 1985; MEYER *ET AL*. 1986; DEXTER *ET AL*. 1996; JONES & MIROCHA 1999). (SCHADE-SCHÜTZE *ET AL*. 2000) demonstrated that thousand-kernel weight of wheat was reduced in a range from 14-61% after artificial inoculation with *F. culmorum*.

Seed quality

FHB also has an impact on seed quality. As the primary inoculum source, infected grain may lead to seedling blight and foot rot (PARRY *ET AL*. 1995). Seed germination and vigour, if not totally inhibited, can be reduced in such dimensions that it is below commercial standards (BECHTEL *ET AL*. 1985; ARGYRIS *ET AL*. 2003).

Grain quality

Technological quality

Furthermore, technological traits of grain can be negatively affected because kernel composition is altered by the fungus indicated by the degradation of cell walls, starch granules and storage

proteins (BECHTEL *ET AL*. 1985; BOYACIOGLU & HETTIARACHCHY 1995; JONES & MIROCHA 1999; SIUDA *ET AL*. 2010.) Since the influence of *Fusarium* infection on grain quality is one key aspect of this study, studies regarding this topic will be reviewed in more detail in chapter 1.1.2.

Mycotoxins

The most crucial aspect of FHB is the association with several mycotoxins that accumulate in the grain already in the field and/or during storage. Mycotoxins in food are secondary metabolites mainly produced by fungi belonging to the three genera *Fusarium*, *Apergillus* and *Penicllium* (MURPHY *ET AL*. 2006). While the biological role of many mycotoxins is still "elusive" (REVERBERI *ET AL*. 2010) the threat they pose to humans and animals is more clear. Dose dependent effects on human and animal health range from skin irritations to neurotoxic, immunosuppressiv, and teratogenic to destruction of vital organs (e.g. liver, kidney) carcinogenic, mutagenic, and death (MURPHY *ET AL*. 2006).

Fusarium mycotoxins usually belong to three main structural groups that are trichothecenes (T-2 toxin (T2), deoxynivalenol (DON), diacetoxyscirpenol (DAS), fusarenone X (FUS), nivalenol (NIV)), zearalenones (zearalenone (ZEA)), and fumonisins (fumonisin B_1 (FB_1)). Moniliformin (MON), beauvericin (BEA), and fusaproliferin (FUP) are in addition increasingly associated with FHB (BOTTALICO 1998). In Europe, DON and its derivates (3-ADON, 15-ADON) and ZEA are the most frequently detected mycotoxins in association with head blight of wheat and other small grain cereals. DON and derivates are produced by *F. graminearum* and *F. culmorum*. ZEA usually co-occurs with DON and its derivates and is mainly produced by *F. graminearum*, *F. culmorum*, *F. equiseti*, and *F. cerealis* (BOTTALICO & PERRONE 2002). Even though, all toxins may represent a hazard to human health, only toxicological aspects of DON and ZEA will be described subsequently in more detail since they are the most prevalent *Fusarium* toxins and were also investigated in this study.

DON, also known as "vomitoxin" due to its emetic effects in pigs, is associated with gastroenteritis in humans. Toxicological aspects of DON were recently reviewed by PESTKA 2010. Feeding trials, with pigs have shown emesis after acute exposure with DON. Chronic low-dose exposure with DON caused anorexia, growth retardation, immunotoxicity, and impaired reproduction and development deriving from maternal toxicity. Pathophysiologic effects are alteration of neuroendocrine signalling, proinflammatory gene induction, disruption of the growth hormone axis, and gut integrity. In cells, DON elicits ribotoxic stress. Even though undesirable as FHB itself, it can be regarded as a fortunate circumstance that DON is the most prevalent trichothecene detected cereals, since it was demonstrated to be 10 to 20 times less toxic than e.g. T2-toxin and NIV (UENO 1983).

Toxicological aspects of ZEA were reviewed by ZINEDINE *ET AL.* 2007. Compared to other toxins, ZEA shows relatively low acute toxicity. Subacute and subchronic toxicity was indicated by interaction of ZEA and metabolites with estrogen receptors, especially sensitive reacted pigs. In rodents, chronic effects were liver lesions with development of hepatocacarcinoma, alterations in several estrogen-related tissues (uterus, mammary glands), myelofibrosis in bone marrow, pituitary adenomas, depression of body weight, inflammation of prostate gland, testicular atrophy, increased nephropathy, retinopathy and cataracts. ZEA also showed haematotoxic effect and is associated with the growth of human breast cancer cells. In cows and pigs, an influence of ZEA on reproduction and development was observed. On the cellular level, ZEA appears to induce DNA-adduct formation, DNA fragmentation, targets mitochondria and/or lysosoms, induces lipid peroxidation, cell death and inhibition of protein and DNA syntheses.

So far, it is not possible to eliminate mycotoxins completely from the products meant for consumption during the different processing steps (cleaning, milling, baking/cooking) of wheat (see chapter 1.1.1). With the intention to protect the consumers from mycotoxins in food the European Union (EU) has established maximum levels (EU_{Max}) for DON and ZEA in cereals and cereal products with Commission regulation (EC) No. 856/2005 of 6th July. Tab 1 lists those concerning foodstuffs produced from wheat. For DON, the EU recommended maximum tolerable daily intake (TDI) is 1 µg kg^{-1} bodyweight (bw), for ZEA 0.2 µg kg^{-1} bw, respectively. Regarding other *Fusarium* associated toxins, maximum levels do not yet exist for wheat or wheat products. In the subsequent text EU_{Max} for DON in unprocessed cereals and cereal flour are abbreviated as EU_{MaxUP} and EU_{MaxCF}, respectively.

Tab 1. EU maximum levels (EU_{MAX}) for *Fusarium* toxins in foodstuffs (in µg kg^{-1}) concerning wheat and wheat products according to Commission regulation (EC) No 856/2005

Product	DON	ZEA
Unprocessed cereals (Durum wheat)	1250 (1750)	100
Cereals intended for direct human consumption, cereal flour	750	75
Pasta	750	-
Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals	500	50
Processed cereal-based foods and baby foods for infants and young children	200	20

1.2.3 Epidemiology and symptoms

Initial point of the epidemic cycle of FHB is an inoculum source (stubble, crop debris) in the soil where *Fusarium* spp. either survive as saprophytic mycelium or as chlamydospores. The fungus may from there infect the seedling, causing seedling blight and foot rot (PARRY *ET AL.* 1994).

However, FHB is primarily a floral disease even though a few studies have also proposed systemic colonization of the plants as the cause for head blight (STACK 2003). This means that wheat plants were observed to be particularly susceptible to *Fusarium* infection during flowering and pre-flowering stages. Airborne and water-splashed (macro-)conidia as well as ascospores released from perithecia infect the wheat florets, resulting in FHB and providing new inoculum (STACK 2003, PARRY *ET AL.* 1995). Especially, warm and moist weather conditions during wheat anthesis and shortly thereafter promote germination of spores and growth of fungal hyphae into grain tissues (OSBORNE & STEIN 2007).

By means of light and electron microscopy KANG & BUCHENAUER 2000 described in detail the process of penetration, infection and invasion of wheat spikes by *F. culmorum*. Referring to their observations, after spore germination the fungus develops a dense hyphal network on the host surface and penetrates the inner surfaces of lemma, glume, palea, and the upper ovary part by infection hyphae. Palea and lemma can occasionally also be invaded through stomata. By intra- and intercellular growth, fungal hyphae spread in parenchyma and vascular bundles of lemma, glume, ovary and rachis causing severe damage to the host cells. In another study the same authors described a degradation in the cell wall components cellulose, xylan, and pectin in infested tissue and concluded that the infection of wheat spikes is facilitated by cell-wall-degrading enzymes that were secreted by the fungus (KANG & BUCHENAUER 2000B). Similar observations were made in wheat spikes infected with *F. graminearum* (WANJIRU *ET AL.* 2002). Further infection within wheat ear occurs vertically through vascular bundles or horizontally through anthers and bracts from primarily infected spikelet to contiguous spikelet (RIBICHICH *ET AL.* 2000).

Several alterations in the xylem vessels and phloem sieves have been mentioned to cause at least partial interruption of nutrient supply which leads to a premature death of the apical spikelets and results in the typical bleaching ("blight") symptoms of the upper part of the wheat ear (KANG & BUCHENAUER 2000; RIBICHICH *ET AL.* 2000; GOSWAMI & KISTLER 2004). Other typical FHB symptoms in wheat are brown, dark purple to black necrotic spots on spikelets ("scab") as well as distorted and downwards curved ears. Infected kernels appear shriveled and discolored. Grain color reaches from white over light brown to pink and salmon-orange, the later resulting from developed conidia. Reddish colored spore packages can also be visible on the outer surface of spikelets and glumes (BOTTALICO & PERRONE 2002, GOSWAMI & KISTLER 2004).

Fig 1. Blighted wheat heads (A) and bleaching of upper spike part (B) caused by naturally occurring FHB in 2007, at trial location Torland (51.36° N, 9.55° E), Pictures: BAADER 2007

1.2.4 Control strategies

Due to the disastrous effect of FHB on grain yield and quality, various attempts have aimed to identify factors that support *Fusarium* infection in the field and develop measures to prevent the frequency and intensity of FHB occurrence. Agronomic practices that control FHB epidemics include choice of (I) pre-crop, (II) tillage system, (III) cultivar, (IV) fungicide application, and (V) fertilizers.

Pre-crop

The pre-crop can facilitate FHB in wheat in the following year in two ways. Either the pre-crop functions as host plant for *Fusarium* spp. and therefore, increases inoculum compared to a non host plant or the pre-crop leaves a large amount of crop debris after harvest in which *Fusarium* spp. survive saprophytically (BEYER *ET AL*. 2006). Maize has been identified as the pre-crop that promotes FHB and DON contamination in wheat the most. First, maize serves as a host for *F. graminearum* which is the main causal agent of FHB, and second, maize leaves plenty of crop debris where *Fusarium* spp. can survive (DILL-MACKY & JONES 2000). Non-cereals such as rapeseed, potato, and sugar beet as well as wheat itself have been demonstrated to cause significantly lower DON levels in subsequent wheat (BECK & LEPSCHY 2000).

Tillage

Ascospores and conidia that are forcibly discharged, wind-dispersed or water splashed coming from colonized crop debris present the primary inoculum source for *Fusarium* infection (XU 2003). Incorporation of *Fusarium* infested crop residues deep in the soil reduced effectively the *F. graminearum* populations (YI *ET AL*. 2002). Consequently, the most effective tillage treatment to remove *Fusarium* spp. colonized crop residues from the soil surface is the application of the moldboard plough. Compared to a chisel plough treatment and a no tillage system the moldboard plough reduced the residuals of corn, wheat, and soybean significantly. Disease incidence and disease severity caused by FHB in wheat in the following year were significantly lower after

moldboard ploughing (DILL-MACKY & JONES 2000). A no-ploughing system after pre-crop maize presents the highest risk factor leading to high DON contamination levels in wheat (BECK & LEPSCHY 2000; BARRIER-GUILLOT *ET AL*. 2004).

Cultivar

Since crop management and agrochemical measures are only partly able to control FHB effectively, the cultivation of resistant wheat cultivars is a key aspect in integrated *Fusarium* control and in prevention mycotoxin accumulation in grains (BUERSTMAYR *ET AL*. 2009). Researcher worldwide agree that "resistant cultivars will provide the most stable and durable solution to the problem of FHB" and "ensure stable yields and high-quality grain free of mycotoxins" (GILBERT & TEKAUZ 2000). Resistance mechanisms to FHB are complex and yet not fully understood. It is assumed that a complex of physiological, biochemical and morphological factors leads to a reduced susceptibility which are expressed in active or passive defense reactions by the wheat plant (MIEDANER 1997).

Two types of resistance to FHB are classically distinguished: resistance to initial penetration (type I) and resistance to spreading of the fungus within infected spike (type II) (SCHROEDER & CHRISTENSEN 1963). Whereas type I resistance is more important in barely, wheat cultivars mainly express type II resistance. So far, the Chinese wheat cultivar Sumai 3 and its derivates have shown the highest degree of type II resistance and at the same time the highest degree of FHB resistance in wheat known so far. Point inoculation in spikelets did not lead to spread of FHB symptoms in the ear. Several infected spikelets within one ear could only be observed under heavy disease pressure in the field (BAI & SHANER 2004). So far, no genotype with complete resistance to FHB has been found, all genotypes investigated proved to be more or less susceptible (MIEDANER 1997).

Two other types of active resistance mechanisms were proposed by MESTERHAZY 1995. On the on hand, resistance to kernel infection which is measured as percentage of infected kernels. On the other hand tolerance which expresses the ability of the plant to endure the FHB infection and disease, measured in relative yield reduction. Nevertheless, these two types of resistance did not become widely accepted for several reasons (BAI & SHANER 2004). Another active resistance mechanism is the resistance to DON accumulation in grain (MILLER *ET AL*. 1985). Usually, in *Fusarium* infected wheat cultivars DON is detectable in the grain. A study of 116 wheat lines of various origins under greenhouse and field conditions inoculated with a mixture of several *F. graminearum* isolates showed that DON levels varied in a wide range between the cultivars: from trace levels up to 283 mg kg^{-1} in the greenhouse test and from 2.8 to 52 mg kg^{-1} in the field (BAI *ET AL*. 2001). Possible explanations for the lower DON content in kernels of some wheat cultivars compared to others grown under identical environmental conditions are: (i) low DON production by the fungus, (ii) enzymatic degradation of DON by the wheat plant during grain development,

and (iii) high accumulation of DON in other tissues of the spike. The resistance to DON accumulation in grain was termed type III resistance (MILLER & ARNISON 1986).

Passive resistance mechanisms against FHB have also been investigated. Morphological features of the wheat plant, such as plant height, awndness, spikelet density as well as flowering habit e.g. avoidance of the infection by escape (flowering in boot stage), flowering period, anther morphology, position and density of florets, and time interval that florets are open have been connected with the disease severity (SCHROEDER & CHRISTENSEN 1963; MESTERHAZY 1995).

Since the phenotypic evaluation of FHB resistance is elaborate and its expression greatly influenced by genotype x environment interactions, breeding efforts for FHB resistance recently concentrate more on the use of molecular markers. FHB resistance is a quantitative trait and to current knowledge quantitative trait loci are present on all of the 21 wheat chromosomes except 7D (KOLB *ET AL.* 2001; BUERSTMAYR *ET AL.* 2009).

Fungicide

The efficacies of various pesticides against FHB has been investigated under controlled conditions (in vitro, greenhouse) as well as in field trials. Results are partly contradictory; therefore, a reduction of mycotoxin content in grain cannot clearly be related to fungicide application (EDWARDS 2004). Triazole fungicides have proven to be the most effective chemical control against FHB so far (BEYER *ET AL.* 2006). Fungicides with metconazole and tebuconazole as active agents reduced FHB infection and DON content in field samples significantly in comparison to an untreated control (EDWARDS *ET AL.* 2001). The newer compound prothioconazole applied at growth stages 31, 39, and 65 reduced FHB symptoms by 50, 58 and 83 % and DON levels by 27, 49, 57 %, respectively (EDWARDS & GODLEY 2010).

The application of strobilurin containing fungicides produced contradicting results. Whereas EDWARDS *ET AL.* 2001 did not find an effect of azoxystrobin on trichothecene-producing *Fusarium* spp. SIMPSON *ET AL.* 2001 reported a risk potential coming from azoxystrobin treatment on the contrary. This resulted from an increase in toxigenic *Fusarium* spp. and increased DON contamination. In a greenhouse trial conducted by PIRGOZLIEV *ET AL.* 2002 different doses of azoxystrobin reduced FHB and DON levels, even though not as efficiently as metconazole.

Besides the choice of the active ingredient the right timing for fungicide application plays a key role in the chemical control of FHB. BEYER *ET AL.* 2006 revised several studies that observed the best efficacies of fungicides when applied during wheat anthesis. Application pre-anthesis or post-anthesis led to a drastically reduced impact of the fungicides.

Fertilizer

The use of different fertilizer regimes are assumed to affect FHB either by (i) influencing the decomposition rate of crop debris, (ii) creation of physiological stress on host plant or (iii) changing construction of cereal canopy (EDWARDS 2004). Nevertheless, the role of fertilization measures, especially nitrogen fertilization, in FHB development is still discussed controversially. A standard management that included a single application of 70 kg ha^{-1} of ammonium nitrate at seeding was compared with the standard management and two supplementary nitrogen applications, 50 kg ha^{-1} each. Supplementary nitrogen fertilization resulted in significantly higher incidence of *Fusarium* spp. in wheat and triticale. The same effect was observed in two-row and six-row barely, even though supplementary nitrogen of 50 kg ha^{-1} was only applied once (MARTIN *ET AL*. 1991). An earlier study compared forms of nitrogen fertilizers and showed lower FHB incidence in wheat where urea was applied compared to the use of ammonium nitrate (TEICH 1987). LEMMENS *ET AL*. 2004 compared different types of mineral and organic nitrogen fertilizers and could not find an effect on FHB. But they observed an increase of FHB intensity with increasing nitrogen input over a range from 0 to 160 kg ha^{-1}. In another experiment, published within the same study, they showed a significant increase of FHB intensity and DON contamination in grain with increasing nitrogen input, but only in wheat with nitrogen deficit (< 80 kg ha^{-1}). At higher input rates, common in agricultural practice, FHB intensity and DON levels remained on a constant level. They concluded that nitrogen fertilization is not a practical control strategy in managing FHB in the field. AUFHAMMER *ET AL*. 2000 and OLDENBURG *ET AL*. 2007A came to the same conclusion after investigating the effect of different types of nitrogen fertilizers, compositions, doses, and applications times on FHB in the field.

Other measures

For the sake of completeness, it should be mentioned that other methods to control FHB and DON contamination are also discussed, such as avoidance of growth regulators, biological measures, weed management, insect control, and transgenic approaches. But so far, there is not enough reliable data to evaluate the possible contribution these measures might have against FHB (EDWARDS 2004; SNIJDERS 2004).

Quantifying the effects of different control strategies affecting FHB and DON

On the data basis of only field trials which were reviewed by BEYER *ET AL*. 2006, the authors made the attempt to evaluate the efficacy of different methods to control FHB and therefore DON contamination in the grain. The model to quantify the effect on DON included previous crop, tillage, choice of cultivar, and fungicide treatment as influencing factors. The outcome is presented in Fig 2.

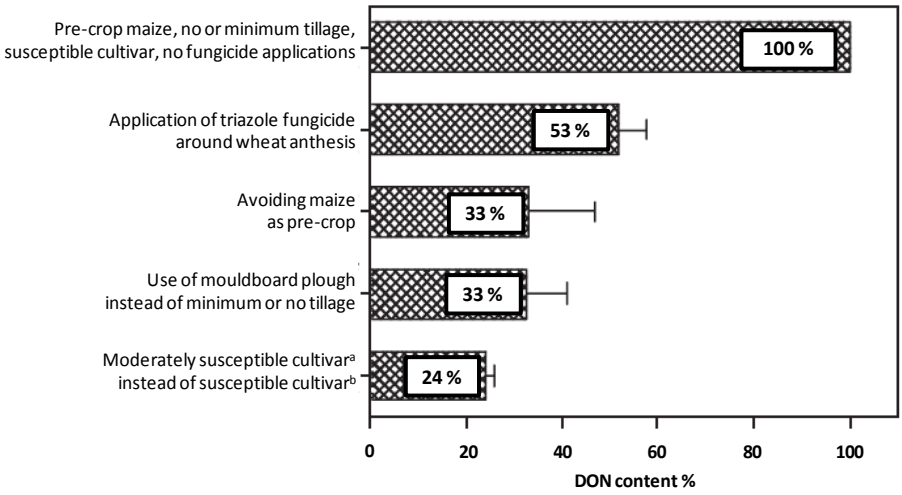

Fig 2. Relative DON content obtained by the use of agronomic methods or fungicides. DON reduction is presented as percentage in relation to the worst case scenario (pre-crop maize, no or minimum tillage, susceptible cultivar, no fungicide application = 100 % DON content), [a] cv. Dekan, [b] cv. Ritmo (BEYER *ET AL.* 2006)

It becomes clear, that to the current state of knowledge the choice of a moderately susceptible cultivar compared to a more susceptible one is the most effective strategy to minimize DON contamination in wheat grain. DON contamination in comparison to the worst case situation was decreased to 24 ± 7% by that measure, followed by ploughing which reduced DON to 33 ± 7%, avoiding maize as previous crop which lowered DON content to 33 ± 11%, and triazole treatment during anthesis which could only decrease DON levels to 53 ± 4% (Fig 2).

The authors of that study assumed that factors such as N fertilization and growth regulators might have indirect effects on DON contamination but due to the limited data available concerning these factors they did not account for them. The results are in accordance with the findings of OBST *ET AL*. 2000 who identified five DON risk factors based on long-term *Fusarium* monitoring of Bavarian wheat: (i) pre-crop maize, (ii) minimum tillage after maize, (iii) cultivation of moderately to highly susceptible wheat varieties, (iv) use of strobilurin fungicides, and (iv) warm and wet weather conditions during wheat anthesis. The accumulation of the factors increased the risk of high DON levels not in an additive, but in a multiplicative way. Therefore, it is assumed that the avoidance of one or more risk factors which can be managed by the farmer may also decrease DON content in a synergistic manner (BEYER *ET AL*. 2006).

1.3 Significance of gluten proteins for wheat processing

The worldwide success of wheat as crop plant does not only derive from its high adaptation capabilities to various climatic conditions and high yield potential but is largely also a result of the unique characteristics of dough produced from wheat flours (SHEWRY 2009). The viscoelastic properties of wheat dough make it suitable for various end-uses (bread, cakes, biscuits, pasta, noodles) of which the processing of leavened bread is particularly important (WEEGELS *ET AL.* 1996; SHEWRY 2009). These properties derive from the wheat storage proteins which are also termed 'gluten' proteins due to their ability to form a network in dough that is able to trap carbon dioxide (SHEWRY 2009). Due to their insolubility in water or dilute saline but their extractability in alcohol-water solutions OSBORNE 1924 classified wheat gluten proteins as 'prolamins'. These can be further separated into gliadins, which are soluble in e.g. 60% ethanol-water solution, and glutenins which need additional reducing agents to become soluble owed to their higher degree of polymerization and connection by interchain disulphide bonds (WIESER *ET AL.* 1998; SHEWRY *ET AL.* 2002). Gluten proteins (gliadin + glutenins) make up for 80-85% of total protein in mature wheat grain, while albumins (water-soluble) and globulins (soluble in salt-water solution) play a minor role and mainly function as metabolic and structural proteins (SHEWRY & HALFORD 2002; GOESAERT *ET AL.* 2005). Prolamins of wheat are exclusively located in the endosperm while e.g. globulins are also present in the outer part of the aleurone layer and in the embryo (SHEWRY & HALFORD 2002).

According to WIESER 2007 and SHEWRY & HALFORD 2002, gliadins and glutenins can be classified into several subgroups (Fig 3) that differ in their proportion of total gluten, molecular weight, amino acid composition (*not shown*), and their ability to form interchain disulfide bonds due to different levels of cysteine. Gliadins, generally monomers with molecular weight (MW) from 28-55 kDa, can be divided into ω-gliadins that lack cysteine and are therefore not able to form disulphide crosslinks between proteins, while a-/ß-gliadins and γ-gliadins contain relatively high contents of cysteine and are able to form intrachain bonds. Glutenins are highly polymerized proteins that can be roughtly divided into low-molecular-weight glutenin subunits (LMW-GS) with MWs from 32-35 kDa and high-molecular-weight glutenin subunits (HMW-GS) with MWs from 67-88 kDa. LMW-GS form interchain bonds, HMW-GS are linked by intra- as well as interchain disulfide bonds. SHEWRY & HALFORD 2002 assign prolamins into a sulfur-rich group (e.g. α-, γ-gliadins, subunits of LMW-GS), a sulfur-poor group (ω-gliadins, subunits of LMW-GS), and HMW-GS that contain also subgroups that are rich in sulfur.

Both, gliadins and glutenins, contribute to the rheological dough characteristics, however in a different manor. Gliadins are less elastic and cohesive than glutenins and rather contribute to viscosity and extensibility of dough while glutenins contribute, on the contrary, to its strength and elastic properties (WIESER & KIEFFER 2001; WIESER 2007). Especially the HMW-GS subgroup has

been demonstrated to be important for rheological gluten and dough properties as well as for baking volume (WIESER & ZIMMERMANN 2000; WIESER & KIEFFER 2001).

Even though wheat protein content is comparatively low with regard to other crops, the remarkable functions of the storage protein fraction make wheat one of the most consumed diets of the world (SHEWRY 2009). Therefore, the influence of *Fusarium* infection on protein content of grain, flour, as well as on content and composition of gluten proteins and subfractions is a key aspect in this study.

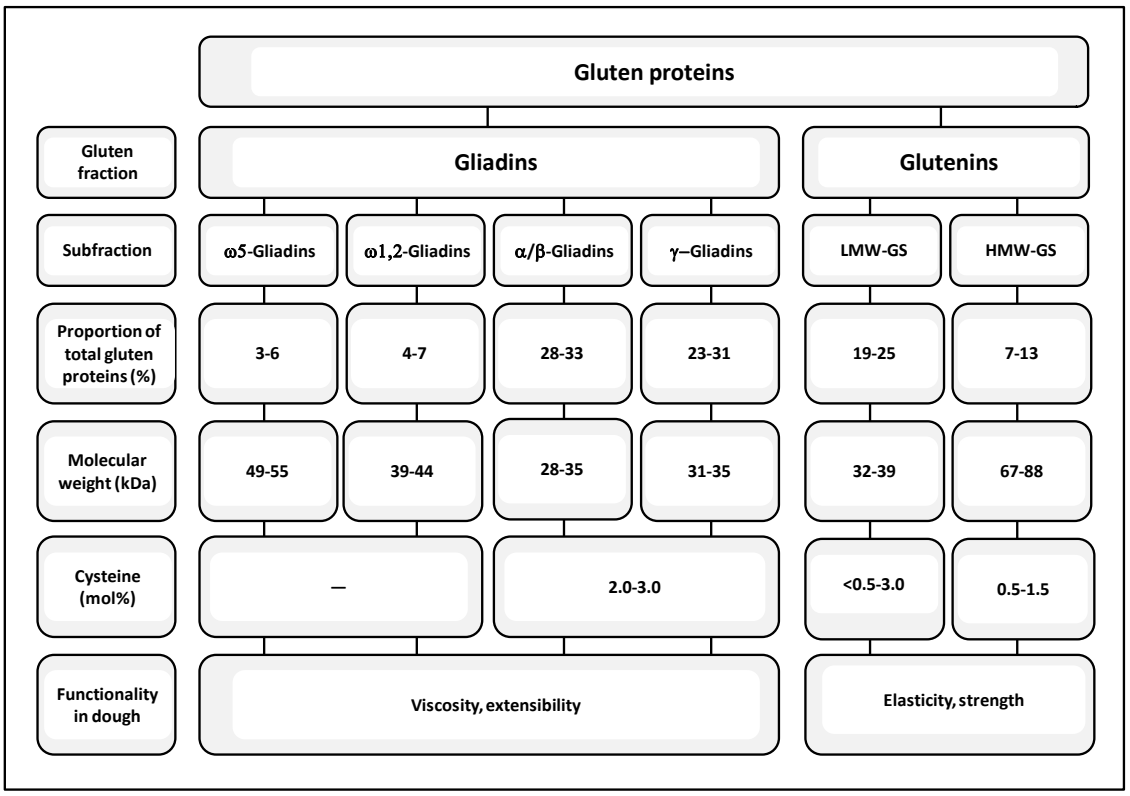

Fig 3. Classification of gluten proteins according to WIESER 2007 and SHEWRY & HALFORD 2002 (modified)

1.4 Evaluation of bread wheat quality

In general, the technological value of wheat is strongly influenced by the functional properties of starch, and protein, particularly gluten protein, as well as by quantity of these components. Both quality and quantity of grain components are strongly linked to each other and primarily depend on genetic background (cultivar) as well as on environmental conditions (climate, soil, fertilization, pathogens). All of these factors influence the final baking quality which is defined by e.g. water absorption of flour, tolerance to mixing (dough), and baking volume (SEIBEL 2005; SELING 2010).

International standards that define terms and methods for wheat grading do not yet exist, even though processing tools are similar and quality of the end product is usually described with the same expressions in different countries (POSNER & HIBBS 2005). Council Regulation (EC) No 1784/2003 of 29th September 2003 is the basis for the common organization of the market in

cereals in the European Union. It describes the intervention system for the internal market and support measures for European cereal products when traded on the world market. Related to this act is Commission Regulation (EC) No 824/2000 of 20th April 2000 which lays down the procedures for the assumption of cereals by intervention agencies and describes methods of analysis for certifying that cereals are "sound, fair and of marketable quality". For durum wheat, common wheat (includes bread wheat), barley, maize, and sorghum the regulation establishes parameters e.g. maximum moisture content, the maximum percentage of impurities and the minimum specific weight. Evaluation of grain by e.g. by determination of besatz, falling number, moisture content, zeleny sedimentation, and protein content base upon the standard methods established by the International Association of Cereal Chemistry (ICC). The requirement for "marketable quality" includes the compliance with legal regulations that rule e.g. the maximum content of mycotoxins in cereals (see chapter 1.2.2, Tab 1).

In Germany, wheat is mainly traded as cultivars or quality groups; in some years a minimum falling number and a minimum protein content is required (SEIBEL 2005). The Federal Office of Plant Varieties (BSA) annually publishes Descriptive Variety Lists that describe the listed and marketed varieties of the most important agricultural and vegetable species regarding cultivation, resistance, quality, and yield. Technological quality of bread wheat is thereby estimated by indirect quality characteristics (protein content, falling number, sedimentation value, pearling index, water absorption of flour), milling properties (ash number, flour yield) and baking properties (baking volume, dough elasticity and surface consistency) (BSA 2007). The assignment of soft wheat cultivars to quality groups (E, A; B, C) is based on defined minimum requirements for the most important quality characteristics whereas baking properties and baking volume in particular play the major role. Together with protein quality and starch properties, protein content particularly determines the quality of wheat for end-use (SELING 2010). While the determination of e.g. protein content, falling number (as a measure of α-amylase activity in grain, indicating sprout damage), and sedimentation value (as a measure of protein swelling in acidy or SDS solution indicating protein quality) are taken as fast inexpensive measures to indicate baking performance, the investigation of e.g. dough mixing properties and the actual baking test (as ultimate method to evaluate quality of end-product) are time consuming and costly for which they are only applied in mills and large bakeries that own the specific equipment (SEIBEL 2005). In this work, both fast standard procedures as well as more elaborate methods for the evaluation of bread wheat quality were used with the objective to gain a better overall picture how *Fusarium* infection influences wheat quality.

1.1 *Fusarium* infection and wheat quality

1.1.1 Fate of DON during wheat processing

Various studies have so far focused on the possible retention of DON, the *Fusarium* mycotoxin with the highest occurrence in wheat, during primary processing (cleaning, milling) and secondary processing (baking, cooking) of wheat. The efficiency to remove DON contaminated kernels from the mixture of healthy and unhealthy kernel of several processes prior to milling –sorting, sieving, and scouring- with varying equipment has been tested. Cleaning was demonstrated to be capable to remove DON partly with varying success. SCOTT *ET AL*. 1983 found the highest DON concentration in dockage and therefore expected at least a slight reduction of DON content in cleaned wheat. Removal of dust and screenings reduced DON content about 7% to 23% (YOUNG *ET AL*. 1984). In cleaned wheat, DON content decreased in a range from 6 to 19%. In other studies, obtained cleaning efficiencies from 48 to 86% depending on the original DON concentration in the grain lot (ABBAS *ET AL*. 1985; SEITZ *ET AL*. 1985; SEITZ *ET AL*. 1986). TKACHUK *ET AL*. 1991 removed shriveled and light severely infected wheat kernels ("tombstone kernels") and therefore kernels with the highest DON concentrations effectively by gravity table. Separation by gravity table is based on the principle that *Fusarium* damaged kernels are of lower density, a principle that has also been used by HUFF & HAGLER 1985 in a less practical treatment which involved the removal of buoyant kernels in water and sucrose solution. DON content in wheat decreased by 68% to 96%. Scouring of wheat reduced DON level by 22% in one wheat cultivar (NOWICKI *ET AL*. 1988).

Most of the wheat meant for human consumption is converted into wheat flour by milling. Since the early 1980s a large amount of studies worldwide have reported on the distribution of DON in milling fractions gained from test mills. Independent of wheat cultivar, DON was distributed in all milling fractions, with the highest amounts usually concentrated in milling fractions containing more of the outer layers (germ and bran) of the kernels (SCOTT *ET AL*. 1983; YOUNG *ET AL*. 1984; ABBAS *ET AL*. 1985; SEITZ *ET AL*. 1985; SEITZ *ET AL*. 1986; TRIGO-STOCKLI *ET AL*. 1996; GARTNER *ET AL*. 2008; LANCOVA *ET AL*. 2008). In flours produced from the inner proportion of the endosperm, DON levels could be reduced by fractionation to some extent (SCOTT *ET AL*. 1983; GARTNER *ET AL*. 2008; LANCOVA *ET AL*. 2008). DEXTER *ET AL*. 1996 reported an average DON reduction of 50% in white flour produced from four wheat cultivars.

Wheat flour is used for the processing of diverse foods e.g. bread, cakes, pasta, and noodles. Therefore, numerous studies have also focused on the retention of DON during cooking processes, investigating the behavior of DON during heating. A few studies showed that DON was neither destroyed nor reduced during baking at temperature between 170 to 350 °C with varying baking times (ELBANNA *ET AL*. 1983; SCOTT *ET AL*. 1983; TANAKA *ET AL*. 1986; SUGITA-KONISHI *ET AL*. 2006; LANCOVA *ET AL*. 2008) which underlines the heat-stability of DON. Other studies report a reduction of DON in bread in a range from 19 to 69% (YOUNG *ET AL*. 1984; ABBAS *ET AL*. 1985).

SAMAR *ET AL.* 2001 observed a reduction of DON by 41% and 56% in French bread and Vienna bread, respectively, during fermentation at 50°C. Two additives, sodium bisulfite and L-cysteine, reduced DON level in bread by 38.0 to 46.0% (BOYACIOGLU *ET AL.* 1993). SEITZ *ET AL.* 1986 showed a decrease of DON level in wheat with lower DON contamination, whereas DON content in bread made from wheat with more than 1.6 mg kg^{-1} DON increased. Studies that investigated the effect of other cooking processes such as frying, spaghetti and noodle cooking, extrusion, and superheated steam treatment have been thoroughly reviewed by KUSHIRO 2008 and will not be mentioned here. It should just be mentioned that DON is highly water-soluble and can therefore be reduced by a large extent from spaghetti and noodles when boiling in water and discarding of cooking water.

In summary, DON can be reduced to some degree and step by step during the different wheat processing steps, but cannot be totally eliminated from the end-products. One crucial problem is that separation of *Fusarium* infected kernels is not easy to solve, since FHB level does not always correlate with DON content in the kernels and FHB/DON ratio varies among cultivars. Also, the observed reduction of DON levels during secondary processing might partly result from the degradation and conjugation of DON. The toxicological effect of such degradation products and conjugated DON is unknown (KUSHIRO 2008).

1.1.2 Impact of *Fusarium* infection on wheat quality and composition

While studies on the pathogenicity and epidemiology of FHB, resistance mechanisms, associated mycotoxins as well as control measures in the field is abundant, literature on the impact of *Fusarium* infection on wheat quality seems comparatively scarce. Although it is understandable, that the management of FHB has in the past primarily focused on food safety and therefore on the avoidance of mycotoxin contamination in grain, the effects of FHB on grain quality are not to be underestimated. Researchers have just recently become aware that quality assurance in FHB affected wheat is essential for wheat marketing. Because the wheat price is directly determined by its processing attributes, influence of FHB on wheat milling performance, flour properties and endproduct quality have just recently confirmed its partially adverse effects (DEXTER & NOWICKI 2003).

Early works on the influence of *Fusarium* infection on processing quality of wheat date back to the 1950s. FINNEY 1954 (cited in SEITZ *ET AL.* 1986) already reported a reduced baking performance for scab infected wheat in the U.S.; in Germany, BOCKMANN 1964 first investigated the influence of *Fusarium* infection on baking performance in detail, later other European researchers such as JOVICEVIC 1972; BEROVA & MLADENOV 1974; ZWATZ 1975; SARIC *ET AL.* 1980 (cited in MEYER *ET AL.* 1986) also reported a loss in wheat quality due to *Fusarium* infection of grain. Since the

1980s scientists worldwide have continuously published results on the influence of *Fusarium* infection on quality parameters of wheat and the effect of the fungus on the composition of flour.

Authors have first of all focused on milling value of wheat (measured as flour yield, ash content, flour color) (HAMILTON & TRENHOLM 1984; BECHTEL *ET AL*. 1985; MEYER *ET AL*. 1986; SEITZ *ET AL*. 1986; TKACHUK *ET AL*. 1991; DEXTER *ET AL*. 1996; MATTHAUS *ET AL*. 2004; GARTNER *ET AL*. 2008). However, even more studies investigated the impact of *Fusarium* infection on quality parameters that give a fast indication of flour properties (protein content, sedimentation value, falling number, wet gluten, water absorption) and are easy to assess (BOCKMANN 1964; HAMILTON & TRENHOLM 1984; BECHTEL *ET AL*. 1985; SEITZ & BECHTEL 1985; MEYER *ET AL*. 1986; SEITZ *ET AL*. 1986; BOYACIOGLU & HETTIARACHCHY 1995; DEXTER *ET AL*. 1996; PAWELZIK *ET AL*. 1998; HERMANN *ET AL*. 1999; NIGHTINGALE *ET AL*. 1999; MATTHAUS *ET AL*. 2004; PRANGE *ET AL*. 2005; WANG *ET AL*. 2005A; WANG *ET AL*. 2005B; TERZI *ET AL*. 2007; GARTNER *ET AL*. 2008; WANG *ET AL*. 2008; SIUDA *ET AL*. 2010). Fewer studies have reported on the impact of *Fusarium* infection on mixing properties of dough (measured as dough development time, dough stability, dough softening) (BOCKMANN 1964; MEYER *ET AL*. 1986; DEXTER *ET AL*. 1996; PAWELZIK *ET AL*. 1998; NIGHTINGALE *ET AL*. 1999; WANG *ET AL*. 2005B; GARTNER *ET AL*. 2008), rheological dough properties (resistance to extension, extensibility, elasticity) (MEYER *ET AL*. 1986; PAWELZIK *ET AL*. 1998; NIGHTINGALE *ET AL*. 1999; ANTES *ET AL*. 2001; PRANGE *ET AL*. 2005; WANG *ET AL*. 2005B; LANCOVA *ET AL*. 2008) as well as other dough properties (measured as dough volume, fermentation time, proofing time, proofing stability) (LANCOVA *ET AL*. 2008). In contrast, more studies investigated baking volume (MEYER *ET AL*. 1986; SEITZ *ET AL*. 1986; DEXTER *ET AL*. 1996; NIGHTINGALE *ET AL*. 1999; ANTES *ET AL*. 2001; PRANGE *ET AL*. 2005; WANG *ET AL*. 2005B; TERZI *ET AL*. 2007; GARTNER *ET AL*. 2008; LANCOVA *ET AL*. 2008), which can be regarded as the ultimate parameter to evaluate baking performance of a flour and end-use quality. A few authors also reported on the influence on further baking properties (crumb and crust color, crumb structure, shape) (BOCKMANN 1964; DEXTER *ET AL*. 1996; WANG *ET AL*. 2005B; TERZI *ET AL*. 2007)

While parameters indicating the processing quality of wheat have been investigated by a bigger number of researchers, only a few of them focused on the impact of *Fusarium* infection on single grain or flour components other than total protein. Most studies have focused on the content and properties of starch e.g. starch damage, pasting properties, amylose content (MEYER *ET AL*. 1986; BOYACIOGLU & HETTIARACHCHY 1995; DEXTER *ET AL*. 1996; PAWELZIK *ET AL*. 1998; MATTHAUS *ET AL*. 2004; WANG *ET AL*. 2005A; SIUDA *ET AL*. 2010) which presents the major component of wheat grain. Others investigated carbohydrates other than starch were sugars ((non-)reducing sugars, maltose, sucrose) (MEYER *ET AL*. 1986; BOYACIOGLU & HETTIARACHCHY 1995; PAWELZIK *ET AL*. 1998; WANG *ET AL*. 2005A), and major cell wall components (cellulose, hemicelluloses) (HAMILTON & TRENHOLM 1984; BOYACIOGLU & HETTIARACHCHY 1995; MATTHAUS *ET AL*. 2004;

WANG *ET AL*. 2005A). A few studies also investigated quantitative and qualitative changes in protein composition whereas the focus mainly lay on gluten composition (BECHTEL *ET AL*. 1985; MEYER *ET AL*. 1986; BOYACIOGLU & HETTIARACHCHY 1995; DEXTER *ET AL*. 1996; NIGHTINGALE *ET AL*. 1999; ANTES *ET AL*. 2001; PRANGE *ET AL*. 2005; WANG *ET AL*. 2005B; EGGERT *ET AL*. 2010). Only four studies reported on changes in lipid content and lipid composition, including single fatty acids (HAMILTON & TRENHOLM 1984; MEYER *ET AL*. 1986; BOYACIOGLU & HETTIARACHCHY 1995; WANG 2004) which is most likely a result of the minor role of lipids in grain (1.5-2.5% of dry matter) however not necessarily insignificant role in gas-holding capacity of dough (BROOKER 1996; BELITZ *ET AL*. 2009).

Since the results of the respective studies are used in the discussion (chapter 5.2 and 5.3) for comparison with our own results and are then for that purpose described in more detail, at this point only a short summary of the most important findings of the above mentioned studies is given.

Several studies observed a reduced milling value of FHB damaged wheat. While flour yield decreases, most researchers found higher ash contents in flours milled from *Fusarium* damaged wheat which resulted in a darker flour color equal to a loss of brightness. MEYER *ET AL*. 1986 even reported an adverse affect on flour odor. Regarding flour properties, results were contradictory. Researcher found an increase, a decrease, as well as no effect of *Fusarium* infection on protein content, sedimentation value, wet gluten, falling number, and water absorption. Mixing properties of dough were generally found to be weaker. In addition, dough was reduced in strength, stickier and therefore more difficult to handle. LANCOVA *ET AL*. 2008 recently recorded higher dough volume and a longer fermentation time in wheat flour with higher DON levels measured with fermentograph. Dough resistance and proofing time recorded with maturograph increased whereas proofing stability, also measured with maturograph, decreased.

As for baking properties, after comparison of results from different studies, it was apparent that impact of *Fusarium* infection is not clear yet, since either an increase or a decrease of baking volume was observed as well as no effect. FINNEY 1954 (cited in SEITZ *ET AL*. 1986) and SEITZ *ET AL*. 1986 described the crumb color of *Fusarium* contaminated bread as unsatisfactory. Crumb and crust color became darker in bread made from heavily *Fusarium* infected wheat compared to less infected samples (BOCKMANN 1964; WANG *ET AL*. 2005B). BOCKMANN 1964 noticed bigger pores in loaves baked from *Fusarium* contaminated wheat whereas DEXTER *ET AL*. 1996 could not observe an effect of *Fusarium* damage on crumb structure. Loaf shape also changed in an undesired manor (WANG *ET AL*. 2005B).

Changes of quality parameters have been tried to explain by changes in the composition of grain and flours after *Fusarium* infection whereas results were not as contradictory as for processing quality, which is probably due to the smaller number of studies published investigating single grain components. Starch content of grain (PAWELZIK *ET AL*. 1998; MATTHAUS *ET AL*. 2004; SIUDA *ET AL*.

2010) and pasting properties of starch were reduced (WANG *ET AL*. 2005A) while content of reducing sugars increased (MEYER *ET AL*. 1986; BOYACIOGLU & HETTIARACHCHY 1995; PAWELZIK *ET AL*. 1998). Regarding protein composition, all investigators observed a quantitative change after *Fusarium* infection, however by means of different separation methods including, SDS-PAGE, Kjedahl principle, RP-HPLC, SE-HPLC, and turbidimetry which makes a direct comparison of investigated protein fractions difficult. None of these studies (except EGGERT *ET AL*. 2010) have investigated gluten fractions and subfractions separated according to the detailed classification of WIESER 2007 (Fig 3) and which have been demonstrated to correspond well to dough, gluten, and baking properties.

Observed changes in wheat quality and biochemical composition of flour have been assumed to have resulted to a large extend from changes in enzyme activity after *Fusarium* infection. Some authors measured the activity of amylase, protease, and cell wall degrading enzymes (cellulase xylanase, 1,3-ß-glucanase) and found it increased in *Fusarium* infected grain (MEYER *ET AL*. 1986; DEXTER *ET AL*. 1996; PAWELZIK *ET AL*. 1998; NIGHTINGALE *ET AL*. 1999; MATTHAUS *ET AL*. 2004; WANG *ET AL*. 2005A; WANG *ET AL*. 2005B). Biochemical changes observed in wheat kernels after *Fusarium* infection were also examined by means of microscopic studies. *Fusarium* infected wheat kernels investigated by electron microscopy showed already in lightly infected kernels the presence of fungal hyphae in pericarp, aleuron layer, endosperm, and the penetration of cell walls. Heavily infected kernels consisted mainly out of the outer kernel layers and showed nearly a complete loss of storage protein and starch granules in endosperm (BECHTEL *ET AL*. 1985; MEYER *ET AL*. 1986; NIGHTINGALE *ET AL*. 1999). A decrease in staining storage proteins and a change in starch granule surface examined with means of light microscopy supported the hypothesis of enzymatic degradation of protein, starch, and other kernel components during *Fusarium* infection (BECHTEL *ET AL*. 1985; SEITZ & BECHTEL 1985; WANG *ET AL*. 2005A).

1.1.3 Assessment of *Fusarium* infection in wheat

One crucial aspect about the investigation of the impact of *Fusarium* infection on wheat quality is how intensity of *Fusarium* infection is defined. Studies that reported on the respective topic have assessed *Fusarium* damage and their investigated material in several ways. This may lead to a limited comparability of the results and may partly explain contradictory results gained for several quality parameters (chapter 1.1.2).

The major proportion of authors cited in chapter 1.1.2 used wheat samples that had been separated into several kernel fractions, according to the occurrence and severity of *Fusarium* symptoms and the resulting change of morphological kernel features such as shape, color, and weight (BECHTEL *ET AL*. 1985; SEITZ & BECHTEL 1985; SEITZ *ET AL*. 1986; BOYACIOGLU & HETTIARACHCHY 1995; DEXTER *ET AL*. 1996; JONES & MIROCHA 1999; NIGHTINGALE *ET AL*. 1999; SIUDA *ET AL*. 2010).

Sample separation into lightly, moderately, and severely infected kernels basically followed individual classification procedures. E.g. BECHTEL *ET AL.* 1985 and BOYACIOGLU & HETTIARACHCHY 1995 each hand-picked three kernel categories while SIUDA *ET AL.* 2010 subdivided four kernel categories by self-defined degree of kernel infestation. Others used composite samples with varying degrees of *Fusarium* damaged kernels (FDK) (SEITZ *ET AL.* 1986; DEXTER *ET AL.* 1996; JONES & MIROCHA 1999). DON levels of the respective samples were determined in most cases additionally. According to these, DON levels of lightly infected samples (kernels) ranged from 0.0-2.5 mg kg^{-1}, in moderately infected samples (kernels) from 0.4-22.7 mg kg^{-1}, and in severely infected samples (kernels) from 22.7-113.0 mg kg^{-1}, indicating that equal sample classification by morphological kernel features resulted in a wide range of DON concentrations in the investigated material between studies. During wheat processing, the removal of severely and moderately infected kernels by cleaning procedures is possible to a certain degree (see chapter 1.1.1.). Therefore, the separate analysis of processing quality and biochemical composition of single moderately to severely infected wheat kernels in comparison to a control or composite samples with high percentage of FDK allows for the exemplified demonstration of *Fusarium* damage on grain but rather lacks practical relevance. First, a grain lot contains a mixture of all kernel classes. Second, marketability of wheat in Northern America and the EU is limited by the percentage of FDK (DEXTER & NOWICKI 2003; COMMISSION REGULATION (EC) NO 824/2000). In addition, in the EU unprocessed wheat must not contain more than 1.25 mg kg^{-1} DON (COMMISSION REGULATION (EC) NO 856/2005). Most of the studies investigated wheat samples and kernels that exceeded these values manifolds. e.g. NIGHTINGALE *ET AL.* 1999 compared clean wheat that contained 0.5 mg kg^{-1} DON with *Fusarium* damaged wheat that comprised 88.0-113.0 mg kg^{-1} DON.

A large number of studies described the *Fusarium* infection exclusively by the DON concentration of grain partly using wheat samples exceeding EU_{MAX} up to 24-fold (HAMILTON & TRENHOLM 1984; ANTES *ET AL.* 2001; MATTHAUS *ET AL.* 2004; PRANGE *ET AL.* 2005; TERZI *ET AL.* 2007; LANCOVA *ET AL.* 2008). A smaller number of authors described *Fusarium* intensity by visual disease assessment in the field by means of different rating scales measuring either disease incidence (MEYER *ET AL.* 1986; PAWELZIK *ET AL.* 1998; HERMANN *ET AL.* 1999) or disease severity (GARTNER *ET AL.* 2008). Corresponding DON levels were only determined by HERMANN *ET AL.* 1999 and GARTNER *ET AL.* 2008. Since DON levels in harvested grain and visual disease are not always closely correlated (DEXTER & NOWICKI 2003) it is difficult to compare the results of these studies with the studies that only detected DON. One working group even used an enzyme-linked immunosorbent assay (ELISA) to determine the degree of *Fusarium* infection by soluble *Fusarium* protein and subdivides sample material by means of this method (WANG 2004; WANG *ET AL.* 2005A; WANG *ET AL.* 2005B; WANG *ET AL.* 2008). However, they neither assessed visual disease nor mycotoxin content of grain which makes comparison with other studies difficult. Yet, since

Fusarium infection of wheat in this study was provoked by artificial inoculation with a high concentration of macroconidia from *F. culmorum*, it can be assumed that DON concentration in grain was also high.

Aside from the aspect of assessment of *Fusarium* infection and preparation of sample material of wheat used for quality determination, two other aspects have to be considered. Most studies used artificial inoculation in order to gain *Fusarium* infected wheat, which is also probably the explanation for partly very high DON levels in these studies. Therefore, generally *F. culmorum (*MEYER ET AL. 1986; PAWELZIK ET AL. 1998; MATTHAUS ET AL. 2004; WANG 2004; WANG ET AL. 2005A; WANG ET AL. 2005B; GARTNER ET AL. 2008; WANG ET AL. 2008; SIUDA ET AL. 2010), a spore mixture (ANTES *ET AL*. 2001; PRANGE *ET AL*. 2005; LANCOVA *ET AL*. 2008) or *F. graminearum* (BOYACIOGLU & HETTIARACHCHY 1995; HERMANN ET AL. 1999; ANTES ET AL. 2001) was used. Since it`s known that different *Fusarium* spp. prefer different growing conditions and also interact with each other (XU *ET AL*. 2007), it can be assumed that fungal biomass differed widely between the respective studies. Furthermore, the major proportion of the studies used wheat samples conducted in field trials with low amount of factor levels for experimental factors involved. In a number of studies just one growing season (and more than one cultivar) (DEXTER *ET AL*. 1996; NIGHTINGALE *ET AL*. 1999; TERZI *ET AL*. 2007; GARTNER *ET AL*. 2008; LANCOVA *ET AL*. 2008) or just one cultivar were investigated (PAWELZIK *ET AL*. 1998; MATTHAUS *ET AL*. 2004; WANG 2004; WANG *ET AL*. 2005A; WANG *ET AL*. 2005B). Other studies used composite samples partly coming from harvest surveys where precise growing conditions are therefore not known (HAMILTON & TRENHOLM 1984; BECHTEL *ET AL*. 1985; SEITZ *ET AL*. 1985; SEITZ *ET AL*. 1986; JONES & MIROCHA 1999). BOYACIOGLU & HETTIARACHCHY 1995 cultivated one spring wheat cultivar under greenhouse conditions. These studies could consequently not consider the abundant number of possible interactions between environmental conditions, cultivars, and agricultural practices such as crop rotation, fungicide treatment, fertilization, and tillage which might have an impact on the occurrence of FHB and on wheat quality and grain composition as well.

All together, this underlines the need for a multifactorial field trial over several growing seasons where natural *Fusarium* infection occurs as a result of different environmental conditions and agricultural practices and where the impact of *Fusarium* infection on quality parameters of wheat and important flour components can be evaluated within the context of these. *Fusarium* infection of wheat should be than be quantified by visual disease in the field, as well as by subsequent mycotoxin concentrations and content of fungal biomass in milling products that were gained from cleaned wheat samples that represent, as common practice, a composite of healthy kernels as well as FDK.

2 Objectives

Research on the influence of *Fusarium* infection on processing quality of wheat and single grain constituents with functional properties seems relatively scarce compared to literature dealing with *Fusarium* mycotoxins, prevention strategies and epidemiology of FHB. The impact of FHB on processing quality and kernel composition was shown to be contradictory and of varying severity when comparing the results of a number of studies (see chapter and 1.1.2 and 1.1.3) which can partly be attributed to the way how *Fusarium* infection was quantified in the different studies and how investigated material was gained. Hardly any study presented data on more than growing season under otherwise identical growing conditions.

Therefore, in the first part of this study the objective was to make a contribution to answering the following questions:

- Which of the experimental factors (environment, cultivar, pre-crop, fungicide) of a field experiment conducted over a time period of three years influence the mycotoxin contamination in milling products (flour Type 550, whole grain flour, bran) gained from cleaned wheat?
- Which *Fusarium* toxins and *Fusarium* spp. are detectable in wheat milling products?
- Can a subsequent mycotoxin contamination of milling products already be estimated from visual rating parameters (disease intensity, disease severity, *Fusarium* head blight index) obtained in the field?
- How are *Fusarium* biomass and produced mycotoxins related in wheat milling products?

In the second part of the study the focus of the investigations lies on the impact of *Fusarium* infection on wheat quality traits –with focus on parameters that are commonly assessed in agricultural trade, mills, and bakeries- and flour composition – with focus on gluten proteins- while taking the following questions into account:

- Which of the experimental factors (environment, cultivar, pre-crop) influence *Fusarium* infection (quantified than as DON concentration), wheat quality, and composition of wheat flour Type 550?
- In what way does *Fusarium* spp. infection have an impact on wheat quality and flour composition (starch, protein, gluten, enzyme activity)?
- Is there a correlation between the investigated wheat quality parameters and flours components e.g. gluten proteins?
- If there is an effect of *Fusarium* spp. infection on wheat quality and flour composition, how high are DON levels in the respective flours? Are these samples, with regard to EU_{MAX}, still suitable for wheat processing and human consumption, respectively?

- If there is an effect of *Fusarium* spp. infection on wheat quality and flour composition, is this impact relevant for practical wheat processing, particularly for bread and roll production?

Fig 4 gives an overview of possible interactions between experimental factors, *Fusarium* infection, chemical flour composition, and wheat quality traits that were investigated within this work. Additional information on grain yield, thousand-kernel weight, and sulfur content of flours are not shown in particular in the results sections but can be looked up in the supplementary material and are partly mentioned in the discussion.

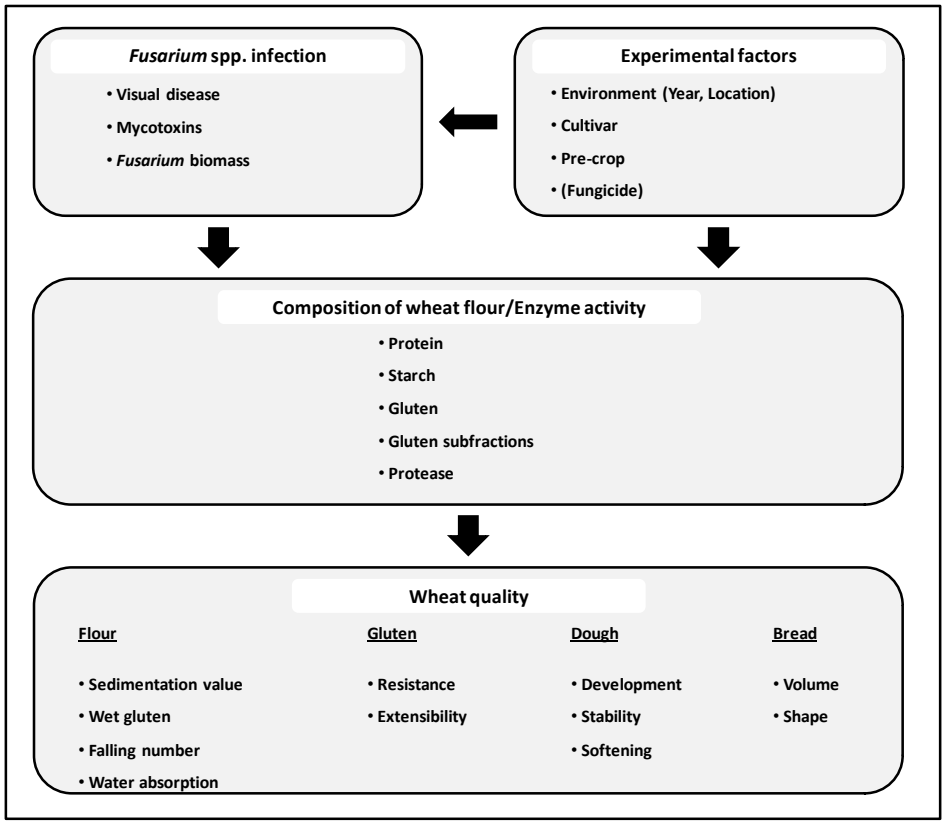

Fig 4. Scheme of investigated interactions between experimental factors, *Fusarium* infection, composition of wheat flour/enzyme activity, and wheat quality parameters within objectives of this study

3 Material and methods

3.1 Plant material

3.1.1 Field experiment

To investigate the above mentioned a field experiment over the time period of three years (2007-2009) was conducted. Trial locations were situated 15 to 20 km north of Göttingen, in the south of Lower Saxony, Germany, and termed Torland (51.36° N, 9.55° E) and Gladebeck (51.37° N, 9.52° E) in the subsequent text. The dominant soil type on these locations is braunerde, either braunerde mixed with lime stone (Torland) or para-braunerde (Gladebeck). Torland lies 140 m above sea level, Gladebeck above 165 m, respectively. The average annual precipitation in this region is 645 mm (WSG 2003). The trial design was conducted as split-split-split plot including pre-crop, cultivar, and fungicide treatment as experimental factors. A detailed description of the trail was already given by GÖDECKE 2010. Detailed information about weather conditions around wheat anthesis (May-July) in the respective years can be viewed in the supplementary material (Fig 30) and was gained from the Weather Station Göttingen (WSG 2010, 51.63° N, 9.86° E, 171 m above sea level). Pre-crops were winter wheat, (forage) maize, and sugar beet which are commonly grown crops in Lower Saxony and Germany. To enhance natural *Fusarium* spp. infection, reduced tillage was applied. Subsequently grown winter wheat cultivars (cv.) were cv. Centrum and cv. Ritmo. Both were classified as bread wheat cultivars (quality class B) and where chosen because they differed strongly in their susceptibility against *Fusarium* infection according to BSA 2007. Cv. Centrum generally showed a low to very low susceptibility against FHB, cv. Ritmo was highly susceptible. Both cultivars differed only slightly in their quality traits (Tab 2).

Tab 2. Selected variety descriptions of winter wheat cultivars Centrum and Ritmo (BSA 2007)

Characteristic trait		Centrum	Ritmo
Susceptibility against FHB		**2**	**7**
Falling number		**6**	**7**
Crude protein		**3**	**3**
Sedimentation value		**6**	**5**
Pearling value		**6**	**5**
Ash		**5**	**5**
Water absorption ability		**5**	**6**
Flour yield T 550		**7**	**6**
Baking volume		**5**	**5**
Dough elasticity	dominating property	**3**	**3**
Surface properties of dough		**3**	**3**

Description of characteristics is based on a 1-9 scale, 1 = characteristic is shown to a very low degree, 9 = characterisitic ist shown to a very high degree, 5 = medium expression of characteristic

Fungicide treatments were applied as described in Tab 3 and Tab 4. Fungicides were applied against fungal leaf pathogens in the conventional way at start (BBCH 31) and end of shooting (BBCH 39). Fungicide treatments were applied according to the project objectives of FAEN project 1 and were explained in more detail by GÖDECKE 2010. However, it should be emphasized at this point that fungicides were not applied against FHB or prevention of the disease but rather used to study the physiological effects of fungicides on the plant (in interaction with a *Fusarium* spp. infection).

Tab 3. Description of fungicides and growth regulator applied in field experiment

Commercial name	Active ingredient	Class	Indication in wheat
Amistar	Azoxystrobin	Strobilurine	DTR, rusts, *Septoria* , Powdery Mildew
Bravo	Chlorthalonil		Rusts, *Septoria* , Powdery Mildew
CCC	Chlorocholine chloride		Growth regulator
Corbel	Fenpropimorph	Morpholine	Rusts, Powdery Mildew
Flexity	Metrafenone		*Pseudocercosporella,* Powdery Mildew
Opus	Epoxiconazol	Azole	Rusts, *Septoria* , Powdery Mildew
Proline	Prothioconazol	Azole	DTR, rusts, *Septoria* , Powdery Mildew, *Fusarium, Pseudocercosporella*

DTR: *Drechslera tritici-repentis*

Tab 4. Fungicide and growth regulator application in field experiment

BBCH*	Triazole		Strobilurine		Chlorthalonil	
	splitted	CCC	splitted	CCC	splitted	CCC
31	0.8	Proline	1.0	Amistar	1.0	Bravo
	1.0	Bravo	1.0	Bravo	1.0	Corbel
			0.4	Flexity		
39	1.0	Opus	1.0	Amistar	1.0	Bravo
	1.0	Bravo	1.0	Bravo	1.0	Corbel

* Development stage of wheat according to Federal Biological Research Centre for Agriculture and Forestry, Federal Office of Plant Varieties and the Chemical Industry, values show the dosage of fungicide and growth regulator in l ha^{-1}

Further details of agricultural measures (seed density, soil preparation, etc.) can be viewed in the thesis of GÖDECKE 2010. Nitrogen fertilization regime can be viewed in the supplementary material of this thesis (Tab 65). Tab 5 gives an overview of all experimental factors and factor levels.

Tab 5. Factors and factor levels of field experiment

	Factor				
	Year	Location	Cultivar	Pre-crop	Fungicide
Factor level	2007	Torland	Centrum	Winter wheat	Strobilurin
	2008	Gladebeck	Ritmo	Maize	Triazole
	2009			Sugar beet	Chlorthalonil

Wheat was harvested during end of July beginning of August at moisture content of 14.5-16.0 %. Each sub-subplot was harvested completely by combine harvester (Farmliner, Deutz-Fahr, Cologne, Germany) and yield was documented. Grain was harvested from 196 plots (25 m²) since each factor combination (cultivar x pre-crop x fungicide) was replicated 4 times. 2.5 kg were taken from each plot as a subsample, representing 15-20 % of the yield coming from one plot.

3.1.2 Post-harvest treatment

Cleaning and sampling

Samples were left to air drying until grain moisture was <14 %. Grain was than cleaned with standard settings of A/S Rationel Kornservice, Esbjerk, Denmark. 1.5 kg of each of the four plots coming from the same treatment (location x pre-crop x cultivar x fungicide) were combined. This created a representative harvest sample for each factor combination and randomized effects of the field trial. Therefore, fungicide application only added to variability of samples within the combination of location x pre-crop x cultivar (2 locations x 3 pre-crops x 2 cultivars x 3 fungicides = 36 samples) and could not be considered in the complete interaction of environment (year x location) x pre-crop x cultivar x fungicide since there existed just one observation (n) of this combination. Grain samples were stored at room temperature in burlap bags until milling.

Milling and preparation of flours and bran (milling products)

After one month of storage 100 g of grain of each sample were milled to whole grain flour (WGF) (ZM 100, Retsch GmbH, Haan, Germany) with a mesh of 0.5 mm. Flour Type 550 was gained by milling 2 kg of grain into several milling fractions (2007: Buhler laboratory mill type MLU 202, Uzwil, Switzerland; 2008/9: Quadrumat Senior 220/380, Brabender, Duisburg, Germany) after tempering grain at room temperature for 12 h to moisture content of 15.5 %. For the determination of mineral (ash) content of milling streams, ICC STANDARD NO 104/1 was slightly modified. 5 g of flour was dried in drying oven (Ecocell 55, MMM Medcenter Einrichtungen GmbH, Munich, Germany) until constant weight and after that incinerated in a muffle furnance (MR 260, Heraeus Holding GmbH; Hanau, Germany) for 4 h at 900°C. Determination was replicated at least two times. Ash content was calculated on the basis of dry matter (DM). Than milling streams were mixed to gain an average ash content of 0.55%. Flours that contain an ash content from 0.51-0.63% are termed flour Type 550 according to DIN 10355. All of the bran (30-35 % of grain) obtained from milling flour Type 550 was collected and milled to smaller particle size (ZM 100, Retsch

GmbH, Haan, Germany) with the mesh of 0.5 mm. Over three years, a total n of 108 WGF, Type 550, and bran were gained each.

Storage

Flours and bran were kept in air-tight bottles (PE) at 4-8 °C in the dark until analysis.

3.2 Quantification of *Fusarium* ssp. infection

3.2.1 Visual disease

Occurrence of *Fusarium* head blight was evaluated approximately 15-20 days after anthesis at BBCH 75 (medium milk). *Fusarium* head blight index (FHB index in %) was calculated as the product of disease incidence (DI), presenting the percentage of spikes infected, and disease severity (DS), presenting the proportion of infected spikelets per infected spike, and was determined as described by WILCOXSON *ET AL.* 1992. FHB index can be interpreted as the average proportion of diseased spikelet per spike (FHB index = DI * DS). DI was determined in all three years for a total of 432 plots. Due to very few symptoms visible, DS was not determined in 2009. Therefore, FHB index could only be calculated in 2007 and 2008 for a total of 288 plots.

3.2.2 Mycotoxins

Mycotoxin determination was performed by means of HPLC-MS-MS by S. Limsuwan and P. Kössler in the laboratory of Molecular Phytopathology and Mycotoxin research, Department of Crop Sciences, University of Göttingen, Germany. 5 g of flours (WGF: n = 108, Type 550: n = 108) and bran (n = 108) were extracted with acetonitrile-water (84:16) in 2007, and methanol-isopropanol-water (80:5:15) in 2008 and 2009 and the extracts were cleared, defatted, concentrated and filtered as described previously by ADEJUMO *ET AL.* 2007. The analytes were eluted and separated by HPLC on polar modified C_{18} phase using a methanol-water gradient containing 5 mM acetic acid. DON, 3-ADON, 15-ADON; NIV, FUSX, and ZEA were detected by tandem mass spectrometry using transitions described by KLOTZEL *ET AL.* 2006 and ADEJUMO *ET AL.* 2007. Calibration curves were prepared in matrix extracts using certified analytical standards and processed in the same way as the samples. For DON, limit of detection (LOD) was 0.015 mg kg^{-1}; limit of quantification (LOQ) was 0.100 (2007), 0.010 (2008), and 0.050 (2009) mg kg^{-1}. For 3-ADON, LOD was 0.015 mg kg^{-1}, LOQ was 0.05 mg kg^{-1}. For ZEA, LOD was 0.002 mg kg^{-1}, LOQ was 0.025 (2007/2008) and 0.005 mg kg^{-1} (2009). LOD and LOQ applied to all milling products including flour Type 550, whole grain flour, and bran. 15-ADON, NIV, and FUSX were not detected in any of the samples. Mycotoxin content of flours and bran is usually presented in mg kg^{-1}, except for Tab 7 where µg kg^{-1} is used in the context of the other toxins. All values are based on DM.

3.2.3 *Fusarium* biomass

Fusarium biomass was assessed as *F. graminearum* and *F. culmorum* DNA (*Fusarium* DNA) by real-time PCR and was performed in the laboratory of Molecular Phytopathology and Mycotoxin research, Department of Crop Sciences, University of Göttingen, Germany. DNA from *F. graminearum* and *F. culmorum* was extracted from 100 mg of flour (WGF: n = 108, Type 550: n = 108) and bran (n = 108) material using the modified CTAB protocol and purified by polyethylene glycol precipitation as described by BRANDFASS & KARLOVSKY 2008. Real-time PCR for *F. culmorum* was performed as described previously by BRANDFASS & KARLOVSKY 2006. The real-time PCR for *F. graminearum* was performed under identical conditions except that the $MgCl_2$ concentration was set to 2.5 mM. Standards of fungal DNA were combined with plant DNA extracted from flour samples free of *Fusarium* spp. in order to simulate the effects of the plant matrix on the PCR. The limit of quantification for *F. culmorum* was 500 pg fungal DNA g^{-1} flour. For *F. graminearum*, the LOQ was the same in 2007 and 2008. In 2009, the LOQ was 300 pg g^{-1} flour. The limit of detection has not been determined. DNA content of *F. graminearum* (*Fg*DNA) and *F. culmorum* (*Fc*DNA) in flours and bran is presented in µg kg^{-1}. All values are based on DM.

3.3 Determination of quality parameters

3.3.1 Protein content

Nitrogen (N) content of WGF and flour Type 550 (2009) was determined according to Dumas principle from 100 mg dried flour by C/N analyzer (Vario MAX CN Elementar Analysensysteme GmbH, Hanau, Germany). N of 100 mg dried of flour Type 550 from 2007 and 2008 was analyzed with elemental analyzer (Elementar Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany). Quantitative N determination was performed in the laboratory of the Institute of Soil Science, Department of Crop Sciences, University of Goettingen, Germany and at the Institute of Soil Science, Leibniz University of Hannover, Germany. Protein content (PC) was calculated according to ICC No. 105/2 (N x 5.7). PC of WHG and flour Type 550 is referred to as PC_{WGF} (n = 108) and $PC_{Type550}$ (n = 108).

3.3.2 Wet gluten content, sedimentation value, falling number

Wet gluten content (WG) of flour Type 550 (n = 108) was determined by following standard procedures described by ICC STANDARD NO. 106/2. Sedimentation value (SV) of flour Type 550 (n = 108) was determined with Sodium Dodecyl Sulfat (SDS) test according to ICC STANDARD NO. 151. Hagberg falling number (FN) of WGFs (n = 108) was measured according to ICC STANDARD NO. 107/1 with Falling Number System FN1200 (Perten Instruments, Huddinge, Sweden).

3.3.3 Water absorption and mixing properties of dough

In 2007, water absorption (WA) and dough properties of flour Type 550 (2007: n = 36, 2008/9: n = 24 each) were obtained by using a valorigraph (Type FQA-205, Metefem, Budapest, Hungary) similar to the Brabender farinograph using modified ICC STANDARD NO. 115/1. Obtained valorigrams were analyzed manually. In 2008 and 2009 dough properties were gained by use of Farinograph (Brabender®, No. 901368, type 810 105 001, Duisburg, Germany). Farinograms were analyzed with Brabender® Farinograph Version 4.0.3. Each measurement was repeated three times. WA of flour is the volume of water required to produce dough with a maximum consistency of 500 VU or 500 FU, respectively. WA is expressed as a percentage (%), meaning ml per 100 g of flour at 14 % moisture content. Mixing behavior of dough prepared from these flours is characterized by dough development time (DDT), dough stability (DST), and degree of softening (DS). DDT (min) indicates the time from the moment where water is added until dough reaches its maximum consistency. The time where the dough maintains its maximum consistency is indicated by DST (min). DS (VU/FU) expresses the difference in dough consistency between maximum consistency and consistency 12 min after maximum.

3.3.4 Microbaking test

Dough production for microbaking test was done according to the following recipe: 50 g flour Type 550 (corrected to 14% moisture content) were mixed with 1% sucrose, 1% fat, 1.5% salt, 5% fresh yeast, and 0.002% L-ascorbic acid based on flour weight. Than the water amount was added that was obtained with Valorigraph or Farinograph (see chapter 3.3.3) before. Dough preparation followed procedures of Rapid-Mix-Test (RMT). Dough was mixed for 2 min at 30 °C and proofed for 20 min at 30 °C in a water saturated atmosphere (Ecocell 55, MMM Medcenter Einrichtungen GmbH, Munich, Germany). Dough was divided into five pieces of equal weight and relaxed for further three minutes. In order to remove air bubbles, dough balls were formed with a noodle machine (model Atlas 150, Marcato, Italy) and rolled into a log shape. Thereafter, pieces were proofed again for 45 min. Baking was performed for 12 min at 240 °C (electric furnance, AEG Haustechnik, Nuernberg, Germany) as described by KIEFFER *ET AL.* 1993. Baking volume (BV) was measured after loaves had cooled by rapeseed displacement (SEIBEL 1985); loaf dimensions, length (LL), height (LH), width (LW) by digital slide gauge (Digital caliper 0-150 mm, WK 1020 SK). BV is expressed in ml 100 g^{-1} flour, loaf dimensions in mm. n was 36 in 2007 and 24 in 2008 and 2009, respectively.

3.3.5 Micro-extension test on gluten

Determination of gluten properties of *Fusarium* infected wheat flour was acquired in the context of the Bachelor thesis of J. Schelle (SCHELLE 2010). For flour Type 550 from 2007, wet gluten was determined according to ICC STANDARD NO. 106/2. Afterwards, wet gluten was centrifuged for 5

min at 5 000 rpm, placed in a teflon form base and relaxed for 40 min at 30°C in water saturated atmosphere (Ecocell 55, MMM Medcenter Einrichtungen GmbH, Munich, Germany) as described by KIEFFER *ET AL*. 1998. Measurement of gluten extensibility was performed with the SMS/Kieffer Doug and Gluten Extensibility Rig and the Texture Analyser (TA) (TA-XT2, Stable Micro Systems, Surrey, England). The test procedure of micro-extension followed basically the description of KIEFFER *ET AL*. 1981 using a 25 kg load cell. The precise settings were: test speed 3.3 mm s^{-1}, distance 140 mm, trigger force 5 g, data acquisition rate: 200 pps.

For each sample wet gluten was extracted twice, and from each wet gluten sample three to four gluten strips were stretched until rupture according to the description of the instruction manual (SMEWING 1995). Obtained graphs of gluten extension were analyzed with Texture Expert Version 1.22. Peak force, which corresponds to resistance to extension (R_{MAX}), and the distance at which the peak force occurs were measured. R_{MAX} is presented in N, extensibility (EXT) in mm. Since gluten partly did not rupture even though stretched to maximum of 140 mm, the ratio of R_{MAX} to EXT (N/m) was calculated to compare and gluten properties anyhow. Stability of measurement was determined by measuring the gluten properties of a commercial wheat flour of type 550. Wet gluten was prepared three times as described before and a total of 12 gluten stripes were stretched. The average relative difference of a single measurement from the mean value of all measurements was 10.0% for R_{MAX}, 10.5% for EXT, and 13.5 for R_{MAX}/EXT.

3.4 Determination of flour composition

3.4.1 Starch

For determination of starch content, flour Type 550 (n = 108) were dissolved in hydrochloric acid according to ICC STANDARD NO. 123/1 and measured by polarimetric method (Polarimeter type V DrNa, Zeiss AG, Jena, Germany). Protein was precipitated with wolframatophosphoric acid. Starch content was calculated with the rotation angle of the filtrate at 589 nm. Each measurement was repeated twice.

3.4.2 Gluten proteins

Extraction

Extraction of gliadins and glutenins from 100 mg of flour Type 550 (n = 108) was conducted with the solvents and procedure according to WIESER 2000. Before each extraction step, solvent and flour or sediment, respectively, were vortexed for two minutes. Albumins/globulins and gliadins were extracted at room temperature with magnet stirring (Variomag Multipoint 15, H+P Labortechnik AG, Oberschleissheim, Germany). Extraction of glutenins was performed for 20 min at 60 °C in a thermomixer (Thermomixer comfort, Eppendorf AG, Hamburg, Germany) at 750 rpm. Suspensions were centrifuged (Centrifuge 5804R, Eppendorf, Hamburg, Germany) at 20 °C

for 15 min at 7500 rpm. Each sample was extracted twice. Protein extracts were stored at -20°C until measurement.

Protein standards

For quantification of protein fractions, gliadin and glutenin standards were prepared from commercially available gluten from wheat (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) as described by EGGERT *ET AL*. 2011. PC of isolated gliadin and glutenin was determined by C/N analyser as described in chapter 1.4.2. PC of gliadin and glutenin standard were 93.41 and 100% of DM, respectively. Gliadin and glutenin standards were solved and determined as described by WIESER 2000.

Detection with RP-HPLC

Quantification of gliadin and glutenin fractions followed with slight modification the description of WIESER *ET AL*. 1998 and EGGERT *ET AL*. 2010. For the RP-HPLC, a PerfectSil 300 C_8 300 x 4.6 column (Machery-Nagel, Dueren, Germany) was used. Mobile phases were A = 0,1 % trifluoroacetic acid (TFA) (Merck, Darmstadt, Germany) in H_2O (v/v) (Chromanorm, VWR, Fontenay-Sous-Bois, France) and B = 0,1 % TFA in acetonitrile (v/v) (HiPerSolv, Chromanorm, VWR; Leuven, Belgium). All solvents were of HPLC grade. 100 µl of the aliquots of gliadin and glutenin were injected. For separation of protein fractions the gradient program shown in Tab 6 was applied.

Tab 6. Gradient program for RP-HPLC for separation of gliadin and glutenin

Time (Min)	% A	% B
0	100	0
5	76	24
50	50	50
54	10	90
59-70	100	0

Flow rate was 1 ml min^{-1}; the column temperature was set at 50 °C. Chromatograms for gliadins (Fig 5) and glutenins (Fig 6) show exemplified the retention times of single subfractions. Gliadin subfractions were separated into ω5-, ω1,2-, α-, γ-gliadins, whereas glutenins were separated into ωb, HMW-GS, and LMW-GS. ωb gliadin represents a small proportion of gliadins that are linked to the glutenins (WIESER & SEILMEIER 1998).

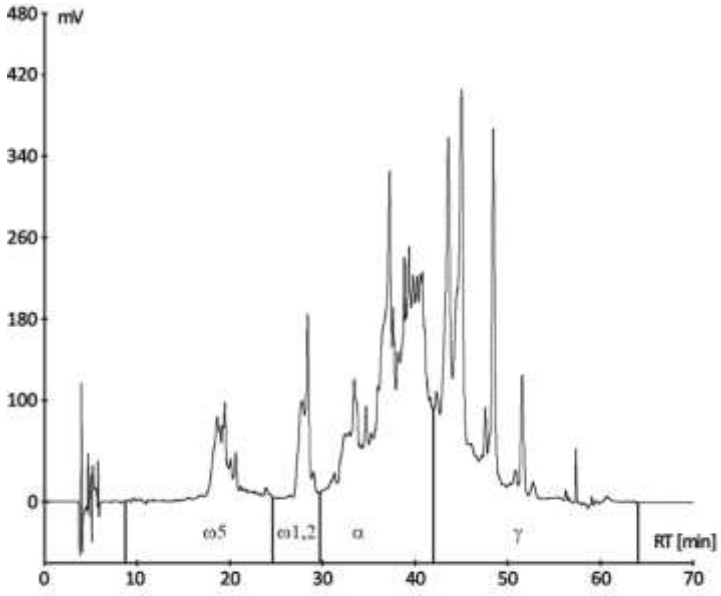

Fig 5. RP-HPLC of gliadin extract (cv. Centrum) with elution ranges for ω5-, ω1,2-, α-, and γ-gliadins

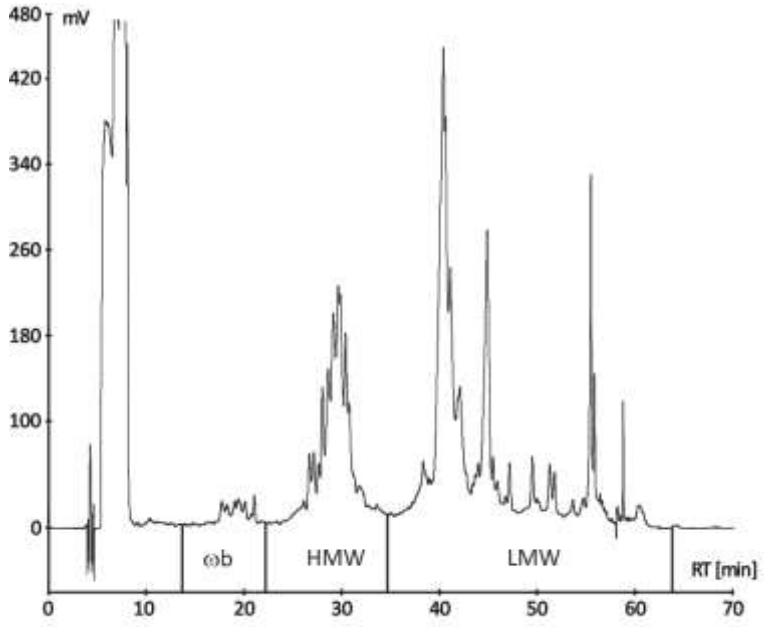

Fig 6. RP-HPLC of glutenin extract (cv. Centrum) with elution ranges for ωb, HMW-GS, and LMW-GS

For the quantification of the protein fractions gliadin and glutenin standards were used. In order to eliminate the effect of starch accumulation and the adverse relationship with PC in grain, quantity of gliadin, glutenin as well as subfractions was calculated as percentage of total protein in flour Type 550 (% Protein).

HPLC system (MZ Analysentechnik, Mainz, Germany) consisted of autosampler (Jasco AS-2051 Plus Intelligent Sampler), oven (Jasco CO-2060 Plus Intelligent Column Thermostat), degaser (Jasco DG-2080-54 4-Line-Degaser), pump (Jasco PU-2080 Plus Intelligent HPLC Pump), gradient mixer (Jasco LG-2080-04 S), and detector (Jasco MD-2015 Plus Multiwavelength Detector).

Obtained chromatograms were analyzed by Jasco ChromPass Chromatography Data Systems Version 1.8.6.1.

3.5 Protease activity

Protease activity (PA) was determined for flours Type 550 from 2007 (n = 36).

Extraction

400 mg of flour Type 550 was extracted with 1.5 ml sodium acetate buffer (50 mM, pH 5.5) at 4°C for 12 h by shaking overhead. After centrifugation (10 000 rpm, 15 Min), aliquots were stored at -20°C. Each sample was extracted at least twice.

Synthesis of azogelatin

Synthesis of azogelatin followed the procedure decribed by JONES *ET AL*. 1998, except that gelatin from porcine skin (high gel strength, Fluka, St. Louis, USA) bloom 250 g was used.

In-solution assay with azogelatin

In-solution assay with azogelatin followed basically the descriptions from JONES *ET AL*. 1998. 0.17 ml of enzyme extract was mixed with 0.17 ml ammonium acetate (0.1 M, pH 5.5), 0.017 ml Cysteine (0.1 M). To start the reaction, 0.5 ml of azogelatin (1 % (w/v)) was added. In order to simulate similar temperature conditions as present during dough mixing in Valorigraph/Farinograph, samples were incubated at 30°C with 300 rpm for 1 h (Thermomixer comfort, Eppendorf AG, Hamburg, Germany). At the end of incubation, reaction was stopped by adding 0.857 ml tricloric acid (25% (v/v) TCA) and incubating samples on ice for 15 min. After centrifugation for 10 min at 14 000 rpm and 4°C (Centrifuge 5804R, Eppendorf, Hamburg, Germany) the supernatant was measured photometrically in a microcuvette at 440 nm (Spectrophotometer, Hewlett Packard 8453, Palo Alto, USA). For blank, solutions were incubated without enzyme extract; enzyme extract was added after TCA had been added. For each extract, one blank was prepared; in-solution assay with enzyme extract was repeated three times.

3.6 Additional analyses

3.6.1 Determination of yield components

Grain yield (GY) (dt ha^{-1}) was calculated by considering the harvest of a total plot (25 m^2). Thousand-kernel weight (TKW) was conducted by measuring the weight of 100 randomly picked intact kernels. Measurement was repeated four times. TKW (g) was calculated on moisture content of 14 %. Both measures were determined as additional information and not presented in the results but in the supplementary material (Tab 66).

3.6.1 Sulfur and sulfur to nitrogen ratio

Sulfur (S) content of flour Type 550 from 2007 (n = 36) and 2008 (n = 36) was analyzed with elemental analyzer (Elementar Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany). Quantitative S determination was performed in the laboratory of the Institute of Soil Science, Leibniz University of Hannover, Germany. S content of flours was determined as additional information and not presented in the results but in the supplementary material (Tab 66). The same applies to the calculated ratio of N/S. The purpose of the investigation was to rule out sulfur deficiency in grain.

3.7 Statistical analysis

Data analyses were performed by SAS version 9.2, SAS Institute, Cary, NC, USA and STATISTICA version 8.0, StatSoft, Inc., Tulsa, OK, USA. Graphs were conducted by means of SigmaPlot version 10.0, Systat Software GmbH, Erkrath, Germany.

To investigate the factors influencing DON and *Fg*DNA, the experimental design was considered to be completely randomized since samples of four plots from each treatment were combined (as described in 3.1.2). Analysis of variance (ANOVA SAS: proc mixed) was conducted with untransformed data restricting higher-order interaction effects to the number of factors minus 1 since no replication for each treatment was available. Assumptions were normal distribution and homogenicity of variance checking residuals graphically (histogram, q-q-plot, residual plot). Main factors that were considered in ANOVA were environment (as a combination of year and location), cultivar, pre-crop, fungicide and milling fraction. Data of flour Type 550, WGF, and bran of each treatment were taken into account as repeated measurement of the same experimental unit. Depending on the outcome of the global *F* test statistic, multiple comparisons of means were applied according to Tukey and Kramer. Unless not stated otherwise the significance level was $\alpha = 0.05$. With the objective to elucidate effects at the level $p < \alpha < 0.05$ effects were sliced into single factors. Data is presented graphically as bar chart with mean values (MV) and standard error (SE) of the respective treatments. For DON, for each experimental factor the severity of effect (SoE) was calculated according to KOCH *ET AL*. 2006, dividing the maximal MV with the minimum MV of each treatment (treatment included data of all other factors) in order to evaluate the relative importance of each factor for occurrence of DON in milling products.

To investigate the influence of environment (year x location), cultivar, and pre-crop on wheat quality and flour composition, ANOVA considered the data of three years. Interactions were calculated to the level of 2. Data in chapter 4.2 and 4.3 is either presented graphically as box plots where one box plots comprises six biological samples (from two locations and three fungicide treatments) or in tables where mean values (MV) and standard deviation (SD) of the respective treatments are given. Multiple comparisons of MVs were generally performed according to Tukey

and Kramer by slicing years. For additional information, ANOVA was also performed for the separate effects of year, location, cultivar, pre-crop, and fungicide up to 3-fold interactions. SoE for the single experimental factors was calculated, as described before, in addition. Both can be looked up in the supplementary material (Tab 54-Tab 66).

In order to investigate how *Fusarium* infection affected wheat quality and flour composition, effect of environment, pre-crop, cultivar, and DON was tested by performing analysis of covariance (ANCOVA) whereas DON, taken as a measure for intensity of *Fusarium* infection, was introduced into the model as covariate and a ANCOVA strategy was performed according to LITTEL *ET AL*. 2006. ANCOVA included the categorical effects of environment (year x location), pre-crop, and cultivar restricting higher-order interaction effects to the number of factors minus 1.

Since in 2008, DON concentrations were very low or below LOD, it was impossible to evaluate the impact of *Fusarium* infection on quality parameters in the respective year. Thus, only data of 2007 and 2009 were included into ANCOVA. Since DON data of 2007 and 2009 did not exhibit normal distribution, DON data was logarithmically transformed (DON_{LOG}). Zero DON values were taken into account by adding a small value (0.01) to the logarithmic function. Normal distribution of quality parameters was checked graphically (see above). If ANCOVA revealed a significant effect ($p < 0.05$) of DON_{LOG} alone or in combination with other factors this effect was further investigated by performing simple linear regressions (proc reg) with DON_{LOG} and the respective parameter separated by the interacting factor or factor combination. Linear regressions are presented if DON_{LOG} explained at least 50% of variability in quality parameters (coefficient of determination $R^2 \geq 0.50$, $p < 0.05$) and contained at least six observations. $R^2 = 0.50$ corresponds to correlation coefficient r of ~ 0.71 and describes a moderate relationship among target and in depended variable. Original DON data (DON_{ORG}) of respective samples was either displayed in the tables or in the text. If there was no significant influence of DON, the covariate DON could be omitted from the model resulting in a simple ANOVA.

Relationships among quality parameters and flour components were investigated by linear regression. If R^2 was ≥ 0.50 at a significance level $p < 0.05$, correlations were presented with trend (+/-) in chapter 4.5. Additionally, complete correlation matrices can be looked up in the supplementary material (Tab 62-Tab 64)

4 Results

4.1 *Fusarium* biomass and mycotoxins in milling products

4.1.1 Occurrence of *Fusarium* DNA and mycotoxins

Depending on year, *Fusarium* ssp. and type of toxin differing levels of *Fusarium* DNA and mycotoxins were detected in flours and bran (Tab 7). Both *Fusarium* DNA and mycotoxin could be detected in all milling products. Highest *Fusarium* DNA and toxin concentrations were detected in 2007.

Tab 7. Range of mycotoxin and *Fusarium* DNA content of wheat milling products (flour Type 550, whole grain flour, bran)

		Milling product								
		Flour Type 550			Whole grain flour			Bran		
Fungal DNA (µg kg^{-1})	Year	Min	Median	Max	Min	Median	Max	Min	Median	Max
F. graminearum	2007	4	42	317	8	119	765	<LOQ	319	2771
	2008	<LOQ	<LOQ	10	<LOQ	<LOQ	18	<LOQ	<LOQ	<LOQ
	2009	<LOQ	<LOQ	39	<LOQ	<LOQ	200	<LOQ	<LOQ	63
F. culmorum	2007	<LOQ	3	19	<LOQ	6	65	<LOQ	<LOQ	134
	2008		-			-			-	
	2009	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Mycotoxin (µg kg^{-1})										
DON	2007	312	1,588	11,843	197	1,260	10,237	506	2,332	16,437
	2008	<LOD	<LOD	109	<LOD	<LOD	283	<LOD	<LOD	227
	2009	<LOD	406	8,416	<LOD	171	4,624	<LOD	309	6,865
3-ADON	2007	<LOD	<LOD	524	<LOD	<LOD	102	<LOD	<LOD	425
	2008	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2009		-		<LOD	<LOD	295		-	
ZEA	2007	<LOD	<LOD	86	<LOD	<LOD	175	<LOD	<LOD	<LOD
	2008	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2009		-		<LOD	<LOD	73		-	

Min: minimum content, Max: maximum content, DON: deoxynivalenol, 3-ADON: 3-acetyldeoxynivalenol, ZEA: zearalenone, LOD: limit of detection, LOQ: limit of quantification, "-" not analyzed, flours and bran per year: n = 36

Total *Fusarium* DNA predominantly consisted of *Fg*DNA (Tab 8). Only in 2007, both *Fusarium* species, *F. graminearum* and *F. culmorum*, could be detected by qPCR. The *Fc*DNA content was significantly lower than *Fg*DNA content of all milling products. In flours and bran, *Fc*DNA just made up an average proportion of approx. 8% of total *Fusarium* DNA. The relationship between the two species was positive, but very weak (R^2 = 0.03-0.12) Therefore, for further analysis *Fc*DNA levels will not be considered.

Tab 8. Proportion of *Fg*DNA and *Fc*DNA from total *Fusarium* DNA (*Fg*DNA + *Fc*DNA = 100%) in milling products (flour Type 550, whole grain flour (WGF), bran) of 2007

	Flour Type 550		WGF		Bran	
	Fg DNA (%)	*Fc* DNA (%)	*Fg* DNA (%)	*Fc* DNA (%)	*Fg* DNA (%)	*Fc* DNA (%)
Median	93	7	93	7	100	0
MV	90	10	91	9	92	5
SD	12	12	11	11	18	9
p	***		***		***	
R^2	0.05		0.12		0.03	

MV mean value SD standard diviation, WGF whole grain flour, flours and bran: n = 36 each, p significance level: *** p < 0.001 according to student`s pairwise t-test, R^2 coefficient of determination

In all milling products, DON was the most frequently occurring and also quantitatively the most important *Fusarium* toxin of all toxins analyzed. Especially in 2007, up to 50% of all Type 550 flours and bran samples surpassed the EU_{MaxCF} (750 µg kg^{-1}) at least twice. In 2007, also 3-ADON could be detected in 14, 2, and 8 samples of flour Type 550, WGF, and bran, respectively. Additionally, ZEA was found in one sample of flour Type 550 and 12 WGFs in levels exceeding the EU_{MaxCF} (75 µg kg^{-1}). In 2008, only DON could be detected in 7, 17, and 15 samples of flour Type 550, WGF, and bran, respectively. In 2009, 13 WGFs contained 3-ADON, three contained ZEA. In none of the years of investigation, 15-ADON, FUSX or NIV could be detected in any of the milling products. Further data analysis will therefore take only DON content of milling products into account.

4.1.2 Factors influencing *Fg*DNA and DON

The results for ANOVA for DON and *F. graminearum* biomass are presented in Tab 9. Most effects that showed a significant influence on DON also affected *Fg*DNA content in milling products. For both, significant effects of environment (as a combination of year and location), pre-crop, cultivar, fungicide, milling fraction, as well as the interactions of environment-pre-crop, environment-cultivar, environment-milling fraction, environment-pre-crop-cultivar, environment-pre-crop-milling fraction, environment-cultivar-milling fraction, environment-pre-crop-cultivar-milling fraction, pre-crop-cultivar, pre-crop-milling fraction, pre-crop-cultivar-fungicide, cultivar-fungicide, and cultivar-milling fraction were observed. Additionally, for DON, the interaction of environment-fungicide, environment-pre-crop-fungicide, environment-cultivar-fungicide, environment-pre-crop-cultivar-fungicide, and pre-crop-fungicide was significant. For *Fg*DNA, also a significant effect of pre-crop-cultivar-milling fraction was detected. Interestingly, interactions with fungicides did not have a significant effect on *Fg*DNA in milling products, except the interaction of cultivar-fungicide, which was usually the case for DON.

Tab 9. ANOVA for effects of environment (year, location), pre-crop, wheat cultivar, fungicide, and milling product on DON and *Fg*DNA content

		DON		*Fg* DNA	
Effect	**DF**	**F**	**p**	**F**	**p**
Environment (E)	5	261.54	***	178.52	***
Pre-crop (P)	2	392.47	***	148.36	***
Cultivar (C)	1	587.01	***	198.93	***
Fungicide (F)	2	36.52	***	3.60	***
Milling product (MP)	2	34.14	***	127.85	***
E x P	10	56.75	***	56.40	***
E x C	5	95.74	***	68.89	***
E x F	10	7.88	***	1.34	**n.s.**
E x MP	10	14.36	***	57.15	***
E x P x C	10	21.94	***	19.94	***
E x P x F	20	3.03	**	0.64	**n.s.**
E x P x MP	20	5.75	***	21.82	***
E x C x F	10	6.23	***	2.05	**(*)**
E x C x MP	10	8.09	***	25.58	***
E x F x MP	20	1.81	**(*)**	0.51	**n.s.**
E x P x C x F	20	2.10	*	1.66	**(*)**
E x P x C x MP	20	3.70	***	8.73	***
E x P x F x MP	40	1.51	**(*)**	0.35	**n.s.**
E x C x F x MP	20	1.56	**n.s.**	1.21	**n.s.**
P x C	2	141.37	***	57.09	***
P x F	4	10.60	***	1.59	**n.s.**
P x MP	4	7.29	***	46.26	***
P x C x F	4	7.98	***	4.14	**
P x C x MP	4	2.47	**(*)**	18.98	***
P x F x MP	8	1.19	**n.s.**	0.55	**n.s.**
P x C x F x MP	8	0.60	**n.s.**	2.01	**(*)**
C x F	2	20.00	***	6.14	**
C x MP	2	10.40	***	56.98	***
C x F x MP	4	0.94	**n.s.**	2.71	**n.s.**
F x MP	4	1.40	**n.s.**	0.97	**n.s.**

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1; and n.s. = not significant, DF degrees of freedom

In the following, the fixed effects of environment, pre-crop, cultivar, fungicide, and milling fraction on DON and *Fg*DNA levels will be described in detail.

Single effects

Environment

Both, DON and *Fg*DNA, were significantly influenced by the environment as a combination of location and year (Fig 7). The highest DON and *Fg*DNA levels in milling products were detected in 2007. Interestingly, while the DON content did not differ significantly between the two locations in this year, samples from Torland showed a significantly higher *Fg*DNA content than those from Gladebeck. In the two following years, milling products showed significantly lower DON and *Fg*DNA levels compared to 2007. As already described by Tab 7, in 2008, in most samples DON levels were below LOD or LOQ for *Fg*DNA, respectively. No difference between the two locations

was observed. In 2009, an effect of location was determined, since samples from Torland contained approx. twice as much DON as those from Gladebeck, while there was no difference in *Fg*DNA content.

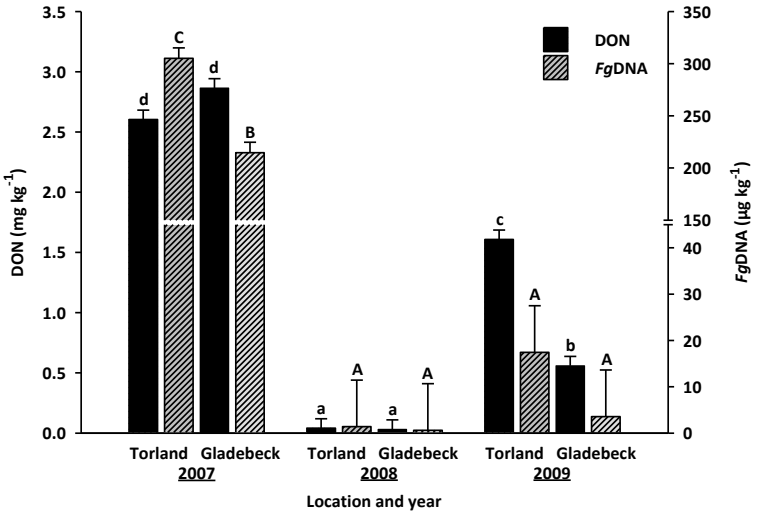

Fig 7. Effect of environment (year, location) on DON and *Fg*DNA content of wheat milling products (flour Type 550, whole grain flour, bran) across all samples. MV (n = 54) with same letters are not significantly different at p < 0.05 according to Tukey test. Error bars indicate SE.

Pre-crop

Looking at DON and *Fg*DNA levels in milling products after three different pre-crops, for both a clear descending order of maize > wheat > sugar beet could be set up (Fig 8). Cereal products milled from wheat grown after maize comprised 3- to 5-fold higher DON levels than from wheat harvested after pre-crop wheat and sugar beet.

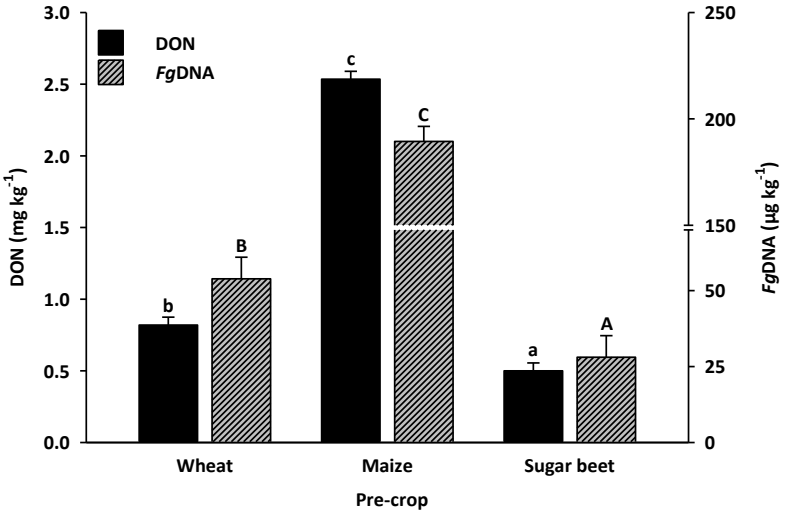

Fig 8. Effect of pre-crop on DON and *Fg*DNA content of wheat milling products (flour Type 550, whole grain flour, bran) across all samples. MV (n = 108) with same letters are not significantly different at p < 0.05 according to Tukey test. Error bars indicate SE.

Cultivar

Susceptibility of cultivars against FHB was reflected by their DON and *Fg*DNA levels (Fig 9). Milling products from cv. Centrum (low susceptibility) contained in average only 25% of the DON and *Fg*DNA levels detected in milling products from cv. Ritmo (high susceptibility).

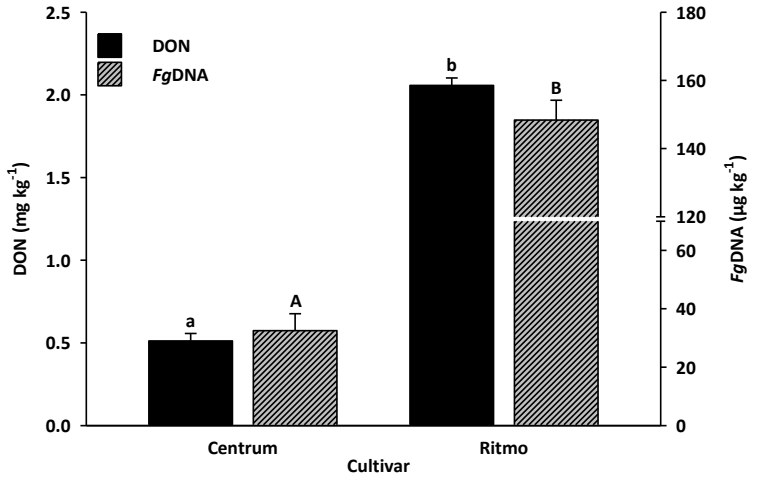

Fig 9. Effect of cultivar on DON and *Fg*DNA content of wheat milling products (flour Type 550, whole grain flour, bran) across all samples. MV (n = 162) with same letters are not significantly different at p < 0.05 according to Tukey test. Error bars indicate SE.

Fungicide

Fungicide application during shooting had an effect on DON and *Fg*DNA levels in the harvested grain and milling products as well (Fig 10). While triazole application clearly resulted in significantly lower DON contents, and when compared to strobilurin treatment, also in significantly less *Fg*DNA, milling products gained from chlorthalonil and strobilurin treatment showed similar amounts of DON and *Fg*DNA.

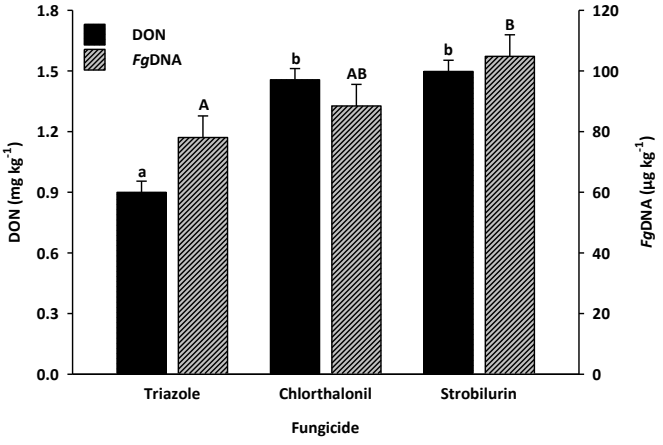

Fig 10. Effect of fungicide treatment on DON and *Fg*DNA content of wheat milling products (flour Type 550, whole grain flour, bran) across all samples. MV (n = 108) with same letters are not significantly different at p < 0.05 according to Tukey test. Error bars indicate SE

DON and *Fg*DNA levels also strongly depended on the milling product (Fig 11). For *Fg*DNA amounts, a clear descending order of bran > WGF > flour Type 550 was determined. For DON levels, rather unexpected, the order was bran = flour Type 550 > WGF. While obviously in bran (0.009) and WGF (0.013), the ratio of DON:DNA (mg DON per µg *Fg*DNA) was equally high, the comparatively high amount of DON in flour Type 550 (0.061) led to a higher DON:DNA ratio in this flour.

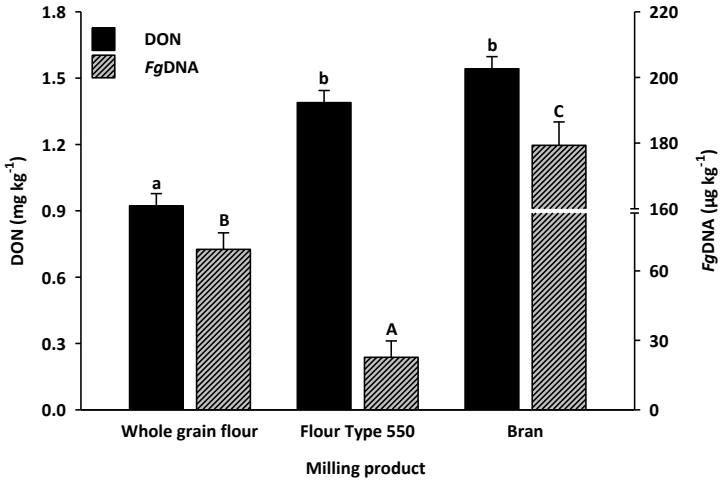

Fig 11. Effect of milling (flour Type 550, whole grain flour, bran) on DON and *Fg*DNA content of milling products. MV (n = 108) with same letters are not significantly different at p < 0.05 according to Tukey test. Error bars indicate SE.

Since flour Type 550 was used for the determination of most quality parameters and gluten proteins, the distribution of DON content in this milling fraction is shown in particular (Fig 12). Approximately 40% of all flours contained DON levels below the EU_{Max} for DON in baby food (0.2 mg kg^{-1} ~ 10%) including samples with DON levels lower than the LOD (~30%). In 29% of the samples, DON contents ranged from 0.20 to less than 1.25 mg kg^{-1}. EU_{Max} for unprocessed cereals (EU_{MaxUP}) (1.25 mg kg^{-1}) was exceeded in 31% of the flours. 50% of these contained up to 2.8 mg kg^{-1} DON (median), 75% of all samples comprised DON levels up to approx. 4 mg kg^{-1}, the remaining of the samples contained more than 4 mg kg^{-1} up to extreme values of 11.8 mg kg^{-1} DON.

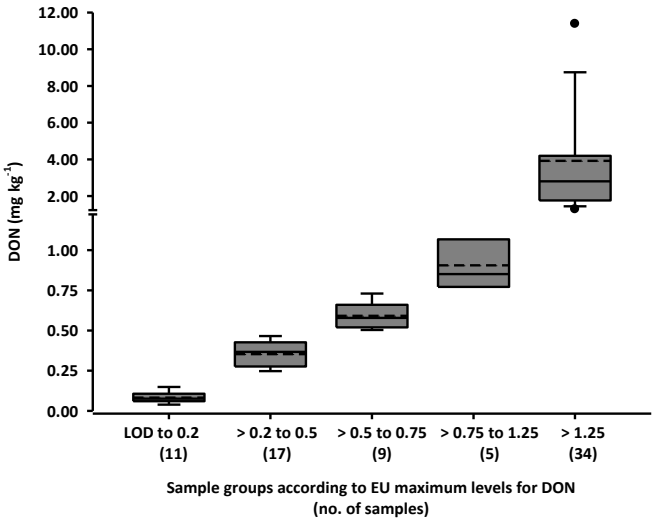

Fig 12. Distribution of DON levels (mg kg^{-1}) of flour Type 550 samples grouped into EU_{Max} for cereal and cereal products (unprocessed cereals: 1.25, Flour: 0.75, bread: 0.5, processed cereals/baby food: 0.2, details: see Tab 1). In brackets: number of samples (total: 108, below limit of detection (LOD): 32). Box plots with median (solid line), mean (short dash), and whiskers indicating 5th/95th percentiles, outliers (dot).

All single factors considered in the ANOVA –year, location (environment), pre-crop, cultivar, fungicide treatment, and milling product- had a significant effect on *Fg*DNA and DON levels in wheat milling products (Tab 9). The previous figures (Fig 7, Fig 8, Fig 9, Fig 10, Fig 11) showed a high positive correspondence between DON and *Fg*DNA regardless of the factors looked upon (except for flour Type 550 in Fig 11).

Interactions

In addition, a large number of significant interactions between the experimental factors were calculated (Tab 9). A short summary on these interactions is given below. Out of all, the interaction of pre-crop – cultivar – fungicide is described in more detail.

Since the environment, especially the year of harvest, played a key role in the occurrence of FHB and associated DON (shown in Fig 7), significant simple, dual, and triple interactions of the environment with all other experimental factors occurred. In 2008, a year with hardly any detectable *Fusarium* infection (measured as *Fg*DNA and DON), a significant difference in *Fg*DNA and DON levels between locations, cultivars, pre-crops, fungicides, and milling products could not be observed, while in 2007 the effect of these factors was distinct. At the same time, the effect of cultivar susceptibility, fungicide treatment, and milling fraction on DON became extraordinarily pronounced after previous crop maize and was less or not at all prevalent after pre-crop sugar beet reflected in significant interactions of these factors with the pre-crop. The same applied to significant interactions with the cultivar. Only the highly susceptible cv. Ritmo showed significant differences in fungicide treatments and milling fractions regarding *Fg*DNA and DON levels (*data not shown*).

Fig 13 shows the details of the interaction pre-crop - cultivar - fungicide subdivided for the effect of pre-crop. This interaction is especially interesting because it includes only factors a farmer can manage. Five things can be affiliated: 1) both cultivars contained highest DON levels after maize pre-crop 2) after pre-crop maize and wheat, susceptible cv. Ritmo comprised significantly more DON than less susceptible cv. Centrum (except for triazole application in pre-crop wheat) 3) strobilurin and chlorthalonil application resulted in higher DON contamination in cv. Ritmo after maize pre-crop compared to triazole treatment; after pre-crop wheat, only strobilurin treatment contained significantly higher DON levels in comparison to triazole 4) in cv. Centrum, fungicides did not show a significant effect on DON 5) after pre-crop sugar beet, neither effect of cultivar nor fungicide was distinct.

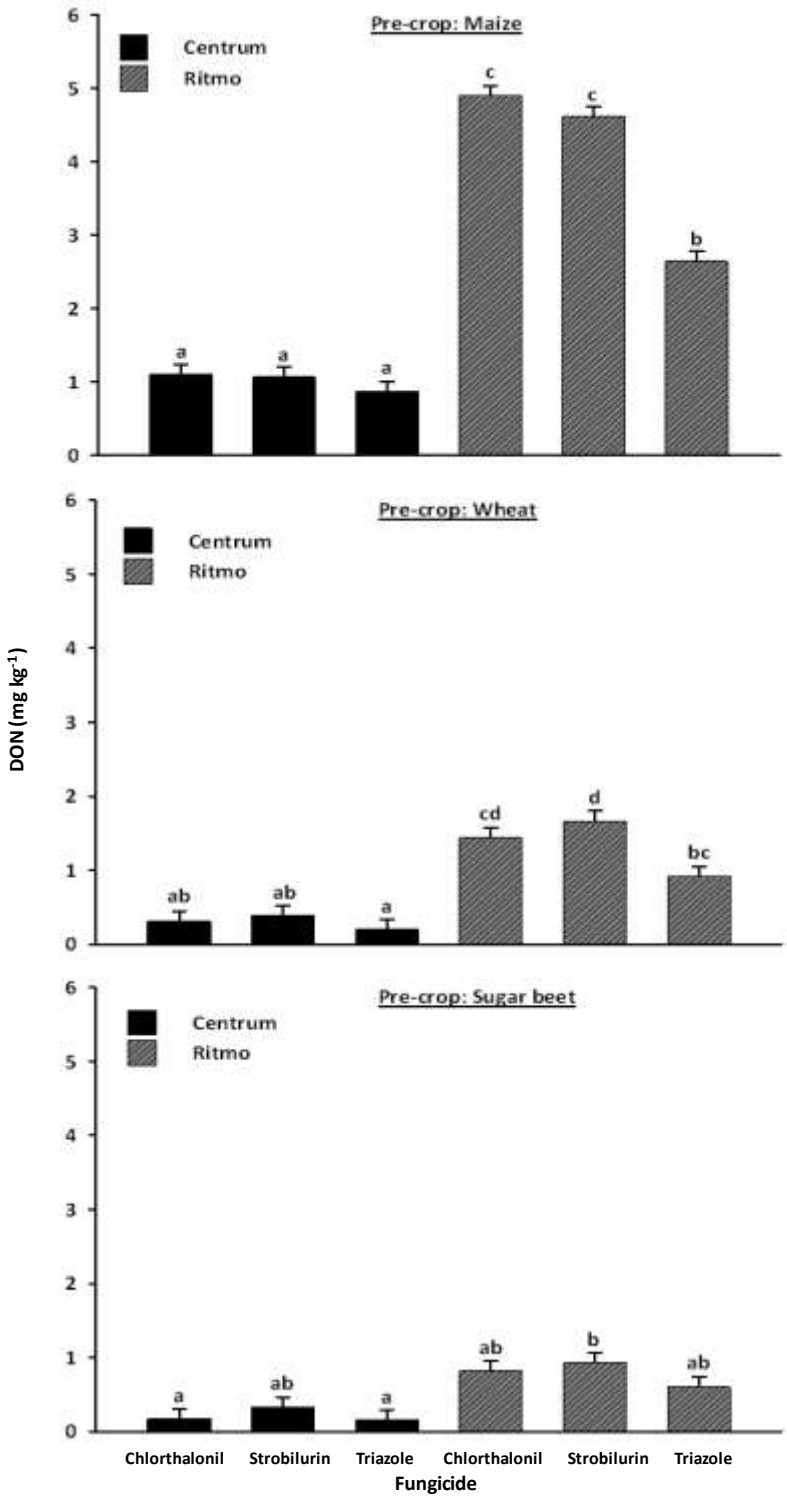

Fig 13. Effect of wheat cultivar (Centrum, Ritmo) and fungicide treatment on DON levels in wheat milling products (flour Type 550, whole grain flour, bran) cultivated after different pre-crops. MV (n = 18) with same letters are not significantly different at p < 0.05 according to Tukey test. Error bars indicate SE.

In the present field trial, maximum DON levels (DON_{Max}) were obtained in 2007 at location Gladebeck, after previous crop maize, susceptible cv. Ritmo, the application of strobilurin (or chlorthalonil in interaction with pre-crop and cultivar), and in bran (Tab 10). Minimum DON levels (DON_{Min}) were present at the same location in 2008, after pre-crop sugar beet, in less susceptible cv. Centrum, triazole treatment, and in WGF.

Evaluation of the single experimental factors regarding their contribution to DON levels in wheat by SoE, it became obvious that environment (year x location) was the most important factor, followed by pre-crop and cultivar. Fungicide treatment and milling product played a comparatively minor role. Out of all interactions, including just agricultural measures, SoE was highest (30.6) within the interaction of pre-crop-cultivar-fungicide.

Tab 10. Specification of maximum and minimum DON levels (DON_{Max}/DON_{min}) (mg kg^{-1}) and severity of effect (SoE of single effects of environment (year x location), pre-crop, cultivar, fungicide, milling product, and in selected interactions

Effect	n	Specification	DON_{Max} (mg kg^{-1})	Specification	DON_{Min} (mg kg^{-1})	SoE
Environment (E)	54	Gladebeck 2007 (GL 07)	**2.86**	Gladebeck 2008 (GL 08)	**0.03**	**90.7**
Pre-crop (P)	108	Maize (M)	**2.53**	Sugar beet (SB)	**0.50**	**5.1**
Cultivar (C)	162	Ritmo (R)	**2.06**	Centrum (C)	**0.51**	**4.0**
Fungicide (F)	108	Strobilurin (S)	**1.50**	Triazole (T)	**0.90**	**1.7**
Milling fraction (MF)	108	Bran (B)	**1.54**	Whole grain flour (WGF)	**0.92**	**1.7**
P x C	54	M x R	**4.05**	SB x C	**0.22**	**18.4**
P x F	36	M x Chlorthalonil (Chl)	**3.00**	SB x T	**0.38**	**7.9**
P x C x F	18	M x R x Chl	**4.90**	SB x C x T	**0.16**	**30.6**
C x F	54	R x S	**2.40**	C x T	**0.41**	**5.8**

n number of observations, SoE severity of effect = DON_{Max}/DON_{Min}, WGF whole grain flour

4.1.3 Relationship of visual disease, DON, and *Fg*DNA

Visual disease

Visual disease in the field was measured as DI, DS, and FHB index (Tab 11). All three parameters were influenced by year and cultivar (neglecting other experimental factors, e.g. pre-crop, fungicide). DI was significantly higher in highly susceptible cv. Ritmo than in cv. Centrum in all three years. DS and FHB index of cv. Ritmo were significantly higher than that of cv. Centrum in 2007, but not in 2008. Highest DI, DS, and FHB index were determined in both cultivars in 2007. Coefficients of correlation for linear regression of DI:DS, DI:FHB, and DS:FHB across 2007, 2008 and cultivars were 0.50 ($p<0.001$), 0.98 ($p<0.001$), and 0.50 ($p<0.001$), respectively.

Tab 11. Disease incidence (DI), disease severity (DS), and FHB index of winter wheat cv. Centrum and Ritmo in 2007, 2008, and 2009[a]

		DI (%)				DS (%)				FHB index (%)			
Year		Centrum		Ritmo		Centrum		Ritmo		Centrum		Ritmo	
2007	Mean	4.571	a	12.440	b	29.876	a	48.819	b	1.448	a	6.766	b
	Median	1.016		3.667		29.125		49.125		0.301		1.762	
2008	Mean	0.002	a	0.005	b	8.611	a	8.646	a	0.001	a	0.001	a
	Median	0.002		0.005		1.250		5.000		0.000		0.001	
2009	Mean	0.045	a	0.705	b	-		-		-		-	
	Median	0.030		0.400		-		-		-		-	

[a] Means and medians for cultivars based on n = 18 per year, "-" not determined, different letters indicate significant differences between cultivars within years with $p < 0.05$ according to Mann-Whitney-U test

Visual disease and DON

Since DI correlated very well with FHB index and DI was determined in all three years, the relationship of DI and DON levels in milling products was investigated (Fig 14). In 2007, the DON content of milling products was best described with a logarithmic function of DI. 80 to 89% of the variability in DON levels could be explained in this way, except for bran and WGF of cv. Ritmo. In 2009, DON levels were best described as a linear function of DI, R^2 was 0.71 to 0.91. In 2008, in most plots DI was zero and only in a few samples, DON could be detected. Therefore, no function for DON was calculated since relationship of DON:DI can only be defined when FHB occurs. Logarithmic functions of FHB for the prediction of DON (calculation only meaningful in 2007) resulted in similar R^2 as the functions of DI (*data not shown*).

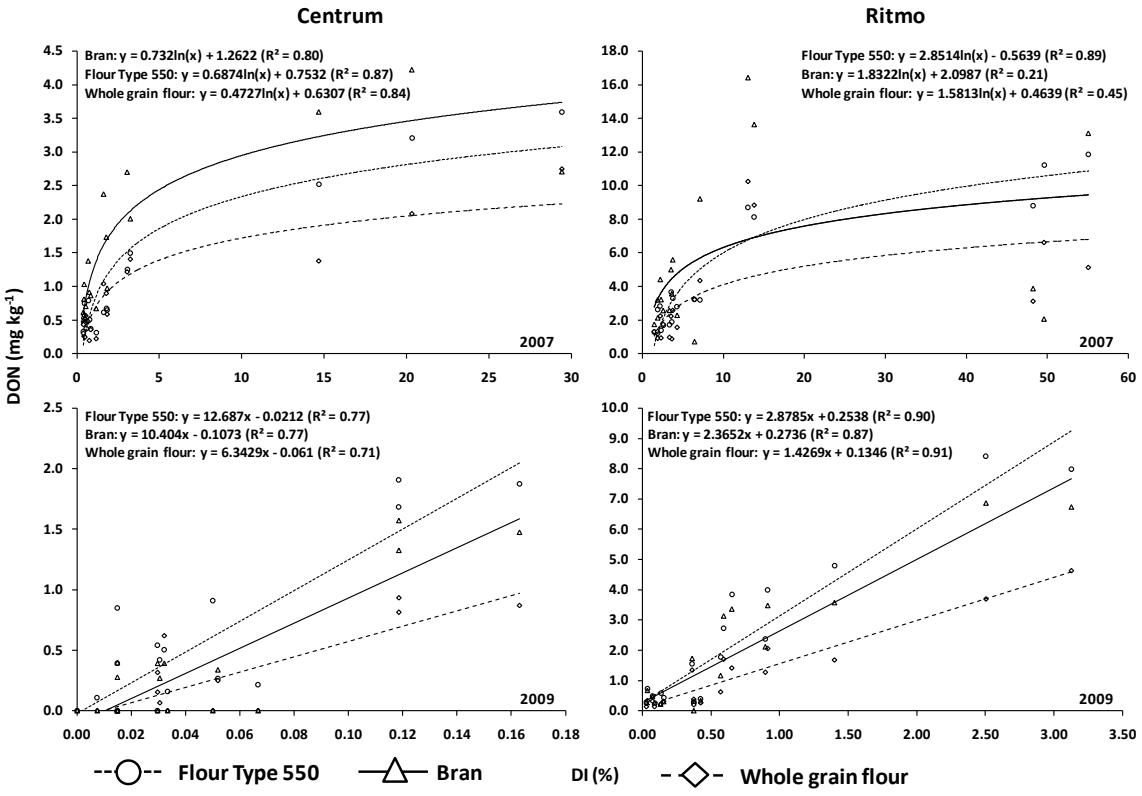

Fig 14. Relationship between disease incidence (DI) and DON content of flours and bran (n = 18 each) milled from two winter wheat cultivars (Centrum, Ritmo) in 2007 and 2009 (Attention: due to otherwise impaired readability of data points, axis of graphs have been set to different scales)

Visual disease and *Fg*DNA

In 2007, relationship of visual disease and *Fg*DNA levels in milling products could be well described with logarithmic functions of DI for both cultivars (Fig 15). DI described 62 to 88% of variability in *Fg*DNA concentrations in flours and bran. In 2008 and 2009, most samples did not contain quantifiable amounts of *Fg*DNA (Tab 7) so that the relationship between both parameters could not be investigated even though DI was not necessarily zero. Since DI correlated highly with FHB, it was not unexpected that *Fg*DNA also related highly to FHB; logarithmic functions of FHB

to estimate *Fg*DNA levels in milling products possessed R²s from 0.66 to 0.87 for both cultivars; the R^2 for logarithmic functions of DS obtained lower R² (0.05-0.57) indicating none to moderate positive correlation with *Fg*DNA in cv. Centrum (R²=0.05-0.26) and none to weak correlation in cv. Ritmo (R²=0.39-0.57) (*data not shown*).

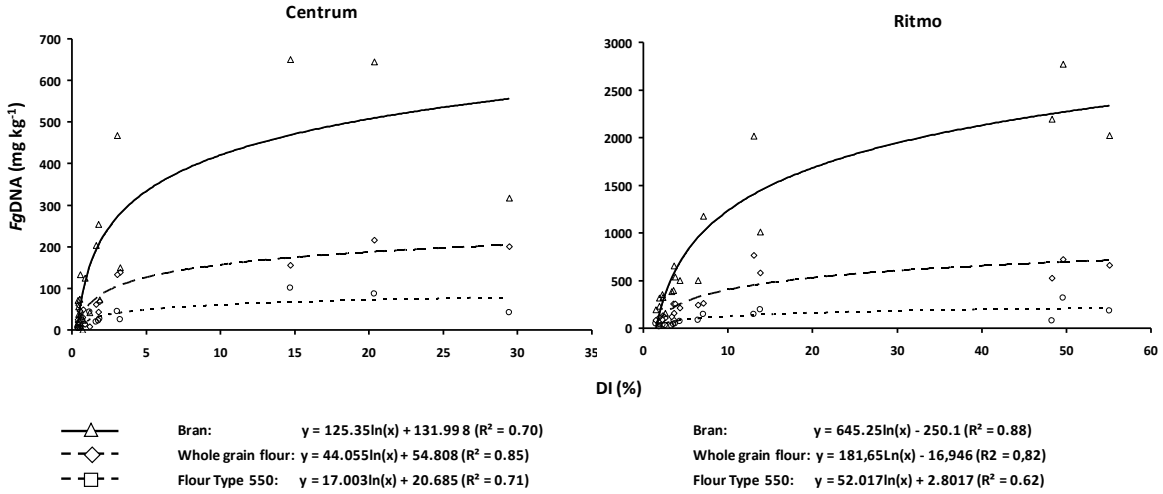

Fig 15. Relationship between disease incidence (DI) and *Fg*DNA content of flours and bran (n = 18 each) milled from two winter wheat cultivars (Centrum, Ritmo) in 2007 (Attention: due to otherwise impaired readability of data points, axis of graphs have been set to different scales)

DON and *Fg*DNA

In a linear model, DON and *Fg*DNA measured in flours and bran across all samples were positively correlated (Fig 16). 65% to 85% of variance of DON content in milling products was explained by the presence of *Fg*DNA. The highest R^2 was gained from the prediction model of WGF. Remarkably, the slope of the regression curve of flour Type 550 was four times as steep (a = 0.04) as the slopes of the curves estimated from WGF and bran (a = 0.01 and 0.007). The DON concentration per µg *Fg*DNA which can be predicted from the slope of the models was similar to DON:DNA ratio calculated from data shown already in Fig 11. A detailed description of differences between cultivars in DON:DNA ratio can be viewed in GÖDECKE 2010.

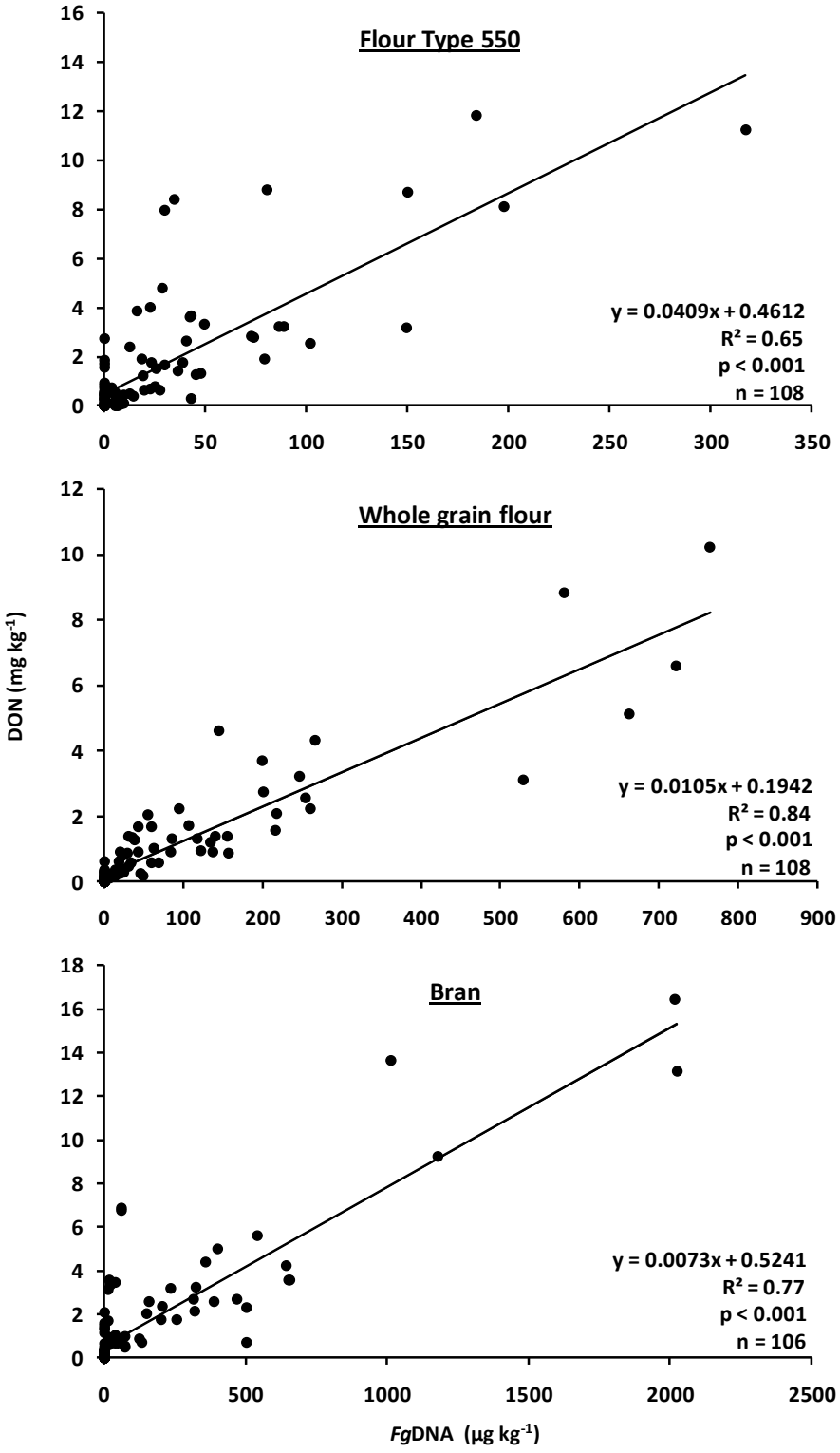

Fig 16. Linear regression of *Fg*DNA and DON content of wheat milling products (flour Type 550, whole grain flour, bran) across all samples (Attention: due to otherwise impaired readability of data points, axis of graphs have been set to different scales)

There also existed a close positive relationship between the DON levels of all milling products (R^2=0.67-0.83) as well as between their *Fg*DNA (R^2=0.81-0.88) contents (Tab 12).

Tab 12. Coefficient of determination (R^2) of linear regression of DON and *Fg*DNA levels of milling products (flour Type 550, whole grain flour (WGF), bran)

	Flour Type 550		WGF	
	DON	*Fg* DNA	DON	*Fg* DNA
WGF	0.79	0.83	-	-
Bran	0.67	0.81	0.83	0.88

WGF whole grain flour, Flours and bran: n = 108 each, **significance of all correlations was p < 0.001**

4.2 *Fusarium* infection and quality parameters

4.2.1 Protein content of flours (PC_{WGF} and $PC_{Type550}$)

PC_{WGF} was mainly influenced by year and pre-crop (Tab 54). In 2007, grain contained significantly more protein (Ø 14.0%) than in 2008 and 2009 where PC averaged only 11.3% and 12.3%, respectively. Across years and cultivars, wheat grown after wheat contained significantly more grain protein (about 4.7 and 4.2%) than grown after maize and sugar beet, respectively. Across all samples, cv. Ritmo (Ø 12.7%) contained significantly more protein than cv. Centrum (Ø 12.4%) which was mainly due to 2007, the only year where a significant difference in PC between the two cultivars was apparent (cv. Centrum: Ø 13.7%, cv. Ritmo: Ø 14.3%).

Within 2007, PC_{WGF} of cv. Centrum was highest after pre-crop sugar beet (Ø 14.1%), cv. Ritmo contained highest PC_{WGF} after pre-crop wheat (14.7%) (Fig 17). Within years 2008 and 2009, both cultivars exhibited higher PC_{WGF} after pre-crop winter wheat (2008: 11.8%, 2009: 12.8%) than after maize (2008: 11.3%, 2009: 12.0%) and sugar beet (2008: 11.0%, 2009: 12.0%), while the difference between the later two pre-crops was not significant.

Milling of flour Type 550 reduced PC in dry matter compared to WGF (Fig 17). Flour Type 550 contained averagely 11.7, 10.2, and 10.4% protein in 2007, 2008, and 2009 which resembled a reduction of 1-2% protein when compared to WGF (Tab 54). Milling smoothed $PC_{Type550}$ which is indicated by narrower percentiles of box plots (Fig 17), nevertheless, the effect of year and pre-crop which was present in WGF mainly remained visible. Both, PC_{WGF} (x) and $PC_{Type550}$ (y) were significantly positively correlated ($y = 0.5933x + 3.342$, $R^2=0.892$, $p < 0.001$, $n = 108$).

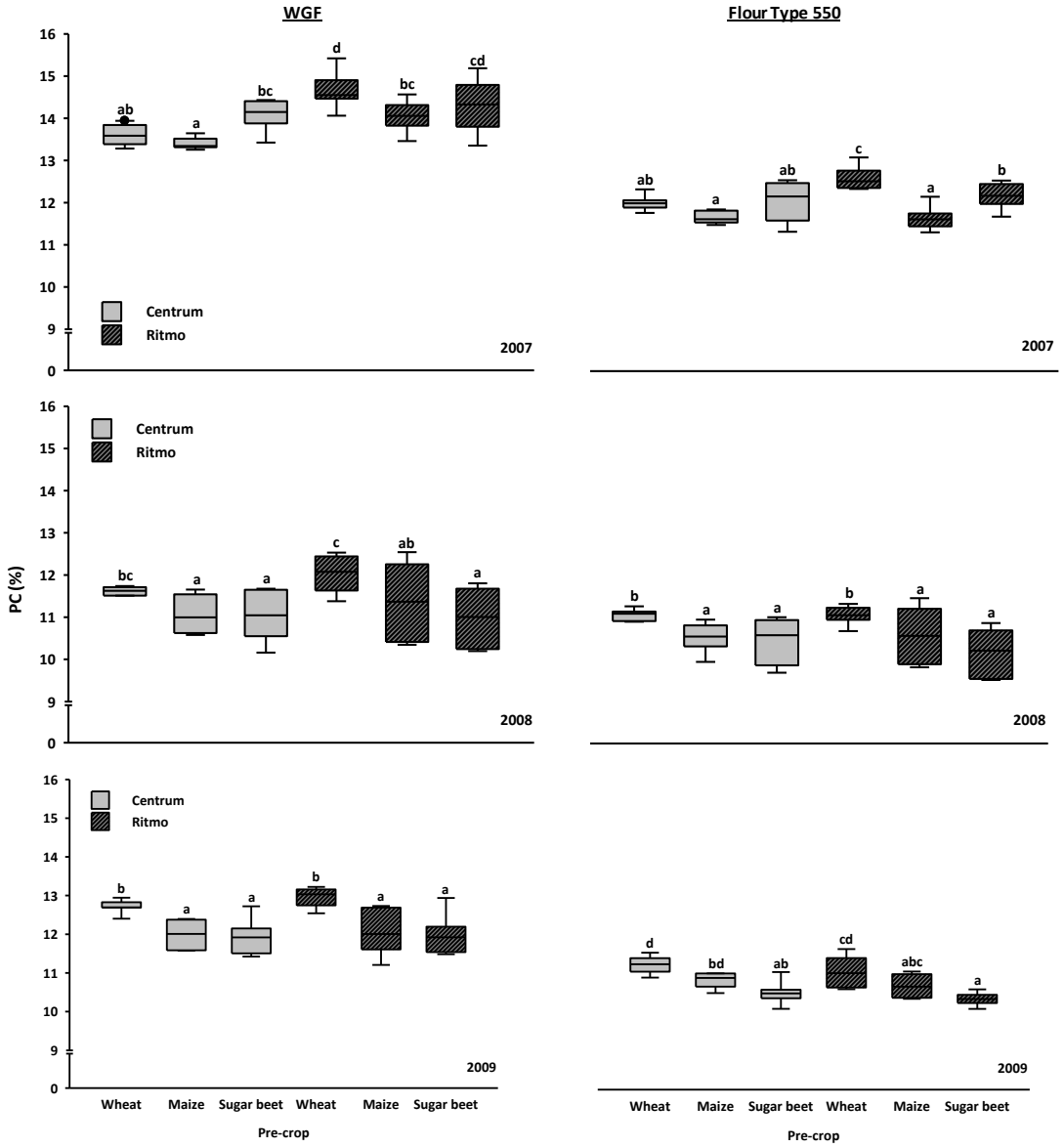

Fig 17. Protein content (PC) of whole grain flour (WGF) and flour Type 550 milled from two winter wheat cv. (Centrum, Ritmo) grown after pre-crop wheat, maize, and sugar beet in 2007, 2008, 2009. Box plots with median (solid line) and whiskers indicating 5th/95th percentiles. Box plots (n = 6) with same letters are not significantly different at p < 0.05 according to Tukey test. Outliers are indicated by a dot.

Obviously, none of the variability in protein data could be sufficiently explained by the concentration of DON in grain (and therefore *Fusarium* infection) (Tab 13). This leads to the conclusion that in WGFs (and grain respectively), PC was not affected by *Fusarium* infection. Subsequently, the model could be reduced (by performing ANOVA) to the main effects environment (year x location), pre-crop, and cultivar which all affected PC_{WGF} significantly (Tab 13, Fig 17).

Tab 13. ANCOVA for effects of environment (E) (year, location), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) and ANOVA for effects of E, P, and C on protein content of whole grain flour

	ANCOVA			ANOVA	
Effect	**DF**	**F**	**p**	**F**	**p**
Environment (E)	3	19.79	***	273.9	***
Pre-crop (P)	2	10.31	***	32.65	***
E x P	6	3.11	*	14.02	***
Cultivar (C)	1	0.32	**n.s.**	36.47	***
E x C	3	1.11	**n.s.**	6.03	**
P x C	2	2.96	**(*)**	5.55	**
E x P x C	4	0.78	**n.s.**	1.87	**n.s.**
DON	1	1.04	**n.s.**		
DON x E	3	0.43	**n.s.**		
DON x P	2	0.57	**n.s.**		
DON x E x P	6	0.94	**n.s.**		
DON x C	1	0.12	**n.s.**		
DON x E x C	3	0.6	**n.s.**		
DON x P x C	2	0.8	**n.s.**		
DON x E x P x C	4	0.24	**n.s.**		

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1; and n.s. = not significant, DF degrees of freedom

In contrast to WGF, for flour Type 550 a significant effect of DON_{LOG} on PC was calculated aside from significant effects of environment and pre-crop (Tab 14).

Tab 14. ANCOVA for effects of environment (E) (year, location), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) on protein content of flour Type 550

Effect	**DF**	**F**	**p**
Environment (E)	3	**18.41**	***
Pre-crop (P)	2	**8.42**	**
E x P	6	**2.25**	**(*)**
Cultivar (C)	1	**0.71**	**n.s.**
E x C	3	1.92	**n.s.**
P x C	2	1.15	**n.s.**
E x P x C	5	0.63	**n.s.**
DON	1	**6.78**	*
DON x E	3	**3.66**	*
DON x P	2	2.59	**(*)**
DON x E x P	6	**3.21**	*
DON x C	1	**5.89**	*
DON x E x C	3	**0.53**	**n.s.**
DON x P x C	2	**0.78**	**n.s.**
DON x E x P x C	5	**1.38**	**n.s.**

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1; and n.s. = not significant, DF degrees of freedom

Further analysis by linear regression showed that $PC_{Type550}$ increased significantly across cultivars with higher DON contents after previous crop wheat in 2007, but decreased in 2009 at location Torland when grown after wheat and sugar beet (Tab 15). In 2007, within the range of 0.31 to 3.65 mg DON kg^{-1} $PC_{Type550}$ increased by 0.58 to 1.09% if DON_{LOG} increased by 1 mg kg^{-1}.

In 2009, $PC_{Type550}$ was reduced about 0.46 and 0.75% per 1 mg DON_{LOG} kg^{-1} within a range of DON_{ORG} similar to 2007.

Tab 15. Parameters of linear regression of protein content of Type 550 (%) and DON_{LOG} (mg kg^{-1}) (DON) in different environments (E) (year, location) and pre-crops (P) across cultivars, and range of original DON values (DON_{ORG})

Effect	Effect level									DON_{ORG} (mg kg^{-1})
	Year	Location	Cultivar	Pre-crop	n	R^2	p	b	a	Min-Max
E x P	2007	TL & GB	Centrum & Ritmo	Wheat	12	0.75	***	11.98	0.85	0.31-3.65
		TL			6	0.78	*	11.91	0.58	0.31-2.75
		GB			6	0.88	**	12.06	1.09	0.37-3.30
	2009	TL	Centrum & Ritmo	Wheat	6	0.68	*	10.51	-0.46	0.42-3.99
				Sugar beet	6	0.88	**	9.60	-0.75	0.16-0.91

n number of observations, p significance: ***, **, and * = p < 0.001, 0.01, 0.05, b constant, a slope, TL Torland, GB Gladebeck

4.2.2 Wet gluten content (WG) and sedimentation value (SV)

WG was significantly influenced by year, pre-crop, and cultivar (Tab 54, Fig 18). Flour Type 550 contained 38.0%, 29.1%, and 26.4% WG across all samples in 2007, 2008, and 2009, respectively. Regarding cultivar, from cv. Ritmo significantly more WG could be gained than from cv. Centrum. The difference in WG between both cultivars was about 21%, 13%, and 10% in 2007, 2008, and 2009, respectively. While WG was hardly influenced by pre-crop in cv. Centrum (except 2009), in cv. Ritmo WG was significantly lower after previous crop maize < sugar beet < wheat in 2007 and in 2009 maize=sugar beet < wheat. The wider range of the box plots in Fig 18 of cv. Ritmo compared to cv. Centrum indicated a stronger variation of WG in this cultivar which may have been caused by other factors, e.g. location or *Fusarium* infection. However, ANCOVA did not reveal a significant effect of DON_{LOG} on WG (Tab 17).

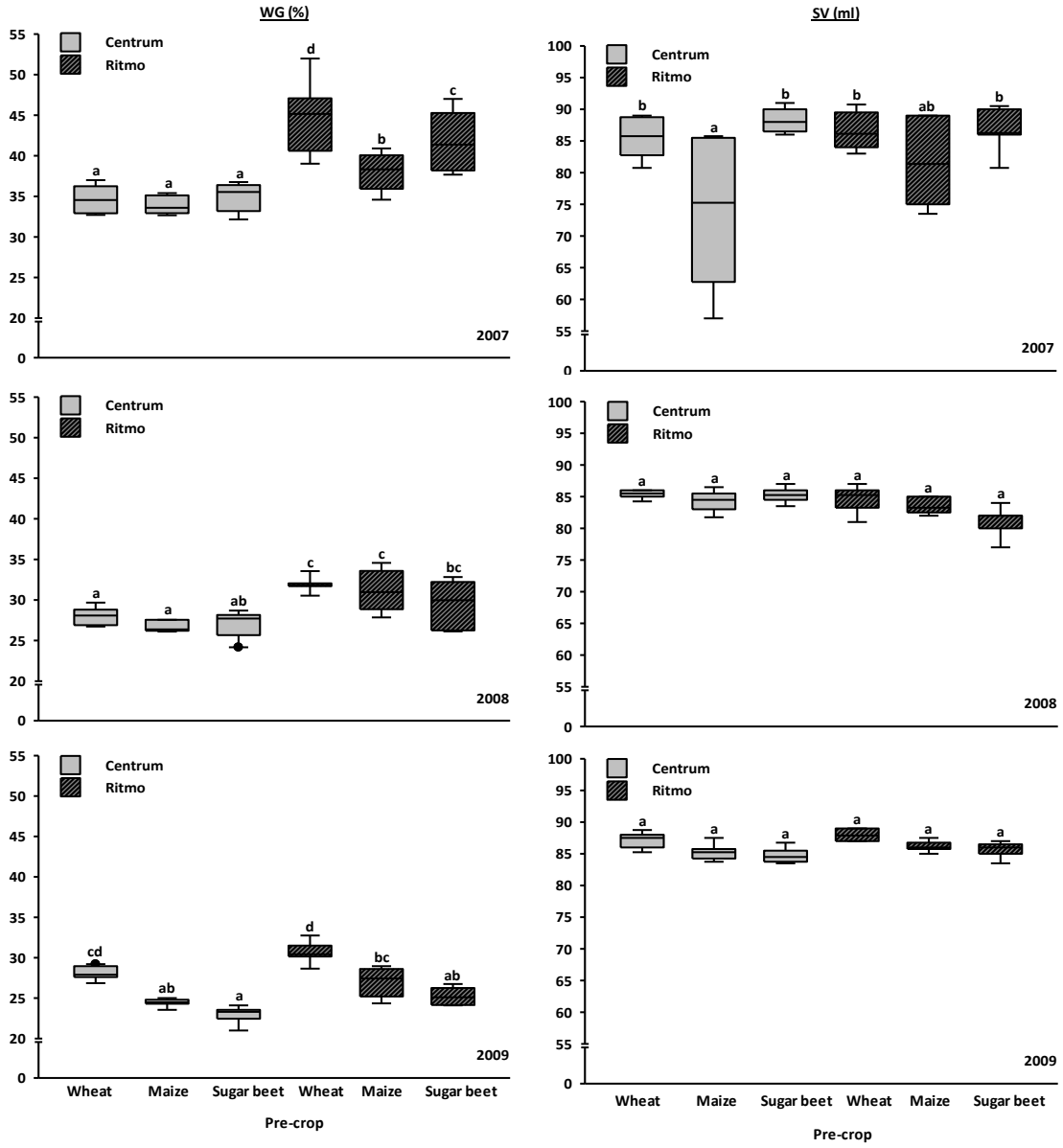

Fig 18. Wet gluten content (WG) and sedimentation value (SV) of flour Type 550 milled from two winter wheat cv. (Centrum, Ritmo) grown after pre-crop wheat, maize, and sugar beet in 2007, 2008, and 2009. Box plots with median (solid line) and whiskers indicating 5th/95th percentiles. Box plots (n = 6) with same letters are not significantly different at p < 0.05 according to Tukey test. Outliers are indicated by a dot.

Tab 17. ANCOVA for effects of environment (E) (year, location), pre-crop (P), wheat cultivar (C), and DON_{LOG}(DON) and ANOVA for effects of E, P, and C on wet gluten content

	ANCOVA			ANOVA	
Effect	**DF**	**F**	**p**	**F**	**p**
Environment (E)	3	23.47	***	420.84	***
Pre-crop (P)	2	1.94	**n.s.**	51.64	***
E x P	6	1.57	**n.s.**	19.72	***
Cultivar (C)	1	2.11	**n.s.**	211.48	***
E x C	3	0.56	**n.s.**	17.39	***
P x C	2	0.65	**n.s.**	7.19	**
E x P x C	6	0.65	**n.s.**	4.77	***
DON	1	0.44	**n.s.**		
DON x E	3	0.48	**n.s.**		
DON x P	2	0.14	**n.s.**		
DON x E x P	6	0.74	**n.s.**		
DON x C	1	0.03	**n.s.**		
DON x E x C	3	0.41	**n.s.**		
DON x P x C	2	0.36	**n.s.**		
DON x E x P x C	5	0.15	**n.s.**		

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1;
and n.s. = not significant, DF degrees of freedom

SV was significantly affected by year and pre-crop (Tab 54, Fig 18). Differences between cultivars were only significant within the interaction of year-pre-crop, yet, not across all samples (cv. Centrum: Ø 84 ml, cv. Ritmo: Ø 85 ml). While SV did not differ significantly between 2007 (Ø 84 ml) and 2008 (Ø 84 ml), it was significantly higher in 2009 (Ø 86 ml). In 2007, SV varied more than in the two following years indicated by wider box plots. In the respective year, SV of both cultivars were lower after pre-crop maize compared than after wheat and sugar beet. In cv. Centrum, the difference was significant. SV of cv. Centrum was reduced on average about 17 and 14% after maize when compared to wheat and sugar beet, respectively, SV of cv. Ritmo after pre-crop maize was only reduced about 6%.

ANCOVA revealed a significant effect of DON on SV across all samples, within environment, pre-crop, as well as in the interaction of environment (year x location) and pre-crop (Tab 18).

Tab 18. ANCOVA for effects of environment (E) (year, location), pre-crop (P), wheat cultivar (C), and DON_{LOG} on sedimentation value

Effect	DF	F	p
Environment (E)	3	15.50	***
Pre-crop (P)	2	17.21	***
E x P	6	8.37	***
Cultivar (C)	1	0.42	n.s.
E x C	3	5.42	**
P x C	2	0.88	n.s.
E x P x C	5	0.55	n.s.
DON	1	7.70	*
DON x E	3	24.36	***
DON x P	2	4.20	*
DON x E x P	6	8.66	***
DON x C	1	1.52	n.s.
DON x E x C	3	0.90	n.s.
DON x P x C	2	0.87	n.s.
DON x E x P x C	5	2.58	(*)

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1; and n.s. = not significant, DF degrees of freedom

Even though ANCOVA revealed that there is a significant effect of DON_{LOG} on SV across all samples and within different environments and pre-crops, only one linear regression out of 36 ones with a very small number of observations had an $R^2 \geq 0.50$ with $p < 0.05$ (Fig 19). In 2009, across cultivars SV increased significantly with increasing DON_{LOG} content after pre-crop maize. DON_{ORG} content in the respective samples ranged from 0.27 to 3.84 mg kg^{-1}. Fig 19 also displays EU_{Max} for DON in cereal flours (EU_{MaxCF}) and unprocessed cereals (EU_{MaxUP})

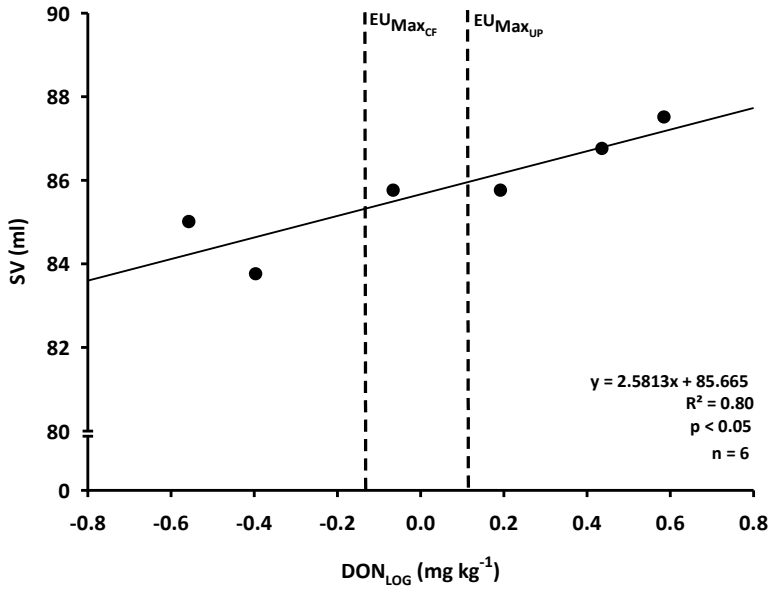

Fig 19. Linear regression of sedimentation value (SV) and DON_{LOG} of flour Type 550 across cultivars (Centrum, Ritmo) in 2009 at location Gladebeck after pre-crop maize, EU_{MaxCF} = 0.75 mg kg^{-1}, EU_{MaxUP} = 1.25 mg kg^{-1}

4.2.3 Falling number (FN)

FN was significantly affected by environment (year, location), pre-crop, and cultivar (Tab 54, Fig 20). Regarding years, FN was highest in 2009 (Ø 406), followed by 2008 (Ø 315) and lowest in 2007 (Ø 249). Across three years, cv. Ritmo showed significantly higher FN than cv. Centrum. The difference to cv. Centrum was 32, 39, and 21% in 2007, 2008, and 2009, respectively. Even though not always statistically significant, FN generally dropped stepwise from pre-crop wheat over maize to sugar beet. This was particularly pronounced in 2007. FN of cv. Centrum was on average 18 and 29% and FN of cv. Ritmo 11 and 23% lower after pre-crop maize and sugar beet, respectively, when compared to wheat as pre-crop.

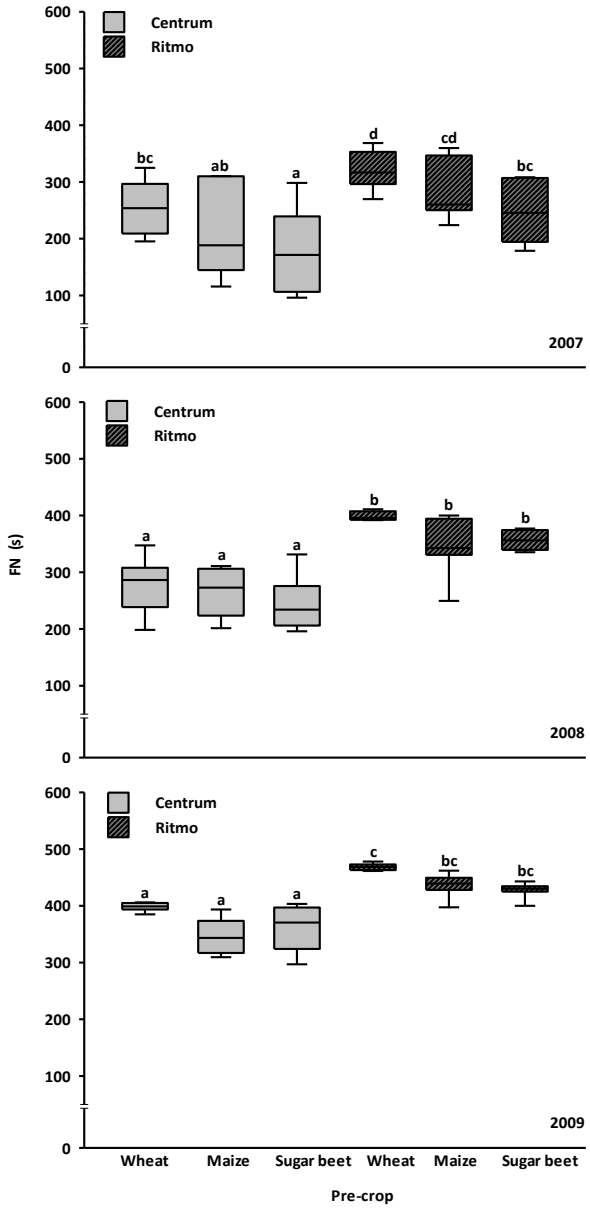

Fig 20. Falling number (FN) of wheat cultivars (Centrum, Ritmo) grown after pre-crop wheat, maize, and sugar beet in 2007, 2008, and 2009. Box plots with median (solid line) and whiskers indicating 5th/95th percentiles. Box plots (n = 6) with same letters are not significantly different at p < 0.05 according to Tukey test.

ANCOVA showed a significant effect of DON within the interaction of environment and pre-crop (Tab 19). Investigation of the effect by means of linear regression showed that FN seemed to increase significantly with increasing DON content across cultivars within pre-crops sugar beet and maize on at least one location each year (Tab 20).

Tab 19. ANCOVA for effects of environment (E) (year, location), pre-crop (P), wheat cultivar (C), and DON_{LOG} on falling number (both measured in whole grain flour)

Effect	DF	F	p
Environment (E)	3	73.90	***
Pre-crop (P)	2	16.07	***
E x P	6	6.59	***
Cultivar (C)	1	14.99	***
E x C	3	7.29	**
P x C	2	3.25	(*)
E x P x C	4	4.60	**
DON	1	1.96	**n.s.**
DON x E	3	2.16	**n.s.**
DON x P	2	2.00	**n.s.**
DON x E x P	6	2.65	*
DON x C	1	0.06	**n.s.**
DON x E x C	3	1.08	**n.s.**
DON x P x C	2	0.13	**n.s.**
DON x E x P x C	4	2.68	(*)

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1; and n.s. = not significant, DF degrees of freedom

Tab 20. Parameters of linear regression of falling number (s) and DON_{LOG} (mg kg^{-1}) (both measured in whole grain flour) in different environments (E) (years, location) and pre-crops (P) across cultivars, and range of original DON values (DON_{ORG})

Effect	Effect level									DON_{ORG} (mg kg^{-1})
	Year	Location	Cultivar	Pre-crop	n	R^2	p	b	a	Min-Max
E x P	2007	TL	Centrum & Ritmo	Maize	6	0.88	**	74.66	240.62	1.38-6.61
				Sugar beet	6	0.81	*	175.54	154.36	0.26-1.74
	2009	TL & GB	Centrum & Ritmo	Sugar beet	12	0.56	**	456.70	47.98	0.00-1.70
		TL	Centrum & Ritmo	Sugar beet	6	0.94	**	451.79	28.07	0.00-0.37
		GB	Centrum & Ritmo	Maize	6	0.88	**	436.73	40.82	0.00-1.70
				Sugar beet	6	0.82	*	463.57	68.54	0.00-0.30

n number of observations, p significance: ***, **, and * = p < 0.001, 0.01, 0.05, b constant, a slope, TL Torland, GB Gladebeck

Since cultivar had a significant influence on FN, regardless of *Fusarium* infection, linear regression was additionally performed within cultivars. Thereafter, only in cv. Ritmo a significant positive correlation between DON_{LOG} and FN could be detected after previous crop maize in 2007 and previous crop wheat in 2009, respectively (Fig 21). DON_{ORG} of WGF from cv. Ritmo ranged from 3.11-10.24 mg kg^{-1} in 2007 after maize and from 0.13 to 2.05 mg kg^{-1} in 2009 after pre-crop wheat.

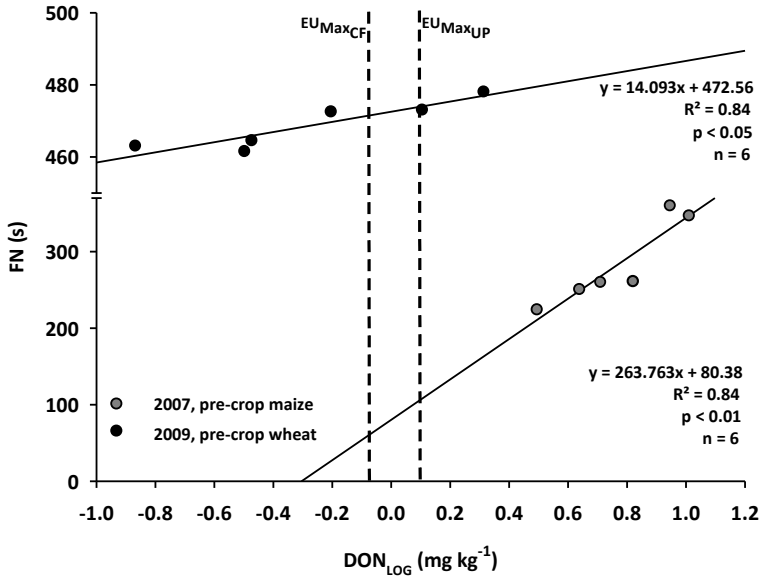

Fig 21. Linear regression between falling number (FN) and DON_{LOG} (both measured in whole grain flour) from cv. Ritmo in 2007 and 2009 grown after pre-crop maize and wheat across locations (Torland, Gladebeck), EU_{MaxCF} = 0.75 mg kg^{-1}, EU_{MaxUP} = 1.25 mg kg^{-1}

4.2.4 Water absorption (WA) and mixing properties of dough (DDT, DST, DS)

WA was primarily influenced by year and pre-crop (Tab 21, Tab 55). In 2007, WA was significantly higher than in the two following years. Flour absorbed on average 63.5, 57.2, and 54.9% water in 2007, 2008, and 2009, respectively. Within 2007, additionally a significant effect of cultivar and pre-crop was observed which could not be detected in the two following years. In 2007, cv. Ritmo exhibited overall higher WA than cv. Centrum, a difference which was significant after pre-crop wheat and sugar beet.

DDT was influenced by year and cultivar (Tab 21, Tab 55). Doughs developed significantly faster in 2007 (Ø 1.4 min), than in 2008 (Ø 1.9 min) and 2009 (Ø 2.0 min). Across years, doughs from cv. Centrum (Ø 1.5 min) developed significantly faster than the respective doughs of cv. Ritmo (Ø 1.9 min). Within years 2008 and 2009, differences between cultivars were not detectable, only cv. Ritmo after pre-crop maize showed significantly higher DDT than the rest of the doughs. In 2007, an effect of pre-crop was observed in cv. Ritmo. After maize, dough developed within significantly less time than after sugar beet and wheat.

DST highly depended on year (Tab 21, Tab 55). Doughs were significantly more tolerant to mixing in 2009 (Ø 7.8 min) than in 2007 (Ø 1.3 min) and 2008 (Ø 2.8 min). Within and across years and cultivars, a significant effect of cultivar and pre-crop was not detectable.

DS was particularly influenced by year (Tab 21, Tab 55). In 2007 (Ø 95 VU) and 2008 (Ø 92 FU) doughs lost significantly more of their consistency after 12 min of mixing than in 2009 (Ø 50 FU). Effect of cultivar depended on environment. Effect of pre-crop depended on cultivar and year.

However across all samples, no significant effect of cultivar and pre-crop on DS could be detected. Interestingly, in 2007 DS showed a very high variability within cultivars and pre-crops (indicated by high SD) which was not so pronounced in the other years. Average coefficient of variation in 2007 was 40% compared to 15 and 32% in 2008 and 2009, respectively.

Tab 21. Water absorption (WA) and mixing properties of dough (dough development time: DDT, dough stability: DST, dough softening: DS) of wheat cv. (Centrum, Ritmo) grown after different pre-crops in 2007, 2008, and 2009 (MV ± SD, n = 6)

Year	Cultivar	Pre-crop	WA (%)	DDT (min)	DST (min)	DS (2007: VU, 2008/9: FU)
2007	Centrum	Wheat	62.5 ± 1.1 **a**	1.1 ± 0.2 **a**	1.4 ± 0.6 **a**	67 ± 28 **a**
		Maize	62.5 ± 0.5 **a**	1.2 ± 0.2 **ab**	0.8 ± 0.4 **a**	98 ± 47 **b**
		Sugar beet	62.8 ± 0.9 **a**	1.3 ± 0.2 **ab**	1.0 ± 0.1 **a**	85 ± 44 **ab**
	Ritmo	Wheat	65.2 ± 0.9 **b**	1.9 ± 0.3 **c**	1.8 ± 0.4 **a**	83 ± 24 **ab**
		Maize	63.0 ± 1.0 **a**	1.1 ± 0.2 **a**	1.2 ± 0.6 **a**	140 ± 51 **c**
		Sugar beet	65.0 ± 1.5 **b**	1.6 ± 0.4 **bc**	1.6 ± 0.2 **a**	97 ± 33 **b**
2008	Centrum	Maize	57.0 ± 0.8 **a**	1.8 ± 0.1 **a**	2.4 ± 0.8 **a**	97 ± 7 **ab**
		Sugar beet	56.4 ± 0.6 **a**	1.7 ± 0.2 **a**	2.4 ± 1.0 **a**	107 ± 13 **b**
	Ritmo	Maize	57.7 ± 0.7 **a**	2.6 ± 1.0 **b**	3.5 ± 1.5 **a**	76 ± 19 **a**
		Sugar beet	57.8 ± 0.6 **a**	2.0 ± 0.3 **a**	2.9 ± 1.3 **a**	83 ± 12 **a**
2009	Centrum	Maize	54.5 ± 0.5 **a**	1.9 ± 0.2 **a**	9.2 ± 7.6 **ab**	33 ± 17 **a**
		Sugar beet	55.0 ± 0.6 **a**	1.9 ± 0.1 **a**	9.4 ± 6.3 **b**	29 ± 12 **a**
	Ritmo	Maize	55.0 ± 0.2 **a**	2.6 ± 0.9 **b**	5.3 ± 2.4 **a**	59 ± 15 **b**
		Sugar beet	55.1 ± 0.3 **a**	2.0 ± 0.3 **a**	7.4 ± 2.7 **ab**	39 ± 5 **ab**

Same letters indicate no differences between cultivars and pre-crops **within** years at a significance level of $p < 0.05$ according to Tukey test

ANCOVA showed that *Fusarium* infection neither explained any of the variance of WA of flour (Tab 22), nor of DDT (Tab 23) or DST (Tab 24) sufficiently. Therefore, ANOVA was adequate for these parameters.

Tab 22. ANCOVA for effects of environment (E) (year. Location), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) and ANOVA for effects of E, P, and C on water absorption

	ANCOVA			ANOVA	
Effect	**DF**	**F**	**p**	**F**	**p**
Environment (E)	3	31.40	***	464.69	***
Pre-crop (P)	2	0.00	**n.s.**	6.81	**
E x P	4	2.45	**(*)**	1.68	**n.s.**
Cultivar (C)	1	0.62	**n.s.**	34.50	***
E x C	3	0.99	**n.s.**	2.61	**(*)**
P x C	2	1.20	**n.s.**	4.16	*
E x P x C	4	0.53	**n.s.**	1.65	**n.s.**
DON	1	0.13	**n.s.**		
DON x E	3	1.51	**n.s.**		
DON x P	2	1.82	**n.s.**		
DON x E x P	4	0.49	**n.s.**		
DON x C	1	0.92	**n.s.**		
DON x E x C	3	0.81	**n.s.**		
DON x P x C	2	0.13	**n.s.**		
DON x E x P x C	4	2.60	**(*)**		

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1;
and n.s. = not significant, DF degrees of freedom

Tab 23. ANCOVA for effects of environment (E) (year, location), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) and ANOVA for effects of E, P, and C on dough development time

	ANCOVA			ANOVA	
Effect	**DF**	**F**	**p**	**F**	**p**
Environment (E)	3	3.06	**(*)**	55.83	***
Pre-crop (P)	2	0.23	**n.s.**	2.53	**(*)**
E x P	4	0.35	**n.s.**	6.91	***
Cultivar (C)	1	0.26	**n.s.**	48.05	***
E x C	3	1.05	**n.s.**	10.82	***
P x C	2	0.03	**n.s.**	7.25	**
E x P x C	4	0.55	**n.s.**	5.17	**
DON	1	0.8	**n.s.**		
DON x E	3	0.57	**n.s.**		
DON x P	2	0.56	**n.s.**		
DON x E x P	4	1.24	**n.s.**		
DON x C	1	0.09	**n.s.**		
DON x E x C	3	0.48	**n.s.**		
DON x P x C	2	0.35	**n.s.**		
DON x E x P x C	4	0.68	**n.s.**		

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1;
and n.s. = not significant, DF degrees of freedom

Tab 24. ANCOVA for effects of environment (E) (year, location), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) and ANOVA for effects of E, P, and C on dough stability

	ANCOVA			ANOVA	
Effect	**DF**	**F**	**p**	**F**	**p**
Environment (E)	3	1.99	**n.s.**	54.57	***
Pre-crop (P)	2	0.06	**n.s.**	0.82	**n.s.**
E x P	4	0.36	**n.s.**	1.21	**n.s.**
Cultivar (C)	1	0.02	**n.s.**	3.30	(*)
E x C	3	0.73	**n.s.**	6.96	***
P x C	2	0.13	**n.s.**	0.34	**n.s.**
E x P x C	4	0.22	**n.s.**	0.89	**n.s.**
DON	1	0.01	**n.s.**		
DON x E	3	0.54	**n.s.**		
DON x P	2	0.04	**n.s.**		
DON x E x P	4	0.03	**n.s.**		
DON x C	1	0.01	**n.s.**		
DON x E x C	3	0.07	**n.s.**		
DON x P x C	2	0.04	**n.s.**		
DON x E x P x C	4	0.38	**n.s.**		

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1;
and n.s. = not significant, DF degrees of freedom

ANCOVA for DS discovered a significant effect of DON_{LOG} within environment and the interaction of environment with pre-crop (Tab 25). Since the slight differences between cultivars (Tab 21) and also environmental effects became insignificant when DON_{LOG} was considered, it seemed legitimate to perform linear regression of DS and DON_{LOG} within pre-crops across cultivars and locations (Tab 26). In pre-crop maize, DON_{LOG} explained 60 and 82% of the variability in DS

in 2007 and 2009. R^2 increased if linear regression was performed separately for locations and pre-crop maize. Linear regression across cultivars and pre-crops within years increased the number of observations but gave weaker R^2 (Tab 26, Fig 22).

Tab 25. ANCOVA for effects of environment (E), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) on dough softening

Effect	DF	F	p
Environment (E)	3	0.44	**n.s.**
Pre-crop (P)	2	3.58	*
E x P	4	2.34	**(*)**
Cultivar (C)	1	0.00	**n.s.**
E x C	3	3.33	*
P x C	2	0.51	**n.s.**
E x P x C	4	2.38	**(*)**
DON	1	0.44	**n.s.**
DON x E	3	5.90	**
DON x P	2	2.34	**n.s.**
DON x E x P	4	3.02	*
DON x C	1	0.83	**n.s.**
DON x E x C	3	0.60	**n.s.**
DON x P x C	2	3.40	**(*)**
DON x E x P x C	4	0.84	**n.s.**

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1; and n.s. = not significant, DF degrees of freedom

Regardless of the factors levels included into linear regression, DS increased significantly with higher DON content of flour Type 550 (Fig 22) which was particularly observed after previous crop maize (Tab 26).

Tab 26. Parameters of linear regression of dough softening (VU/FU) and DON_{LOG} (mg kg^{-1}) within different environments (E) (year, location) and pre-crops (P) across cultivars, and range of original DON values (DON_{ORG})

Effect	Effect level									DON_{ORG} (mg kg^{-1})
	Year	Location	Cultivar	Pre-crop	n	R^2	p	b	a	Min-Max
E	2007	TL	Centrum & Ritmo	Wheat & Maize & Sugar beet	18	0.57	***	112.68	55.07	0.31-11.84
		GB			18	0.59	***	57.67	34.77	0.33-8.70
	2009	TL	Centrum & Ritmo	Maize & Sugar beet	12	0.69	***	44.19	24.69	0.16-8.42
E x P	2007	TL & GB	Centrum & Ritmo	Maize	12	0.60	**	61.63	98.21	0.37-11.84
	2009				12	0.82	***	34.13	39.78	0.39-8.42
	2007 & 2009	TL	Centrum & Ritmo	Sugar beet	12	0.51	**	105.48	104.16	0.16-1.73
		GB		Maize	12	0.74	***	43.04	54.76	0.27-8.70
	2007	TL	Centrum & Ritmo	Maize	6	0.88	**	92.31	91.73	2.52-11.84
		GB			6	0.80	*	56.15	48.85	0.67-8.70
	2009	GB			6	0.81	*	32.94	37.14	0.27-3.84

n number of observations, p significance: ***, **, and * = p < 0.001, 0.01, 0.05, b constant, a slope, TL Torland, GB Gladebeck

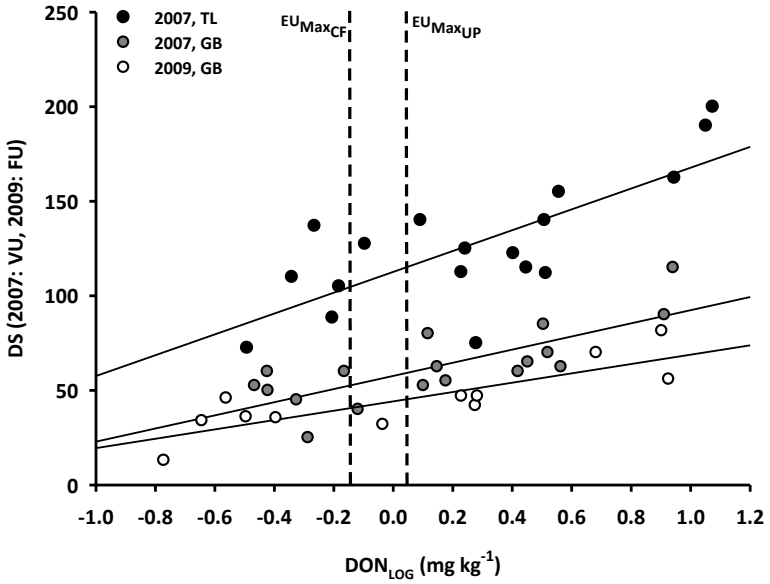

Fig 22. Linear regression of dough softening (DS) and DON_{LOG} in 2007 within locations Torland (TL) and Gladebeck (GB) and within location GB in 2009 across cultivars and pre-crops (for regression parameters see Tab 26), EU_{MaxCF} = 0.75 mg kg^{-1}, EU_{MaxUP} = 1.25 mg kg^{-1}

4.2.5 Baking properties (BV, LH, LL, LW)

BV was predominately influenced by year (Fig 23, Tab 28, Tab 56). In 2007 (Ø 408 ml 100 g^{-1} flour), BV was significantly higher than in 2008 (Ø 305 ml 100 g^{-1} flour) and in 2009 (Ø 270 ml 100 g^{-1} flour). Only in 2007, baking properties showed significant differences between cultivars. Cv. Ritmo (Ø 428 ml 100 g^{-1} flour) showed on average a 10% higher BV than cv. Centrum (Ø 388 ml 100 g^{-1} flour) while there were no differences between pre-crops within cultivars. Regarding loaf shape, all measures, LH, LL; LW, were also significantly affected by year (Fig 23, Tab 28, Tab 56). Loaves in 2007 were significantly longer, wider, and flatter than in the two following years. While there were no significant differences in LL, LH or LW detectable between cultivars and pre-crops in 2008 and 2009, in 2007 differences of shape could be observed in cv. Ritmo. Loaves produced from wheat grown after maize expressed reduced height when compared to sugar beet, reduced length when compared to pre-crop wheat, and increased width when compared to both (Fig 23, Fig 24).

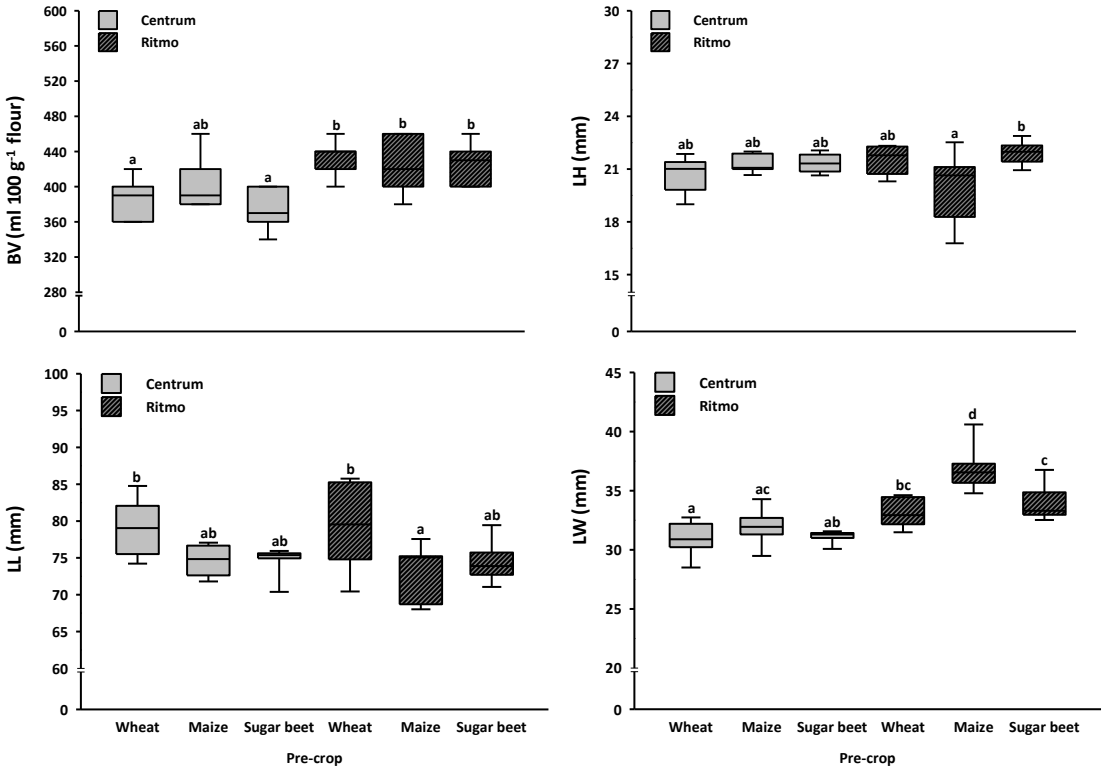

Fig 23. Baking properties (baking volume: BV, loaf heigth: LH, loaf length: LL, loaf width: LW) of two winter wheat cultivars (Centrum, Ritmo) grown after pre-crop wheat, maize, and sugar beet in 2007. Box plots with median (solid line) and whiskers indicating 5th/95th percentiles. Box plots (n = 6) with same letters are not significantly different at p < 0.05 according to Tukey test.

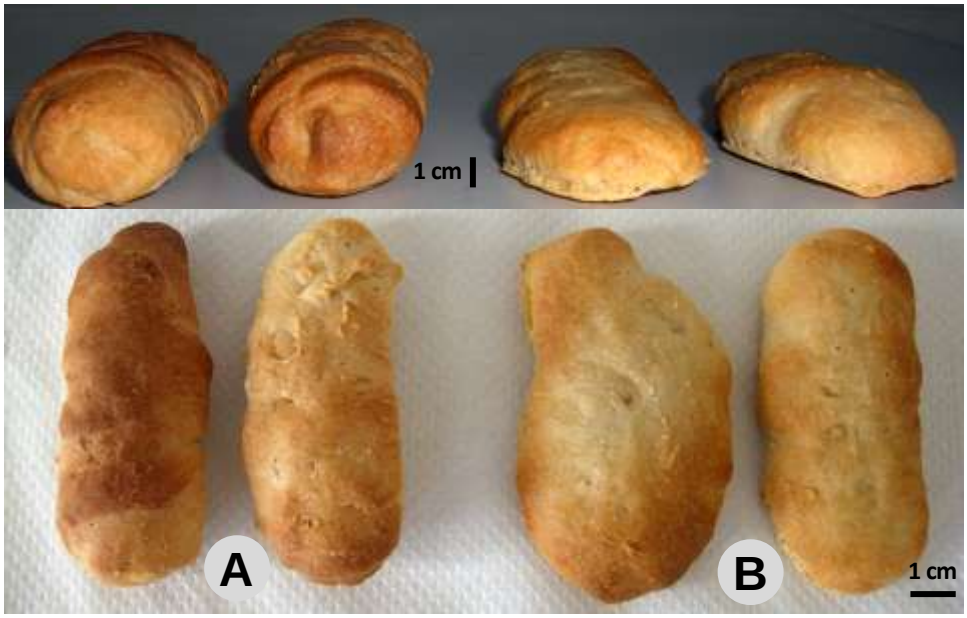

Fig 24. Loaves prepared from flour Type 550 from cv. Ritmo grown after pre-crop sugar beet (A) and pre-crop maize (B) in 2007, Pictures: KREUZBERGER 2008

Tab 28. Baking properties(baking volume: BV, loaf length: LL, loaf height: LH, loaf width: LW) wheat cv. (Centrum, Ritmo) grown after pre-crop maize and sugar beet in 2008 and 2009 (pre-crop wheat: not analyzed) (MV ± SD, n = 6)

Year	Cultivar	Pre-crop	BV (ml 100 g^{-1} flour)				LL (mm)				LH (mm)				LW (mm)			
2008	Centrum	Maize	312	±	48	**ab**	64.5	±	1.7	**a**	24.2	±	1.6	**a**	30.8	±	1.2	**a**
		Sugar beet	312	±	50	**ab**	64.6	±	2.1	**a**	25.2	±	1.1	**a**	31.2	±	0.6	**a**
	Ritmo	Maize	318	±	37	**b**	67.0	±	1.5	**a**	24.2	±	1.0	**a**	30.7	±	1.0	**a**
		Sugar beet	278	±	37	**a**	67.2	±	1.6	**a**	23.9	±	0.8	**a**	29.2	±	1.3	**a**
2009	Centrum	Maize	260	±	14	**a**	66.7	±	1.3	**a**	23.1	±	0.8	**a**	28.6	±	0.6	**a**
		Sugar beet	262	±	12	**a**	66.6	±	0.8	**a**	23.6	±	0.4	**a**	28.7	±	0.2	**a**
	Ritmo	Maize	283	±	5	**a**	69.4	±	1.4	**a**	23.8	±	0.6	**a**	29.4	±	0.9	**a**
		Sugar beet	277	±	10	**a**	68.7	±	1.3	**a**	23.1	±	0.7	**a**	29.4	±	0.6	**a**

Same letters indicate no differences between cultivars and pre-crops **within** years at a significance level of $p < 0.05$ according to Tukey test

ANCOVA detected an effect of DON_{LOG} on BV and LH (Tab 29). However, linear regression of DON_{LOG} and LH did not reveal a significant relationship between the two parameters ($R^2 = 0.05$, $p > 0.05$, $n = 72$). ANCOVA did not reveal a significant impact of DON on LL (Tab 30) and LW (Tab 31). In summary, there seemed to be no impact of *Fusarium* infection (on the basis of DON) on loaf shape.

Tab 29. ANCOVA for effects of environment (E) (year, location), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) on baking volume (BV) and loaf height (LH)

		BV		LH	
Effect	**DF**	**F**	**p**	**F**	**p**
Environment (E)	3	9.88	***	4.95	**
Pre-crop (P)	2	0.39	**n.s.**	2.21	**n.s.**
E x P	4	1.34	**n.s.**	2.30	**(*)**
Cultivar (C)	1	7.89	*	2.87	**n.s.**
E x C	3	2.71	**(*)**	1.52	**n.s.**
P x C	2	1.09	**n.s.**	1.21	**n.s.**
E x P x C	4	3.31	*	2.16	**n.s.**
DON	1	0.27	**n.s.**	5.39	*
DON x E	3	2.98	**(*)**	0.58	**n.s.**
DON x P	2	4.46	*	1.93	**n.s.**
DON x E x P	4	1.40	**n.s.**	1.37	**n.s.**
DON x C	1	1.80	**n.s.**	0.42	**n.s.**
DON x E x C	3	4.36	*	1.58	**n.s.**
DON x P x C	2	2.12	**n.s.**	0.24	**n.s.**
DON x E x P x C	4	5.48	**	2.37	**(*)**

p significance: ***, **, and * = $p < 0.001$, 0.01, 0.05, respectively; (*) = $p < 0.1$; and n.s. = not significant, DF degrees of freedom

Tab 30. ANCOVA for effects of environment (E) (year, location), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) and ANOVA for effects of E, P, and C on loaf length

	ANCOVA			ANOVA	
Effect	**DF**	**F**	**p**	**F**	**p**
Environment (E)	3	3.12	*	23.82	***
Pre-crop (P)	2	3.36	(*)	13.09	***
E x P	4	1.49	n.s.	3.30	*
Cultivar (C)	1	0.76	n.s.	1.57	n.s.
E x C	3	1.66	n.s.	1.57	n.s.
P x C	2	0.64	n.s.	0.13	n.s.
E x P x C	4	0.78	n.s.	0.94	n.s.
DON	1	1.60	n.s.		
DON x E	3	1.12	n.s.		
DON x P	2	0.88	n.s.		
DON x E x P	4	0.47	n.s.		
DON x C	1	0.10	n.s.		
DON x E x C	3	0.73	n.s.		
DON x P x C	2	0.94	n.s.		
DON x E x P x C	4	0.70	n.s.		

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1;
and n.s. = not significant, DF degrees of freedom

Tab 31. ANCOVA for effects of environment (E), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) and ANOVA for effects of E, P, and C on loaf width

	ANCOVA			ANOVA	
Effect	**DF**	**F**	**p**	**F**	**p**
Environment (E)	3	0.06	n.s.	63.21	***
Pre-crop (P)	2	0.04	n.s.	10.28	***
E x P	4	0.69	n.s.	2.30	(*)
Cultivar (C)	1	0.83	n.s.	41.20	***
E x C	3	0.52	n.s.	7.64	***
P x C	2	0.96	n.s.	3.45	*
E x P x C	4	0.08	n.s.	1.90	n.s.
DON	1	0.02	n.s.		
DON x E	3	1.42	n.s.		
DON x P	2	3.07	(*)		
DON x E x P	4	0.40	n.s.		
DON x C	1	1.10	n.s.		
DON x E x C	3	0.86	n.s.		
DON x P x C	2	0.13	n.s.		
DON x E x P x C	4	0.71	n.s.		

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1;
and n.s. = not significant, DF degrees of freedom

Linear regression between DON and BV in significant effects of ANCOVA revealed a significant moderate to strong increase of BV with increasing DON_{LOG} in cv. Centrum across all pre-crops in 2007 at location Torland and within maize across both locations (Tab 32). Correlation of DON_{LOG} and BV within pre-crop wheat/sugar beet across years, locations, and cultivars revealed the same trend.

Tab 32. Parameters of linear regression of baking volume (ml 100 g^{-1} flour) and DON_{LOG} in different environments (E) (year, location), cultivars (C), and pre-crops (P), and range of original DON values (DON_{ORG})

Effect	Effect level									DON_{ORG} (mg kg^{-1})
	Year	Location	Cultivar	Pre-crop	n	R^2	p	b	a	Min-Max
P	2007	TL & GB	Centrum & Ritmo	Wheat	12	0.51	**	407.89	55.72	0.31-3.65
	2007 & 2009			Sugar beet	24	0.53	***	366.55	84.67	0.33-2.82
E x C	2007	TL	Centrum	Wheat & Maize & Sugar beet	9	0.66	**	400.75	58.47	0.31-3.59
E x P x C		TL & GB		Maize	6	0.66	*	378.52	94.21	0.67-3.59

n number of observations, p significance: ***, **, and * = p < 0.001, 0.01, 0.05, b constant, a slope, TL Torland, GB Gladebeck

4.2.1 Gluten properties (R_{Max}, EXT, R_{Max}/EXT)

A micro-extension experiment on wet gluten from flour Type 550 in 2007 showed that both cultivars differed significantly in their gluten properties (Tab 33, Tab 59). While gluten stripes from cv. Centrum ruptured at an average force of 0.64 N and could be stretched until this point to an average length of 109 mm, gluten stripes of cv. Ritmo stayed intact until technical limitation of stretching (140 mm). Therefore, the two parameters –R_{MAX} and EXT- which typically characterize the micro-extension experiment on dough and gluten, could not be used to compare the two cultivars directly except by a qualitative statement. In order to find a common quantitative measure to compare both cultivars, R_{MAX}/EXT was calculated. It became clear that significantly less force (about 66%) was necessary to extend gluten of cv. Ritmo (Ø 2.1 N m^{-1}) to the same length as gluten of cv. Centrum (Ø 6.0 N m^{-1}).

Tab 33. Gluten properties (resistance to extension: R_{MAX}, extensibility: EXT) of wheat cv. (Centrum, Ritmo) grown after pre-crop wheat, maize, and sugar beet in 2007 measured with SMS/Kieffer Dough and Gluten Extensibility Rig with Texture Analyzer (MV ± SD, n = 6)

Year	Cultivar	Pre-crop	R_{MAX} (N)	EXT (mm)	R_{MAX}/EXT	Rupture of gluten
2007	Centrum	Wheat	0.64 ± 0.05 **a**	113 ± 9 **a**	5.78 ± 0.69 **a**	yes
		Maize	0.60 ± 0.03 **a**	108 ± 9 **a**	5.62 ± 0.62 **a**	
		Sugar beet	0.69 ± 0.06 **a**	105 ± 7 **a**	6.63 ± 0.62 **a**	
	Ritmo	Wheat	0.31 ± 0.03	138 ± 1	2.26 ± 0.18 **b**	no
		Maize	0.24 ± 0.04	137 ± 2	1.73 ± 0.29 **b**	
		Sugar beet	0.29 ± 0.04	136 ± 3	2.17 ± 0.29 **b**	

Same letters indicate no differences **within** cultivar Centrum between pre-crops for resistance to extension and extensibility and **between** cultivars and pre-crops for ratio of resistance to extension/extensibility at a significance level of p < 0.05 according to Tukey test

ANCOVA for R_{MAX}/EXT showed no significant effect of DON_{LOG} on the same and demonstrated again the highly significant effect of cultivar on the properties of gluten (Tab 34).

Tab 34. ANCOVA for effects of location (L), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) and ANOVA for effects of L, P, and C on R_{MAX}/EXT of wet gluten extracted from flour Type 550 in 2007

	ANCOVA			ANOVA	
Effect	**DF**	**F**	**p**	**F**	**p**
Location (L)	1	0.55	**n.s.**	0.01	**n.s.**
Pre-crop (P)	2	0.20	**n.s.**	2.39	**n.s.**
L x P	2	0.77	**n.s.**	2.11	**n.s.**
Cultivar (C)	1	19.48	***	194.27	***
L x C	1	0.16	**n.s.**	0.01	**n.s.**
P x C	2	0.22	**n.s.**	0.69	**n.s.**
L x P x C	2	0.50	**n.s.**	1.95	**n.s.**
DON	1	0.01	**n.s.**		
DON x L	1	0.01	**n.s.**		
DON x P	2	0.92	**n.s.**		
DON x L x P	2	0.20	**n.s.**		
DON x C	1	0.12	**n.s.**		
DON x L x C	1	1.73	**n.s.**		
DON x P x C	2	0.75	**n.s.**		

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1; and n.s. = not significant, DF degrees of freedom

4.3 *Fusarium* infection and flour composition

4.3.1 Starch

Starch content in flour Type 550 was primarily influenced by year and pre-crop (Tab 35, Tab 59). Starch content varied significantly between all years. Lowest starch content was measured in 2007 (Ø 73.5 %), followed by 2008 (Ø 75.7%) and 2009 (Ø 77.7%). While differences between cultivars were not significant across all samples, pre-crop had an effect on starch content. In 2008 and 2009, pre-crop maize led to higher starch contents of flours in both cultivars compared to previous crop wheat and sugar beet resulting in a significant effect of pre-crop across all three years. This effect was not present within 2007.

Tab 35. Starch content of flour Type 550 of wheat cultivars (Centrum, Ritmo) grown after different pre-crops in 2007, 2008, and 2009 (MV ± SD, n = 6)

Year	Cultivar	Pre-crop	Starch (%)			
2007	Centrum	Wheat	73.0	±	0.8	ab
		Maize	74.0	±	0.9	ab
		Sugar beet	74.1	±	0.8	b
	Ritmo	Wheat	72.6	±	0.5	a
		Maize	73.7	±	1.4	ab
		Sugar beet	73.4	±	0.7	ab
2008	Centrum	Wheat	74.7	±	0.7	a
		Maize	78.1	±	1.6	b
		Sugar beet	74.1	±	1.0	a
	Ritmo	Wheat	74.8	±	1.3	a
		Maize	78.5	±	2.2	b
		Sugar beet	74.2	±	0.8	a
2009	Centrum	Wheat	76.5	±	1.1	a
		Maize	79.3	±	0.8	b
		Sugar beet	76.8	±	0.5	a
	Ritmo	Wheat	76.7	±	0.3	a
		Maize	79.8	±	0.8	b
		Sugar beet	77.0	±	0.7	a

Same letters indicate no differences between cultivars and pre-crops **within** years at a significance level of $p < 0.05$ according to Tukey test

ANCOVA did not reveal a significant effect of DON_{LOG} on starch content on flour Type 550 (Tab 36). Therefore, ANOVA described variability in starch content adequately.

Tab 36. ANCOVA for effects of environment (E) (year, location), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) and ANOVA for effects of E, P, and C on starch content (%) of flour Type 550

	ANCOVA			ANOVA	
Effect	DF	F	p	F	p
Environment (E)	3	12.66	***	247.70	***
Pre-crop (P)	2	2.77	(*)	57.51	***
E x P	6	1.31	n.s.	12.62	***
Cultivar (C)	1	0.16	n.s.	0.38	n.s.
E x C	3	1.11	n.s.	2.89	*
P x C	2	0.71	n.s.	0.43	n.s.
E x P x C	5	0.80	n.s.	0.63	n.s.
DON	1	1.26	n.s.		
DON x E	3	0.18	n.s.		
DON x P	2	0.15	n.s.		
DON x E x P	6	0.70	n.s.		
DON x C	1	1.65	n.s.		
DON x E x C	3	0.47	n.s.		
DON x P x C	2	0.07	n.s.		
DON x E x P x C	5	0.60	n.s.		

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1; and n.s. = not significant, DF degrees of freedom

4.3.2 Gluten proteins

4.3.2.1 Total gluten

More than 75% of $PC_{Type550}$ of cv. Ritmo and cv. Centrum consisted of gluten forming proteins whereas gliadin proteins made up for the major proportion (Fig 25). Total gluten content was primarily influenced by year and pre-crop (Tab 38, Tab 57). Across all samples, total gluten content was highest in 2009 and after pre-crop sugar beet. However, differences between years as well as pre-crops only averaged 2-3%. Within years, differences between pre-crops were in most cases not significant. In general, there were no significant differences between cultivars across or within years.

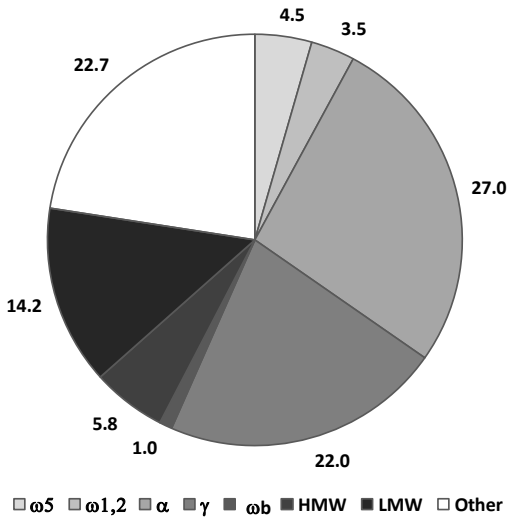

Fig 25. Average relative proportion (%) of gliadins (ω5, ω1,2, α, γ), glutenins (ωb, HMW-GS, LMW-GS), and residual (other) protein in total protein of flour Type 550 across all investigated samples (n =108)

Tab 38. Total gluten content (% Protein) of wheat cultivars (Centrum, Ritmo) grown after pre-crops wheat, maize, and sugar beet in 2007, 2008, and 2009 (MV ± SD, n =6)

Year	Cultivar	Pre-crop	Total gluten (% Protein)			
2007	Centrum	Wheat	75.4	±	2.2	ab
		Maize	78.1	±	5.8	ab
		Sugar beet	81.7	±	5.1	b
	Ritmo	Wheat	75.3	±	3.7	ab
		Maize	70.1	±	4.7	a
		Sugar beet	79.7	±	2.8	b
2008	Centrum	Wheat	69.9	±	3.7	a
		Maize	77.3	±	3.1	ab
		Sugar beet	79.0	±	1.8	b
	Ritmo	Wheat	75.7	±	4.5	ab
		Maize	79.3	±	1.8	b
		Sugar beet	80.6	±	4.8	b
2009	Centrum	Wheat	84.4	±	1.4	c
		Maize	73.1	±	2.7	ab
		Sugar beet	80.4	±	2.0	bc
	Ritmo	Wheat	80.4	±	1.5	bc
		Maize	80.8	±	1.8	bc
		Sugar beet	71.0	±	9.2	a

Same letters indicate no differences between cultivars and pre-crops **within** years at a significance level of $p < 0.05$ according to Tukey test

ANCOVA showed a significant effect of DON_{LOG} on total gluten content within the effect of environment and the interaction of environment and pre-crop (Tab 39). Linear regression revealed trends in both directions, either total gluten content increased with increasing DON_{LOG} or the opposite effect. Strength and direction of the effect depended on year, pre-crop, location, and the number of observations included (Tab 40).

Tab 39. ANCOVA for effects of environment (E) (year, location), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) and ANOVA for effects of E, P, and C on total gluten content

Effect	DF	F	p
Environment (E)	3	4.03	*
Pre-crop (P)	2	4.66	*
E x P	6	4.68	**
Cultivar (C)	1	0.44	n.s.
E x C	3	2.70	(*)
P x C	2	0.96	n.s.
E x P x C	5	4.19	**
DON	1	0.76	n.s.
DON x E	3	3.56	*
DON x P	2	1.20	n.s.
DON x E x P	6	4.09	**
DON x C	1	0.00	n.s.
DON x E x C	3	1.81	n.s.
DON x P x C	2	0.09	n.s.
DON x E x P x C	5	1.08	n.s.

p significance: ***, **, and * = $p < 0.001, 0.01, 0.05$, respectively; (*) = $p < 0.1$; and n.s. = not significant,

Tab 40. Parameters of linear regression of total gluten (% Protein) and DON_{LOG} (mg kg^{-1}) within environments (E) (year, location), cultivars (C), and pre-crops (P), and range of original DON values (DON_{ORG})

Effect	Effect level									DON_{ORG} (mg kg^{-1})
	Year	Location	Pre-crop	Cultivar	n	R^2	p	b	a	Min-Max
E x P	2009	TL & GB	Maize	Centrum & Ritmo	12	0.68	**	74.57	8.18	0.39-8.42
	2007 & 2009	GB	Wheat		12	0.62	**	75.69	-4.19	0.00-3.99
	2007		Maize		6	0.99	***	79.87	-14.17	0.67-8.70
	2009	GB	Wheat	Centrum & Ritmo	6	0.88	**	78.57	-2.69	0.00-0.73
			Maize		6	0.74	*	75.64	10.41	0.27-3.84

n number of observations, p significance: ***, **, and * = p < 0.001, 0.01, 0.05, b constant, a slope, TL Torland, GB Gladebeck

4.3.2.2 Total gliadin and subfractions

Across all investigated samples, total gliadin (sum of all gliadin subfractions) made up the major proportion of total protein in flour Type 550 (Ø 56.9 % Protein) (Fig 25). The total gliadin content was significantly influenced by year and cultivar (Tab 41, Tab 57). Total gliadin content differed significantly between all three years and averaged 57.0, 52.4, and 61.3% in 2007, 2008, and 2009. While within years not always apparent, across years cv. Ritmo (Ø 57.9% Protein) contained about 4% more total gliadin than cv. Centrum (Ø 55.9% Protein), a slight but significant difference. Pre-crop did not influence gliadin content significantly, only within some combinations of year and cultivar.

ω5- and ω1,2-gliadin presented the smallest proportion of total gliadin (Fig 25). Both subfractions made up only for about 4.5 and 3.5% of total protein in flour, respectively. Both ω5-gliadin and ω1,2-gliadin content differed significantly between all the years (Tab 41, Tab 57). In 2007, flours contained significantly more ω-gliadins than in 2009 and 2008. Within the years, neither significant differences between cultivars or pre-crops could be observed for both gliadin fractions in 2008, however, data of 2007 and 2009 showed more variation. While in 2007, cv. Centrum expressed slightly more ω5-Gliadin (Ø 4.9 % Protein) than cv. Ritmo (Ø 4.5% Protein) across pre-crops, the effect of cultivar was adverse (Ø cv. Centrum: 4.2% Protein, Ø cv. Ritmo: 4.7% Protein) in 2009. The effect of pre-crop depended on cultivar and year. Across all years cv. Ritmo (Ø 4.6% Protein) contained significantly more ω5-gliadin than cv. Centrum (Ø 4.4% Protein). For ω1,2-gliadin content there was not significant difference between cultivars across year, but a significant effect of pre-crop. Across years and cultivars, significantly higher ω1,2-gliadin content was observed after pre-crop wheat (3.7% protein) when compared to maize (3.4% protein), and sugar beet (3.3% protein). Effect of pre-crop and cultivar depended on year and on the interaction of pre-crop and cultivar.

α-gliadin was quantitatively the biggest fraction of total gliadin (Fig 25). Across all samples α-gliadin averaged 27.0% of total protein. The content varied only slightly between the years 2007 (Ø

26.4%) and 2008 (Ø 26.0%) and was significantly higher in 2009 (Ø: 28.5%) (Tab 41, Tab 57). Within and across the years no significant differences between cultivars and pre-crops could be observed.

γ-gliadin presented the second biggest proportion of total gliadin (Fig 25). Across the years it made up for averagely 22.0% of total protein. Differences between the years were significant (Tab 41, Tab 57). Highest γ-gliadin content was observed in 2009 (Ø 24.8% protein), followed by 2007 (Ø 22.1% protein), and 2008 (Ø 19.0% protein). Across all years, cv. Ritmo (Ø 22.7% protein) contained significantly more γ-gliadin than cv. Centrum (Ø 21.3% protein), also this difference was not so apparent within the single years. Across all years and cultivars, pre-crop showed a significant effect on γ-gliadin content. Content was on average highest after sugar beet (23.1%), followed by maize (22.3%) and significantly lower in wheat (20.6%).

Tab 41. Content of total gliadin and subfractions (ω5, ω1,2, α, γ) (% Protein) of flour Type 550 from wheat cultivars (Centrum, Ritmo) grown after pre-crop wheat, maize, and sugar beet in 2007, 2008, and 2009 (MV ± SD, n = 6)

Year	Cultivar	Pre-crop	Total				ω5				ω1,2				α				γ			
2007	Centrum	Wheat	57.0	±	3.4	ab	5.2	±	0.5	bc	4.0	±	0.3	c	27.2	±	1.8	a	20.3	±	2.2	ab
		Maize	60.7	±	2.7	b	5.5	±	0.9	c	3.8	±	0.3	ac	27.5	±	1.4	a	23.9	±	0.6	bc
		Sugar beet	52.9	±	1.9	a	4.0	±	0.2	a	3.2	±	0.2	a	24.6	±	1.0	a	21.1	±	0.9	ab
	Ritmo	Wheat	56.2	±	2.6	ab	4.6	±	0.7	ab	3.8	±	0.6	ac	26.6	±	1.5	a	21.4	±	0.3	ab
		Maize	54.8	±	1.0	ab	4.3	±	0.2	a	3.4	±	0.1	ab	26.1	±	0.5	a	21.1	±	0.6	ab
		Sugar beet	60.3	±	2.2	b	4.6	±	0.2	ac	3.9	±	0.2	bc	26.8	±	1.3	a	25.0	±	0.7	c
2008	Centrum	Wheat	49.9	±	4.5	a	4.0	±	0.5	a	3.3	±	0.4	a	25.7	±	2.7	a	16.9	±	1.3	a
		Maize	51.8	±	3.0	ab	4.0	±	0.4	a	3.4	±	0.3	a	25.8	±	1.5	a	18.5	±	1.6	ab
		Sugar beet	52.7	±	5.2	ab	3.9	±	0.4	a	3.1	±	0.3	a	26.5	±	2.6	a	19.2	±	2.2	ab
	Ritmo	Wheat	49.2	±	3.1	a	4.2	±	0.3	a	3.1	±	0.2	a	24.8	±	1.6	a	17.1	±	1.7	a
		Maize	54.1	±	3.6	ab	4.4	±	0.3	a	3.0	±	0.2	a	25.8	±	2.0	a	20.9	±	1.4	b
		Sugar beet	56.8	±	5.0	b	4.7	±	0.3	a	3.2	±	0.3	a	27.6	±	2.9	a	21.2	±	1.7	b
2009	Centrum	Wheat	62.2	±	3.8	b	4.7	±	0.3	bc	3.9	±	0.2	b	29.6	±	1.3	a	24.0	±	2.9	ab
		Maize	55.5	±	2.9	a	3.7	±	0.2	a	3.1	±	0.1	a	26.7	±	0.9	a	22.1	±	1.9	a
		Sugar beet	60.1	±	2.8	ab	4.2	±	0.3	ab	3.2	±	0.2	a	27.1	±	1.5	a	25.6	±	0.9	bc
	Ritmo	Wheat	62.9	±	1.3	b	5.1	±	0.3	c	3.9	±	0.3	b	29.9	±	1.3	a	23.9	±	0.8	ab
		Maize	64.5	±	1.3	b	4.7	±	0.1	bc	3.5	±	0.1	ab	29.3	±	0.8	a	27.0	±	0.7	c
		Sugar beet	62.6	±	3.0	b	4.4	±	0.1	ac	3.3	±	0.1	ab	28.5	±	1.2	a	26.4	±	1.8	bc

Same letters indicate no differences between cultivars and pre-crops **within** years at a significance level of $p < 0.05$ according to Tukey test

ANCOVA for total gliadin (Tab 42) and α-gliadin (Tab 43) showed that there was no significant effect of DON_{LOG} on both. Therefore, ANOVA was sufficient to explain variability of data.

Tab 42. ANCOVA for effects of environment (E) (year, location), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) and ANOVA for effects of E, P, and C on total gliadin content

	ANCOVA			ANOVA	
Effect	**DF**	**F**	**p**	**F**	**p**
Environment (E)	3	3.94	*	23.24	***
Pre-crop (P)	2	0.22	**n.s.**	0.63	**n.s.**
E x P	6	1.47	**n.s.**	1.45	**n.s.**
Cultivar (C)	1	2.07	**n.s.**	15.68	***
E x C	3	0.52	**n.s.**	5.24	**
P x C	2	1.66	**n.s.**	7.13	**
E x P x C	5	3.82	*	11.67	***
DON	1	2.90	**n.s.**		
DON x E	3	1.64	**n.s.**		
DON x P	2	1.01	**n.s.**		
DON x E x P	6	1.76	**n.s.**		
DON x C	1	0.36	**n.s.**		
DON x E x C	3	0.55	**n.s.**		
DON x P x C	2	0.45	**n.s.**		
DON x E x P x C	5	1.31	**n.s.**		

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1;
and n.s. = not significant, DF degrees of freedom

Tab 43. ANCOVA for effects of environment (E) (year, location), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) and ANOVA for effects of E, P, and C on content of α-gliadin

	ANCOVA			ANOVA	
Effect	**DF**	**F**	**p**	**F**	**p**
Environment (E)	3	3.64	*	16.99	***
Pre-crop (P)	2	0.12	**n.s.**	10.00	***
E x P	6	0.98	**n.s.**	1.09	**n.s.**
Cultivar (C)	1	1.41	**n.s.**	6.78	*
E x C	3	0.50	**n.s.**	3.80	*
P x C	2	2.00	**n.s.**	3.57	*
E x P x C	5	2.19	**(*)**	3.11	*
DON	1	3.04	**(*)**		
DON x E	3	2.15	**n.s.**		
DON x P	2	0.45	**n.s.**		
DON x E x P	6	2.03	**(*)**		
DON x C	1	0.47	**n.s.**		
DON x E x C	3	0.48	**n.s.**		
DON x P x C	2	0.45	**n.s.**		
DON x E x P x C	5	0.95	**n.s.**		

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1;
and n.s. = not significant, DF degrees of freedom

For ω5- and γ-gliadin ANCOVA showed a slight significant effect of DON_{LOG} within environment (Tab 44). However, linear regression did not reveal a single significant correlation between these protein fractions and DON_{LOG}. Out of eight possible linear regressions for each subfraction nearly

all showed $R^2 < 0.05$ ($p > 0.05$). The same applied to ω1,2-gliadin where ANCOVA calculated a significant effect of DON_{LOG} across all samples and within environment (Tab 44). Therefore, it can be summarized that DON concentration did not have linear relationship with the content of any of the three gliadin subfractions and ANOVA with main effects of environment, pre-crop, and cultivar was sufficient to describe variability of data.

Tab 44. ANCOVA for effects of environment (E) (year, location), pre-crop (P), cultivar (C), and DON_{LOG} (DON) on content of ω5-gliadin, ω1,2-gliadin, and γ-gliadin

		ω5-Gliadin (% Protein)		ω1,2-Gliadin (% Protein)		γ-Gliadin (% Protein)	
Effect	**DF**	**F**	**p**	**F**	**p**	**F**	**p**
Environment (E)	3	1.95	**n.s.**	1.21	**n.s.**	8.02	***
Pre-crop (P)	2	1.59	**n.s.**	1.57	**n.s.**	6.34	**
E x P	6	1.14	**n.s.**	1.22	**n.s.**	2.81	*
Cultivar (C)	1	0.30	**n.s.**	2.88	**n.s.**	2.62	**n.s.**
E x C	3	0.86	**n.s.**	0.35	**n.s.**	2.09	**n.s.**
P x C	2	1.52	**n.s.**	3.17	**(*)**	0.44	**n.s.**
E x P x C	5	1.17	**n.s.**	0.75	**n.s.**	6.10	***
DON	1	3.33	**(*)**	5.09	*	0.63	**n.s.**
DON x E	3	5.01	**	4.14	*	3.55	*
DON x P	2	0.88	**n.s.**	2.07	**n.s.**	2.47	**n.s.**
DON x E x P	6	1.57	**n.s.**	1.75	**n.s.**	2.22	**(*)**
DON x C	1	0.05	**n.s.**	0.01	**n.s.**	0.38	**n.s.**
DON x E x C	3	0.55	**n.s.**	0.85	**n.s.**	2.29	**n.s.**
DON x P x C	2	0.57	**n.s.**	0.41	**n.s.**	1.81	**n.s.**
DON x E x P x C	5	0.76	**n.s.**	0.89	**n.s.**	1.86	**n.s.**

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1; and n.s. = not significant, DF degrees of freedom

4.3.2.3 Total glutenin and subfractions

Compared to total gliadin total glutenin presented a significantly smaller fraction of total protein (Fig 25). Across all samples, total glutenin content averaged 20.9% of protein. Year had a significant effect on glutenin content (Tab 45, Tab 58). In 2008 (Ø 24.5% protein), across all samples, flour contained a significantly higher glutenin proportion than in 2007 (Ø 19.8% protein) and 2009 (Ø 18.5% protein). Across all years and pre-crops, cv. Ritmo (Ø 20.0% protein) contained about 8.4% less glutenin than cv. Centrum (Ø 21.8% protein). Differences between cultivars were not significant within years. Across years and cultivars, total protein of flours consisted of about 14% less glutenin when gained from wheat grown after maize (Ø 19.5% Protein) compared to pre-crop sugar beet (Ø 22.6% Protein), which meant a significant difference. Compared to pre-crop wheat the difference was only 5.1% and not significant. Differences between pre-crops within in years were not significant (except for cv. Centrum after sugar beet in 2007). Interestingly, glutenin contents varied more in 2007 than in the following years, indicated by relatively high SD.

ωb was the smallest proportion of glutenin and made up averagely 1% of total protein and was therefore the smallest subfraction of all gluten forming proteins (Fig 25). ωb content was significantly influenced by year (Tab 45, Tab 58). There were no significant differences between the two cultivars and pre-crops within years In 2009, across all samples protein consisted of significantly less ωb (0.7%) than in the other two years (2007: 1.1%, 2008, 1,2%). Across years and pre-crops, cv. Ritmo contained significantly less ωb-GS (Ø 0.9% protein) than cv. Centrum (Ø 1.1 % protein). The difference was 15% on average. Pre-crops also did not influence ωb-GS content across years.

On average, protein consisted of 5.8% HWW-GS across all samples (Fig 25). Across all samples, HMW-GS content was significantly influenced by year and pre-crop, however not by cultivar (Tab 45, Tab 58). In 2008 (Ø 6.6% protein), flours contained significantly more HMW-GS than in 2007 (Ø 5.5% protein) and 2009 (Ø 5.6% protein). After pre-crop maize, HMW-GS content was on average significantly reduced about 12% when compared to sugar beet and 10% when compared to wheat. Within years, the effect of pre-crop was not significant (Tab 45, Tab 58). Across and within years, no significant differences between the two cultivars were observed. Protein of cv. Ritmo and cv. Centrum contained averagely 5.7% and 6.0% HMW-GS, respectively.

LMW-GS made up the biggest proportion of the glutenin subfractions (Fig 25). On average 14.2% of protein consisted of LMW-GS (Fig 25). Looking at all samples, year, cultivar, and pre-crop influenced LMW-GS content significantly (Tab 45, Tab 58). As described for HMW-GS, LMW-GS content was significantly higher in 2008 (Ø 16.9% protein), than in the other two years (2007: 13.2% protein, 2009: 12.2% protein). Across all samples, cv. Ritmo (Ø 13.4% protein) comprised significantly less LMW-GS than cv. Centrum (Ø 14.8% protein). Average difference was 9.3%. After pre-crop maize (Ø 13.2% Protein) and wheat (Ø 13.6% Protein) flours contained significantly less LMW-GS than after pre-crop sugar beet (15.3%). Within years, neither differences of cultivars nor pre-crops were significant.

Tab 45. Content of total glutenin and subfractions (ωb, HMW-GS, LMW-GS) (% Protein) of flour Type 550 from wheat cultivars (Centrum, Ritmo) grown after pre-crop wheat, maize, and sugar beet in 2007, 2008, and 2009 (MV ± SD, n = 6)

Year	Cultivar	Pre-crop	Total				ωb				HMW-GS				LMW-GS			
2007	Centrum	Wheat	18.4	±	1.3	a	0.8	±	0.3	a	5.4	±	0.5	ab	12.2	±	1.3	a
		Maize	17.4	±	8.1	a	1.1	±	0.7	a	4.7	±	2.3	a	11.7	±	5.2	a
		Sugar beet	28.8	±	4.9	b	1.8	±	0.5	b	7.1	±	1.3	b	19.9	±	3.2	b
	Ritmo	Wheat	19.1	±	2.9	a	1.1	±	0.1	a	5.6	±	1.1	ab	12.5	±	1.8	a
		Maize	15.3	±	4.6	a	0.8	±	0.2	a	4.5	±	1.7	a	10.0	±	2.8	a
		Sugar beet	19.4	±	2.5	a	1.0	±	0.1	a	5.6	±	1.1	ab	12.8	±	1.4	a
2008	Centrum	Wheat	20.0	±	1.6	a	0.8	±	0.1	a	5.8	±	0.5	a	13.3	±	1.1	a
		Maize	25.5	±	2.3	a	1.3	±	0.2	a	6.8	±	0.5	a	17.4	±	1.7	ab
		Sugar beet	26.3	±	3.6	a	1.3	±	0.3	a	6.7	±	0.6	a	18.4	±	2.8	b
	Ritmo	Wheat	26.4	±	4.2	a	1.4	±	0.4	a	6.9	±	0.6	a	18.1	±	3.3	ab
		Maize	25.2	±	2.6	a	1.3	±	0.2	a	6.4	±	0.6	a	17.5	±	1.9	ab
		Sugar beet	23.8	±	1.6	a	1.1	±	0.1	a	6.3	±	0.4	a	16.5	±	1.3	ab
2009	Centrum	Wheat	22.2	±	3.5	a	1.0	±	0.3	b	6.6	±	0.7	a	14.6	±	2.6	a
		Maize	17.6	±	1.6	a	0.7	±	0.1	ab	5.0	±	0.5	a	11.9	±	1.3	a
		Sugar beet	20.3	±	1.6	a	0.9	±	0.2	b	5.8	±	0.5	a	13.6	±	1.1	a
	Ritmo	Wheat	17.5	±	1.4	a	0.7	±	0.1	ab	5.6	±	0.5	a	11.2	±	0.9	a
		Maize	16.3	±	1.1	a	0.6	±	0.1	ab	5.0	±	0.6	a	10.7	±	0.5	a
		Sugar beet	16.9	±	1.4	a	0.3	±	0.3	a	5.2	±	0.7	a	11.2	±	0.7	a

Same letters indicate no differences between cultivars and pre-crops **within** years at a significance level of $p < 0.05$ according to Tukey test

ANCOVA detected a significant and a highly significant effect of DON_{LOG} on total glutenin content and HMW-GS content, respectively, within environment (Tab 46).

Tab 46. ANCOVA for effects of environment (E) (year, location), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) on content of total glutenin and HMW-GS

	Glutenin (% Protein)			HMW-GS (% Protein)	
Effect	**DF**	**F**	**p**	**F**	**p**
Environment (E)	3	5.40	**	6.15	**
Pre-crop (P)	2	2.56	(*)	4.02	*
E x P	6	2.14	(*)	3.33	*
Cultivar (C)	1	2.80	n.s.	2.25	n.s.
E x C	3	0.43	n.s.	2.95	(*)
P x C	2	0.49	n.s.	0.58	n.s.
E x P x C	4	0.29	n.s.	0.41	n.s.
DON	1	1.36	n.s.	1.80	n.s.
DON x E	3	3.24	*	8.30	***
DON x P	2	0.33	n.s.	1.38	n.s.
DON x E x P	6	1.92	n.s.	2.07	(*)
DON x C	1	0.48	n.s.	0.45	n.s.
DON x E x C	3	0.72	n.s.	0.98	n.s.
DON x P x C	2	0.03	n.s.	0.05	n.s.
DON x E x P x C	4	0.48	n.s.	0.67	n.s.

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1;
and n.s. = not significant, DF degrees of freedom

Linear regression between DON_{LOG} and total glutenin content revealed that even though all possible correlations within different environments showed without exceptions a negative trend (average R^2 of 20 linear regression curves was 0.27), only two showed a significant $R^2 \geq 0.5$, $p<0.05$

(Fig 26). In 2007, DON_{LOG} was very highly negatively related to total glutenin content in cv. Ritmo at location Gladebeck. DON_{ORG} in these samples ranged from 1.30 to 8.70 mg kg^{-1}. In 2009, across cultivars, DON_{LOG} was highly adversely correlated to total glutenin content at the same location. DON_{ORG} of the respective samples ranged from 0.00-3.84 mg kg^{-1}. The same significant negative trend of similar strength could be observed between HMW-GS content and DON_{LOG} for the identical samples (Fig 26). In general, as shown for total glutenin content, a negative relationship between HMW-GS content and DON_{LOG} could be observed within more environments, yet, R^2 was below 0.50 (average R^2 of 20 linear regression curves was 0.24).

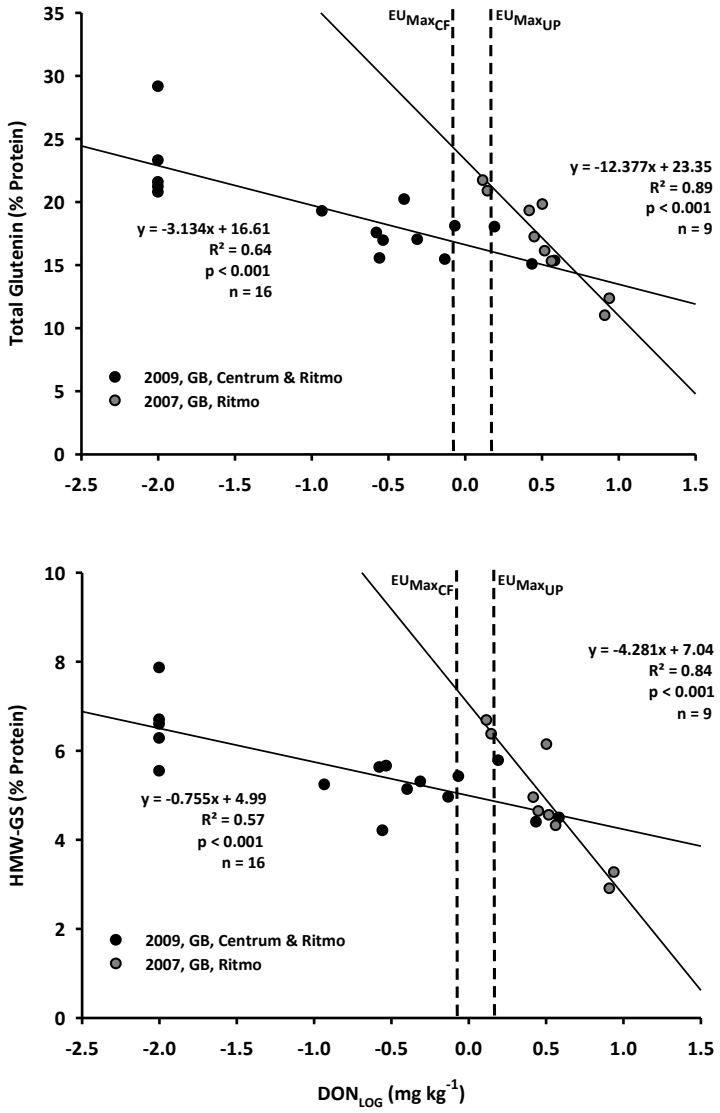

Fig 26. Linear regression of total glutenin content and DON_{LOG} and HMW-GS content and DON_{LOG} of flour Type 550 within cv. Ritmo in 2007 and across cultivars (Centrum, Ritmo) in 2009 at location Gladebeck (GB), EU_{MaxCF} = 0.75 mg kg^{-1}, EU_{MaxUP} = 1.25 mg kg^{-1}

ANCOVA did not find a significant effect of DON_{LOG} on content of ωb (Tab 47) and LMW-GS (Tab 48), therefore ANOVA with effects of environment, cultivar, and pre-crop described variance in these parameters sufficiently.

Tab 47. ANCOVA for effects of environment (E) (year, location), pre-crop (P), cultivar (C), and DON_{LOG} (DON) and ANOVA for effects of E, P, and C on ωb content

	ANCOVA			ANOVA	
Effect	**DF**	**F**	**p**	**F**	**p**
Environment (E)	3	4.58	*	9.94	***
Pre-crop (P)	2	1.03	**n.s.**	3.12	**(*)**
E x P	6	1.21	**n.s.**	2.83	*
Cultivar (C)	1	0.85	**n.s.**	16.13	***
E x C	3	0.52	**n.s.**	0.91	**n.s.**
P x C	2	0.26	**n.s.**	7.29	**
E x P x C	5	0.67	**n.s.**	1.44	**n.s.**
DON	1	1.01	**n.s.**		
DON x E	3	1.16	**n.s.**		
DON x P	2	0.07	**n.s.**		
DON x E x P	6	1.67	**n.s.**		
DON x C	1	0.18	**n.s.**		
DON x E x C	3	0.98	**n.s.**		
DON x P x C	2	0.06	**n.s.**		
DON x E x P x C	5	0.46	**n.s.**		

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1;
and n.s. = not significant, DF degrees of freedom

Tab 48. ANCOVA for effects of environment (E) (year, location), pre-crop (P), cultivar (C), and DON_{LOG} (DON) and ANOVA for effects of E, P, and C on LMW-GS content

	ANCOVA			ANOVA	
Effect	**DF**	**F**	**p**	**F**	**p**
Environment (E)	3	5.30	**	1.12	**n.s.**
Pre-crop (P)	2	2.24	**n.s.**	9.21	***
E x P	6	1.97	**n.s.**	1.89	**n.s.**
Cultivar (C)	1	3.16	**(*)**	17.63	***
E x C	3	0.16	**n.s.**	0.65	**n.s.**
P x C	2	0.44	**n.s.**	2.89	**(*)**
E x P x C	4	0.34	**n.s.**	1.81	**n.s.**
DON	1	1.25	**n.s.**		
DON x E	3	2.43	**(*)**		
DON x P	2	0.15	**n.s.**		
DON x E x P	6	1.92	**n.s.**		
DON x C	1	0.49	**n.s.**		
DON x E x C	3	0.76	**n.s.**		
DON x P x C	2	0.07	**n.s.**		
DON x E x P x C	4	0.47	**n.s.**		

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1;
and n.s. = not significant, DF degrees of freedom

4.3.2.4 Gluten composition

Across all samples of three years, gliadin/glutenin ratio averaged 2.9. Year and pre-crop showed a significant effect on the ratio (Fig 27, Tab 58). In 2008 (Ø 2.2), ratio was significantly smaller than in the other two years (2007: 3.3, 2009: 3.4). Regarding pre-crop across three years, gliadin/glutenin ratio was significantly higher after pre-crop maize (Ø 3.3) than after pre-crop sugar beet (Ø 2.7). The difference of maize to pre-crop wheat (Ø 2.8) was not significant. Across the years, cultivars did not appear to differ significantly within gliadin/glutenin ratio (cv. Centrum: Ø 2.8, cv. Ritmo: Ø 3.1). Within years, effect of pre-crop was not significant in 2008 and 2009. In 2007, however, cv. Centrum showed a significantly higher gliadin/glutenin ratio after pre-crop maize (Ø 4.7) than after pre-crop sugar beet (Ø 1.9).

Samples comprised an average LMW/HMW ratio of 2.4. The ratio was significantly affected by year, cultivar, and pre-crop (Fig 27, Tab 58). Differences between the years were overall significant. Highest ratio was on average present in 2008 (Ø 2.6), followed by 2007 (Ø 2.4), and 2009 (Ø 2.2). Across all samples, cv. Ritmo (Ø 2.4) showed a 4.4% smaller ratio than cv. Centrum (Ø 2.5), which made up a significant difference. A significantly smaller ratio was detected after pre-crop wheat (Ø 2.3) than after the other two pre-crops (Ø maize: 2.5, Ø sugar beet: 2.5). Significant effects of cultivar and pre-crops could not be detected within years, except for cv. Centrum in 2007, where pre-crop sugar beet (Ø 2.8) led to a significantly higher LMW/HMW ratio than pre-crop wheat (Ø 2.3).

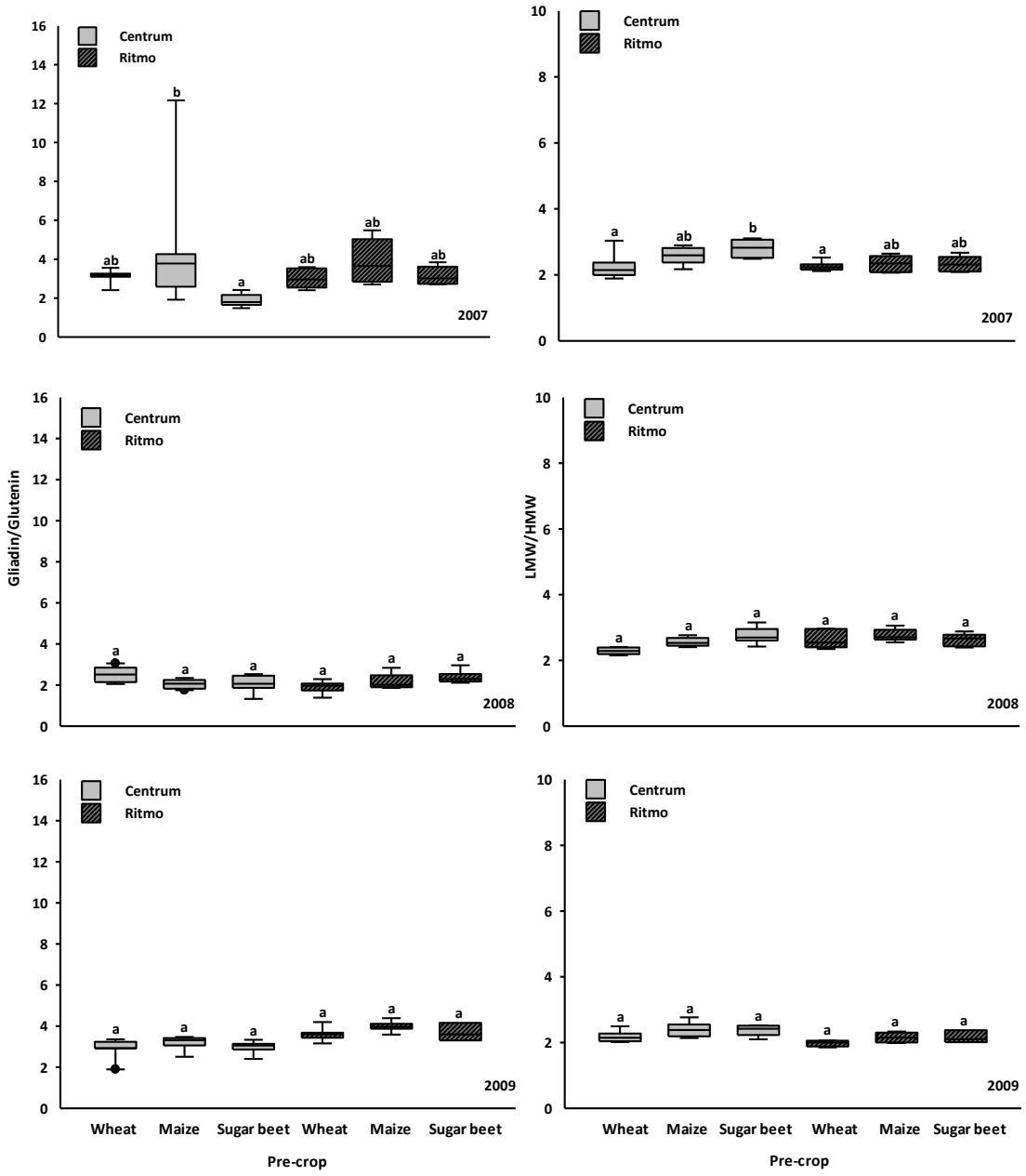

Fig 27. Gliadin/Glutenin ratio and LMW/HMW ratio of wheat cultivars (Centrum, Ritmo) grown after pre-crop wheat, maize, and sugar beet in 2007, 2008, and 2009. Box plots with median (solid line) and whiskers indicating 5th/95th percentiles. Box plots (n = 6, except 2009, Ritmo, pre-crop sugar beet: n =3) with same letters are not significantly different at p < 0.05 according to Tukey test. Outliers are indicated by a dot.

ANCOVA revealed a significant effect of DON_{LOG} on gliadin/glutenin ratio across all samples, within environment, and within environment and pre-crop (Tab 49). For LMW/HMW ratio, a significant influence of DON_{LOG} within environment, pre-crop, and the interaction of environment and pre-crop were calculated.

Tab 49. ANCOVA for effects of environment (E) (year, location), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) on ratio of gliadin/glutenin and LMW/HMW

	Gliadin/Glutenin			LMW/HMW	
Effect	**DF**	**F**	**p**	**F**	**p**
Environment (E)	3	10.63	***	5.40	**
Pre-crop (P)	2	8.25	**	3.85	*
E x P	6	7.37	***	5.56	**
Cultivar (C)	1	0.27	**n.s.**	0.87	**n.s.**
E x C	3	0.58	**n.s.**	11.37	***
P x C	2	0.90	**n.s.**	0.37	**n.s.**
E x P x C	4	0.24	**n.s.**	2.58	**(*)**
DON	1	4.68	*	1.12	**n.s.**
DON x E	3	9.57	***	19.30	***
DON x P	2	2.62	**(*)**	6.12	**
DON x E x P	6	6.68	***	1.84	**n.s.**
DON x C	1	1.52	**n.s.**	0.02	**n.s.**
DON x E x C	3	0.80	**n.s.**	3.34	*
DON x P x C	2	0.71	**n.s.**	2.19	**n.s.**
DON x E x P x C	4	2.62	**(*)**	2.31	(*)

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1;
and n.s. = not significant, DF degrees of freedom

Linear regression showed a strong and highly significant positive correlation of DON_{LOG} with gliadin/glutenin ratio in 2007 and 2009 at location Gladebeck across pre-crops and cultivars (Fig 28). DON_{ORG} ranged from 0.33-8.70 mg kg^{-1} and 0.00-3.84 mg kg^{-1} in 2007 and 2009, respectively, in these samples. Further linear regressions where $R^2 \geq 0.50$ are presented in Tab 50. All of them showed that gliadin/glutenin ratio increased with increasing DON concentration in flours. This was also the general trend across all 27 possible linear regression curves (R^2 averaged 0.36 with r = 0.43). An exception was observed 2007 at location Torland after pre-crop wheat where the effect appeared to be adverse (Tab 50).

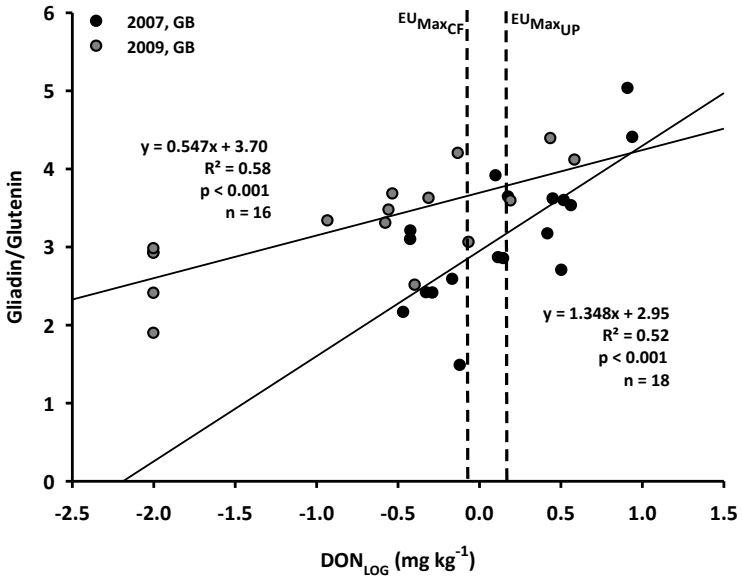

Fig 28. Linear regression of gliadin/glutenin ratio and DON_{LOG} (mg kg^{-1}) at location Gladebeck (GB) across cultivars and pre-crops in 2007 and 2009 (for other significant linear regressions see Tab 50), EU_{MaxCF} = 0.75 mg kg^{-1}, EU_{MaxUP} = 1.25 mg kg^{-1}

Out of 28 linear regression curves for DON_{LOG} and LMW/HMW only two were significant (Tab 50). At location Gladebeck, across all pre-crops, within cv. Ritmo with increasing DON content a significant increase of LMW/HMW ratio was observed across 2007 and 2009 as well as within 2009.

Tab 50. Parameters of linear regression of gliadin/glutenin ratio and LMW/HMW ratio and DON_{LOG} (mg kg^{-1}) of flour Type 550 within environments (E), cultivars (C), and pre-crops (P), and range of original DON values (DON_{ORG})

Ratio	Effect	Effect level									DON_{ORG} (mg kg^{-1})
		Year	Location	Pre-crop	Cultivar	n	R^2	p	b	a	Min-Max
Gliadin/Glutenin	E x P	2007	TL & GB	Sugar beet	Centrum & Ritmo	12	0.51	**	2.53	1.79	0.33-2.82
			TL	Wheat		6	0.66	*	3.05	-0.88	0.31-3.24
		2009	TL & GB	Maize		12	0.51	**	3.35	0.79	0.27-8.42
			TL			6	0.87	**	3.13	0.93	1.68-8.42
LMW/HMW	E x C	2007 & 2009	GB	Wheat & Maize & Sugar beet	Ritmo	16	0.61	***	2.12	0.42	0.26-8.70
		2009				7	0.69	*	2.09	0.29	0.26-3.84

n number of observations, p significance: ***, **, and * = p < 0.001, 0.01, 0.05, b constant, a slope, TL Torland, GB

4.4 Protease activity (PA)

PA (only analyzed in 2007) of flours was significantly influenced by location, pre-crop and *Fusarium* infection (Tab 51, Tab 59). Extinctions at 440 nm, indicating PA, were on average 26% higher in cv. Ritmo (Ø 0.61 AU) than in cv. Centrum (Ø 0.45 AU). However, this difference was not significant (except for cv. Centrum after sugar beet compared to cv. Ritmo after pre-crop

maize). Regardless of location and cultivar PA was highest after maize, followed by wheat and sugar beet. Within cultivars, a significant effect of pre-crop was not observed.

Tab 51. Protease activity (AU 440 nm) in flour Type 550 from wheat cultivars (Centrum, Ritmo) grown after pre-crops wheat, maize, and sugar beet in 2007 (MV ± SD, n = 6)

Year	Cultivar	Pre-crop	Protease activity (AU 440 nm)			
2007	Centrum	Wheat	0.49	±	0.17	ab
		Maize	0.48	±	0.15	ab
		Sugar beet	0.38	±	0.14	a
	Ritmo	Wheat	0.62	±	0.15	ab
		Maize	0.70	±	0.20	b
		Sugar beet	0.51	±	0.15	ab

Same letters indicate no differences between cultivars and pre-crops at a significance level of $p < 0.05$ according to Tukey test

ANCOVA revealed a significant effect of DON_{LOG} on PA across all samples and within location and pre-crop (Tab 52). Across all 36 samples, linear regression of both parameters demonstrated a positive but rather moderate correlation ($R^2 = 0.35$, $p < 0.001$).

Out of six further linear regression curves within the two locations and the three pre-crops, only across cultivars within pre-crop maize at location Torland a strong significant increase of PA with increasing DON_{LOG} was observed (Fig 29). Linear regression of protease activity and DON_{LOG} curve could be described with $y = 0.50x + 0.34$, $R^2 = 0.69$, $p < 0.05$ across these six observations. DON_{ORG} of these samples ranged from 2.52 to 11.84 mg kg^{-1}. However, the trend within all correlations was without exception positive (R^2 averaged 0.30 with $r = 0.53$).

Tab 52. ANCOVA for effects of location (L), pre-crop (P), wheat cultivar (C), and DON_{LOG} (DON) on protease activity

Effect	DF	F	p
Location (L)	1	8.92	*
Pre-crop (P)	2	7.24	**
L x P	2	6.96	**
Cultivar (C)	1	2.86	n.s.
L x C	1	1.14	n.s.
P x C	2	1.06	n.s.
L x P x C	2	12.79	***
DON	1	7.30	*
DON x L	1	2.63	n.s.
DON x P	2	0.56	n.s.
DON x L x P	2	18.53	***
DON x C	1	0.06	(*)
DON x L x C	1	1.34	n.s.
DON x P x C	2	2.83	(*)

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1; and n.s. = not significant, DF degrees of freedom

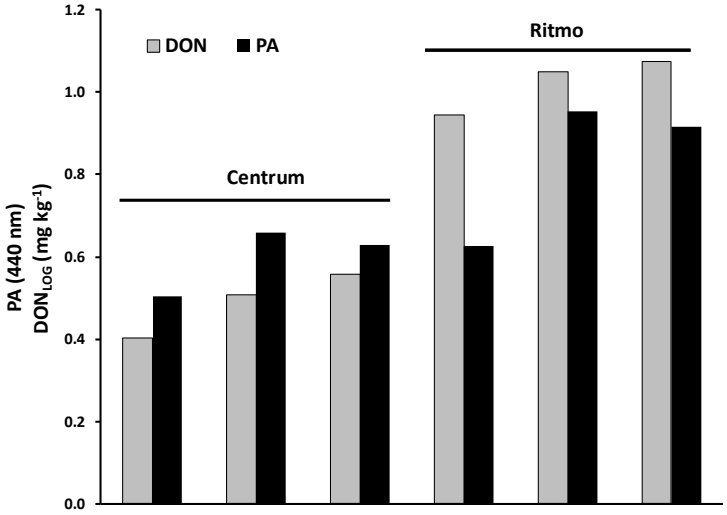

Fig 29. Protease activity (PA) and DON_{LOG} of flour Type 550 from wheat (Centrum, Ritmo) grown after pre-crop maize at location Torland in 2007. Each bar represents the MV of the technical measurements of one flour sample

4.5 Relationship among quality parameters and flour components

Correlation studies showed that both PC_{WGF} and $PC_{Type550}$ were highly positively correlated to WG, WA, BV and loaf shape (LL) (Tab 53). Both parameters were strongly positively related among each other. Furthermore, both WG and WA showed a strong positive relationship with BV, while BV was apparently higher if LW increased. LH and LL were inversely related. Gluten property (R_{MAX}/EXT) was inversely related to LW. Interestingly, none of the measured single flour components was significantly correlated to any of the quality parameters, except for PC and starch content which showed a moderate negative trend with WA. Main gluten fractions, gliadin and glutenin, as well as single subfractions merely showed significant correspondence among each other and with gliadin/glutenin ratio. The complete correlation matrices for all quality parameters and flour components can be viewed in the supplementary material (Tab 62, Tab 63, Tab 64)

Tab 53. Linear relationships among quality parameters and total gluten subfractions where $R^2 \geq 0.50$ and $p < 0.05$

Dependent variable	n	Independent variable	Trend	R^2	p
PC_{WGF}	108	$PC_{Type550}$	+	0.83	***
	108	WG	+	0.63	***
	84	WA	+	0.69	***
	84	BV	+	0.66	***
	84	LH	-	0.54	***
	84	LL	+	0.59	***
$PC_{Type550}$	108	WG	+	0.67	***
	108	WA	+	0.73	***
	84	BV	+	0.72	***
	84	LL	+	0.55	***
WG	84	WA	+	0.83	***
	84	BV	+	0.75	***
	84	LL	+	0.53	***
WA	84	BV	+	0.79	***
	84	LL	+	0.54	***
	84	Starch	-	0.55	***
BV	84	LW	+	0.57	***
LH	84	LL	-	0.54	***
LW	36	R_{MAX}/EXT	-	0.54	***
R_{MAX}/EXT	18	R_{MAX}	+	0.54	***
Total Gliadin	108	α-Gliadin	+	0.78	***
	108	γ-Gliadin	+	0.81	***
ω5-Gliadin	108	ω1,2-Gliadin	+	0.66	***
Total Glutenin	105	ωb-GS	+	0.78	***
	105	HMW-GS	+	0.82	***
	105	LMW-GS	+	0.99	***
	105	Gliadin/Glutenin	-	0.67	***
ωb-GS	105	LMW-GS	+	0.79	***
HMW-GS	105	LMW-GS	+	0.73	***
	105	Gliadin/Glutenin	-	0.66	***
LMW-GS	105	Gliadin/Glutenin	-	0.64	***

n number of observations, p significance: *** = $p < 0.001$, PC_{WFG} protein content of whole grain flour, $PC_{Type550}$ protein content of flour Type 550, WG wet gluten, WA water absorption, BV baking volume, LH loaf height, LW loaf width, R_{MAX}/EXT ratio of resistance to extension (R_{MAX}) and extensibility (EXT)

5 Discussion

5.1 *Fusarium* biomass and mycotoxins

5.1.1 Occurrence of *Fusarium* DNA and mycotoxins

The occurrence of the two most prevalent *Fusarium* spp., *F. graminearum* and *F. culmorum*, that are commonly associated with FHB in wheat in the Europe and northern America were investigated in milling products of wheat by qPCR with the objective to determine fungal abundance over a time period of three years. The results showed that both species at the same time could only be detected in 2007 (Tab 7), where humid weather conditions during anthesis enhanced a natural *Fusarium* infection of wheat spikes. While *Fg*DNA could also be detected in 2008 and 2009 in lower concentrations in a few samples, *Fc*DNA was not found in 2009 in any of the flours and bran (2008: not analyzed). Subsequently, in 2009, *Fg*DNA made up 100% of total *Fusarium* DNA (if only *Fg*DNA and *Fc*DNA were considered). In 2007, *Fg*DNA accounted for a significant proportion of over 90% of total *Fusarium* DNA (Tab 8) which indicated the predominant existence of *F. graminearum* on and within the wheat kernels. This finding is supported by GÖDECKE 2010 who isolated *Fusarium* spp. from the resembling samples and identified at least seven different species, including *F. culmorum*, *F. tricinctum*, *F. compactum*, *F. poae*, *F. avenaceum*, and *Microdochium nivale*. However, *F. graminearum* was the most frequently isolated pathogen in 2007 and 2009. In 2008, hardly any *Fusarium* spp. could be detected, *F. graminearum* isolates made up < 5%. In 2007, *F. culmorum* accounted for up to 10% of all fungal isolates whereas it could not be isolated at all in 2008 and 2009. This confirms the results of the qPCR. These species were not considered in our investigation, but are also assumed to be part of the FHB pathogen complex in wheat (BOTTALICO & PERRONE 2002; LIDELL 2003). However, it`s likely that infected samples contained more *Fusarium* DNA aside from *F. graminearum* and *F. culmorum* DNA since coexistence of different *Fusarium* spp. at field sites is well known (KLIX *ET AL.* 2008; XU *ET AL.* 2008A).

The presence and the predominance of *Fusarium* pathogens in wheat are first of all assumed to be determined by climatic conditions, primarily temperature and humidity (PARRY *ET AL.* 1995). While the occurrence of *F. graminearum* was particularly verified in warmer and humid environmental conditions, *F. culmorum* seems to prefer a cooler and also humid/wet climate (PARRY *ET AL.* 1995; XU *ET AL.* 2008A). In Germany, several studies that focused on the pathogen spectrum of *Fusarium* spp. in wheat demonstrated a differing predominance of single species depending on the geographical region of investigation and its climatic conditions. In southern Germany (Bavaria) *F. poae* was the most prevalent specie followed by *F. graminearum* and *F. avenaceaum* while *F. culmorum* and other species played a minor role (BÜTTNER 2006). In Rhineland, characterized by a cooler climate than Bavaria, *F. avenaceum* was the most frequently isolated pathogen, followed by

F. culmorum, *F. graminearum* and *F. poae* (LIENEMANN *ET AL*. 2001). During the 1980s, in northern Germany, characterized by cooler and partly maritime climatic conditions, *F. culmorum* was the dominant specie while *F. graminearum* was hardly detected (SCHLÜTER *ET AL*. 2006). A more recent study demonstrated that *F. graminearum* was the most frequently detected specie in Schleswig-Holstein followed by *F. poae*, *F. avenaceum* and *F. culmorum* despite wet and cool and maritime conditions that would favor the development of *F. culmorum* (KLIX *ET AL*. 2008). The predominance of *F. graminearum* over *F. culmorum* as DON-producing specie in the south of Lower Saxony, geographically placed between the northern and southern part of Germany, as shown with our field trial, is supported by several other investigations that described a shift in FHB incidence caused nowadays rather rarely by *F. culmorum* but more often by *F. graminearum* in European countries with differing climatic conditions (PARRY *ET AL*. 1995; WAALWIJK *ET AL*. 2003; XU *ET AL*. 2005). Three main reasons are discussed for this phenomenon. First, the increasing production area for maize which is apparently an optimal host plant for *F. graminearum* and has been demonstrated to increase FHB incidence and severity in wheat significantly when compared to other pre-crops (BECK & LEPSCHY 2000; RINTELEN 2000; STACK 2000; LOGRIECO *ET AL*. 2002). Second, XU *ET AL*. 2005 assumed that *F. graminearum* becomes steadily adapted to cooler geographical regions. Third, a possible climate change caused former cooler regions to become warmer (XU *ET AL*. 2005; BÜTTNER 2006). While the later two arguments can neither be proven nor rejected for the region of south Lower Saxony since they were not in the focus of this investigation, the first argument -intensified maize cultivation- has to be considered. Due to the increasing demand for substrate meant for biogas production, from 1999 to 2007 the maize production area of Lower Saxony increased in average about 5%, in some areas about 10 to 15%. This led to a change in crop rotation from the typical winter wheat/winter wheat/winter barely/winter rapeseed (sugar beet) or winter wheat/winter wheat/sugar beet to a less wheat dominated maize/winter wheat/winter barely/rapeseed (sugar beet) rotation. Some farmers even reduced their crop spectrum and applied a maize/winter wheat/winter wheat and maize/winter wheat/sugar beet rotation (KARPENSTEIN-MACHAN & WEBER 2010). With the objective to investigate FHB in wheat under typical agronomical practices for Lower Saxony, pre-crop maize and minimum tillage was integrated into the 3-year-field experiment. *Fg*DNA was most abundant in wheat milling products with pre-crop maize (Fig 8). In our investigation, *Fg*DNA levels after maize pre-crop were approx. 7-fold and 3.5-fold higher than after pre-crop sugar beet and winter wheat.

Mycotoxins

The focus of our qPCR study did not only lay on *F. graminearum* and *F. culmorum* because of their worldwide distribution and connection to FHB, but primarily because they have been associated with the presence of mycotoxins in grain including trichothecenes (DON, ADON, NIV, ANIV, FUS X), ZEA and zearalenol of which all of them present a greater concern to human

health (BOTTALICO 1998; LIDELL 2003). Since at least *Fg*DNA could be detected in a bigger amount of samples, the presence of these mycotoxins could also be expected. Of all mycotoxins, only DON, 3-ADON, and ZEA could be detected (Tab 7) while zearalenol was not investigated.

The absence of NIV and FUS X in all samples and the simultaneous contamination with DON and 3-ADON suggests a predominant infection of the grain with *Fusarium* genotypes that were only able to produce DON and its acetyl derivate 3-ADON. The ability of *F. graminearum* strains to produce exclusively DON and 3-ADON or NIV and FUS X was verified by ICHINOE *ET AL.* 1983 and led to the classification of "DON chemotypes" and "NIV chemotypes" whereas 3-ADON and FUS X can be assumed to be precursors within the biosynthesis pathway of the later, respectively. These findings supported the results of *in vitro* assays conducted from 42 *F. culmorum* isolates that either synthesized DON or NIV but not both toxins together (GANG *ET AL.* 1998). The reports are contradicted by SUGIURA *ET AL.* 1990 who firstly demonstrated a cross-production of both toxins in *F. graminearum* strains. A further division of the DON chemotype (chemotype I) into subgroups IA (DON, 3-ADON) and IB (DON, 15-ADON) that characterized the formation of only one specific DON acetyl derivate by MILLER *ET AL.* 1991 and which may explain the particular detection of 3-ADON but not 15-ADON in our samples was rejected by Sugiura *et al.* 1990. They attributed the prevalence of either 3-ADON or 15-ADON to the environmental temperature that either enhances or inhibits the enzymatic catalysation of 3-ADON into 15-ADON. The exclusive presence of DON and 3-ADON in our samples suggested an infection by *F. graminearum* (and *F. culmorum* in 2007) of chemotype IA or environmental conditions that favored the production of 3-ADON rather than 15-ADON. In a recent study, 26 isolates of *F. graminearum* from Hungary were mainly of chemotype IB (TOTH *ET AL.* 2005). Both TOTH *ET AL.* 2005 and PERKOWSKI *ET AL.* 1997 cited several studies conducted in Europe, including Germany, and worldwide which demonstrated the co-existence of DON, NIV and their derivates in harvested grain. The reasons for the predominance of single chemotypes and therefore their produced toxins in different geographical regions are not clear yet. An influence of host, specie, temperature and soil type are assumed (TOTH *ET AL.* 2005). After all, only about 15% (37/252) of all samples analyzed in our study contained 3-ADON, all of them below the EU_{MAX} for DON in flours. In our study, only 6% (16/252) of all samples analyzed were contaminated with ZEA. ZEA was frequently detected together with DON (LOGRIECO *ET AL.* 2002; XU *ET AL.* 2008B). Since the occurrence of other *Fusarium* ssp. was likely (GÖDECKE 2010) and only a small range of the total mycotoxin spectrum that can be produced by *Fusarium* spp. was analyzed, the presence of other mycotoxins in milling products could not be excluded (BOTTALICO & PERRONE 2002).

5.1.2 Factors influencing *Fg*DNA and DON

As has been described in several previous studies (OBST *ET AL.* 2000; SCHAAFSMA *ET AL.* 2001; BEYER *ET AL.* 2006), all factors (environment, pre-crop, cultivar, fungicide) that were investigated

in our field experiment had a significant influence on the DON levels in grain and their afterwards produced milling products. Obviously, even though grain had been cleaned to a certain degree, effects of the agricultural practices on DON levels were still clearly visible. This study is the first, that also takes the abundance of the most prevalent DON-producing *Fusarium* specie, *F. graminearum*, measured as *Fg*DNA into account and therefore allows for conclusion of the influence of agricultural practices on fungal abundance and DON contamination.

Environment

The tremendous significance of weather conditions during the time period around and during wheat anthesis for successful *Fusarium* infection has been reported before (LACEY *ET AL.* 1999). *F. graminearum* forms perithecia on crop debris that forcibly release ascospores into the air and conidia which are transported by rain splashes up to the wheat spike. Both inoculum sources are critically influenced by temperature and moisture (XU & NICHOLSON 2009). While different FHB pathogens seem to require different temperature conditions for the process of colonization and infection (XU *ET AL.* 2008A), generally all of them need wet or moist conditions before and during anthesis as well as during the early grain development stages (DE WOLF *ET AL.* 2003).

*Fg*DNA and DON levels ranged widely between the six environments (Fig 7). The weather conditions during the years 2007, 2008 and 2009 varied widely between the years, which may explain the significant differences of DON levels and amount of *Fg*DNA in milling products (Fig 30). In 2007, the average daily precipitation in May (153.4 mm) and June (141.6 mm) was at least twice as high as in the same months in 2008 and 2009 while average daily temperatures were comparatively warm in June. A few heavy rain incidences with more than 10 mm/h precipitation prior to anthesis and during anthesis led in average to 218- and 358-fold higher *Fg*DNA amounts and to 65 and 95-fold higher DON levels in 2007 at the locations Torland and Gladebeck, respectively, when compared to the same locations in 2008. Samples in 2007 also contained 18- and 59-fold more *Fg*DNA and 1.6- and 5.1-fold higher DON amounts when compared to the respective samples in 2009. In 2008, a long dry period during May (average precipitation only 6.1 mm) with only 4 rain fall events (< 2mm precipitation) and wheat anthesis may have prevented the development of ascospores within perithecia on crop debris which is favored by high humidity and their dispersal which is often connected to rainfall events (XU & NICHOLSON 2009). This was reflected by hardly detectable DON concentrations in only a few samples, and rarely detectable *Fg*DNA (Tab 7). In 2009, average precipitation was higher than in 2008 and also were there a few more rainfall events pre-anthesis and also during flowering, but average daily temperature in June 2009 (14.8 °C) was 1-2 °C lower than in 2008 and 2007 which was mainly due to daily minimum temperatures down to 5°C. Development of perithecia and sexual development of *F. graminearum* occurs between 15-29°C (PARRY *ET AL.* 1995). The optimum temperatures for ascospore production are 15-20°C (XU & NICHOLSON 2009). Lower temperatures and precipitation in 2009 prevented an

epidemic as in 2007 but obviously supported the fungal growth and DON accumulation in grain better than the weather conditions in 2008 since DON levels were 4- and 19-fold higher at location Torland and location Gladebeck, respectively than in the previous year, and wheat contained 12- and 6-fold higher *Fg*DNA contents.

In general, the effects of the environment on *Fg*DNA amount corresponded very well to the effects on DON. The more *Fg*DNA was detected the higher the DON levels were found in milling products. The effect of location on both parameters was not clear. While *Fg*DNA was clearly more abundant in wheat from location Torland in 2007, DON levels were not significantly different between the two locations. This is contradictory to the results of GÖDECKE 2010 who reported 3-fold higher DON contents in wheat samples from location Torland in 2007 and concluded that there is clear difference between the two locations in potential to accumulate DON in grain even though they are geographically not far apart. The discrepancy of the results might have derived from the pre-harvest cleaning of our grain samples prior to further processing which was not applied for the samples of GÖDECKE 2010. It is known from literature that wheat kernel with very high DON concentrations are usually lower in weight (TKACHUK *ET AL*. 1991). It is thinkable that the actually heavier DON contaminated but lighter wheat kernels from location Torland might have been removed during cleaning because the sample cleaning machine included an aspiration to remove light grains, chaff and dust by air. Therefore, samples from the two locations apparently contained equal DON levels.

In 2009, DON was 3-fold more abundant at Torland while there was not a significant difference in *Fg*DNA levels between the two locations. The differences in *Fg*DNA and DON between the locations might have resulted from small differences in temperature and water availability (MAGAN *ET AL*. 2002), soil conditions influencing the presence of crop debris (OLDENBURG *ET AL*. 2007B), neighboring crops that function as hosts for *F. graminearum* such as maize, differences in mycoflora composition (DOOHAN *ET AL*. 2003) or oxidative stress that is present at one site and enhances toxin production more than at the other site (PONTS *ET AL*. 2009; REVERBERI *ET AL*. 2010). The interactions of factors that lead to DON accumulation are complex and yet not fully understood.

The results demonstrate once more the importance of specific weather conditions during the susceptible stage of wheat flowering for the successful colonization of the wheat heads by *F. graminearum*, the development of FHB, and the subsequent contamination of harvested grain with DON which has been stressed by many other studies before (PARRY *ET AL*. 1995; MCMULLEN *ET AL*. 1997; MEIER *ET AL*. 2000; OBST *ET AL*. 2000; DE WOLF *ET AL*. 2003). DE WOLF *ET AL*. 2003 stated that FHB epidemics are generally associated with the longer periods of temperatures between 15 to 30° and extended periods of relative high humidity (≥90%) before or during flowering. In a four year survey conducted by SCHAAFSMA *ET AL*. 2001, who analyzed the effect of

different agronomic practices on DON content in wheat, 48% of the total variation of DON levels was explained by the influence of the year, which represented of all the most important factor. This agrees with our result that the environment and the year in particular showed of all experimental factors the highest effect on DON content in milling products reflected by highest SoE (Tab 10)

Regardless of environmental conditions, which cannot (weather) or hardly be influenced (location), and type of milling product which is in the hands of the miller, for the farmer it`s crucial to know which agronomical measures avoid high DON levels in harvested wheat.

Pre-crop

Pre-crop maize generally led to higher contents of DON and *Fg*DNA in milling products than wheat and sugar beet (Fig 8). The high potential risk that pre-crop maize presents for the accumulation of DON in following wheat especially after minimum tillage (as applied in our study) or non tillage systems has been reported many times before (DILL-MACKY & JONES 2000; BEYER *ET AL*. 2006). BECK & LEPSCHY 2000 gave three main reasons for this. First, maize is a host of *F. graminearum*. Second, maize leaves plenty of colonized debris after harvest where the fungus survives saprophytically. Third, the rot of maize debris proceeds rather slowly due to comparatively wide C/N ratio. While KOCH *ET AL*. 2006 also reported significantly reduced DON contents (by a factor of 4.3) in wheat after pre-crop sugar beet when compared to pre-crop wheat, which supports our own results, BECK & LEPSCHY 2000 found higher DON contents in wheat after sugar beet. GODECKE & VON TIEDEMANN 2011 concluded that the effect of the year/weather conditions influences strongly how the pre-crop affects DON contamination in wheat, which was also reflected in the significant interactions between environment and pre-crop in our study (Tab 9). In years with severe natural infection, e.g. 2007, there might not be a difference between wheat and sugar beet, because inoculum might not primarily result from the debris of pre-crop at site but rather from conidia spread by wind from neighboring host plants.

The difference between pre-crop wheat and sugar beet as a potential inoculum source for *Fusarium* infection might be a qualitative as well as a quantitative one. While the infection of wheat and maize with *F. graminearum*, the most important causal pathogen of FHB, is well studied, the *Fusarium* spp. composition in sugar beet is not well researched and therefore the possible carryover of single species on the following crop is still a matter of discussion. CHRIST *ET AL*. 2011A have shown that the infection of wheat with *Fusarium* spp. isolated from sugar beet is possible under greenhouse conditions. In a further study the authors demonstrated that stored sugar beets (which can be regarded comparable to sugar beet crop debris left on the field) contained *Fusarium* spp. that are in Europe part of the FHB complex and therefore sugar beet debris in the field cannot be ruled out as primary inoculum source (CHRIST *ET AL*. 2011B). Furthermore, compared to wheat (3.7 t ha^{-1}) and maize (9.2 t ha^{-1}) (BUYANOVSKY & WAGNER 1986), sugar beet leaves less crop residues in the soil, only up to 1.8 t ha^{-1} (ZANDSTRA & SQUIRE 2007). OLDENBURG *ET AL*. 2007B showed a

strong relation between the remaining amount of maize residues on the soil surface and their content of *Fusarium* biomass, whereas the DON contamination in wheat particularly depended on the susceptibility of cultivar against FHB.

Cultivar

Since the two winter wheat cultivars were primarily chosen because of their difference in susceptibility against FHB out of all varieties available grouped in quality class B (Tab 2), it was not surprising that the highly susceptible cv. Ritmo (grade 7) comprised significantly higher DON levels in milling products that less susceptible cv. Centrum (grade 2) (Fig 9). Additionally, cv. Ritmo also contained considerably more *Fg*DNA than cv. Centrum. These results are confirmed by the findings of GOSMAN 2001 (cited in NICHOLSON *ET AL*. 2003) who also reported higher DON levels and higher *Fusarium* DNA in the grain of the susceptible cultivar. Since we did not have any information about the types of resistance obtained by the cultivars (see chapter 1.2.4), it can just be concluded that neither cultivar is completely resistant to spread of the fungus *F. graminearum* within the kernels, nor DON/trichothecenes accumulation within the kernels. It can be assumed that cv. Centrum is less susceptible to disease spread (type II resistance according to SCHROEDER & CHRISTENSEN 1963) and to DON accumulation within kernels (type III resistance according to MILLER & ARNISON 1986, type V resistance according to MESTERHAZY 2003) than cv. Ritmo. Especially, the reason for the later is not known. One explanation could be that there was less DON in cv. Centrum because there was also less *Fg*DNA. Another could be that there was less DON because cv. Centrum is capable of modifying the trichothecene chemically (type V class 1 resistance) or because cv. Centrum is able to inhibit trichothecene synthesis (type V class 2 resistance) to a certain degree (MESTERHAZY 2003; BOUTIGNY *ET AL*. 2008). In addition to the active resistance mechanisms summarized by FOROUD & EUDES 2009, passive mechanisms as described by SCHROEDER & CHRISTENSEN 1963 and MESTERHAZY 1995 might contribute to the different reaction of the two cultivars towards FHB or be an indicator for the FHB resistance. E.g. plant height of cv. Ritmo was visibly less than that of cv. Centrum. HILTON *ET AL*. 1999 found a negative correlation between straw height and the presence of FHB symptoms. This suggested a possible escape of the spikes due to the greater distance to the inoculum on the ground, e.g. conidia which are splash dispersed to wheat heads as suggested by HORBERG 2002. However, the reason for the correlation seems to be rather due to an overlapping of QTLs for FHB resistance and plant height as well as with other plant features (time of anthesis, awnedness) which were assumed to influence disease incidence (MESTERHAZY 1995; GERVAIS *ET AL*. 2003). Similar to the results of BEYER *ET AL*. 2006 who demonstrated that the use of a moderately susceptible cultivar (cv. Dekan) reduced DON content about 76% when compared to cv. Ritmo, in our study DON content of cv. Centrum was only 25% of that in cv. Ritmo which emphasizes once more the key role of cultivar choice within the management of FHB which has been discussed before (OBST *ET AL*. 2000;

SCHAAFSMA *ET AL*. 2001; BEYER *ET AL*. 2006). After, environment and pre-crop, cultivar was the most important factor to influence DON content of milling products (Tab 10). A significant interaction between environment and cultivar (susceptibility) for DON and *Fg*DNA as shown in Tab 9 has also been observed by MIEDANER *ET AL*. 2001 who demonstrated a strong interaction between FHB resistance in wheat, rye, and triticale cultivars and environmental conditions.

Fungicide

Even though efficacy of tebuconazole in reducing FHB and mycotoxin levels in grain has been demonstrated to vary (PAUL *ET AL*. 2007) it`s common census that at present triazole fungicides present the most effective available chemical control of FHB and DON contamination (MATTHIES & BUCHENAUER 2000; BEYER *ET AL*. 2006; PAUL *ET AL*. 2007; XU & NICHOLSON 2009). Triazole inhibit the C_{14} demethylase in microsomes that catalyze an intermediate step of the ergosterol biosynthesis, with ergosterol being an essential part of the fungal cell well (HALLMANN *ET AL*. 2007). While the direction of the effect of triazoles on DON content in grain is clear, the treatment with strobilurins have produced contradictory results (see chapter 1.2.4). Strobilurins are single site inhibitors of the mitochondrial respiratory chain within the fungal cell (HALLMANN *ET AL*. 2007). Except for their direct effect on the fungal cell, an indirect impact of the fungicides on disease development can also be expected since WU & VON TIEDEMANN 2001 have shown a significant delay of senescence after application of azoxystrobin and epoxyconazole at GS 31/32 and GS 59/61. To gain more knowledge how fungicide application in the stage of shooting (GS 31-39) might indirectly affect FHB development was the main objective of the study of GÖDECKE 2010, one of our cooperation partners within the FAEN joint project, therefore fungicides were not applied at wheat anthesis (GS 61-69) in order to prevent/reduce FHB development and DON accumulation, but at GS 31-39 (stage of shooting). Additionally to strobilurin and triazole, chlorthalonil, a fungicide of which a senescence delaying effect was not known, was applied. Chlorthalonil is a phthalonitrile impairing fungal growth by reacting with glutathione and coenzyme A in fungal metobolism (ANONYMUS 2011). Since the results are discussed in detail by GÖDECKE 2010, at this point will be no further discussion concerning the possible reasons for the effects of the applied fungicides on DON and *Fg*DNA (Fig 10). It should just be concluded, that these fungicides significantly contributed to the variance within *Fg*DNA and DON levels in milling products (Tab 9).

Since also fungicide treatment influenced DON concentration of milling products significantly, yet not as strong as pre-crop and cultivar, it seem reasonable that the combination of the "worst" factor combinations (maize x susceptible cultivar (cv. Ritmo) x strobilurin/chlorthalonil application) of agricultural measures led to the highest DON concentrations in milling products whereas the combination of sugar beet x cultivar with low susceptibility (cv. Centrum) x triazole application minimized the risk of DON accumulation (Tab 10).

Milling product

As described in chapter 1.1.1 further processing of harvested grain leads to a reduction of DON/trichothecenes to a certain degree. Several studies reviewed by KUSHIRO 2008 have shown that DON contamination of the grain leads to a distribution of DON in all milling fractions, but that there is in general at least a slight reduction of DON in flours milled from endosperm, the more inner part of the kernel (straight-grade flour, extraction flour, break flour), when compared to the milling products containing more of the kernel`s outer layers (bran, shorts). In our study, DON content in bran was in average 1.7-fold higher than in WGF (cleaned grain) (Fig 11). Increased DON levels in a similar range were also observed by HART & BRASELTON 1983, TANAKA *ET AL*. 1986, TRIGO-STOCKLI *ET AL*. 1996, and LANCOVA *ET AL*. 2008 who observed 2.3, 2.7, 1.2, and 2.0 times higher DON content in bran than in the original wheat. Unlike other studies (YOUNG *ET AL*. 1984; ABBAS *ET AL*. 1985; SEITZ *ET AL*. 1985; SEITZ *ET AL*. 1986; NOWICKI *ET AL*. 1988; DEXTER *ET AL*. 1996; TRIGO-STOCKLI *ET AL*. 1996; LANCOVA *ET AL*. 2008), we could not observe a reduction of DON in flour Type 550 by milling. Instead, DON concentrations in flour Type 550, which resembles 75% yield straight-grade flour, were 150% of the DON content of WGF and not significantly different from DON levels in bran. At the same time, *Fg*DNA content in flour Type 550 was only 33% of *Fg*DNA contained in WGF and 13% of *Fg*DNA content in bran, suggesting the dominant prevalence of *F. graminearum* within the outer layers of the kernels and a less dense colonization of the endosperm. The stepwise reduction of *Fusarium* DNA during wheat cleaning and processing has been reported by TERZI *ET AL*. 2007. They showed that cleaned grain contained 60%, extraction flour 20%, and bread just 4% of the original amount of *Fusarium* DNA measured in uncleaned grain. In our study, the increased DON level and the reduced *Fg*DNA content in flour Type 550 when compared to WGF and bran, resulted in similar DON:DNA ratios in WGF (0.013) and bran (0.009) and a significantly higher ratio in flour Type 550 (0.061). This result was confirmed by the linear regressions between DON levels and *Fg*DNA of milling products in (Fig 16). Regression curves of WGF and bran had similar slopes (a=0.01 and 0.007) but for flour Type 550 we estimated about 4 times more DON per µg *Fg*DNA (a=0.04) (see also chapter 5.1.3). DON concentration per µg *Fusarium* DNA in different milling products gives an interesting inside where the fungus is located within the kernels and associated toxins at the same time. The higher DON content in flour Type 550 compared to WGF deserves consideration. HART & BRASELTON 1983 made the same observation and gave two possible explanations 1) the higher recovery of DON from milled fractions, 2) fungal growth and subsequent DON production during one week of tempering prior to milling. Even though matrices effects interfering with the detection of mycotoxins are known from other studies (ZHOU *ET AL*. 2007; VENDL *ET AL*. 2009), this explanation seems unlikely for our data, since DON quantification was done by means of matrix-matched calibration (see chapter 3.2.2) and would certainly not account for the double amount of DON in flour Type 550. The term ‘tempering’ describes the process of incubating grain with a defined

amount of water for a particular time in order to reach the same moisture content and physical condition within all kernels with the objective to "toughen the bran and mellow the endosperm" (POSNER & HIBBS 2005). In our case, fungal growth and associated DON accumulation within 12h of tempering cannot be excluded, but does not explain the higher DON:DNA ratio in white flour compared to bran if we assume that the production rate of DON per unit *Fg*DNA is equal in all kernel parts. Therefore, it`s suggested that there had been a translocation of DON from the outer parts of the kernels into the endosperm at some point. DON is well water soluble (EMAN 2011). The translocation might have occurred already in the field while moisture content in grain is still high as described by SNIJDERS & KRECHTING 1992, and/or during storage as well as during the tempering process itself. DELGADO *ET AL*. 2010 discussed the possibility of diffusion of DON produced by *F. graminearum* from rotten tissue of potato tubers into surrounding asymptomatic tissue during storage. The authors came to the conclusion that warmer temperatures (≥20°C) during storage might enhance the diffusion of DON into adjacent tissue while cooler storage temperatures (10-12°C) retard the process. Such a diffusion process could also be thinkable for grain, even though harvested grain contains considerably less water than potato. In the present study, DON obviously diffused more rapidly into the endosperm than it was penetrated by the fungus. This process could have been enhanced by the warm ambient temperatures during storage until milling (>20°C) or/and during tempering (18-20°C). XU *ET AL*. 2007 demonstrated that mycotoxin production by *Fusarium* spp. increased with high temperatures (≥20°C) during the initial infection period. Optimal production of type B trichothecenes occurs at warm (25-28°C) and humid (a_w = 0.97) conditions (DOOHAN *ET AL*. 2003). HOMDORK *ET AL*. 2000 found a significant increase of DON levels in low to moderately *F. culmorum* infected wheat when grain was stored for 6-8 weeks under warm and humid conditions (25°C, 90% RH). Therefore we conclude, that tempering can be seen as short term storage (12-16h) which provides optimal conditions for mycotoxin production and translocation, since water content of grain is raised from 8-10% storage moisture up to 15-16% milling moisture under ambient temperature (18-20°C). This might have caused DON levels of flour Type 550 to exceed DON levels of WGF and to be similar high as DON contents of bran.

DON levels of flour Type 550

The large number of experimental factors (year, location, pre-crop, cultivar, fungicide) that had been shown to influence DON and *Fg*DNA content in flours and bran significantly, led to a wide spectrum of DON levels in flour Type 550, ranging from below LOD up to extreme values of 11.8 mg kg^{-1} (Fig 12). In our study, flour type 550 was primarily investigated because it`s most commonly used for the production of bread and bakery products in Germany. In order to have a more practical approach, flour was produced from grain that was cleaned by a single passage through a sample cleaner (see chapter 3.1.2) where a removal of the most contaminated kernels (light, shrunken) could have been expected. Nevertheless, 31% of all flours contained more DON

than the EU_{MaxUP}. It's obvious that these flours would not have been suitable for further processing of food and that the corresponding grain would have been very likely discarded before milling. 5% comprised 0.75 to 1.25 mg kg^{-1} DON. 64% of flours contained less than 0.75 mg kg^{-1} DON and would have been suitable for further processing into cereal products as well as for direct consumption by adults.

Since no considerable amounts of other toxins were found, DON levels in flours would be from the toxicological and legal point of view the most decisive criterion of all for further use. Therefore, in the chapters 5.2 and 5.3 the impact of DON concentration of flours on quality parameters of wheat flours and flour composition will be discussed.

5.1.3 Relationship of visual disease, DON, and *Fg*DNA

Visual disease

In the field, FHB intensity in wheat may be estimated visually by DI, DS, or FHB index whereas the index is assumed to be the most useful parameter since it is the product of DI and DS. High DI might not always correspond with high DS (WILCOXSON *ET AL*. 1992). Nevertheless, in 2009, DS was so low that we chose to determine only DI, the easier and often more accurate parameter to obtain (Tab 11) (PAUL *ET AL*. 2005A). In 2008, both were measured but were so low that the index was in most cases very close to zero or zero, respectively. Therefore, in 2008 and 2009, where *Fusarium* infection was low to moderate (see chapter 5.1.2) in both cultivars a low DI also corresponded with a low DS, implicating that at the time of visual rating not only the primary infection did rarely occur but also the spread of the FHB causing pathogens within the spikes was exceptional. In 2007, were all three parameters could be easily obtained, high DI corresponded with high DS and therefore high FHB indices in both cultivars. The measurement of the visual disease reflected very well the susceptibility of the cultivars against FHB (see chapter 5.1.2). Independent of *Fusarium* infection level, DI was always significantly higher in highly susceptible cv. Ritmo than in less susceptible cv. Centrum, indicating that cv. Ritmo is less resistant to infection than cv. Centrum (type I resistance) and also less resistant to spread of the infection within the spikes (type II resistance) which was implied by a significantly higher DS in 2007 (Tab 11).

It is agreed that generally there exists a positive relationship of DI and DS, however, the modeling of this relationship is still discussed (GROTH *ET AL*. 1999; PAUL *ET AL*. 2005A). The easiest model, the linear regression with untransformed data, as applied in our study and resulting in a moderate correlation coefficient ($R^2 = 0.25$), may not be the best-fitted. PAUL *ET AL*. 2005A found that DS was more precisely estimated at lower DI values than at higher values in a model based on complementary log-log transformation of both visual estimates.

Visual disease and DON

The most interesting question regarding visual FHB usually is whether possible mycotoxin occurrence, especially contamination with DON, can be predicted from it. In our study, in general, DI was highly positively associated with DON levels in milling products gained from cleaned (!) wheat (Fig 14). However, the type of equation for predicting DON levels with a high R^2 was dependent on the infection intensity of the years. In 2009, where FHB intensity was moderate (with DI up to 0.17% in cv. Centrum and up to 3.2% in cv. Ritmo) linear functions of DI described DON contents in flours and bran sufficiently (DON = a*DI + b). Interestingly, higher DI in cv. Centrum (a = 6.3, 10.4, 12.7) corresponded with a higher DON accumulation in milling products than in cv. Ritmo (a = 1.4, 2.4, 2.9). In 2007, DI was many-fold higher than in 2009 with DI up to 30% in cv. Centrum, and up to 55% in cv. Ritmo. DI values >13% were without exception determined in plots with pre-crop maize. DON levels of milling products from these samples would have been highly overestimated if predicted with a linear model. Logarithmic functions of DI described the correlation with DON best (DON = a ln(DI) + b). With higher DI, cv. Centrum responded with less DON (a = 0.5, 0.7, 0.7) accumulation in milling product than cv. Ritmo (a = 1.6, 1.8, 2.9). Yet, it has to be considered that DON levels in WGF (R^2=0.45) and bran (R^2=0.21) of cv. Ritmo did not correlated well with DI. Inserting EU_{MAXUP} (1.25 mg kg^{-1}) and EU_{MaxCF} (0.75 mg kg^{-1}) into these prediction models gave an estimate of maximum DI. For EU_{MAXUP}, DI could not have been higher than 0.63% in 2007 and 0.10% in 2009 so that all milling products of both cultivars stayed still within the tolerable amounts of DON. Regarding the EU_{MaxCF}, DI could not have been higher than 0.48% in 2007 and 0.06% in 2009 in order that all milling fractions of both cultivars contained no more than 0.75 mg kg^{-1} DON. These values indicate two things. First, prediction of DON from DI may work well within the years but not between years. Second, in years of epidemic occurrence of FHB it`s unrealistic to stay within the range of EU_{Max} for DON without further prevention management (e.g. triazole application during anthesis) or thorough cleaning of the grain, noting that far less than 1 spike per 100 spikes could show symptoms without considerable subsequent DON contamination. In 2008, the overall low DI indicated very low to zero DON values very precisely.

A meta-analysis performed by PAUL *ET AL*. 2007 over a total of 163 studies revealed a general positive correlation between DON and visual estimates for FHB, including DI (r = 0.52), DS (r = 0.53), FHB index (r = 0.62), and *Fusarium* damaged kernels (FDK) (r = 0.73) which supports our own results regarding relationship between DON:DI and DON:FHB. Other studies did not observe a relation between visual disease in the field and DON levels in grain (LIU *ET AL*. 1997; EDWARDS *ET AL*. 2001). Yet, the prevalence and strength of the correlation may depend on cultivars (susceptibility), environment (weather condition, location), pathogen complex, crop production, disease management (fungicides) and other unknown effects (PAUL *ET AL*. 2007; XU *ET AL*. 2007).

Overall, it cannot be recommended to predict DON levels in milling products on the sole parameter of DI, particularly if models contain rather small amount of data (n = 18) as it was the case in our study. The cleaning of the grain and the further processing (tempering, milling) represent further effects which seem to influence relationship between visual disease and DON, which appeared to be positively correlated, but may influence the precision of the prediction (R^2 = 0.21 to 0.91 (Fig 14).

Visual disease and *Fg*DNA

For 2007, we found low to strong positive correlations of visual disease assessment and presence of *F. graminearum* depending on the parameter used. DI (Fig 15) and FHB predicted *Fg*DNA concentrations in milling products very well, probably because the two disease estimates (DI and FHB) were also very strongly related to each other, while DS did not provide such a good prediction model. Our results are supported by ZHANG *ET AL*. 2009 and BURLAKOTI *ET AL*. 2007 who reported a strong positive association between DS and *Fg*DNA. XU *ET AL*. 2007 demonstrated a consistent positive relationship between incidence of diseased spikelets and amount of DNA of several *Fusarium* spp., particularly *Fg*DNA, under controlled environment conditions. Whereas EDWARDS *ET AL*. 2001 did not find a correlation between visual disease (assessed as DS) and concentration of *F. graminearum* and *F. culmorum* (measured as DNA content) in harvested grain gained from a field trial.

The contradiction might result from the different species composition in the field and specific isolates used in the trials, differing in their capability to produce FHB symptoms by the time of disease assessment and colonizing the grain until harvest. XU *ET AL*. 2007 demonstrated that inoculation with *F. graminearum* resulted in highest DS but grain infected with *F. culmorum* contained highest fungal DNA amounts, indicating that the same DS value might not result in the same amount of species specific DNA.

***Fg*DNA and DON**

While studies on the relationship between visual disease and *Fusarium* DNA are rare, the association between fungal biomass and DON contamination has gained more attention in research. Our results revealed a moderate positive linear correlation in flour Type 550 and bran (R^2=0.65 and 0.77) and a strong correlation in WGF (R^2=0.84) between *Fg*DNA and DON levels (Fig 16). The positive correlation between colonization/fungal biomass (measured as ergosterol or DNA content) and DON levels in grain has been reported by several authors (MILLER *ET AL*. 1985; EDWARDS *ET AL*. 2001; XU *ET AL*. 2007; ZHANG *ET AL*. 2009, GANG *ET AL*. 1998; MIEDANER *ET AL*. 2000; BURLAKOTI *ET AL*. 2007). We could show that this relationship is not only true for the harvested whole grain but also for milling fractions (flour Type 550 and bran) derived from it. Our results are supported by TERZI *ET AL*. 2007 who reported a consistent positive linear regression for *Fusarium*

DNA and DON levels in (not cleaned) grain (R^2=0.97), WGF (R^2=0.89), 60-63% yield flour (R^2=0.99), and bread (R^2=0.87). Additionally, we could also show that there was a close positive relationship between DON and *Fg*DNA in WGF with DON and *Fg*DNA levels in bran and flour Type 550 (Tab 12) indicating that DON levels and *Fg*DNA amounts of other kernel parts (outer layers, endosperm) could be easily estimated from the WGF which would shorten the detection procedure and reduce costs.

The possible reasons for the 4 times higher DON:DNA ratio of flour Type 550 when compared to WGF and bran, indicated by the slopes of the regression curves, has been discussed before (see chapter 5.1.2). Even though it has not been discussed by TERZI *ET AL*. 2007, they also reported a higher DON content per amount of *Fusarium* DNA in extraction flour (a = 12.6) than in corresponding WGF (a = 7.99) which represented a 1.6-fold higher DON:DNA ratio. The reasons might be the same as discussed in chapter 5.1.2 (tempering, DON translocation), yet, the discrepancy might not have been as distinct as in our study, since they used a flour with a lower extraction rate than ours and also tempering conditions were different. Tempering to a moisture content of 16% and for 36h might have given not only DON translocation but also the trichothecenes-producing fungi (*F. graminearum* and *F. culmorum*) more time to grow into the inner parts of the kernels than during our grain preparation (15.5% moisture, 12h tempering).

Our results demonstrate that not only cultivar resistance (NICHOLSON *ET AL*. 2003), environmental conditions, species composition (XU *ET AL*. 2007), and fungicides (EDWARDS *ET AL*. 2001) might influence the DON:DNA ratio in wheat but also further processing steps such as tempering pre milling.

The good correlation between DON and *Fg*DNA even across years and cultivars, especially in WGF, compared to the comparatively weaker correlation of visual disease and DON/*Fg*DNA when determined across all samples (*data not shown*) likely resulted from the differences in tissue and time they were measured. Visual disease was assessed from floral tissue 14-20 days after anthesis (milky stage) while *Fg*DNA and DON were determined in harvested grain which had been roughly cleaned. From the initial location of infection and colonization (glumes) the fungus (and DON) would have to spread through several tissues layers into the kernel (PAUL *ET AL*. 2005B). Since fungal growth and mycotoxin production highly depends on environmental/weather conditions and also time (LACEY *ET AL*. 1999; XU *ET AL*. 2007) discrepancy between visual symptoms and final fungal colonization of grain and toxin accumulation might occur (PAUL *ET AL*. 2005B). In 2008 and 2009, we could often not measure any FHB symptoms (DI) but still found insignificant levels of DON in WGF, which speaks for a development of the fungus and DON production post time of visual rating. This has also been reported by LACEY *ET AL*. 1999.

5.2 *Fusarium* infection and quality parameters

5.2.1 Protein content (PC_{WGF}, $PC_{Type550}$)

Our results showed the significant effect of year on PC_{WGF} and $PC_{Type550}$ (Fig 17, Tab 54). Higher daily average temperatures and higher precipitation during grain filling period in 2007 (Fig 30) obviously favored the accumulation of proteins in kernels more than in the two following years. The importance of environmental conditions on PC e.g. moisture and temperature, has been demonstrated before (WILLIAMS *ET AL*. 2008., ABEDI *ET AL*. 2011, DANIEL & TRIBOI 2000). Higher PC_{WGF} resulted in higher $PC_{Type550}$ (Fig 17, Tab 53). The close linkage between PC of grain and flour has also been observed by (BHATT & DERERA 1975). Slightly reduced $PC_{Type550}$ compared to PC_{WGF} as demonstrated is the consequence of separating the protein-rich aleuronlayer with bran from the endosperm by milling (POMERANZ 1988). While the potential of both cultivars to accumulate protein was rated to be equal according to BSA (Tab 2), cv. Ritmo showed the potential to accumulate more protein under favorable weather conditions as present in 2007 than cv. Centrum which emphasizes the importance of genotype x environment interaction which has been reported before (BHATT & DERERA 1975; GRAUSGRUBER *ET AL*. 2000, ZECEVIC *ET AL*. 2009). There was a slight but significant influence of pre-crop on PC across all samples (Tab 54). PC_{WGF} and $PC_{Type550}$ after pre-crop wheat was 4-5% higher when compared to pre-crop maize and sugar beet. Within years, this effect was in general also visible, depending on cultivar and year (Fig 17). This effect might have resulted from a fertilization effect. Next to other factors, N fertilization plays a key role in the accumulation of grain protein (WILLIAMS *ET AL*. 2008., ABEDI *ET AL*. 2011, DANIEL & TRIBOI 2000). After wheat, soil contained 15 to 24 kg N ha^{-1} (only analyzed in 2008 and 2009) more than after pre-crop maize and sugar beet (Tab 60). N fertilization in our trail did not consider the different N_{min} contents subsequent to different pre-crops and also not the possible N mineralization during vegetation coming from the different crop debris, which differed very much in amount and quality (see chapter 5.1.2). Therefore, it is possible that wheat plants grown after wheat had at least up to 15 to 24 kg ha^{-1} more N available than cultivated after maize and sugar beet. Higher N availability usually does not only promote PC accumulation in grain but also grain yield (GY) (DANIEL & TRIBOI 2000, ABEDI *ET AL*. 2011). However, GY and PC are associated adversely with each other (CAMPBELL *ET AL*. 1977; CLARKE *ET AL*. 1990; GAUER *ET AL*. 1992). This trend we could also observe in our study (Tab 62). Across all years, GY was indeed significantly lower after pre-crop wheat (74.5 dt ha^{-1}) than after pre-crop maize (76.6 dt ha^{-1}) and pre-crop sugar beet (84 dt ha^{-1}) (Tab 59). N fertilization in our study was conducted in three rates, 1st at middle of tillering (GS 23), 2nd at end of tillering (GS 29), and 3rd at end of stem elongation (GS 39) or at end of booting (GS 49) (Tab 65). While the first dressings (during tillering) rather promotes vegetative growth and yield (ABEDI *ET AL*. 2011), later applications near anthesis have been demonstrated to promote grain PC more effectively (FINNEY *ET AL*. 1957; BLY & WOODARD 2003). Therefore, in

addition to the different N levels available for the plants even though N application was the same for each treatment, also the aspect of time needs to be considered. Different crop residues may have delivered mineralized N at different time points promoting either GY or PC.

Aside from N also S plays an important role in wheat plant metabolism and the accumulation of grain proteins since S is part of cysteine which crosslinks protein polymers (see chapter 1.3). The S nutrition of plants has in the past decade gained more and more attention primarily due to a reduction of atmospheric S emissions of industrial origin (ZORB *ET AL*. 2010). Wheat requires 15-20 kg ha^{-1} sulfur for optimum growth (ZHAO *ET AL*. 1999). Since S_{min} after the different pre-crops was not analyzed and S fertilization was not applied in our study, we analyzed S content of flours and calculated N/S ratio to rule out S deficiency in impaired grain protein accumulation. According to RANDALL *ET AL*. 1981 S content <0.12% and N/S ratio >17/1 of grain indicates a S-deficient plant status. However, in our study, S content of flour Type 550 averaged 0.138% and N/S ratio was 14/1 (Tab 59, Tab 66). Therefore, S deficiency influencing wheat quality could be excluded. Significant differences in S content between pre-crops within years and across years could not be detected. However, significantly higher S content of flours in 2007 than in 2008 corresponded with higher PC of flours and higher BV in the respective year (Tab 67) which emphasizes the importance of S supply for wheat quality.

Fusarium infection did not have an effect on PC_{WGF} (Tab 13), which is supported by the findings of other studies (SEITZ *ET AL*. 1986; DEXTER *ET AL*. 1996; PRANGE *ET AL*. 2005; WANG *ET AL*. 2005B; TERZI *ET AL*. 2007). However, there was an effect of *Fusarium* infection on $PC_{Type550}$ (Tab 14). While in a small amount of samples $PC_{Type550}$ was shown to increase significantly with increasing DON concentration of flour Type 550 in 2007, this effect was adverse in 2009 (Tab 15). Both effects have already been described by other studies e.g. MEYER *ET AL*. 1986; BOYACIOGLU & HETTIARACHCHY 1995; PAWELZIK *ET AL*. 1998; MATTHAUS *ET AL*. 2004; SIUDA *ET AL*. 2010 found an increase of PC after severe *Fusarium* infection. BECHTEL *ET AL*. 1985; NIGHTINGALE *ET AL*. 1999; PRANGE *ET AL*. 2005; GARTNER *ET AL*. 2008 observed the opposite effect. Yet, one has to be aware that correlations between $PC_{Type550}$ and DON_{LOG} in 2007 after pre-crop wheat could have been spurious since they were developed across cultivars. Cv. Ritmo contained significantly more $PC_{Type550}$ than cv. Centrum grown after wheat (average difference 0.6% protein) (Fig 17). At the same time cv. Ritmo showed about 5.8-fold higher DON content than cv. Centrum in this treatment. Consequently, there might be two possible explanations for the increased $PC_{Type550}$ in cv. Ritmo after wheat. On the one hand, higher intensity of *Fusarium* infection of cv. Ritmo caused higher $PC_{Type550}$ due to a change of starch to protein ratio in favor of protein as supposed by PAWELZIK *ET AL*. 1998. For this phenomenon three possible explanations are suggested in literature. The first reason could be the consumption of carbohydrates by the fungus (HAMILTON & TRENHOLM 1984; BOYACIOGLU & HETTIARACHCHY 1995; HERMANN *ET AL*. 1999; WANG *ET AL*.

2005B). The second reason could be temporally earlier accumulation of storage proteins during grain-filling. Thus, proteins might not be influenced by an emergency maturation due to *Fusarium* infection to the same extend as starch (MEYER *ET AL*. 1986; MATTHAUS *ET AL*. 2004). Third, some studies also suggest that fungal protein might contribute additionally to total grain PC (HAMILTON & TRENHOLM 1984; BOYACIOGLU & HETTIARACHCHY 1995), since *Fusarium* mycelium comprised about 42% protein when grown in liquid media. However, the latter explanation was neglected by EGGERT *ET AL*. 2010 who reported that only 0.3% of total PC in *Fusarium* infected wheat was of fungal origin. Aside from the influence of fungal infection, cv. Ritmo might have been able to use higher N availability after pre-crop wheat more efficiently for the accumulation of storage protein than cv. Centrum leading to significantly higher $PC_{WGF}/PC_{Type550}$ in in 2007. This argument is supported by the observation that the two cultivars, with this one exception, did generally not differ significantly in $PC_{Type550}$ within years. The second argument is supported by the fact, that cv. Ritmo showed significantly lower GY (Ø 55 dt ha^{-1}) in this treatment than cv. Centrum (69 dt ha^{-1}) (Tab 66) which might have corresponded with the comparatively higher PC. After all, within the other two pre-crops, cultivar differences were not significant even though DON content of cv. Ritmo was 4.2- and 1.6-fold that of cv. Centrum after maize and sugar beet, respectively. The effect of *Fusarium* infection cannot be cleared doubtlessly with as much as six biological samples per treatment, particularly because both pre-crop and cultivar might influence PC in the same direction as these factors might promote *Fusarium* infection and as *Fusarium* infection might influence PC in the same. Nevertheless, a few things became obvious. First, the effect of *Fusarium* infection on PC depended on flour (WGF/flour Type 550). Second, the direction of effect (increase/decrease/no effect) of *Fusarium* on PC depended on environment including not only year but also location (which was indicated by an improved R^2 if locations were investigated separately), pre-crop, and intensity of *Fusarium* infection (indicated by varying ranges of DON). Discrepancy between the effect of *Fusarium* infection on PC_{WGF} and $PC_{Type550}$ might have resulted from the different composition of WGF and flour Type 550. Flour Type 550 generally contains a higher starch content and a lower PC than flours with a higher extraction rate (BELITZ *ET AL*. 2009). In addition, $PC_{Type550}$ mainly consists of gluten proteins which have been shown to be digested by *Fusarium* spp. in particular (chapter 1.1.2).

Third, year, location, and pre-crop, (and cultivar) might all contribute to different protein levels in flours as well as to different intensity of *Fusarium* infection so that it is not easy to separate the effects from each other. Other studies also found that the effect of *Fusarium* infection on PC depended on cultivar (DEXTER *ET AL*. 1996; NIGHTINGALE *ET AL*. 1999; PRANGE *ET AL*. 2005 and location (HERMANN *ET AL*. 1999).

However, a possible change of % PC in flour from 0.46 to 1.09% protein in one or the other direction due to *Fusarium* infection of grain is not crucial for wheat of quality group B since 1%

PC more/less only increases/decreases baking volume about 25 ml 100 g^{-1} flour (SELING 2010). This would make up only about 4% of the minimum baking volume required for bread wheat (580 ml 100 g^{-1} flour) (SEIBEL 2005). In this context, also the other significant differences in PC that we observed in our study and that were caused by pre-crop, cultivar, as well as location should be evaluated (Tab 54). Even though significant (because of high n across years), quantitative differences of <1% protein, e.g. between pre-crop maize and wheat, might not be relevant for baking quality. However, only few decimals of PC might decisively influence wheat classification and price (HARTL 2010). Finally, it should be considered that a linear relationship between $PC_{Type550}$ and DON_{LOG} was primarily measured within flour samples that contained DON levels in a range from below EU_{Max} to highly exceeding these values which would exclude a bigger number of these samples from being processed at all (Tab 13).

5.2.2 Wet gluten content (WG) and sedimentation value (SV)

WG

Our results showed that WG was most influenced by year and cultivar (Fig 18). The strong influence of environment and cultivar on WG has already been demonstrated by others (GRAUSGRUBER *ET AL.* 2000; MLADENOV *ET AL.* 2001; DENCIC *ET AL.* 2011). These authors also reported a strong positive correlation between WG and PC which confirms our results (Tab 53). WG was highest in 2007 were also PC was highest out of three years. This positive relationship seems reasonable since gluten forming proteins make up the major proportion of total wheat protein (see chapter 1.3). Therefore, the cause for differences in WG between pre-crops might be the same as for PC (see chapter 5.2.1). According to SEIBEL 2005, WG of our flour samples can be evaluated as high to very high in 2007. In both cultivars WG was >30%. WG was normal (25-30%) to high (>30%) in 2008 and low (20-24%) to normal (>25-30%) in 2009. From this it could be expected that in samples from 2009, WA of flour, gas retention and tolerance to fermentation might have already been reduced leading to quality losses in baking product (SEIBEL 2005). This is supported by our result that WG was highly positively correlated to WA and BV (Tab 53).

No effect of *Fusarium* infection on WG could be observed in our study (Tab 17). This result is supported by DEXTER *ET AL.* 1996 who investigated WG in flour composites of naturally *Fusarium* infected wheat with DON levels varying from 0.4 to 6.3 mg kg^{-1}. Only in flour milled from hand-picked FDK a decline of WG could be observed compared to a clean fraction which accounted culivar-dependent for 3 to 14% WG. However, DON content in these flours exceeded current legal thresholds drastically, ranging from 35 to 62 mg kg^{-1}. This later finding of DEXTER *ET AL.* 1996 agrees with the observations of other studies (MEYER *ET AL.* 1986; BOYACIOGLU & HETTIARACHCHY 1995; PAWELZIK *ET AL.* 1998) who found WG reduced in artificially *Fusarium*

infected wheat in comparison to a control or less infected samples (determined by DI). In contrast, SIUDA *ET AL*. 2010 reported an increase of WG in samples with higher kernel DI.

SV

Protein and gluten quality, respectively, are evaluated with the standard procedure of the sedimentation test. The test measures the sedimentation volume of wheat flour that is suspended in an acidified solution which is generally either Zeleny reagent (according to ICC standard 116/1) or an SDS containing solution as we applied in our study (see chapter 3.3.2) (ECKERT *ET AL*. 1993). Agricultural contractors usually refer to Zeleny sedimentation when setting up the requirement for a specific wheat quality group. Wheat for intervention has to have at least a minimum Zeleny sedimentation volume of 22 ml (Regulation (EC) No 824/2000 of 20 April 2000). SDS-SV has been shown to predict BV more precisely than Zeleny sedimentation by AXFORD *ET AL*. 1979 which was the reason for the determination in our study. The comparison of the two methods showed that SDS-SV were approximately 20 ml higher than Zeleny volumes (AXFORD *ET AL*. 1979) which has been later explained by the greater swelling of glutenins in SDS solution by (ECKERT *ET AL*. 1993). Keeping the quantitative difference between Zeleny and SDS-SV in mind, all flour samples exhibited high to very high protein quality, even in 2007 after pre-crop maize were SV was particularly reduced in cultivar cv. Centrum (Fig 18). The higher SV the higher is gas retention in dough, dough stability, and after all baking volume (ECKERT *ET AL*. 1993; SEIBEL 2005). The small variation of SV between years within cultivars suggests a stronger influence of genotype on the parameter than environment in our study which has been also reported by WILLIAMS *ET AL*. 2008. Even though rating of the two cultivars by BSA (Tab 2) indicated a slightly higher zeleny SV of cv. Centrum compared to cv. Ritmo this difference could not be seen across the years, which emphasizes the importance of environmental conditions in interaction with cultivar as observed by others (e.g. MLADENOV *ET AL*. 2001; ZECEVIC *ET AL*. 2009) for SV.

Though literature describes an overall positive relationship between flour sedimentation and PC of grain or flour, the strength of this relationship varies between studies. e.g. PRESTON *ET AL*. 1982 reported a low to moderate correlation (R^2 ~0.40 and 0.55) between SDS volume and flour protein. DEXTER & MATSUO 1977 found a moderate to strong correlation (R^2~0.57 to 0.88) within durum wheat, whereas MLADENOV *ET AL*. 2001 did not find a significant relationship (R^2~ 0.08). In our study no correlation between SV and PC_{WGF} and $PC_{Type550}$, respectively, existed (Tab 62) which was most likely due to the little variation of PC after all and cultivars belonging to the same quality group.

In 2007, where *Fusarium* infection was severe and associated with the highest DON levels in grain and flour of all years (see chapter 4.1.2), no linear relationship between SV and DON_{LOG} could be detected, even though SV varied more widely in this year than in the others. Nevertheless, SV was lowest in the flours gained from wheat grown after maize which also contained the highest DON

concentrations. However, when looking at linear regression of SDS sedimentation volume and BV reported by AXFORD *ET AL.* 1979, the reduction of SV about absolutely 12-15 ml (in cv. Centrum after maize) does not mean a relevant loss in quality if SV is overall very high (>50 ml). The same applies to the seemingly "improved" SV after severe *Fusarium* infection in 2009 after pre-crop maize which could be shown in six samples across two cultivars (Fig 19). The demonstrated incline of SV included at least three flour samples that exceeded EU_{MaxUP} or EU_{MaxCF} by far. However, an absolute difference in SV of about 4 ml in the respective samples can be considered insignificant. While to our knowledge no study so far has reported an increase of SV with higher *Fusarium* infection intensity, several authors have reported a decrease of SV with increased intensity of *Fusarium* infection (MEYER *ET AL.* 1986; BOYACIOGLU & HETTIARACHCHY 1995; WANG *ET AL.* 2005B; TERZI *ET AL.* 2007; GARTNER *ET AL.* 2008). It could be possible that a light degradation of proteins by *Fusarium* proteases might lead to higher swelling of protein in SDS. In contrast, SIUDA *ET AL.* 2010 did not find an effect of *Fusarium* infection on SV which supports our own observations for the epidemic year of 2007.

5.2.3 Falling number (FN)

FN is influenced significantly by climatic conditions (environment) (SMITH & GOODING 1999, cultivar (GRAUSGRUBER *ET AL.* 2000; DENCIC *ET AL.* 2011), nitrogen fertilization (SZENTPETERY *ET AL.* 2005), and even fungicide treatment in interactions with cultivar and environment (DIMMOCK & GOODING 2002). The significantly differing FNs between the years in our study are supported by these findings (Fig 20). Significantly higher FNs across years in cv. Ritmo compared to cv. Centrum are not surprising since they have been described by BSA (Tab 2) in this way. The wide range of data (Fig 20), which became especially obvious in 2007, indicated that FNs was also influenced by other factors which were included in box plot data. ANOVA showed that also location had a significant influence on FN (Tab 54, Tab 60). An effect of location on FN has also been reported by WANG *ET AL.* 2008.

Slight differences of FN within cultivars between pre-crops might be to some degree explained by the slightly different flour composition e.g. $PC_{Type550}$.WANG *ET AL.* 2008 reported that nitrogen fertilization increased PC and subsequently FN. Indeed, across all samples, FN was significantly higher after pre-crop wheat were also $PC_{Type550}$ was higher than after pre-crop maize and sugar beet (Tab 54). Increased FN with rising PC was also reported by AYOUB *ET AL.* 1994. Changes of functional properties of proteins (solubility, water absorption) might contribute to the different gelatinization properties of the flour-water suspension. Additionally, also TKW and the content of starch, ash, pentosans, as well as other hemicelluloses (dietary fiber) might correspond to FN (WANG *ET AL.* 2008). However, no significant correlation between FN and other quality parameters or flour components could be observed in our study (Tab 62, Tab 63).

From the perspective of indicating quality, FN was below the required 220s (Commission Regulation (EC) No 824/2000 of 20 April 2000, RAIFFEISEN 2011) in cv. Centrum in more than 50% of the flours in 2007, and in a few samples in 2008. FNs < 200s in flour Type 550 generally indicate grain damage by sprouting and result in soft doughs with small proofing stability and sticky and unelastic crumb (SEIBEL 2005). Most of the samples of cv. Centrum in 2008 and samples of cv. Ritmo in 2007 were in a medium range of 220 to 320s. Loosening of crumb in these samples can be expected to be optimal (SEIBEL 2005). FN > 320 in WGF, as measured in most of the samples of both cultivars in 2009 and cv. Ritmo in 2008, indicated low enzyme activity, low raise, small volume and light crumb color among other negative consequences for baking product (SEIBEL 2005). However, FN only showed a weak negative relationship to BV in our study (Tab 62).

Significant positive relationships between FN and DON_{LOG} in various environments as presented in Tab 20 have to be very likely to be interpreted as spurious correlations since the more susceptible cv. Ritmo also displayed generally higher FNs than cv. Centrum independently from the occurrence of *Fusarium* infection. This became clear when looking at FNs in 2008 (Fig 20) where there was hardly any *Fusarium* infection. Additionally, ANCOVA results showed a significant effect of cultivar in 2007 and 2009 independently from effect of DON (Tab 19). Therefore, it can be concluded that correlations within pre-crops and environments was rather present due to cultivar effects than to *Fusarium* infection. When linear regression was performed separately for the two cultivars (Fig 21) only the highly susceptible cv. Ritmo displayed a higher FN with higher DON concentration. Yet, one has to be aware of two things: First, DON_{ORG} in 2007 highly exceeded EU_{Max}, so that grain with an increase of FN about 100% (as indicated by slope) in the range of 3 to 10 mg kg^{-}would not need to be taken into account for processing quality. The same applies to the detected slight increase of FN about 20s below DON concentrations of 2.0 mg kg^{-1} (approx. difference between FN of DON_{Max} and DON_{Min}) which would also not be relevant on an overall very high level of FN as present in 2009. Second, due to lack of observations within cultivars, correlations could only be performed across locations which had been demonstrated to have also a significant effect on FN (Tab 54, Tab 60) and which might result in spurious high R^2.

However, the effect of increasing FN with increasing intensity of *Fusarium* infection, as in our samples, has also been reported by others (WANG *ET AL.* 2005A; WANG *ET AL.* 2008 and GARTNER *ET AL.* 2008). PAWELZIK *ET AL.* 1998 and HERMANN *ET AL.* 1999 observed the opposite effect. MEYER *ET AL.* 1986 observed both effects, depending on year of observation. According to the test principle of HAGBERG 1960, which implies a negative correlation of FN and α-amylase activity in grain, and according the findings of various authors (DEXTER *ET AL.* 1996; PAWELZIK *ET AL.* 1998; NIGHTINGALE *ET AL.* 1999; MATTHAUS *ET AL.* 2004; WANG *ET AL.* 2005A; WANG *ET AL.* 2008) who described an increase of α-amylase activity in grain after *Fusarium* infection, a negative

relationship between FN and *Fusarium* infection would seem reasonable. WANG *ET AL*. 2008 supposed that FN method does not display activity of fungal α-amylase activity properly and suggested a longer incubation period of the pasting starch solution. Another reason for increased FN after *Fusarium* infection could be the earlier maturation of infected kernels (KANG & BUCHENAUER 2000; RIBICHICH *ET AL*. 2000; GOSWAMI & KISTLER 2004). Since weather conditions during grain maturation are essential for the level of HFN of harvested grain -higher temperatures in late-season are associated with higher FN (SMITH & GOODING 1999)- it might be possible that infected kernels "escape" cooler and moist conditions possibly present during late ripening period of healthy kernels.

5.2.4 Water absorption (WA) and mixing properties of dough (DDT, DST, DS)

WA of flour is an indicator for dough yield and therefore bread yield (WEBB & OWENS 2003). Higher WA of flour leads to higher dough yield. In general flours of Type 550 take up water in a range from 53 to 66% to reach a dough consistency of 500 FU. Optimal WA of flour Type 550 for roll production lays between >58 to 61% (SEIBEL 2005). Regarding these standard values, flours Type 550 of all three years were in this range, however, only flours from 2008 showed an optimum WA for roll production (Tab 21). As for DDT, flours in our study were in the typical range of 1-8 min for flours of Type 550 (SEIBEL 2005). However, optimal DDT between 2-6 min was with a few exceptions not reached by flours, and also a preferable DST exceeding 4 min (according to SEIBEL 2005) was only observed in 2009 (Tab 21). Low WA (<58%), low DDT (<2 min), and DST (< 4 min) indicated weak gluten properties of the flours investigated in our study (BELITZ *ET AL*. 2009). DS of flour Type 550 generally ranges from 0 to 130 FU with an optimum of 80 FU (according to SEIBEL 2005). Except in 2009 were reduction of dough consistency was <80 FU after 12 min of mixing, doughs generally exceeded this value by far, particularly in 2007 (Tab 21).

All farinograph parameters –WA, DDT, DST, and DS – were significantly influenced by year. DDT was also influenced by cultivar. Most of them showed significant interactions of cultivar with year (WA, DDT, DS) and pre-crop (WA, DDT, DS) (Tab 60). The importance of environment, cultivar, and environment-cultivar interactions for farinograph properties of wheat has been demonstrated before (GRAUSGRUBER *ET AL*. 2000; MLADENOV *ET AL*. 2001; DENCIC *ET AL*. 2011) though the proportion of variance that was explained by these factors differed widely between the studies. The more prominent influence of year observed in all parameters when compared to the effect of cultivar (Tab 55), which has been also observed by GRAUSGRUBER *ET AL*. 2000, can in our study be explained by the choice of cultivars coming from the same quality group and have been shown to express similar dough properties (Tab 2).

WA was strongly positively correlated with PC_{WGF}, $PC_{Type550,}$ and WG (Tab 53), which might explain the overall higher WA after pre-crop wheat when compared to the other two pre-crops (Tab

55). During dough preparation, primarily gluten proteins take up water and develop a protein network during kneading which results in the typical cohesive, viscous and elastic properties of dough (WIESER 2007; BELITZ *ET AL*. 2009).

We did not find an effect of *Fusarium* infection on WA (Tab 22) which is supported by the same observation made by BOCKMANN 1964; GARTNER *ET AL*. 2008 and DEXTER *ET AL*. 1996. However, other studies showed at least a slight increase of WA with increasing intensity of *Fusarium* infection (MEYER *ET AL*. 1986; PAWELZIK *ET AL*. 1998; WANG *ET AL*. 2005B), which might have resulted from a higher proportion of damaged starch granules in *Fusarium* infected kernels. DEXTER *ET AL*. 1996 observed a decrease of WA about 1.5 to 3.2% when they compared flour from exclusively FDK with a control. Since in the cold phase of breadmaking only damaged starch and gluten proteins are able to absorb water, this might have already hinted to a decrease in storage PC or loss of protein functionality due to fungal invasion into the grain. DON contents of flours in the study of DEXTER *ET AL*. 1996 exceeded DON_{Max} values of flours used in our study 3- to 6-fold and therefore also EU_{Max} for DON. The less contaminated flours (1.10-6.30 mg DON kg^{-1}) in the study of DEXTER *ET AL*. 1996 also neither showed an effect of *Fusarium* infection on WA, DDT nor DST in comparison to a control (0.40-1.60 mg kg^{-1}) which confirms our own results (Tab 23, Tab 24). However, other studies reported a decrease of DDT (PAWELZIK *ET AL*. 1998; NIGHTINGALE *ET AL*. 1999; WANG *ET AL*. 2005B), and DST (MEYER *ET AL*. 1986; PAWELZIK *ET AL*. 1998; NIGHTINGALE *ET AL*. 1999; WANG *ET AL*. 2005B) with increasing intensity of *Fusarium* infection. GARTNER *ET AL*. 2008 observed cultivar-depended an increase, a decrease as well as no impact of *Fusarium* infection on DST; also an increase of DS after *Fusarium* infection was observed. The possible explanation for the contracting results is given in chapter 1.1.3.

In our study DS increased linearly with increasing DON_{LOG} of flours (Tab 26, Fig 22). Compared to the contradictory results on the impact of *Fusarium* infection on other quality traits, e.g. WA, FN, the results of all other studies (BOCKMANN 1964; MEYER *ET AL*. 1986; PAWELZIK *ET AL*. 1998; NIGHTINGALE *ET AL*. 1999; WANG *ET AL*. 2005B; GARTNER *ET AL*. 2008) agreed with our own observations for DS and *Fusarium* infection. In a wide range of DON_{ORG} (0.16 to 11.84 mg kg^{-1}) DS was, depending on factors included in regression, more or less intense as can be seen from the wide range of slopes (a = 24.69-104.16) in Tab 26. Especially in 2007, the year with the most severe *Fusarium* infection out of three cultivation years, at location Torland after pre-crop maize, an increase of DON content led in some samples to a DS exceeding the optimal value of 80 FU for rolls by more than a 100%. The improved R^2 of correlations that was gained when separating for locations emphasized the significant influence of location on DS which was observed when performing ANOVA where environment was separated into year and location (Tab 60). Therefore, the relatively high variation of DS data, particularly in 2007, when compared to the other farinogram parameters, can be explained both by impact of *Fusarium* infection and significant

differences between locations. A possible interference of DON_{LOG} and DS within linear regression across all pre-crops within years with pre-crop effects that did not result from *Fusarium* infection cannot be ruled out since ANCOVA also showed a significant fixed effect of pre-crop (Tab 25). The decrease of DDT and DST in other investigations as well as the increase of DS in our study and in other studies due to *Fusarium* infection might have either resulted from reduced glutenin content in infected grain due to impaired synthesis in later stages of grain development (HUEBNER *ET AL*. 1990) or was a consequence of enzymatic degradation of proteins and starch by the fungus. A possibly increased protease activity might have led to an earlier destruction of gluten network and therefore to less tolerance during dough mixing in severely *Fusarium* infected samples (PAWELZIK *ET AL*. 1998; NIGHTINGALE *ET AL*. 1999).

5.2.5 Baking properties (BV, LH, LL, LW)

Baking performance of flours was determined by RMT. The assessment of the actual end-product, which resembled in our study a mini bread loaf or roll (< 10 g), can be regarded as the ultimate evaluation of wheat quality (WILLIAMS *ET AL*. 2008). We characterized bread loaves by their BV and form (LH, LL, LW). Wheat cultivars meant for baking purposes (B quality and higher) aside from cookies should at least have a minimum BV of 592 ml $100g^{-1}$ flour (SEIBEL 2005) which resembles grade 4 according to BSA. Even though both cultivars were rated into quality group B with grade 5 for BV according to BSA (Tab 2), in all cultivation years loaves showed BV below 480 ml $100g^{-1}$ flour (Fig 23, Tab 28). Since BV is the most important criterion for the grouping of quality classes, this would degrade both cultivars to the status of feed (C) quality or make it suitable the production of cookies (C_K quality).

This result emphasizes the importance of interaction between environmental conditions, cultivation practices and cultivar on BV as demonstrated by others (GRAUSGRUBER *ET AL*. 2000; MLADENOV *ET AL*. 2001; DENCIC *ET AL*. 2011). While in some studies the single effect of cultivar was more decisive for BV than environment and environment-cultivar interaction (MLADENOV *ET AL*. 2001; DENCIC *ET AL*. 2011), our results rather revealed the significant effect of year (Tab 56), since both cultivars belonged to the same quality group. Interaction of cultivar and environment became obvious in 2007. Cv. Ritmo showed a significantly higher average BV than cv. Centrum which also corresponded to the significantly higher PC_{WGF} and $PC_{Type550}$ in the respective year. The dependency of BV on PC is well known (SELING 2010). Already FINNEY & BARMORE 1948 showed that rising PC resulted in increased BV, however, this intensity of increase depended on cultivar. This confirms our own observations of a moderate, but highly significant correlation of PC_{WGF} and an even higher correlation with $PC_{Type550}$ across the two cultivars and years (Tab 53). With the objective to minimize time and costs at grain trade, BV is generally hoped to be precisely predicted from PC and SV which have been shown to be cultivar-specific and more or less closely related to the baking performance (SEIBEL 2005; LINDHAUER 2009). In our study, BV was not

significantly related to SV (Tab 62) which might have resulted from the little overall variation of SV throughout the whole investigation (Tab 54).

However, low BV indicated that cv. Ritmo and cv. Centrum did not meet B quality according to the criterion of BV. Obviously weather conditions in the combination with agricultural practices in our field experiment (for details see appendix in GÖDECKE 2010) did not meet the demands of the two cultivars to develop their potential as bread wheats. Since N fertilization is regarded as the most important factor to influence PC and wheat quality directly (ABEDI *ET AL.* 2011), most likely N regime in our study did rather promote GY than PC. N fertilization management has the objective to optimize yield and grain PC at the same time and is consequently complex, involving the consideration of rate, timing, splitting, as well as source of application (GRANT *ET AL.* 2001, FUERTES-MENDIZABAL *ET AL.* 2010). Since late N application (close to anthesis) enhances protein concentration in grain, it can be only assumed that either N rate was to low (60 kg N ha^{-1}) or the timing of the 3rd N application was not suitable. The 3rd N donation was applied rather early in our study (during end of stem elongation or booting). Common is a 3rd donation during ear emergence. Additionally, also S fertilization could have enhanced protein concentration as well as protein quality and therefore BV (ZORB *ET AL.* 2009). Low FN in 2007 and very high FN in 2008 and 2009 might have also contributed to low BV as well as the fact that all doughs were mixed for 2 min according to RMT and not according to their optimal DDT. However, the latter procedure could have improved BV as demonstrated by KIEFFER *ET AL.* 1998

Linear regression showed that BV increased with increasing DON content of flours (Tab 32). However, correlations across years and cultivars were most likely spurious since ANCOVA (Tab 29) showed a fixed significant effect of both environment and cultivar regardless of DON_{LOG}. Therefore, only two correlations of BV and DON_{LOG} with a small number of observations in cv. Centrum most likely reflected the impact of *Fusarium* infection on BV. According to these, an increase of DON_{LOG} about 1 mg kg^{-1} increased BV about 58 and 94 ml depending on regression curve within the range of DON_{ORG} from 0.31-3.59 mg kg^{-1}. However, the major amount of samples clearly exceeded EU_{MaxUP}. An increase of BV after *Fusarium* infection was also observed by MEYER *ET AL.* 1986; DEXTER *ET AL.* 1996; PRANGE *ET AL.* 2005; WANG *ET AL.* 2005B; GARTNER *ET AL.* 2008 and LANCOVA *ET AL.* 2008. MEYER *ET AL.* 1986 stated that the effect of *Fusarium* infection on BV depended on various factors, such as growing season, infection severity, PC and protein quality. Higher BV was observed after low infection degree probably because of low PC and therefore short gluten and resulting in higher dough elasticity which very much resembles the situation of cv. Centrum in 2007 when compared to cv. Ritmo. Also DEXTER *ET AL.* 1996 explained the increase in BV after *Fusarium* infection with an improved viscoelastic balance of gluten due to moderate increase of gliadins. In contrast, some of these authors also observed a decrease of BV (FINNEY 1954; MEYER *ET AL.* 1986; SEITZ *ET AL.* 1986; DEXTER *ET AL.* 1996; NIGHTINGALE *ET AL.*

1999; GARTNER *ET AL*. 2008) in some cultivars, and no effect in others (ANTES *ET AL*. 2001; TERZI *ET AL*. 2007; GARTNER *ET AL*. 2008) which highlights once more that the effect of *Fusarium* infection depends on the complex interaction of cultivar, intensity of *Fusarium* infection, growing conditions, PC and protein composition. The latter observation supports our own result that *Fusarium* infection showed no impact on BV of cv. Ritmo but on cv. Centrum even though the latter cultivar was less severely contaminated with DON since it expressed lower susceptibility towards FHB.

Regarding shape of loaves no linear relationship between DON_{LOG} and LL, LW, as well as LH could be detected, even though ANCOVA for LH stated otherwise (Tab 29-Tab 31). However, multiple comparisons revealed that in the year 2007 with severe *Fusarium* infection the highly susceptible cv. Ritmo showed on average a clearly reduced LH, increased LW and decreased LL after pre-crop maize when compared to the other pre-crops (Fig 23, Fig 24). This factor combination had caused the highest DON contamination in wheat samples after all. Such a loss of loaf shape after *Fusarium* infection was also reported by BOCKMANN 1964 and WANG *ET AL*. 2005B. These authors also reported on the wet and sticky to gluey dough properties we also observed during dough handling for RMT which made it partly impossible to bring doughs into the "log" shape by noodles machine as required. This observation is supported by the increased DS after *Fusarium* infection (Fig 22), particularly after maize pre-crop in 2007, which has been discussed before. We suggest that the plastic deformation of dough, which was already visible after the first leavening, was due to enzymatic activity during dough processing, as reported by PAWELZIK *ET AL*. 1998 and NIGHTINGALE *ET AL*. 1999. However, enzymatic activity might not have been linearly correlated to DON_{LOG} content.

5.2.6 Gluten properties (R_{Max}, EXT, R_{Max}/EXT)

Micro-extension tests clearly showed that there were significant differences in the properties of WG between the two cultivars (Tab 33). Whereas cv. Ritmo comprised very soft gluten indicated by high EXT, gluten of cv. Centrum showed significantly higher R_{MAX} and ruptured –adversely to cv. Ritmo- within the technical limitation of our TA settings. The determination of the functionality of gluten by R_{MAX}, EXT, and R_{MAX}/EXT is a helpful tool for the estimation of baking volume, particularly if CP and WG are considered (KIEFFER *ET AL*. 1998). Generally, R_{MAX} and EXT are inversely correlated while R_{MAX} and R_{MAX}/EXT are positively correlated with BV. That there did not exist a significant correlation between BV and gluten properties in our study (Tab 53) might have resulted from the little variation of gluten properties within the two cultivars. However, that gluten properties may differ strongly between cultivars even though their PC only differs about a few decimal as in our study, has been demonstrated by (KIEFFER *ET AL*. 1998). The reasons for the softer gluten of cv. Ritmo, which could also be a reason for the loss of loaf shape, as discussed before, are not clear. On the one hand cv. Ritmo was the more severely *Fusarium* affected cultivar;

on the other hand ANCOVA did not reveal an effect of DON_{LOG} on R_{MAX}/EXT (Tab 34). Therefore, it is possible that either gluten properties where cultivar-specific, which might have been demonstrated easily in a year with no or low occurrence of FHB (e.g. 2008) or that gluten of cv. Ritmo softened to a higher degree than gluten of cv. Centrum during 40 min of incubation due to increased proteolytic activities resulting from higher *Fusarium* infection level.

A higher EXT of dough as well as a reduced R_{Max} after *Fusarium* infection has been demonstrated by NIGHTINGALE *ET AL*. 1999 and PRANGE *ET AL*. 2005. Since the EXT and R_{MAX} of dough generally corresponds very well to the analog parameters of resembling gluten (KIEFFER *ET AL*. 1998, also the explanation for these observations from the respective authors might apply to gluten samples of cv. Ritmo in our study. NIGHTINGALE *ET AL*. 1999 supposed that gluten softens due to activity of protein hydrolyzing enzymes. Indeed, the characteristics of *Fusarium* proteases described by WANG *ET AL*. 2005B regarding their temperature and pH optimum, allow the conclusion that the incubation conditions of wet gluten for micro-extension test, which is actually meant for gluten relaxation, might also provide optimal conditions for the degradation of gluten proteins by *Fusarium* proteases leading in the end to a loss of gluten functionality

5.3 *Fusarium* infection and flour composition

5.3.1 Starch

Starch is the most abundant component of wheat flour (~75% of dry matter), *Fusarium* infection does influence its content (Tab 36). This was also reported by WANG *ET AL*. 2005A while other studies found a decrease of starch content after *Fusarium* infection (PAWELZIK *ET AL*. 1998; MATTHAUS *ET AL*. 2004; SIUDA *ET AL*. 2010). However, these studies investigated WGF and did not remove small and shriveled kernels (as we did by cleaning) resulting from FHB which might both be reasons for the contradicting results.

Next to storage proteins, starch is other main component of cereal grain whose functional properties are of major importance within the production of bread and bakery products. During baking, starch granules take up approx. 45% water and gelatinize (BELITZ *ET AL*. 2009). Yet, not only starch content is important but also starch properties. Even though *Fusarium* infection must not result in a detectable change of starch content, it might cause a change of starch composition indicated by a change of pasting properties (DEXTER *ET AL*. 1996; WANG *ET AL*. 2005A), amylose extractability as a measure of starch damage (DEXTER *ET AL*. 1996; PAWELZIK *ET AL*. 1998), amylose content (BOYACIOGLU & HETTIARACHCHY 1995), and content of free sugars (MEYER *ET AL*. 1986; BOYACIOGLU & HETTIARACHCHY 1995; PAWELZIK *ET AL*. 1998; WANG *ET AL*. 2005A). All might result from amolytic activity of the fungus which is generally signified by an increase of amylase activitiy in grain (DEXTER *ET AL*. 1996; PAWELZIK *ET AL*. 1998; NIGHTINGALE *ET AL*. 1999; MATTHAUS *ET AL*. 2004; WANG *ET AL*. 2005A). However, since the focus of this study lay on the

storage proteins, starch properties in our study were only measured indirectly by falling number which was discussed before and had indicated a change in starch quality by *Fusarium* infection in some severely infected flours.

Our results showed that starch content is primarily influenced by environmental conditions (year, location) and cultivation practices (pre-crop) as has been demonstrated for other wheat quality traits already (WILLIAMS *ET AL.* 2008). Correlation studies showed a weak negative trend with PC_{WHG}, $PC_{TYPE550}$, and WG, illustrating the competitive relationship between storage proteins and starch in endosperm and also explaining some of the variance in starch content between years and pre-crops (Tab 35). On the other hand, there was a strong positive correlation with WA, emphasizing the importance of starch content for dough yield and therefore also BV (Tab 53).

5.3.2 Gluten proteins

$PC_{Type550}$ was composited as described by other studies: about 75% was made up by gluten proteins (Fig 25), the residual proteins -referred to as "other"- most likely consisted of albumins and globulins (SHEWRY & HALFORD 2002; GOESAERT *ET AL.* 2005; WIESER 2007) as well as glutenin macropolymers (GMP) which are insoluble in the used extraction solutions (KOH *ET AL.* 2010). The major proportion of total gluten was made up by gliadins, whereas α- and γ-gliadins represented the major proportions. This also agreed with an earlier investigation of just gliadin subgroups by WIESER *ET AL.* 1994. Glutenins made up only half the proportion of gliadins, whereas LMW-GS represented the major proportion. Ratio of gliadin/glutenin and LMW/HMW averaged 2.9 and 2.4, respectively. This composition of gluten is typical for flour Type 550 as demonstrated by WIESER & KIEFFER 2001.

WIESER & KIEFFER 2001 demonstrated that gluten content might differ between cultivars either due to genotype or growing conditions. In our study, both cultivars did not differ significantly in total gluten content, but a significant effect of year, pre-crop, which can both be considered as "growing condition", as well as the interaction of year, pre-crop and cultivars on total gluten content were detected (Tab 57, Tab 61). However, quantitative differences were insignificant (2-3%). Even though across all samples after pre-crop sugar beet significantly more gluten could be extracted (Tab 57) a significant effect of pre-crop within years and across cultivars could not be demonstrated (Tab 38).

An effect of growing season on protein composition and quantity might result to a large extent from differences in nitrogen and water supply, as well as temperature conditions during grain filling (WIESER & SEILMEIER 1998; DANIEL & TRIBOI 2000; TRIBOI *ET AL.* 2003). An effect of pre-crop as apparent across all investigated samples (Tab 57) might have resulted from different N availability in the soil (as discussed in 5.2.1). WIESER & SEILMEIER 1998 showed that higher amounts of N fertilizer (40 kg N ha^{-1} vs. 200 kg N ha^{-1}) increased total PC and gluten about 57%

and 67%, respectively. However, total gluten per gram protein was not as severely affected and increased only about 6% in the treatment with the higher N rate. Even though N_{min} and differing mineralization of crop debris in our study might have led to slightly different N availability after different pre-crops for subsequent wheat plants, N supply did in any case not differ about 160 kg N ha^{-1} as in the study of WIESER & SEILMEIER 1998 which represents extremely different N regimes. Therefore, it is reasonable that differences between pre-crops in our study were small and rather due to natural variation. This emphasizes that the proportion of gluten in total protein is rather determined by the genetic background than by growing conditions (TRIBOI *ET AL*. 2003).

The gliadins and glutenins as well as the subfractions (ω5, ω1,2, α, γ; ω5, HMW-GS, LMW-GS) were significantly influenced by year (Tab 57, Tab 58). SoE indicated that year affected gluten subfractions more than all other experimental factors. While for total gluten the effect of year (SoE 1.02) was relatively small, the effect of year within single gluten subfractions, particularly in glutenin (SoE 1.33) and LMW-GS (SoE 1.39), was more apparent (SoE 1.10-1.39). This indicated that gluten compositions seemed to be more variable due to environmental conditions than total gluten proportion. The biosynthesis of gluten fractions is strongly influenced by N availability, temperatures and water supply during grain filling (TRIBOI *ET AL*. 2003). Since weather conditions during grain-filling period varied greatly between the three years (Fig 30) this might explain the strong influence of the year. While TRIBOI *ET AL*. 2000 observed no influence of the year on gliadins, glutenins were found to be affected which supports our own observations of a stronger influence of the year on glutenins and subfractions than on gliadins and subfractions. Since both total gliadin and total glutenin were changed by growing season, year also had a significant effect on gluten composition. Gliadin/glutenin ratio was significantly higher in 2007 and 2009 than in 2008 and therefore reflected the adverse presence of gliadins and glutenins in the single years. LMW/HMW ratio was analog to the single subfractions significantly higher in 2008 than in 2007 and in 2009. In contrast, TRIBOI *ET AL*. 2000 could not observe an effect of year on gliadin/glutenin ratio and LMW/HMW ratio. The contradiction in our results in comparison to the findings of TRIBOI *ET AL*. 2000 demonstrated the importance of interaction between growing conditions and cultivars.

Furthermore, a significant effect of cultivar across years could be observed on gluten fractions and some of the subfractions which were less apparent than the effect of year (Tab 57, Tab 58). Cv. Ritmo contained significantly more total gliadin, ω5-gliadin, and γ-gliadin and significantly less total glutenin, ωb, and LMW-GS than cv. Centrum. Also the ratio of LMW/HMW was significantly lower in cv. Ritmo than in cv. Centrum. Differences in gliadin and glutenin content and subfractions as well as gluten composition between cultivars were reported before (WIESER *ET AL*. 1994; TRIBOI *ET AL*. 2000; WIESER & KIEFFER 2001).

Even though significant differences between pre-crops could be detected across all samples for ω5-gliadin, ω1,2-, γ-gliadin, total glutenin, HMW-GS, LMW-GS and subsequently for ratio of gliadin/glutenin and LMW/HMW (Tab 57, Tab 58), these differences depended on year and cultivar. Slight differences between pre-crops might, as already discussed for total gluten, have resulted from differing N availability after different pre-crops. WIESER & SEILMEIER 1998 found that gliadins were more severely affected by N fertilization than glutenins and that main gluten fractions (γ-gliadins, LMW-GS) were more severely affected than minor fractions (HMW-GS, ω-gliadins). However, we could not observe such clear differences between pre-crops concluding that effect of N on gluten composition was insignificant. This is further supported by the fact that we did not find any moderate to strong relationships between proportions of gluten fractions and subfractions with PC_{WGF} or $PC_{Type550}$ indicating that the effect of N on gluten fractions was rather quantitative than qualitative (Tab 63). This agrees with the findings of TRIBOI *ET AL.* 2003 who reported that differences in protein fraction composition mainly depends on total N quantity of mature grain. High correlations between content of gluten proteins and subfractions have been demonstrated by WIESER & KIEFFER 2001, however gluten proteins in their study were calculated on DM basis and did not consider total PC.

In three out of five significant correlations between content of total gluten and DON_{LOG} the trend was negative (Tab 40), which is in agreement with other studies that reported a reduction of total gluten protein after *Fusarium* infection (NIGHTINGALE *ET AL.* 1999; ANTES *ET AL.* 2001; EGGERT *ET AL.* 2010). However, the correlation across 2007 and 2009 might have been spurious since ANCOVA also revealed a significant relationship of environment regardless of *Fusarium* infection. Nevertheless, the effect was present in samples where DON levels either highly exceeded EU_{Max} e.g. after pre-crop maize in 2007 or where DON levels were below <0.75 mg kg^{-1} e.g. after wheat in 2009. In contrast to these observations, we also found an increase of total gluten protein after pre-crop maize in 2009 across locations and even more pronounced for location Gladebeck separately. DON levels of these samples mostly exceeded EU_{Max}. This effect has not been reported in other studies before. A possible explanation might be a higher extraction rate of gliadins and glutenins due to higher solubility of these gluten fractions in their respective extraction solution. Enzymatic degradation of GMPs by *Fusarium* proteases might have led to this phenomenon. GMPs are generally not soluble with the common extraction solutions for gliadins and glutenins (KOH *ET AL.* 2010), however, protease activity might change this. Summarizing, the effect of *Fusarium* infection on total gluten protein did not become clear in our study and further investigations with a higher number of observations and differing DON levels are needed to elucidate the impact of the fungus in more detail.

Our results showed that *Fusarium* infection did not influence total gliadin or gliadin subfractions, neither in 2007 where DON contents of glour Type 550 ranged from 0.31 to 11.84 mg kg^{-1}, or in

2009 where both DI and DON contamination were less severe (Tab 42-Tab 44). This finding is supported by results of BOYACIOGLU & HETTIARACHCHY 1995 who investigated three categories of artificially infected FDK, by DEXTER *ET AL.* 1996 whose flours composed DON levels in the range of our samples and significantly more (> 35 mg kg^{-1}), and more recently by PRANGE *ET AL.* 2005 where DON concentration of samples were 0.06-12.30 mg kg^{-1}. On the contrary, WANG *ET AL.* 2005B observed a slight increase in gliadin content of severely infected kernels of one wheat cultivar, and also EGGERT *ET AL.* 2010 observed a slight increase of total gliadin content and all gliadin subfractions in one spring wheat cultivar when naturally infected samples (DON content: 0.0-0.3 mg kg^{-1}) were compared to artificially infected ones (DON content: 2.1-3.6 mg kg^{-1}). The intensity of increase of gliadin content in the latter study strongly depended on location and subfraction (3-57%). The increase of gliadin content was explained by the degradation of glutenin subfractions by *Fusarium* proteases. It was assumed that the solubility of digested glutenin fractions increased and extraction occurred with the gliadins. In our study, either digestion of glutenins did not lead to detectable increase in gliadin concentration or digested glutenin fractions were already extracted with the albumin-globulin fraction. The possibility of this has been demonstrated by EGGERT *ET AL.* 2011 in an *in vitro* experiment were *Fusarium* proteases digested gliadin and glutenin fractions separately.

In our study, an increase of total gliadin content and single subfractions did neither occur in flours with lower nor higher DON levels than EU_{Max}. Therefore, a resulting loss of flour quality due to increased gliadin content was insignificant. WIESER *ET AL.* 1994 demonstrated that if e.g. content of ω1,2-gliadin increased it is correlated with decreased SDS sedimentation value, reduced dough resistance and EXT, as well as decreased BV. This is further supported by our observation that total gliadin as well as gliadin subfractions showed no to very weak correlation with quality parameters (Tab 63). The insignificant influence of just gliadins on gluten, dough and baking properties was confirmed by WIESER & KIEFFER 2001. However, gliadins played a significant role together with glutenins.

Regarding total glutenin and subfractions, for total glutenin and HMW-GS an effect of *Fusarium* infection could be detected (Tab 46) while there was no effect on ωb-GS (Tab 47) and LMW-GS content (Tab 48). In 2007, total glutenin content of cv. Ritmo decreased from 21.7% Protein to 12.3% from lowest to highest DON level which resembled a reduction of over 50% (Fig 26). Since ANCOVA showed no significant effect of pre-crop, a spurious correlation due to effects of pre-crops was unlikely. Therefore, we can conclude that much of the variation in glutenin content as particularly apparent in 2007 can be explained by *Fusarium* infection. However, there was a significant effect of environment regardless of *Fusarium* infection which cannot be just explained by the effect of year. Obviously location had an effect since significant regression curves could only be calculated for the location Gladebeck. In 2009, where *Fusarium* infection was less severe

than in 2007, the reduction of glutenin with increasing DON content was less intense. However, linear regression could be performed across cultivars without likely interference of pre-crops and cultivars since ANCOVA did not show a significant effect for these factors. Again, only at location Gladebeck this trend could be observed. In 2007 and 2009, the identical samples showed as similar decrease of HMW-GS content with increasing DON_{LOG} (Fig 26). Therefore, we conclude that the decrease of total glutenin content was mainly due to the decrease of HMW-GS content.

A decrease of total glutenin and HMW-GS content (measured by RP-HPLC) after *Fusarium* infection of wheat was also detected by WANG *ET AL*. 2005B and EGGERT *ET AL*. 2010, however, they also observed an increase of LMW-GS content which we could not see. BOYACIOGLU & HETTIARACHCHY 1995 and DEXTER *ET AL*. 1996 also observed a reduction of total glutenin content after *Fusarium* infection but did not investigate the impact on single glutenin subgroups, whereas PRANGE *ET AL*. 2005 did not find an effect in flours that contained similar high DON levels as our investigated flours. A decrease of high molecular weight gluten proteins (BECHTEL *ET AL*. 1985; MEYER *ET AL*. 1986; NIGHTINGALE *ET AL*. 1999) and a slight increase of low molecular weight gluten proteins (MEYER *ET AL*. 1986; NIGHTINGALE *ET AL*. 1999) after *Fusarium* infection has also been demonstrated by others, however they used other separation methods for gluten proteins (SDS-PAGE, SE-HPLC) and therefore protein fractions investigated by these methods cannot be without exceptions compared to fractions separated by RP-HPLC.

Total glutenin content and HMW-GS content in particular were demonstrated to be moderately to strongly positively correlated to DDT, R_{MAX} of gluten and dough, as well as to BV (WIESER & KIEFFER 2001). Though the observed reduction of total glutenin and HMW-GS content due to *Fusarium* infection was not insignificant when looking at the possible consequences for baking quality one has to consider two things. First, the major number of flours where an effect of *Fusarium* infection could be demonstrated would not have been suitable for processing since EU_{Max} for DON levels were exceeded in most cases. Second, neither glutenin content nor subfractions showed any considerable correlation with quality parameters in our study (Tab 63). From this we can conclude that neither *Fusarium* infection nor any other experimental factors caused such variation in gluten composition that quality parameters such as BV were significantly influenced.

Further analysis of impact of *Fusarium* infections showed, that presence of the fungus did not only cause quantitative changes, as demonstrated for total glutenin and HMW-GS, but also influenced ratios of gliadin/glutenin and LMW/HMW (Tab 49). Several significant correlations across cultivars, across/within environments, and across/within pre-crops showed that in general gliadin/glutenin ratio increased significantly with increasing DON content of flours (Fig 28, Tab 50). However, intensity of increase differed, particularly between years. Some of the correlations, especially those across locations and pre-crops, were possibly spurious since ANCOVA (Tab 49) also showed a significant effect of the respective factors on gliadin/glutenin ratio regardless of

DON. Again, the effect of location was important since R^2 improved when correlations were performed for locations separately. Since total gliadin content was not affected by *Fusarium* infection in our study, it can be concluded that the increase of gliadin/glutenin ratio resulted from the decrease of total glutenin and subsequently from the reduction of HMW-GS with higher DON content of flour.

Furthermore, in flours of the highly susceptible cv. Ritmo an increase of LMW/HMW ratio with increasing DON content could be observed in the year 2009, where infection level was moderate (Tab 50). A significant correlation could just be detected for the location Gladebeck. The linear relationship between LMW/HMW and DON_{LOG} across years showed a similar slope as the correlation for just 2009, however, it can most likely be interpreted as spurious correlation since ANCOVA revealed a significant effect of environment on LMW/HMW ratio regardless of *Fusarium* infection. Since LMW-GS was not affected by *Fusarium* infection, it can be concluded that the increase of LMW/HMW ratio resulted from the decrease in HMW-GS content.

Our results, that gluten composition can be changed significantly after *Fusarium* infection, are supported by DEXTER *ET AL*. 1996; WANG *ET AL*. 2005B and EGGERT *ET AL*. 2010 who also reported an increase of gliadin/glutenin ratio in infected samples. However, no study has so far explicitly described also a change in glutenin composition as indicated by raising LMW/HMW ratio after *Fusarium* infection as shown in our results. Nevertheless, MEYER *ET AL*. 1986 and NIGHTINGALE *ET AL*. 1999 indicated a change of gluten protein composition when they observed an increase of HMW-gluten proteins and a decrease LMW-gluten proteins. Yet, whether increase and decrease happened to the same extent was not reported since SDS-PAGE only provided semi-quantitative information.

WIESER & KIEFFER 2001 showed that a wider ratio of gliadin/glutenin and LMW/HMW led to reduced dough, gluten, and baking properties. Therefore, the increase of gliadin/glutenin and LMW/HMW ratio after *Fusarium* infection cannot be considered insignificant when looking at quality. However, a strong detectable increase could only be detected in flours where DON levels already exceeded EU_{Max}. Additionally, neither gliadin/glutenin ratio nor LMW/HMW ratio showed a moderate or strong relationship with any of the quality parameters measured (Tab 63).

5.4 Protease activity (PA)

In order to determine if differences in gluten quantity and composition resulted from proteolytic activity, we measured PA in flours Type 550 in the year 2007 where FHB was most severe of all three years (Tab 51). Since cereal PA is low at harvest (SHI & XU 2009), we assume that measured PA mainly resulted from endoproteases secreted into the kernel by *Fusarium* spp. as reported by PEKKARINEN *ET AL*. 2000 and PEKKARINEN & JONES 2002. Nevertheless, enzyme assay was not

specific to *Fusarium* proteases; therefore it was possible that both, cereal and fungal proteases were detected together.

Even though average DON_{ORG} content in flour Type 550 of susceptible cv. Ritmo (4.42 mg kg^{-1}) was about 4-fold higher than of cv. Centrum (1.05 mg kg^{-1}), PA in cv. Ritmo was not significantly higher than in cv. Centrum (Tab 51) which one would expect since it is known from other studies that PA in wheat kernels increases with increasing *Fusarium* infection level (e.g.MEYER *ET AL.* 1986; PAWELZIK *ET AL.* 1998; MATTHAUS *ET AL.* 2004; WANG *ET AL.* 2005B). However, these authors only measured PA in one cultivar each, except for MEYER *ET AL.* 1986 who investigated 20 cultivars, but did not report on differences in PA between cultivars. Differences in PA between cultivars could be expected if cultivars obtained different potential to withstand proteolytic activity of fungus, e.g. by production of protease inhibitors (CHILOSI *ET AL.* 2000; ROBERTS & HEJGAARD 2008). However, that was obviously not the case since cultivar effect was insignificant in ANCOVA (Tab 52). Therefore slight differences between cultivars were exclusively due to the levels of *Fusarium* infection. Yet, there was a significant effect of location and pre-crop which could not be explained by $DON_{LOG.}$ Obviously, physiological status of wheat plant differed between locations and pre-crops making the plants more or less "susceptible" to fungal proteolytic activity. The difference between locations is also the reason why no significant differences between pre-crops within cultivars could be detected even though there were differences across cultivars. As expected, PA was highest after pre-crop maize where also *Fusarium* infection was highest in both cultivars followed by wheat and sugar beet, the latter pre-crop causing the least infection levels and DON contaminations in grain. A strong significant positive correlation between DON_{LOG} and PA was detected within few flours (Fig 29), however, these exceeded EU_{Max} many-fold. Nevertheless, the slight increase of PA with increasing DON content which was observed across all samples agreed with the findings of other studies (MEYER *ET AL.* 1986; PAWELZIK *ET AL.* 1998; MATTHAUS *ET AL.* 2004; WANG *ET AL.* 2005B).

Degradation of wheat proteins by fungal proteases could be the reason for quantitative and qualitative changes of gluten (e.g. total glutenin and HMW-GS) and related quality traits (e.g. SV, DS) as discussed before and suggested by others (e.g. NIGHTINGALE *ET AL.* 1999; WANG *ET AL.* 2005B). Nonetheless, correlation of PA with content of gluten fractions as well as quality parameters was, if at all present, very weak (Tab 63, Tab 64). There could be more than one explanation for this. First of all, it could be argued that variation within PA was so insignificant that it could not cause any significant measurable changes in the quality traits or gluten proteins. Second, assay conditions for the determination of PA did not reflect the conditions (substrate, ph, temperature, incubation time) present in the field, during storage, or processing of wheat where fungal proteases might be active (JONES 2005). Third, PA and DON_{LOG} were not correlated linearly. The first argument neglects the significant effect of *Fusarium* proteases on wheat quality

in our study, the latter two arguments do not rule out significant changes by proteases. Consequently, further experiments with assay conditions more adapted to field and processing conditions should be carried out in future to investigate the influence of *Fusarium* protease on gluten proteins during grain-filling and dough processing. For example, a substrate which is closer to the composition of wheat storage proteins than azogelatin would be more suitable.

5.5 How does *Fusarium* infection influence wheat quality?

Two major reasons might be the cause for biochemical changes in grain composition and therefore subsequent changes in wheat quality traits: fungal enzymes and impaired synthesis of grain components (growth impaired, DON).

Fungal enzymes, especially carbohydrases and proteases, are assumed to cause the major changes in flour composition and processing quality (DEXTER & NOWICKI 2003). An increased activity of amylase, xylanase, cellulase, and glucanase in flours from *Fusarium* infected grain could partly explain weaker and stickier dough properties, lower water uptake and poorer baking quality of these flours in comparison to less severely infected flours (DEXTER *ET AL*. 1996; PAWELZIK *ET AL*. 1998; NIGHTINGALE *ET AL*. 1999; MATTHAUS *ET AL*. 2004; WANG *ET AL*. 2005A). It is assumed that the fungus secretes these enzymes during the invasion of the kernel thus degrading starch as well as cell wall components. WANG *ET AL*. 2005A characterized α-amylase of *F. culmorum* and demonstrated that it was active in a wide pH range from 5.0 to 8.5 and at temperatures from 10 to 100 °C, enabling the enzymes to have an adverse effect on dough and baking properties, particularly in baking procedures involving longer proofing times. Even more important might be the activity of fungal proteases. According to DEXTER & NOWICKI 2003, proteases of fungal origin are the most reasonable explanation for poorer dough and baking qualities of *Fusarium* infected wheat in comparison to healthy wheat. PEKKARINEN *ET AL*. 2000 showed that *F. graminearum*, *F. culmorum* and *F. poea* were able to produce acid, neutral or alkaline proteases when grown on gluten media. Several studies showed that PA in severely *Fusarium* infected wheat samples was higher than in a control or less infected samples (PAWELZIK *ET AL*. 1998; MATTHAUS *ET AL*. 2004; WANG *ET AL*. 2005B). The authors suggested that the digestion of storage proteins during processing could lead to change in dough properties e.g. such as reduced R_{MAX} and loss of loaf shape during baking as we have also observed in our study. Also the weak and soft consistence of WG, as described by DEXTER *ET AL*. 1996, could be explained by proteolytic activity. With means of SE-HPLC NIGHTINGALE *ET AL*. 1999 showed a time dependent degradation of high molecular weight proteins concomitant with an increase in low molecular weight proteins resulting from proteolysis. A recent *in vitro* experiment conducted by EGGERT *ET AL*. 2011 with *F. graminearum* proteases on wheat gluten and isolated gluten subfractions demonstrated that fungal proteases were able to degrade both gliadins and glutenins, but degraded HMW-GS in particular. The authors

suggested that the high affinity of *Fusarium* proteases for the HMW-GS was supposed to result from higher lysin content of the respective subfraction which is, next to arginin, one of the important intersections of trypsin-like serine proteases produced by *Fusarium* spp. (PEKKARINEN & JONES 2002; PEKKARINEN *ET AL*. 2007). This is especially crucial for baking purposes since HMW-GS contribute highly to the elastic properties of dough and correspond to a high BV (ANJUM *ET AL*. 2007). A higher content of free amino acids in flour (MEYER *ET AL*. 1986; WANG *ET AL*. 2005B) and an increase of low molecular weight gluten proteins (MEYER *ET AL*. 1986; NIGHTINGALE *ET AL*. 1999) supports the assumption of protein digestion through fungal proteases. WANG *ET AL*. 2005B characterized conditions for the PA of *F. culmorum* and showed that fungal protease was, similar to fungal amylase, active in a wide range of pH (from 4.5 to 8.5) and as well very thermostable. Fungal protease stayed active, even though impaired, up to 100 °C. These findings and the observations of other authors suggests a possible degradation of wheat proteins, particularly storage proteins such as HMW-GS, and starch subsequent to *Fusarium* infection in the field, during dough processing as well as during the first phases of baking.

Besides enzymatic degradation of starch, storage proteins and other flour components another reason for changed flour, dough and baking properties after *Fusarium* infection might be the incomplete accumulation of kernel constituents. One reason for this could be the mechanical blocking of vascular bundles by fungal mycelium, another reason could be the impaired synthesis of grain components due to the presence of mycotoxins.

As already described in chapter 1.2.3 fungal mycelium growth within xylem and phloem may inhibit the nutrient supply for developing spikelets and leading to their premature death (KANG & BUCHENAUER 2000; RIBICHICH *ET AL*. 2000; GOSWAMI & KISTLER 2004). The interruption of assimilate transport within the spike impairs the normal development of kernels and leads to the typically shrunken and shriveled kernels of *Fusarium* damaged grain (MEYER *ET AL*. 1986) and the reduction of TKW and therefore also test weight (see chapter 1.2.2). Since biosynthesis of storage proteins sets in earlier during grain-filling than starch synthesis, it might be that starch content and composition might be affected more severely than PC and protein composition. Depending upon initial infection time, seed development can be already stopped by fungal infection process at early milk stage (SIMMONDS 1968) which is congruent with the observations of BECHTEL *ET AL*. 1985 who observed the most severe *Fusarium* damage in wheat kernels between the second and third week after anthesis. This might also be the reason why glutenins were often found to be more affected by *Fusarium* infection than gliadins. Based on the findings of HUEBNER *ET AL*. 1990 who observed that glutenins compared to gliadins are more rapidly synthesized in the later stages of kernel development, WANG *ET AL*. 2005B concluded that gliadins possibly "escape" the attack of *Fusarium* spp. while accumulation of glutenins stays incomplete due to emergency maturation.

Furthermore, the incomplete or halted synthesis of grain components could be the result of the presence of trichothecenes such as DON. In experiments with leaf tissue of maize and wheat CASALE & HART 1988 demonstrated that DON effectively inhibited the incorporation of the amino acid ^{3}H-leucine and therefore concluded that DON might be a potent inhibitor of protein synthesis in plant cells as well as in other eukaryotic cell. The mode of action is the binding of DON to the peptidyl transferase as a part of the 60S ribosomal subunit (ERIKSEN & PETTERSSON 2004).

6 Conclusions and outlook

Due to various interactions between experimental factors, wheat quality parameters, and *Fusarium* infection, evaluation of bread wheat quality was complex. In general all experimental factors – environment (year, location), cultivar, pre-crop, and fungicide - had an impact on the intensity of *Fusarium* spp. infection in harvested grain and therefore in the respective milling products (chapter 4.1.2 and 5.1.2). At the same time, generally at least one or more of these factors (fungicide not considered) had an effect on the investigated quality parameters and the composition of flour, if not alone, than at least in interaction with other factors. This made the identification of the effect of *Fusarium* infection on wheat flour composition and wheat quality traits particularly complex. In order to identify this effect, we used ANCOVA were DON content of flour was introduced as covariate. It can be summarized that DON as covariate was in no case able to explain the complete variability in data alone, nevertheless, for a few parameters, $PC_{Type550}$, SV, FN, DS, BV, total gluten, total glutenin, HMW-GS and gluten composition (gliadin/glutenin, LMW/HMW), and PA, a significant effect of *Fusarium* infection in interaction with other experimental factors and could be detected. However, the application of ANCOVA strategy requires that the covariate is in the "best case" completely independent from all other factors included in the model (LITTEL *ET AL*. 2006). This requirement was not fulfilled which may therefore have led to interpretation bias of the output. This might be the explanation why in some cases e.g. $PC_{Type550}$, FN, investigation of relationship between target variable and DON_{LOG} might have revealed significant linear regressions where correlations were most likely spurious. In these cases the model failed to separate the effects of experimental factors and *Fusarium* infection on target variable clearly. In an experiment that suited ANCOVA requirements in a better way, DON should vary within the experiment independently from all other experimental factors and not because of the included experimental factors. Such a situation is generally not given in a field experiment as ours which was based on the variation of natural *Fusarium* infection due to the experimental factors. It would rather be present in an artificial inoculation experiment under completely controlled conditions where inoculum concentration determines the intensity of *Fusarium* infection and subsequent mycotoxin contamination alone.

With regard to our interest in mycotoxin occurrence in wheat milling products, EU_{Max} for DON, and lack of other mycotoxin data, we decided on DON as a covariate. Nevertheless, the question arises whether DON is a suitable parameter to correlate with quality parameters, content of flour components as well as PA, respectively. DON concentration of flours might not be the best way to quantify the impact of *Fusarium* infection on wheat flour. First, other mycotoxins might be present as well and therefore "total mycotoxin content" might be more suitable. Second, and probably even more important, DON is generally positively correlated to fungal biomass (see chapter 5.1.3), however DON to fungal biomass ratio might differ. Yet, biochemical changes in flour properties

and subsequent changes in quality traits after *Fusarium* spp. infection might rather correspond to intensity of fungal growth and therefore *Fusarium* biomass in grain and subsequent excretion of fungal enzymes. Thus, fungal biomass quantified as *Fusarium* DNA as in our study or *Fusarium* soluble proteins as used by WANG *ET AL.* 2005B and WANG *ET AL.* 2005A might be better fitting covariates. However, this requires a high sensitivity of these methods. In our study, we did not decide for *Fg*DNA as covariate because particularly in 2009 *Fg*DNA content of most flours was below LOQ and did not represent an appropriate measure for the quantification of *Fusarium* infection; however DON content was in most cases above LOQ.

Furthermore, it has to be considered that ANCOVA only investigates the linear relationship between target variable and covariate. Thus, other possible correlations e.g. exponential or log functional are not taken into account. Consequently, other relationships between quality parameters, flour components, and DON_{LOG} aside from linear ones possibly existed. Further considerations refer to EU_{Max} and practical relevance of measured significant changes caused by *Fusarium* spp. infection in wheat quality. If a linear relationship between DON_{LOG} and target variable could be detected in our study, it generally included to a major proportion flour samples that exceeded EU_{Max} by far and would therefore be neither suitable for direct human consumption nor for further processing into cereal products. Additionally, in most cases linear regression curves with at least moderate R^2 could only be calculated within very low number of observations ($n \leq 12$). Functions gained from these curves are not suitable for predicting a trend safely or making a solid forecast of expected quality changes in flours from DON content alone, but rather fulfill the purpose of illustration.

After all, many significant differences in wheat quality parameters and flour composition between various treatment levels were observed. Nevertheless, if enough observations are made, all detected differences become significant at some point, whether of practical relevance or not (ANONYMUS 2001). Consequently, it is necessary that these differences are evaluated critically with respect to a relevant practical meaning. This proposes a big challenge, considering the broad range of end-uses for wheat (cookies, noodles, bread kinds, cakes) and the possible and nowadays common use of bakery improvers which might balance these flaws in raw material (BELITZ *ET AL.* 2009). Our own observations suggest a rather insignificant impact of *Fusarium* infection on quality parameters determined in samples that are within the admissible range of EU_{Max}. However, further investigations with samples from various environments and cultivars differing in baking quality and DON levels below EU_{Max} should be performed to support our results.

Even though we could detect a few effects of *Fusarium* spp. infection on gluten subgroups, e.g. reduction of total glutenin and HMW-GS, we did not observe any moderate to strong significant linear relationships between quality parameters and gluten protein and subfractions (Tab 63). This leads to the conclusion that in our study neither environmental conditions, or agricultural practices,

or *Fusarium* infection caused such a variation in gluten content and composition that it led to a significant effect on wheat quality. Significant differences within quality parameters were primarily caused by year which was demonstrated by higher SoE when compared to other effects as pre-crop, cultivar, and location (Tab 54-Tab 59).

For future research, which has the objective to elucidate more of the complex interaction between flour composition, quality parameters, and *Fusarium* infection, we would suggest a less complex field experiment with a 2-factorial design (cultivar x inoculation) including at least two cultivars from each quality group according to BSA (E, A, B), differing in their PC as well as in their BV. These should be grown after just one pre-crop where the *Fusarium* inoculum potential coming from the soil is considered to be low e.g. sugar beet or rapeseed. Cultivars should than be treated with *Fusarium* macroconidia suspension with differing in density of spores during wheat anthesis. As control one could use untreated plots (natural *Fusarium* infection) as well as plots where triazole fungicide is applied directly after artificial inoculation. Under favorable weather conditions for *Fusarium* spp. infection this treatment should lead to varying degrees of fungal growth and subsequent mycotoxin production within grain of the different cultivars. Variation within grain components is than mainly due to genotype and intensity of *Fusarium* infection and relationships can then be easily calculated without interference of other factors. Especially interesting would be the "production" and investigation of infected grain were DON levels are below $\leq$1.25 mg kg^{-1}.

Further research should also focus on the direct causes for changes in wheat quality after *Fusarium* infection. In chapter 5.5 three possible reasons (premature death of spikelet, fungal enzymes, mycotoxin) were discussed how *Fusarium* infection might have an impact on flour composition and subsequently on quality parameters. At least one of them or all three at the same time most likely led to observed changes in $PC_{Type550}$, SV, FN, DS, BV, total gluten, total glutenin, HMW-GS gliadin/glutenin and LMW/HMW. It would be interesting, to clear the separate effect of the possible causes. E.g. over the period of grain-filling, the first-time occurrence of *Fusarium* DNA, mycotoxins, and fungal enzymes could be investigated and observed together with the development of important grain components such as starch and gluten protein (and subfractions). At the same time the moment where grain stops developing should be observed and compared to non-infected wheat spikes. This could provide further inside to whether e.g. gluten proteins are already affected in the field by fungal infection or whether changes in gluten composition are primarily due to enzymatic degradation during processing. In addition, the impact of *Fusarium* spp. that do not produce DON (e.g. *F. avenaceum*, *F. equiseti*, or "knock-out mutants" of *F. graminearum*) on grain quality could be interesting and would elucidate the significance of DON in impairing biosynthesis of kernel constituents more.

7 Summary

Fusarium spp. infection of wheat, also known as *Fusarium* head blight (FHB), is a worldwide occurring disease with a disastrous impact on both grain yield and wheat quality in years of severe epidemics. Wheat quality is primarily degraded because several *Fusarium* spp. associated to FHB are capable to produce mycotoxins which occur in the harvested grain and pose a threat to human as well as animal health. Aside from the toxicological risk coming from mycotoxin contamination of grain, *Fusarium* infection changes grain composition affecting proteins, starch, and other grain constituents. Numerous studies on FHB have rather focused on the pathogen complex, epidemiology, *Fusarium* mycotoxins, and control strategies than on impact on technological quality of wheat. Studies that have been published in this field demonstrated the severe impact of FHB in rather less practical samples, either in just severely *Fusarium* damaged kernels (FDK) or composite samples that exceeded legal thresholds for mycotoxin content of wheat e.g. deoxynivalenol (DON) many-fold. Therefore, it was the key objective of this study to investigate the impact of *Fusarium* infection on bread wheat that was cultivated under agricultural measures (pre-crop, cultivar, fungicide) that are known to have an influence on the intensity of natural *Fusarium* infection. Therefore, a field experiment was conducted over the time period of 2007-2009 where two bread wheat cultivars that differed widely in their susceptibility against FHB but hardly in their quality attributes were grown after pre-crop winter wheat, (forage) maize, and sugar beet using minimum tillage. Strobilurin, chlorthalonil, and triazole application during growth stage 31-39 was expected to add to natural variation within FHB disease parameters and subsequent mycotoxin levels in grain. Different milling products (whole grain flour (WGF), flour Type 550, bran) were gained from composite samples of cleaned wheat and investigated for *Fusarium* biomass, measured as *Fusarium* DNA of the most prevalent *Fusarium* spp. in Europe, *F. graminearum* (*Fg*DNA) and *F. culmorum* (*Fc*DNA*)*. Furthermore, mycotoxin contents (DON, 3-ADON, 15-ADON, NIV, FUSX, ZEA) of milling products were analyzed. *Fg*DNA made up the major proportion of total *Fusarium* DNA in all milling product; DON was the most frequently detected and quantitatively most important mycotoxin. 3-ADON and ZEA were detected sporadically. The occurrence and quantity of *Fg*DNA and DON in wheat milling products highly depended on environment (year, location), pre-crop, susceptibility of cultivar, fungicide application, and milling product. FHB occurred only in two out of three cultivations years, demonstrating the significance of weather condition during anthesis. Cultivation of the less susceptible cultivar after pre-crop sugar beet and application of triazole fungicide during shooting represented the most effective measure to avoid high DON levels in milling products. *Fg*DNA content was highest in bran, followed by WGF and flour Type 550. On the contrary, DON content was equally high in bran and flour Type 550 and lowest in WGF. Even though, grain was cleaned, DON levels in flour Type 550 ranged from < LOQ to 11.84 mg kg^{-1}. Possible reasons e.g.

tempering for this contradiction are discussed. The correlation of *Fg*DNA, DON, and visual disease parameters was investigated. *Fg*DNA and DON content of all milling products generally correlated strongly with visual disease as well as with/among each other.

In the second part of this study, the influence of environment (year, location), pre-crop, cultivar, and *Fusarium* infection on wheat quality was investigated in flour Type 550 (and partly WGF) by the determination of commonly used parameters that evaluate bread wheat quality. Several quality traits were supposed to assess the properties of flour (SV, WG, FN, WA), gluten (R_{Max}, EXT), dough (DDT, DST, DS), and bread (BV, shape). Furthermore, the impact of the respective factors on selected flour components (PC, starch, total gluten, gliadin [ω5, ω1,2, α, γ], glutenin [ωb, HMW-GS, LMW-GS]) as well as protease activity (PA) in flour was determined. Since *Fusarium* infection had been demonstrated to be influenced by all experimental factors and wheat quality and flours composition was expected to be as well influenced by environment and agricultural measures, ANCOVA was used to investigate the effect of *Fusarium* infection on the latter. DON was chosen as covariate that quantified *Fusarium* infection in flours since EU_{Max} for DON represent the most important criterion for further use of wheat. In general, all investigated parameters were particularly influenced by year, independently of *Fusarium* infection. Slight quantitative differences e.g. in PC, WG, FN, SV due to pre-crop could generally only be detected across samples of all years and interacted with cultivar and year. Even though quality traits of both cultivars were classified to be similar according to BSA, in interaction with environment, differences between cultivars e.g. in PC, FN became visible. However, most differences appeared to be insignificant from the perspective of processing. Investigation of the relationship between DON and quality parameters as well as flour components by ANCOVA and subsequent linear regression demonstrated a significant effect of *Fusarium* infection on $PC_{Type550}$, SV, FN, DS, BV, total gluten, total glutenin, HMW-GS and gluten composition (gliadin/glutenin, LMW/HMW) as well as on PA. The direction and strength of effects is discussed as well as the possibility of spurious correlation due to interference with other experimental factors. In general, a clear effect of *Fusarium* infection on wheat quality could only be demonstrated within few flour samples highly exceeding $EU_{Max.}$ Consequently, these flours were not relevant for further processing and human consumption. In addition, the quantitative changes in wheat quality caused by fungal infection were overall insignificant. This was emphasized by the lack of correlation between flour components, particularly gluten proteins, as well as PA with quality parameters. Based on our observations, the impact of *Fusarium* infection on technological wheat quality and flour composition seems insignificant in samples that contain less DON than demanded by EU. However, further investigations with more cultivars differing in their susceptibility against FHB and differing in baking performance should be carried out. The key objective should be the investigation of wheat samples that contain less than 1.25 mg kg^{-1} DON which represents EU_{MAX} for unprocessed cereals.

8 Supplementary material

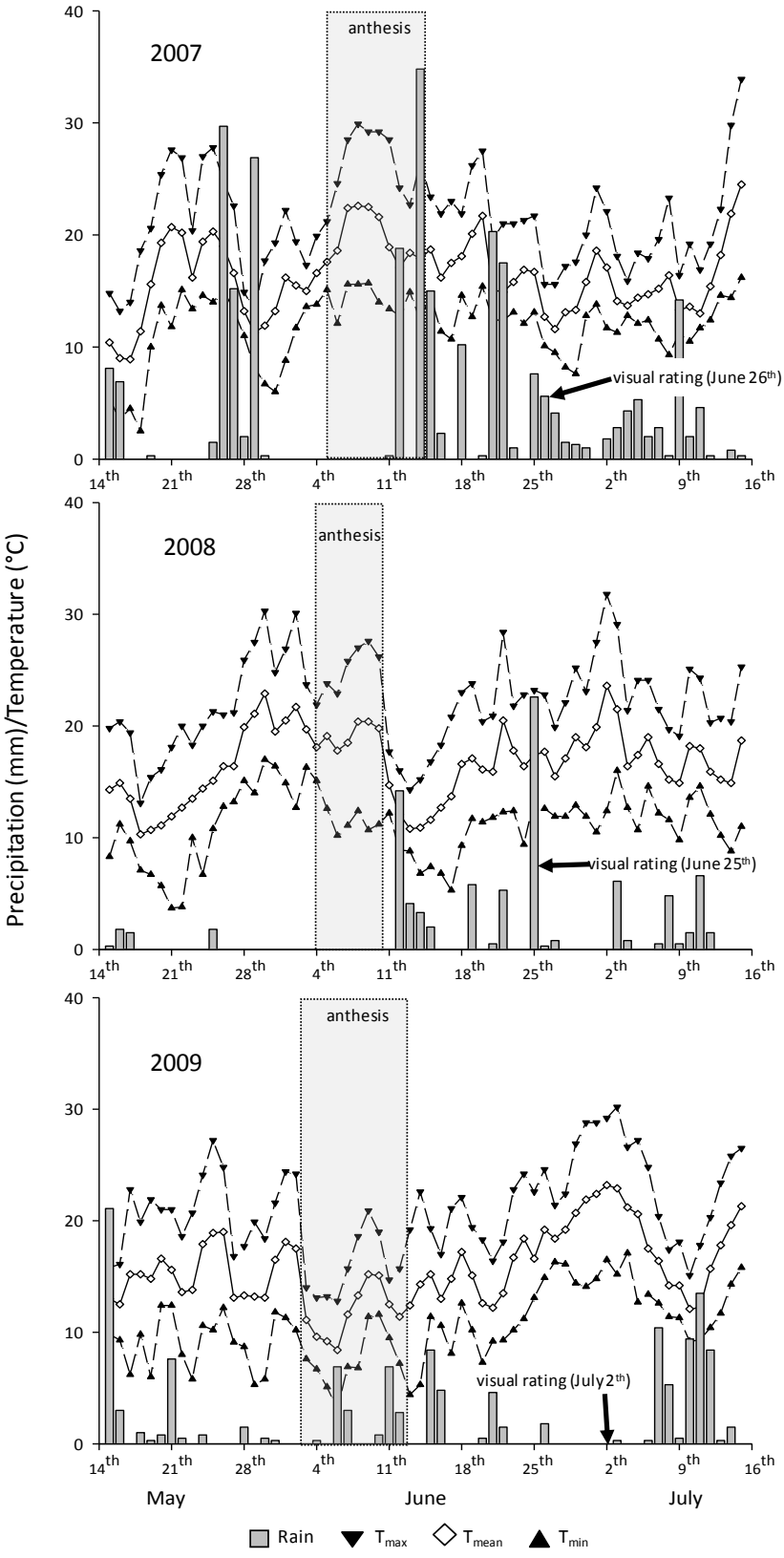

Fig 30. Weather conditions from May 14th to July 16th in 2007, 2008, and 2009: rain incidences with precipitation (mm) and maximum/average/minimum daily temperatures (T_{max}/T_{mean}/T_{min}) (°C) (WSG 2010)

Tab 54. Descriptive statistic, multiple comparison of treatment mean value (MV) for effect of year, location, pre-crop, cultivar, and fungicide as well as severity of effect (SoE) for PC_{WGF}, $PC_{Type550}$, WG, SV, and FN

		PC_{WGF}		$PC_{Type\ 550}$		WG		SV		FN	
Dimension		**%**		**%**		**%**		**ml**		**s**	
n		108		108		108		108		108	
MV		12.5		10.8		31.2		84.6		323.3	
SD		1.3		0.8		6.2		4.9		91.7	
CV (%)		10.1		7.7		19.8		5.7		28.4	
Effect	**Factor level**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**
Year	**2007**	14.0 c	**1.23**	11.7 c	**1.14**	38.02 c	**1.44**	83.67 a	**1.03**	249 a	**1.63**
	2008	11.3 a		10.2 a		29.07 b		84.03 a		315 b	
	2009	12.3 b		10.4 b		26.39 a		86.17 b		406 c	
Location	**Gladebeck**	12.4 a	**1.02**	10.7 a	**1.02**	31.12 a	**1.00**	84.34 a	**1.01**	334 b	**1.07**
	Torland	12.7 b		10.9 b		31.20 a		84.91 a		312 a	
Pre-crop	**Maize**	12.3 a	**1.05**	10.6 a	**1.05**	30.19 a	**1.09**	82.40 a	**1.05**	314 a	**1.17**
	Sugar beet	12.4 a		10.6 a		30.25 a		85.28 b		303 a	
	Wheat	12.9 b		11.2 b		33.04 b		86.19 b		353 b	
Cultivar	**Centrum**	12.4 a	**1.03**	10.8 a	**1.00**	28.97 a	**1.15**	84.38 a	**1.01**	282 a	**1.29**
	Ritmo	12.7 b		10.8 a		33.35 b		84.87 a		365 b	
Fungicide	**Chlorthalonil**	12.6 b	**1.01**	10.8 b	**1.01**	31.22 a	**1.01**	84.17 a	**1.01**	333 b	**1.05**
	Strobilurin	12.6 b		10.8 b		31.26 a		84.94 a		321 ab	
	Triazole	12.4 a		10.7 a		31.00 a		84.76 a		316 a	

n number of observations, MV mean value, SD standard diviation, CV coefficient of variation, SoE severity of effect, PC_{WGF} protein content of whole grain flour, $PC_{Type550}$ protein content of flour Type 550, WG wet gluten, SV sedimentation value, FN falling number, same letters indicate no differences between treatment levels at a significance level of $p<0.05$ according to Tukey test

Tab 55. Descriptive statistic, multiple comparison of treatment mean value (MV) for effect of year, location, pre-crop, cultivar, and fungicide as well as severity of effect (SoE) for WA and dough properties (DDT, DST, DS)

		WA		DDT		DST		DS	
Dimension		**%**		**min**		**min**		**FU/VU**	
n		84		84		84		84	
MV		59.2		1.7		3.6		78.1	
SD		4.0		0.6		3.9		39.1	
CV (%)		6.7		35.1		109.5		50.1	
Effect	**Factor level**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**
Year	**2007**	63.5 c	**1.16**	1.4 a	**1.53**	1.3 a	**5.99**	95.0 b	**2.38**
	2008	57.2 b		2.0 b		2.8 a		90.7 b	
	2009	54.9 a		2.1 b		7.8 b		40.0 a	
Location	**Gladebeck**	59.0 a	**1.01**	1.8 a	**1.05**	4.4 a	**1.55**	65.4 a	**1.39**
	Torland	59.5 a		1.7 a		2.8 a		90.7 b	
Pre-crop	**Maize**	58.3 a	**1.10**	1.9 a	**1.25**	3.7 a	**2.66**	83.8 a	**1.18**
	Sugar beet	58.9 a		1.7 a		4.3 a		70.7 a	
	Wheat	63.8 b		1.5 a		1.6 a		74.8 a	
Cultivar	**Centrum**	58.6 a	**1.02**	1.5 a	**1.27**	3.8 a	**1.19**	73.4 a	**1.14**
	Ritmo	59.9 a		1.9 b		3.2 a		83.5 a	
Fungicide	**Chlorthalonil**	59.3 a	**1.01**	1.8 a	**1.07**	3.5 a	**1.10**	81.2 a	**1.06**
	Strobilurin	58.8 a		1.8 a		3.8 a		76.5 a	
	Triazole	59.4 a		1.7 a		3.5 a		77.6 a	

n number of observations, MV mean value, SD standard diviation, CV coefficient of variation, SoE severity of effect, WA water absorption, DDT dough development time, DST dough stability, DS dough softening, same letters indicate no differences between treatment levels at a significance level of $p<0.05$ according to Tukey test

Tab 56. Descriptive statistic, multiple comparison of treatment mean value (MV) for effect of year, location, pre-crop, cultivar, and fungicide as well as severity of effect (SoE) for baking properties (BV, LH, LL, LW)

		BV		LH		LL		LW	
Dimension		ml 100 g^{-1} flour		mm		mm		mm	
n		84		84		84		84	
MV		339.2		22.7		70.7		31.1	
SD		69.3		1.8		5.5		2.5	
CV (%)		20.4		7.8		7.8		7.9	
Effect	**Factor level**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**
Year	**2007**	408 c	**1.51**	21 a	**1.15**	76 b	**1.15**	33 c	**1.14**
	2008	305 b		24 c		66 a		30 b	
	2009	270 a		23 b		68 a		29 a	
Location	**Gladebeck**	328 a	**1.07**	23 a	**1.02**	71 a	**1.02**	31 a	**1.02**
	Torland	350 a		22 a		70 a		31 a	
Pre-crop	**Maize**	345 a	**1.28**	23 b	**1.10**	69 a	**1.14**	32 a	**1.05**
	Sugar beet	321 a		23 b		69 a		31 a	
	Wheat	410 b		21 a		79 b		32 a	
Cultivar	**Centrum**	330 a	**1.06**	23 a	**1.01**	70 a	**1.02**	30 a	**1.04**
	Ritmo	349 a		23 a		71 a		32 b	
Fungicide	**Chlorthalonil**	339 a	**1.01**	23 a	**1.02**	31 a	**1.01**	71 a	**1.01**
	Strobilurin	341 a		22 a		31 a		71 a	
	Triazole	337 a		23 a		31 a		70 a	

n number of observations, MV mean value, SD standard diviation, CV coefficient of variation, SoE severity of effect, BV baking volume, LH loaf heigth, LL loaf length, LW loaf width, same letters indicate no differences between treatment levels at a significance level of p<0.05 according to Tukey test

Tab 57. Descriptive statistic, multiple comparison of treatment mean value (MV) for effect of year, location, pre-crop, cultivar, and fungicide as well as severity of effect (SoE) for total gluten, total gliadin, and gliadin subfractions (ω5, ω1,2, α, γ)

		Total Gluten		Gliadin		ω5-Gliadin		ω1,2-Gliadin		α-Gliadin		γ-Gliadin	
Dimension		% Protein		% Protein		% Protein		% Protein		% Protein		% Protein	
n		84		108		108		108		108		108	
MV		77.8		56.9		4.5		3.5		27.0		22.0	
SD		5.0		5.4		0.6		0.4		2.1		3.3	
CV (%)		6.4		9.5		13.8		12.1		7.9		14.8	
Effect	**Factor level**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**
Year	**2007**	76.7 a	**1.02**	57.0 b	**1.17**	4.7 c	**1.12**	3.7 c	**1.14**	26.4 a	**1.10**	22.1 b	**1.31**
	2008	77.0 ab		52.4 a		4.2 a		3.2 a		26.0 a		19.0 a	
	2009	78.4 b		61.3 c		4.5 b		3.5 b		28.5 b		24.8 c	
Location	**Gladebeck**	77.1 a	**1.01**	56.7 a	**1.01**	4.5 a	**1.02**	3.5 a	**1.01**	27.0 a	**1.00**	21.6 a	**1.03**
	Torland	77.6 a		57.1 a		4.4 a		3.4 a		26.9 a		22.3 b	
Pre-crop	**Maize**	76.5 a	**1.03**	56.9 a	**1.02**	4.4 ab	**1.08**	3.4 a	**1.11**	26.9 a	**1.02**	22.3 b	**1.12**
	Sugar beet	78.8 b		57.5 a		4.3 a		3.3 a		26.8 a		23.1 b	
	Wheat	76.9 a		56.2 a		4.6 b		3.7 b		27.3 a		20.6 a	
Cultivar	**Centrum**	77.7 a	**1.01**	55.9 a	**1.04**	4.4 a	**1.04**	3.4 a	**1.00**	26.7 a	**1.02**	21.3 a	**1.06**
	Ritmo	77.0 a		57.9 b		4.6 b		3.5 a		27.3 a		22.7 b	
Fungicide	**Chlorthalonil**	77.5 a	**1.01**	56.6 a	**1.01**	4.5 a	**1.02**	3.4 a	**1.02**	26.9 a	**1.01**	21.9 a	**1.01**
	Strobilurin	77.4 a		56.8 a		4.4 a		3.4 a		27.0 a		21.9 a	
	Triazole	77.1 a		57.3 a		4.5 a		3.5 a		27.2 a		22.2 a	

n number of observations, MV mean value, SD standard diviation, CV coefficient of variation, SoE severity of effect, same letters indicate no differences between treatment levels at a significance level of p<0.05 according to Tukey test

Tab 58. Descriptive statistic, multiple comparison of treatment mean value (MV) for effect of year, location, pre-crop, cultivar, and fungicide as well as severity of effect (SoE) for total glutenin, glutenin subfractions (ωb, HMW-GS, LMW-GS), gliadin/glutenin ratio, and LMW/HMW ratio

		Glutenin			ωb			HMW-GS			LMW-GS			Gliadin/Glutenin			LMW/HMW		
Dimension		% Protein			% Protein			% Protein			% Protein								
n		105			105			105			105			105			105		
MV		21.0			1.0			5.8			14.2			2.9			2.4		
SD		5.1			0.4			1.2			3.7			1.2			0.3		
CV (%)		24.1			43.1			20.1			25.9			41.5			13.1		
Effect	**Factor level**	**MV**		**SoE**	**MV**		**SoE**	**MV**		**SoE**	**MV**		**SoE**	**MV**		**SoE**	**MV**		**SoE**
Year	**2007**	19.8	a	**1.33**	1.1	b	**1.73**	5.5	a	**1.18**	13.2	a	**1.39**	3.30	b	**1.56**	2.4	b	**1.19**
	2008	24.5	b		1.2	b		6.5	b		16.9	b		2.18	a		2.6	c	
	2009	18.4	a		0.7	a		5.6	a		12.1	a		3.41	b		2.2	a	
Location	**Gladebeck**	21.1	a	**1.02**	1.0	a	**1.09**	5.7	a	**1.03**	14.3	a	**1.04**	2.88	a	**1.06**	2.5	b	**1.06**
	Torland	20.7	a		1.0	a		5.9	a		13.8	a		3.05	a		2.3	a	
Pre-crop	**Maize**	19.5	a	**1.15**	1.0	a	**1.12**	5.4	a	**1.14**	13.2	a	**1.16**	3.34	b	**1.23**	2.5	b	**1.09**
	Sugar beet	22.6	b		1.1	a		6.1	b		15.3	b		2.72	a		2.5	b	
	Wheat	20.6	ab		1.0	a		6.0	b		13.6	a		2.84	ab		2.3	a	
Cultivar	**Centrum**	21.8	b	**1.09**	1.1	b	**1.17**	6.0	b	**1.06**	14.8	b	**1.11**	2.82	a	**1.10**	2.5	b	**1.05**
	Ritmo	20.0	a		0.9	a		5.7	a		13.3	a		3.11	a		2.3	a	
Fungicide	**Chlorthalonil**	21.2	a	**1.04**	1.0	a	**1.10**	5.9	a	**1.03**	14.3	a	**1.04**	2.85	a	**1.11**	2.4	a	**1.00**
	Strobilurin	21.1	a		1.0	a		5.9	a		14.2	a		2.87	a		2.4	a	
	Triazole	20.4	a		0.9	a		5.7	a		13.7	a		3.17	a		2.4	a	

n number of observations, MV mean value, SD standard diviation, CV coefficient of variation, SoE severity of effect, same letters indicate no differences between treatment levels at a significance level of $p<0.05$ according to Tukey test

Tab 59. Descriptive statistic, multiple comparison of treatment mean value (MV) for effect of year, location, pre-crop, cultivar, and fungicide as well as severity of effect (SoE) for gluten properties (R_{MAX}, EXT, R_{MAX}/EXT), starch content, PA, yield parameters (TKW, GY), S content, and N/S ratio of flour Type 550

		R_{MAX}*		EXT*		R_{MAX}/EXT		Starch		PA		TKW		GY		S		N/S	
Dimension		**N**		**mm**		**N/m**		**%**		**AU 440 nm**		**g**		**dt ha^{-1}**		**%**			
n		18		18		36		108		36		108		108		72		72	
MV		0.64		109		4.0		75.6		0.53		45.7		78.4		0.138		14.0	
SD		0.06		8.8		2.1		2.4		0.18		6.8		20.3		0.010		0.7	
CV (%)		8.95		8.1		51.8		3.2		34.4		15.0		25.9		7.1		5.1	
Effect	**Factor level**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**	**MV**	**SoE**
Year	**2007**	-		-		-		73.5 a	**1.06**	-		42.7 b	**1.33**	64.8 a	**1.60**	0.145 b	**1.11**	14.1 b	**1.03**
	2008	-		-		-		75.7 b		-		53.8 c		103.5 c		0.131 a		13.8 a	
	2009	-		-		-		77.7 c		-		40.5 a		67.0 b		-		-	
Location	**Gladebeck**	0.66 a	**1.05**	109 a	**1.00**	4.1 a	**1.04**	75.6 a	**1.00**	0.51 a	**1.08**	45.0 a	**1.03**	81.4 b	**1.08**	0.137 a	**1.02**	13.7 a	**1.04**
	Torland	0.63 a		109 a		4.0 a		75.7 a		0.55 b		46.3 b		75.4 a		0.139 a		14.2 b	
Pre-crop	**Maize**	0.60 a	**1.15**	108 a	**1.08**	3.7 a	**1.20**	77.2 b	**1.03**	0.59 b	**1.33**	45.5 b	**1.09**	76.6 b	**1.13**	0.137 a	**1.01**	13.7 a	**1.05**
	Sugar beet	0.69 b		105 a		4.4 a		74.9 a		0.44 a		47.7 c		84.2 c		0.139 a		13.7 a	
	Wheat	0.64 ab		113 a		4.0 a		74.7 a		0.55 b		43.8 a		74.5 a		0.138 a		14.4 b	
Cultivar	**Centrum**	-		-		6.0 b	**2.92**	75.6 a	**1.00**	0.45 a	**1.35**	48.1 b	**1.11**	83.1 b	**1.13**	0.139 a	**1.01**	13.8 a	**1.02**
	Ritmo	-		-		2.1 a		75.6 a		0.61 b		43.2 a		73.8 a		0.138 a		14.1 a	
Fungicide	**Chlorthalonil**	0.66 a	**1.05**	112 a	**1.05**	4.0 a	**1.04**	75.5 a	**1.00**	0.54 b	**1.15**	44.8 a	**1.04**	76.9 a	**1.05**	0.138 a	**1.01**	14.0 a	**1.00**
	Strobilurin	0.63 a		108 a		3.9 a		75.6 a		0.56 b		45.6 b		77.4 a		0.139 a		13.9 a	
	Triazole	0.64 a		107 a		4.1 a		75.8 a		0.49 a		46.7 c		81.0 b		0.137 a		14.0 a	

* only measurable for cultivar Centrum, n number of observations, MV mean value, SD standard diviation, CV coefficient of variation, SoE severity of effect, R_{MAX} resistance to extension, EXT extensibility, PA protease activity, TKW thousand kernel weight, GY grain yield, S sulfur, N/S ratio of nitrogen (N) and sulfur, same letters indicate no differences between treatment levels at a significance level of $p<0.05$ according to Tukey test

Tab 60. Significance of effects from ANOVA for indirect quality parameters (PC_{WGF}, $PC_{Type550}$, WG, SV, FN, WA), dough properties (DDT, DST, DS), baking properties (BV, LH, LL, LW), gluten properties (R_{MAX}, EXT, R_{MAX}/EXT), and starch content including all analyzed samples

Effect	PC_{WGF}	$PC_{Type\ 550}$	WG	SV	FN	WA	DDT	DST	DS	BV	LH	LL	LW	R_{MAX}	EXT	R_{MAX}/EXT	Starch
Year (Y)	***	***	***	**	***	***	***	***	***	***	***	***	***				***
Location (L)	***	***	n.s.	n.s.	***	*	n.s.	**	***	**	n.s.	*	(*)	n.s.	n.s.	n.s.	n.s.
Y x L	***	***	**	n.s.	***	*	***	***	***	**	n.s.	n.s.	n.s.				**
Pre-crop (P)	***	n.s.	***	n.s.	***	(*)	**	n.s.	***	(*)	(*)	*	*	n.s.	n.s.	(*)	***
Y x P	***	***	***	*	*	(*)	**	n.s.	**	n.s.	(*)	n.s.	(*)				***
L x P	***	n.s.	n.s.	n.s.	(*)	n.s.	n.s.	n.s.	**	(*)	*	*	n.s.	n.s.	n.s.	n.s.	(*)
Y x L x P	***	**	**	n.s.	*	n.s.	**	(*)	*	n.s.	n.s.	n.s.	n.s.				***
Cultivar (C)	***	***	***	***	***	**	***	n.s.	**	*	n.s.	*	*			***	n.s.
Y x C	***	***	***	***	(*)	(*)	*	**	***	*	n.s.	(*)	*				n.s.
L x C	**	***	***	n.s.	*	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.			n.s.	n.s.
Y x L x C	***	(*)	***	n.s.	*	n.s.	***	*	n.s.	(*)	*	n.s.	n.s.				n.s.
P x C	***	*	*	*	n.s.	(*)	**	n.s.	**	n.s.	(*)	n.s.	(*)			n.s.	n.s.
Y x P x C	*	*	**	(*)	n.s.	n.s.	**	n.s.	*	*	*	n.s.	n.s.				n.s.
L x P x C	n.s.	n.s.	*	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.			n.s.	n.s.
Y x L x P x C	**	*	*	n.s.	(*)	n.s.	**	(*)	(*)	n.s.	(*)	n.s.	n.s.				n.s.
Fungicide (F)	***	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Y x F	n.s.	(*)	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.				n.s.
L x F	*	n.s.	n.s.	**	n.s.	n.s.	*	*	*	n.s.	n.s.	(*)	n.s.	n.s.	n.s.	n.s.	n.s.
Y x L x F	*	*	n.s.	***	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.				n.s.
P x F	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Y x P x F	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.				n.s.
L x P x F	(*)	n.s.	n.s.	(*)	n.s.	n.s.	(*)	n.s.	(*)	n.s.	(*)	n.s.	n.s.			n.s.	n.s.
Y x L x P x C	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	*	(*)	n.s.	n.s.	n.s.	n.s.				n.s.
C x F	*	n.s.	n.s.	n.s.	n.s.	n.s.	(*)	n.s.	*	n.s.	n.s.	n.s.	n.s.			n.s.	n.s.
Y x C x F	(*)	(*)	n.s.	n.s.	(*)	n.s.	(*)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.				n.s.
L x C x F	*	*	n.s.	*	n.s.	n.s.	(*)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.			n.s.	n.s.
Y x L x C x F	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	(*)	n.s.	n.s.	n.s.	n.s.	n.s.				n.s.
P x C x F	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.			n.s.	n.s.
Y x P x C x F	(*)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.				n.s.
L x P x C x F	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	(*)	n.s.				n.s.

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1; and n.s. = not significant, PC_{WGF} protein content of whole grain flour, $PC_{Type550}$ protein content of flour Type 550, WG wet gluten, SV sedimentation value, FN falling number, WA water absorption, DDT dough development time, DST dough stability, DS dough softening, BV baking volume, LH loaf height, LL loaf length, LW loaf width, R_{MAX} resistance to extension, EXT extensibility

Tab 61. Significance of effects from ANOVA for total gluten, total gliadin, gliadin subfractions (ω5, ω1,2, α, γ), total glutenin, glutenin subfractions (ωb, HMW-GS, LMW-GS, gliadin/glutenin ratio, LMW/HMW ratio, protease activity, TKW, grain yield, S content and N/S ratio including all analyzed samples

Effect	Total Gluten	Gliadin	ω5-Gliadin	ω1,2-Gliadin	α-Gliadin	γ-Gliadin	Glutenin	ωb	HMW-GS	LMW-GS	Gliadin/Glutenin	LMW/HMW	PA	TKW	GY	S	N/S
Year (Y)	*	***	**	***	**	***	***	***	***	***	**	***		***	***	***	*
Location (L)	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	***	*	***	***	n.s.	**
Y x L	*	n.s.	n.s.	n.s.	n.s.	(*)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		***	***	n.s.	(*)
Pre-crop (P)	**	n.s.	*	***	n.s.	***	**	n.s.	**	**	*	***	***	***	***	n.s.	**
Y x P	***	(*)	**	*	(*)	n.s.	**	*	**	*	(*)	n.s.		***	***	(*)	n.s.
L x P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***	***	***	(*)	n.s.
Y x L x P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		*	***	(*)	*
Cultivar (C)	n.s.	*	*	n.s.	n.s.	**	*	*	(*)	*	n.s.	**	***	***	***	n.s.	n.s.
Y x C	**	n.s.	***	(*)	n.s.	n.s.	*	*	n.s.	*	n.s.	***		***	***	(*)	n.s.
L x C	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***	***	n.s.	n.s.
Y x L x C	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	(*)		n.s.	n.s.	n.s.	n.s.
P x C	*	(*)	*	**	n.s.	(*)	*	**	n.s.	*	n.s.	**	*	n.s.	**	n.s.	(*)
Y x P x C	***	*	**	*	n.s.	**	*	n.s.	*	*	n.s.	(*)		n.s.	**	n.s.	n.s.
L x P x C	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Y x L x P x C	**	n.s.	n.s.	n.s.	n.s.	(*)	n.s.	n.s.	n.s.	n.s.	n.s.	(*)		n.s.	*	n.s.	n.s.
Fungicide (F)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	***	***	n.s.	n.s.
Y x F	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		**	**	n.s.	n.s.
L x F	n.s.	n.s.	**	**	n.s.	*	*	n.s.	***	n.s.	(*)	***	**	(*)	*	n.s.	n.s.
Y x L x F	**	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	**	*	(*)	*		n.s.	*	n.s.	n.s.
P x F	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	(*)	n.s.	n.s.
Y x P x F	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.
L x P x F	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	(*)	n.s.	n.s.	n.s.	***	n.s.	n.s.	n.s.	n.s.
Y x L x P x C	(*)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	(*)	n.s.	n.s.	n.s.		(*)	n.s.	n.s.	n.s.
C x F	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.
Y x C x F	(*)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.
L x C x F	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	(*)	n.s.	n.s.
Y x L x C x F	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.
P x C x F	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Y x P x C x F	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.
L x P x C x F	(*)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, respectively; (*) = p < 0.1; and n.s. = not significant, PA protease activity, TKW thousand kernel weight, GY grain yield, S sulfur, N/S ratio of nitrogen (N) and sulfur

Tab 62. Correlation matrix for yield components and quality parameters of flour (+/- direction of trend, R^2, significance) for all analyzed samples

	GY			TKW			PC_{WGF}			$PC_{Type550}$			SV			WG			FN			WA			DDT			DST			DS			BV			LH			LL			LW			R_{MAX}/EXT			R_{MAX}			EXT		
TKW	+	0.75	***																																																	
PC_{WGF}	-	0.54	***	-	0.29	***																																													
$PC_{Type550}$	-	0.33	***	-	0.10	***	+	0.83	***																																										
SV	-	0.02	n.s.	-	0.01	n.s.	+	0.00	n.s.	+	0.00	n.s.																																							
WG	-	0.18	***	-	0.07	**	+	0.63	***	+	0.67	***	-	0.00	n.s.																																					
FN	-	0.03	n.s.	-	0.14	***	-	0.07	**	-	0.14	***	+	0.01	n.s.	-	0.10	***																																	
WA	-	0.21	***	-	0.06	**	+	0.69	***	+	0.73	***	-	0.00	n.s.	+	0.83	***	-	0.33	***																														
DDT	+	0.01	n.s.	+	0.01	n.s.	-	0.07	*	-	0.06	*	+	0.02	n.s.	-	0.04	n.s.	+	0.34	***	-	0.19	***																										
DST	+	0.01	n.s.	-	0.00	n.s.	-	0.05	*	-	0.10	**	+	0.01	n.s.	-	0.22	***	+	0.23	***	-	0.31	***	+	0.15	***																				
DS	+	0.00	n.s.	+	0.03	n.s.	+	0.05	*	+	0.06	*	-	0.00	n.s.	+	0.15	***	-	0.41	***	+	0.23	***	-	0.19	***	-	0.36	***																		
BV	-	0.26	***	-	0.06	**	+	0.66	***	+	0.72	***	-	0.00	n.s.	+	0.75	***	-	0.24	***	+	0.79	***	-	0.13	***	-	0.26	***	+	0.21	***														
LH	+	0.40	***	+	0.27	***	-	0.54	***	-	0.44	***	+	0.00	n.s.	-	0.31	***	+	0.14	***	-	0.45	***	+	0.21	***	+	0.09	**	-	0.09	**	-	0.36	***												
LL	-	0.39	***	-	0.32	***	+	0.59	***	+	0.55	***	+	0.00	n.s.	+	0.53	***	-	0.08	**	+	0.54	***	-	0.13	***	-	0.12	**	+	0.02	n.s.	+	0.44	***	-	0.54	***								
LW	-	0.11	***	-	0.03	n.s.	+	0.34	***	+	0.30	***	-	0.00	n.s.	+	0.46	***	-	0.17	***	+	0.50	***	-	0.13	***	-	0.20	***	+	0.43	***	+	0.57	***	-	0.17	***	+	0.11	**						
R_{MAX}/EXT	+	0.36	***	+	0.30	***	+	0.24	**	-	0.04	n.s.	-	0.00	n.s.	-	0.43	***	-	0.19	**	-	0.31	***	-	0.14	*	-	0.17	*	-	0.11	*	-	0.38	***	+	0.00	n.s.	+	0.01	n.s.	-	0.54	***						
R_{MAX}	+	0.09	***	+	0.02	**	-	0.15	**	-	0.01	n.s.	-	0.09	n.s.	-	0.06	***	-	0.02	*	-	0.05	***	-	0.16	*	-	0.03	*	-	0.07	*	-	0.08	***	+	0.00	n.s.	+	0.09	n.s.	-	0.09	***	+	0.54	***			
EXT	-	0.06	***	-	0.27	***	+	0.03	**	+	0.03	n.s.	+	0.00	n.s.	+	0.01	***	+	0.06	**	+	0.04	**	+	0.11	*	+	0.06	**	+	0.00	n.s.	+	0.03	***	+	0.01	n.s.	-	0.01	n.s.	+	0.12	***	-	0.47	***	-	0.00	***

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, and n.s. = not significant, GY grain yield, TKW thousand-kernel weight, PC_{WGF} protein content of whole grain flour, $PC_{Type550}$ protein content of flour Type 550, SV sedimentation value, WG wet gluten, FN falling number, WA water absorption, DDT dough development time, DST dough stability, DS dough softening, BV baking volume, LH loaf height, LL loaf length, LW loaf width, R_{MAX} resistance to extension, EXT extensibility

Tab 63. Correlation matrix for yield components, quality parameters of flour, flour components, and PA (+/- direction of trend, R^2, significance) for all analyzed samples

	GY		TKW		PC_{WGF}		$PC_{Type550}$		SV		WG		FN		WA		DDT		DST		DS		BV		LH		LL		LW		R_{MAX}/EXT		R_{MAX}		EXT	
Starch	+	0.00	**n.s.**	-	0.00	**n.s.**	-	0.28	***	-	0.33	***	+	0.02	**n.s.**	-	0.43	***	+	0.17	***	-	0.55	***	+	0.15	***	+	0.17	***	-	0.09	**	+	0.47	***	+	0.20	***	-	0.32	***	+	0.22	***	+	0.06	**n.s.**	+	0.00	**n.s.**	-	0.01	**n.s.**
Total Gluten	-	0.01	**n.s.**	-	0.02	**n.s.**	-	0.01	**n.s.**	-	0.02	**n.s.**	+	0.08	**	-	0.02	**n.s.**	+	0.02	**n.s.**	-	0.04	**n.s.**	+	0.01	**n.s.**	-	0.00	**n.s.**	-	0.00	**n.s.**	-	0.06	*	+	0.09	**	-	0.02	**n.s.**	-	0.11	**	+	0.13	*	+	0.03	*	-	0.01	**n.s.**
Total Gliadin	-	0.26	***	-	0.38	***	+	0.02	**n.s.**	-	0.00	**n.s.**	-	0.00	**n.s.**	-	0.01	**n.s.**	+	0.11	***	-	0.02	**n.s.**	+	0.01	**n.s.**	+	0.02	**n.s.**	-	0.05	*	-	0.01	**n.s.**	-	0.00	**n.s.**	+	0.01	**n.s.**	-	0.02	**n.s.**	-	0.01	**n.s.**	-	0.00	**n.s.**	+	0.07	**n.s.**
ω5	-	0.01	**	-	0.17	***	+	0.07	**	+	0.04	*	-	0.19	***	+	0.07	**	+	0.01	**n.s.**	+	0.11	**	-	0.03	**n.s.**	-	0.06	*	+	0.00	**n.s.**	+	0.08	*	-	0.05	*	+	0.13	***	-	0.02	**n.s.**	+	0.03	**n.s.**	+	0.19	**n.s.**	-	0.09	**n.s.**
ω1,2	-	0.20	***	-	0.20	***	+	0.17	***	+	0.16	***	-	0.01	**n.s.**	+	0.11	***	-	0.00	**n.s.**	+	0.24	***	-	0.09	**	-	0.08	**	+	0.01	**n.s.**	+	0.22	***	-	0.12	**	+	0.23	***	+	0.10	**	-	0.01	**n.s.**	-	0.20	**n.s.**	+	0.19	**n.s.**
α	-	0.09	**	-	0.19	***	-	0.03	**n.s.**	-	0.02	**n.s.**	-	0.00	**n.s.**	-	0.03	**n.s.**	+	0.13	***	-	0.06	*	+	0.00	**n.s.**	+	0.02	**n.s.**	-	0.02	**n.s.**	-	0.04	**n.s.**	+	0.02	**n.s.**	-	0.00	**n.s.**	+	0.02	**n.s.**	-	0.01	**n.s.**	-	0.19	**n.s.**	+	0.13	**n.s.**
γ	-	0.30	***	-	0.37	***	+	0.28	*	-	0.00	**n.s.**	+	0.00	**n.s.**	-	0.01	**n.s.**	+	0.09	**	-	0.04	**n.s.**	+	0.03	**n.s.**	+	0.06	*	-	0.08	**	-	0.02	**n.s.**	+	0.00	**n.s.**	+	0.00	**n.s.**	-	0.03	**n.s.**	-	0.04	**n.s.**	-	0.12	**n.s.**	+	0.00	**n.s.**
Total Glutenin	+	0.22	***	+	0.26	***	-	0.08	**	-	0.02	**n.s.**	+	0.11	***	-	0.01	**n.s.**	-	0.04	*	-	0.01	**n.s.**	+	0.00	**n.s.**	-	0.02	**n.s.**	+	0.01	**n.s.**	-	0.03	**n.s.**	+	0.08	*	-	0.04	**n.s.**	-	0.04	**n.s.**	+	0.16	*	+	0.07	*	-	0.05	*
ωb-GS	+	0.07	**	+	0.14	***	+	0.00	**n.s.**	+	0.03	**n.s.**	+	0.01	**n.s.**	+	0.05	*	-	0.14	***	+	0.08	**	-	0.02	**n.s.**	-	0.09	**	+	0.06	*	+	0.03	**n.s.**	-	0.00	**n.s.**	+	0.00	**n.s.**	+	0.00	**n.s.**	+	0.13	*	+	0.08	*	-	0.07	*
HMW-GS	+	0.11	***	+	0.15	***	-	0.05	*	-	0.01	**n.s.**	+	0.28	***	-	0.01	**n.s.**	-	0.01	**n.s.**	+	0.01	**n.s.**	+	0.00	**n.s.**	-	0.02	**n.s.**	+	0.02	**n.s.**	-	0.02	**n.s.**	+	0.05	*	-	0.02	**n.s.**	-	0.03	**n.s.**	+	0.07	**n.s.**	-	0.03	**n.s.**	-	0.02	**n.s.**
LMW-GS	+	0.26	***	+	0.30	***	-	0.10	***	-	0.04	**n.s.**	+	0.08	**	-	0.01	**n.s.**	-	0.04	*	-	0.01	**n.s.**	+	0.00	**n.s.**	-	0.02	**n.s.**	+	0.01	**n.s.**	-	0.04	**n.s.**	+	0.10	**	-	0.06	*	-	0.05	*	+	0.19	**	-	0.03	**n.s.**	-	0.06	*
Gliadin/Glutenin	-	0.15	***	-	0.20	***	+	0.07	**	+	0.02	**n.s.**	-	0.29	***	+	0.01	**n.s.**	+	0.00	**n.s.**	+	0.01	**n.s.**	-	0.01	**n.s.**	+	0.00	**n.s.**	+	0.00	**n.s.**	+	0.03	**n.s.**	-	0.06	*	+	0.02	**n.s.**	+	0.03	**n.s.**	-	0.02	**n.s.**	-	0.10	**n.s.**	-	0.01	**n.s.**
LMW/HMW	+	0.20	***	+	0.20	***	-	0.05	*	-	0.02	**n.s.**	-	0.15	***	-	0.00	**n.s.**	-	0.08	**	-	0.00	**n.s.**	+	0.00	**n.s.**	-	0.00	**n.s.**	-	0.00	**n.s.**	-	0.02	**n.s.**	+	0.04	**n.s.**	-	0.07	*	-	0.01	**n.s.**	+	0.16	*	+	0.09	*	-	0.09	**
PA	-	0.08	**n.s.**	-	0.41	***	+	0.05	**n.s.**	+	0.00	**n.s.**	+	0.00	**n.s.**	+	0.04	**n.s.**	+	0.07	**n.s.**	+	0.05	**n.s.**	+	0.04	**n.s.**	-	0.01	**n.s.**	+	0.07	**n.s.**	+	0.00	**n.s.**	-	0.24	**	-	0.00	**n.s.**	+	0.13	*	-	0.28	**	-	0.10	**	+	0.06	**
S	-	0.56	***	-	0.37	***	+	0.62	***	+	0.62	***	+	0.01	**n.s.**	+	0.49	***	-	0.17	***	+	0.57	***	-	0.17	**	-	0.22	***	+	0.03	**n.s.**	+	0.50	***	-	0.39	***	+	0.41	***	+	0.27	***	-	0.02	**n.s.**	-	0.09	**n.s.**	+	0.10	**n.s.**
N/S	-	0.14	**	-	0.07	*	+	0.21	***	+	0.30	***	+	0.00	**n.s.**	+	0.16	***	+	0.02	**n.s.**	+	0.23	***	+	0.04	**n.s.**	+	0.01	**n.s.**	-	0.06	**n.s.**	+	0.25	***	-	0.07	*	+	0.12	**	+	0.00	**n.s.**	-	0.00	**n.s.**	+	0.02	**n.s.**	+	0.09	**n.s.**

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, and n.s. = not significant, GY grain yield, TKW thousand-kernel weight, PC_{WGF} protein content of whole grain flour, $PC_{Type550}$ protein content of flour Type 550, SV sedimentation value, WG wet gluten, FN falling number, WA water absorption, DDT dough development time, DST dough stability, DS dough softening, BV baking volume, LH loaf height, LL loaf length, LW loaf width, R_{MAX} resistance to extension, EXT extensibility, PA Protease activity, S sulfur content of flour Type 550, N/S ratio of nitrogen (N) and sulfur content of flour Type 550

Tab 64. Correlation matrix for flour components, and PA (+/- direction of trend, R^2, significance) for all analyzed samples

	Starch			Total Gluten			Total Gliadin			ω5			ω1,2			α			γ			Total Glutenin			ωb-GS			HMW-GS			LMW-GS			Gliadin/Glutenin			LMW/HMW			PA			S	
Total Gluten	+	0.02	n.s.																																									
Total Gliadin	+	0.05	n.s.	+	0.28	***																																						
ω5	-	0.03	n.s.	+	0.06	**	+	0.39	***																																			
ω1,2	-	0.05	*	+	0.06	*	+	0.34	***	+	0.66	***																																	
α	+	0.05	*	+	0.21	***	+	0.78	***	+	0.38	***	+	0.41	***																														
γ	+	0.08	**	+	0.25	***	+	0.81	***	+	0.12	***	+	0.07	**	+	0.38	***																											
Total Glutenin	-	0.00	n.s.	+	0.18	***	-	0.30	***	-	0.18	***	-	0.16	***	-	0.24	***	-	0.21	***																							
ωb-GS	-	0.07	**	+	0.12	***	-	0.29	***	-	0.04	*	-	0.04	*	-	0.27	***	-	0.24	***	+	0.78	***																			
HMW-GS	-	0.00	n.s.	+	0.25	***	-	0.15	***	-	0.20	***	-	0.10	***	-	0.12	***	-	0.09	**	+	0.82	***	+	0.47	***															
LMW-GS	-	0.00	n.s.	+	0.15	*	-	0.33	***	-	0.18	***	-	0.18	***	-	0.25	***	-	0.24	***	+	0.99	***	+	0.79	***	+	0.73	***												
Gliadin/Glutenin	+	0.00	n.s.	-	0.06	n.s.	+	0.29	***	+	0.30	***	+	0.17	***	+	0.21	***	+	0.20	***	-	0.67	***	-	0.44	***	-	0.66	***	-	0.64	***									
LMW/HMW	-	0.01	n.s.	-	0.00	*	-	0.23	***	-	0.01	n.s.	-	0.09	**	-	0.19	***	-	0.21	***	+	0.20	***	+	0.37	***	+	0.00	n.s.	+	0.30	***	-	0.04	n.s.							
PA	+	0.00	n.s.	-	0.12	n.s.	-	0.01	***	-	0.01	n.s.	+	0.00	n.s.	-	0.00	n.s.	-	0.03	n.s.	-	0.06	n.s.	-	0.02	n.s.	-	0.05	n.s.	-	0.06	n.s.	+	0.00	n.s.	-	0.00	n.s.				
S	-	0.19	***	-	0.01	n.s.	+	0.04	n.s.	+	0.01	n.s.	+	0.10	**	-	0.01	n.s.	+	0.12	**	-	0.07	*	+	0.00	n.s.	-	0.05	n.s.	-	0.09	*	+	0.05	n.s.	-	0.05	n.s.	+	0.01	n.s.		
N/S	-	0.11	**	-	0.00	n.s.	+	0.02	n.s.	+	0.02	n.s.	+	0.07	*	+	0.01	n.s.	+	0.02	n.s.	-	0.03	n.s.	-	0.02	n.s.	-	0.00	n.s.	-	0.04	n.s.	+	0.02	n.s.	-	0.10	**	-	0.00	n.s.	-	0.01	n.s.

p significance: ***, **, and * = p < 0.001, 0.01, 0.05, and n.s. = not significant, PA Protease activity, S sulfur content of flour Type 550, N/S ratio of nitrogen (N) and sulfur content of flour Type 550

Tab 65. Mineral N (N_{min}) content of soil (90 cm depth) after different pre-crops (MV of two locations) and fertilization regime with urea-ammonium nitrate (UAN) at different growth stages (GS) according to BBCH

N partition	Pre-crop	2007	2008	2009
N_{min} (kg ha^{-1})	Wheat	-	71	84
	Maize	-	51	69
	Sugar beet	-	55	60
1st N donation	GS	29	23	21
	UAN (kg ha^{-1})	60	60	60
2nd N donation	GS	31/32	29	27
	UAN (kg ha^{-1})	60	60	70
3rd N donation	GS	39	39	49
	UAN (kg ha^{-1})	65	60	60
Total N (kg ha^{-1}) without N_{min}		185	180	190

Tab 66. Grain yield (GY), thousand-kernel weight (TKW), sulfur content (S), and ratio of nitrogen:sulfur content (N/S) of flour Type 550 (MV ± SD, n=6) from cv. Centrum and cv. Ritmo grown after different pre-crops in 2007, 2008, and 2009

Year	Cultivar	Pre-crop	GY (dt ha^{-1})				TKW (g)				S (%)				N/S			
2007	Centrum	Wheat	69.3	±	2.8	bc	42.7	±	2.1	bc	0.143	±	0.008	a	14.4	±	0.7	bc
		Maize	75.1	±	4.9	c	43.6	±	2.4	c	0.142	±	0.003	a	14.0	±	0.2	ab
		Sugar beet	68.5	±	8.5	bc	46.4	±	1.9	d	0.148	±	0.007	a	13.9	±	0.1	ab
	Ritmo	Wheat	55.1	±	8.3	a	39.3	±	2.7	a	0.144	±	0.006	a	15.1	±	0.4	c
		Maize	58.2	±	8.2	a	40.4	±	2.2	ab	0.147	±	0.005	a	13.5	±	0.4	a
		Sugar beet	62.6	±	6.5	ab	43.9	±	2.9	c	0.149	±	0.006	a	13.9	±	0.4	ab
2008	Centrum	Wheat	108.2	±	6.9	bc	55.9	±	0.9	b	0.136	±	0.008	b	13.8	±	0.6	ab
		Maize	101.1	±	9.6	ab	57.1	±	1.0	b	0.130	±	0.002	ab	13.7	±	0.6	ab
		Sugar beet	111.2	±	3.9	c	56.3	±	2.0	b	0.133	±	0.006	ab	13.3	±	0.9	a
	Ritmo	Wheat	99.5	±	5.1	a	49.7	±	3.0	a	0.130	±	0.001	ab	14.4	±	0.3	b
		Maize	93.7	±	9.4	a	51.6	±	2.7	a	0.131	±	0.007	ab	13.7	±	1.2	ab
		Sugar beet	107.4	±	2.4	bc	52.0	±	2.0	a	0.124	±	0.006	a	13.8	±	0.3	b
2009	Centrum	Wheat	60.7	±	3.2	a	40.7	±	1.1	b		-				-		
		Maize	70.6	±	7.2	b	43.1	±	1.6	b		-				-		
		Sugar beet	82.9	±	7.8	c	47.1	±	1.1	c		-				-		
	Ritmo	Wheat	54.1	±	5.1	a	34.7	±	0.6	a		-				-		
		Maize	60.8	±	4.7	a	37.2	±	1.4	a		-				-		
		Sugar beet	72.7	±	6.0	b	40.5	±	1.1	b		-				-		

GY grain yield, TKW thousand-kernel weight, S sulfur content of flour Type 550, N/S ratio of nitrogen (N) and sulfur (S) content of flour Type 550, same letters indicate no differences between cultivars and pre-crops **within** years at a significance level of $p < 0.05$ according to Tukey test

Tab 67. Linear relationship between quality parameters and sulfur content (S) of flour Type 550 and grain yield (GY)

Dependent variable	n	Independent variable	Trend	R^2	p
PC_{WGF}	72	S	+	0.62	***
$PC_{Type550}$	72		+	0.62	***
WA	60		+	0.57	***
BV	60		+	0.50	***
GY	108	TKW	+	0.75	***
	108	PC_{WGF}	-	0.54	***
	72	S	-	0.56	***

n number of observations, p significance: *** = $p < 0.001$, PC_{WFG} protein content of whole grain flour, $PC_{Type550}$ protein content of flour Type 550, WA water absorption, BV baking volume, S sulfur, GY grain yield

9 References

Anonymus (2001) Primer on statistical significance and P values *Effective clinical practice* 4: 183-184.

Anonymus (2011) Gladio® Bravo ® Pack (Syngenta) Retrieved 03/03/2011 from http://www.syngenta.de/syngenta_infos/pdf_dateien/prd_ga/ga_gladio_bravo_pack_de.pdf

Abbas, H. K., C. J. Mirocha, R. J. Pawlosky and D. J. Pusch (1985) Effect of cleaning, milling, and baking on deoxynivalenol in wheat *Applied and Environmental Microbiology* 50: 482-486.

Abedi, T., A. Alemzadeh and S. A. Kazemeyni (2011) Wheat yield and grain protein response to nitrogen amount and timing *Australian Journal of Crop Science* 5: 327-333.

Adejumo, T. O., U. Hettwer and P. Karlovsky (2007) Survey of maize from south-western Nigeria for zearalenone, alpha- and beta-zearalenols, fumonisin B-1 and enniatins produced by Fusarium species *Food Additives and Contaminants* 24: 993-1000.

Anjum, F. M., M. R. Khan, A. Din, M. Saeed, I. Pasha and M. U. Arshad (2007) Wheat gluten: high molecular weight glutenin subunits-structure, genetics, and relation to dough elasticity *Journal of Food Science* 72: R56-R63.

Antes, S., B. Birzele, A. Prange, J. Krämer, A. Meier, H.-W. Dehne and P. Köhler (2001) Rheological and breadmaking properties of wheat samples infected with Fusarium spp. *Mycotoxin Research* 17: 76-80.

Argyris, J., D. Van Sanford and D. TeKrony (2003) *Fusarium graminearum* infection during wheat seed development and its effect on seed quality *Crop Science* 43: 1782-1788.

Aufhammer, W., E. Kübler, H.-P. Kaul, W. Hermann, D. Höhn and C. Yi (2000) Ährenbefall mit Fusarien (*F. graminearum, F. culmorum*) in Deoxynivalenolgehalt in Korngut von Winterweizen in Abhängigkeit von der N-Düngung Pflanzenbauwissenschaften 4: 72-78.

Axford, D. W. E., E. E. McDermott and D. G. Redman (1979) Sodium dodecyl sulfate test of breadmaking quality - comparison with Pelshenke and Zeleny tests *Cereal Chemistry* 56: 582-584.

Ayoub, M., S. Guertin, J. Fregeaureid and D. L. Smith (1994) Nitrogen fertilizer effect on breadmaking quality of hard red spring wheat in eastern Canada *Crop Science* 34: 1346-1352.

Bai, G. H., R. Plattner, A. Desjardins and F. Kolb (2001) Resistance to *Fusarium* head blight and deoxynivalenol accumulation in wheat *Plant Breeding* 120: 1-6.

Bai, G. H. and G. Shaner (1994) Scab of Wheat - Prospects for Control *Plant Disease* 78: 760-766.

Bai, G. H. and G. Shaner (2004) Management and resistance in wheat and barley to *Fusarium* head blight *Annual Review of Phytopathology* 42: 135-161.

Barrier-Guillot, B., M. Delambre, A. Morel, C. Maumere, H. Gouet, F. Grosjean and M. Leuillet (2004). Occurrence of deoxynivalenol in wheat (*Triticum aestivum*) grown in France and identification of factors involved in content variations. In: *An Overview on Toxigenic Fungi and Mycotoxins in Europe*. (Ed.) A. Logrieco and A. Visconti. Dordrecht, The Netherlands, Kluwer Academic Publishers: 101-108.

Bechtel, D. B., L. A. Kaleikau, R. L. Gaines and L. M. Seitz (1985) The Effects of *Fusarium graminearum* Infection on Wheat Kernels *Cereal Chemistry* 62: 191-197.

Beck, R. and J. Lepschy (2000). Ergebnisse aus dem *Fusarium*-Monitoring 1989-1999-Einfluss der produktionstechnischen Faktoren Fruchtfolge und Bodenbearbeitung. In: *Schriftenreihe der Bayerischen Landesanstalt für Bodenkultur und Pflanzenbau*. (Ed.) Bayerische Landesanstalt für Bodenkultur und Pflanzenbau. Freising und München: 39-47.

Belitz, H.-D., W. Grosch and P. Schieberle (Ed.) (2009). Food Chemistry. Berlin Heidelberg, Springer-Verlag: 670-745

Berova, S. and M. Mladenov (1974) Influence of wheat ear and grain Fusariosis (*Fusarium graminearum* Schwabe) on the chemical, technological and baking qualities (in Bulgarian) *Rastenievudni Nauki* 11: 125-133.

Beyer, M., M. B. Klix, H. Klink and J. A. Verreet (2006) Quantifying the effects of previous crop, tillage, cultivar and triazole fungicides on the deoxynivalenol content of wheat grain - a review *Journal of Plant Diseases and Protection* 113: 241-246.

Bhatt, G. M. and N. F. Derera (1975) Genotype x environment interactions for, heritabilities of, and correlations among quality traits in wheat *Euphytica* 24: 597-604.

Bly, A. G. and H. J. Woodard (2003) Foliar nitrogen application timing influence on grain yield and protein concentration of hard red winter and spring wheat *Agronomy Journal* 95: 335-338.

BMELV (2009). Statistisches Jahrbuch über Ernährung, Landwirtschaft und Forsten 2009 Bremerhaven, Wirtschaftverlag NW GmbH.

Bockmann, H. (1964) Qualität und Backfähigkeit von Weizen bei Befall von *Septoria nodorum* Berk. und *Fusarium culmorum* Link (in German) *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* 16: 5-10.

Bottalico, A. (1998) *Fusarium* diseases of cereals: Species complex and related mycotoxin profiles, in Europe *Journal of Plant Pathology* 80: 85-103.

Bottalico, A. and G. Perrone (2002) Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in *Europe European Journal of Plant Pathology* 108: 611-624.

Boutigny, A. L., F. Richard-Forget and C. Barreau (2008) Natural mechanisms for cereal resistance to the accumulation of *Fusarium* trichothecenes *European Journal of Plant Pathology* 121: 411-423.

Boyacioglu, D. and N. S. Hettiarachchy (1995) Changes in some Biochemical Components of Wheat Grain that was infected with *Fusarium graminearum Journal of Cereal Science* 21: 57-62.

Boyacioglu, D., N. S. Hettiarachchy and B. L. Dappolonia (1993) Additives affect deoxynivalenol (vomitoxin) flour during breadbaking *Journal of Food Science* 58: 416-418.

Brandfass, C. and P. Karlovsky (2006) Simultaneous detection of *Fusarium culmorum* and *F-graminearum* in plant material by duplex PCR with melting curve analysis *Bmc Microbiology* 6.

Brandfass, C. and P. Karlovsky (2008) Upscaled CTAB-Based DNA Extraction and Real-Time PCR Assays for *Fusarium culmorum* and *F. graminearum* DNA in Plant Material with Reduced Sampling Error *International Journal of Molecular Sciences* 9: 2306-2321.

Brooker, B. E. (1996) The Role of Fat in the Stabilisation of Gas Cells in Bread Dough *Journal of Cereal Science* 24: 187-198.

BSA (2007). Beschreibende Sortenliste Getreide, Mais, Ölfrüchte, Leguminosen (grobkörnig), Hackfrüchte (außer Kartoffeln). Hannover, Deutscher Landwirtschaftsverlag.

Buerstmayr, H., T. Ban and J. A. Anderson (2009) QTL mapping and marker-assisted selection for *Fusarium* head blight resistance in wheat: a review *Plant Breeding* 128: 1-26.

Burlakoti, R. R., R. Estrada, V. V. Rivera, A. Boddeda, G. A. Secor and T. B. Adhikari (2007) Real-time PCR quantification and mycotoxin production of *Fusarium graminearum* in wheat inoculated with isolates collected from potato, sugar beet, and wheat *Phytopathology* 97: 835-841.

Büttner, P. (2006) Das Artenspektrum der Gattung *Fusarium* an Weizen und Roggen in Bayern in den Jahren 2003 und 2004 *Gesunde Pflanzen* 58: 28-33.

Buyanovsky, G. and G. Wagner (1986) Post-harvest residue input to cropland *Plant and Soil* 93: 57-65.

Campbell, C. A., D. R. Cameron, W. Nicholaichuk and H. R. Davidson (1977) Effects of fertilizer N and soil moisture on growth, N content, and moisture use by spring wheat *Canadian Journal of Soil Science* 57: 289-310.

Casale, W. L. and L. P. Hart (1988) Inhibition of 3H-leucine incorporation by trichothecene mycotoxins in maize and wheat tissue *Phytopathology* 78: 1673-1677.

Chilosi, G., C. Caruso, C. Caporale, L. Leonardi, L. Bertini, A. Buzi, M. Nobile, P. Magro and V. Buonocore (2000) Antifungal activity of Bowman-Birk-type trypsin inhibitor from wheat kernel *Journal of Phytopathology* 148: 477-481.

Christ, D. S., R. Goedeck, A. von Tiedemann and M. Varrelmann (2011a) Pathogenicity, symptom development, and mycotoxin formation in wheat by *Fusarium* species frequently isolated from sugar beet *Phytopathology* in press.

Christ, D. S., B. Maerlaender and M. Varrelmann (2011b) Characterization and mycotoxigenic potential of Fusarium species in freshly-harvested and stored sugar beet in Europe *Phytopathology* in press.

Clarke, J. M., C. A. Campbell, H. W. Cutforth, R. M. Depauw and G. E. Winkleman (1990) Nitrogen and phosphorus uptake, translocation, and utilizatiob efficiency of wheat in relation to environment and cultivar yield and protein levels *Canadian Journal of Plant Science* 70: 965-977.

Daniel, C. and E. Triboi (2000) Effects of temperature and nitrogen nutrition on the grain composition of winter wheat: Effects on gliadin content and composition *Journal of Cereal Science* 32: 45-56.

De Wolf, E. D., L. V. Madden and P. E. Lipps (2003) Risk assessment models for wheat *Fusarium* head blight epidemics based on within-season weather data *Phytopathology* 93: 428-435.

Delgado, J. A., P. B. Schwarz, J. Gillespie, V. V. Rivera-Varas and G. A. Secor (2010) Trichothecene Mycotoxins Associated with Potato Dry Rot Caused by *Fusarium graminearum Phytopathology* 100: 290-296.

Dencic, S., N. Mladenov and B. Kobiljski (2011) Effects of genotype and environment on breadmaking quality in wheat International Journal of Plant Production 5: 71-81.

Dexter, J. E., R. M. Clear and K. R. Preston (1996) *Fusarium* head blight: Effect on the milling and baking of some Canadian wheats *Cereal Chemistry* 73: 695-701.

Dexter, J. E. and R. R. Matsuo (1977) Influence of protein content on some durum wheat quality parameters *Canadian Journal of Plant Science* 57: 717-727.

Dexter, J. E. and T. W. Nowicki (2003). Safety Assurance and Quality Assurance Issues Associated with Fusarium Head Blight in Wheat.In: *Fusarium* Head Blight of Wheat and Barley. (Ed.) K. J. Leonard and W. R. Bushnell. St. Paul, APS Press: 420-460.

Dill-Macky, R. and R. K. Jones (2000) The effect of previous crop residues and tillage on *Fusarium* head blight of wheat *Plant Disease* 84: 71-76.

Dimmock, J. and M. J. Gooding (2002) The effects of fungicides on Hagberg falling number and blackpoint in winter wheat *Crop Protection* 21: 475-487.

Doohan, F. M., J. Brennan and B. M. Cooke (2003) Influence of climatic factors on *Fusarium* species pathogenic to cereals *European Journal of Plant Pathology* 109: 755-768.

Eckert, B., T. Amend and H. D. Belitz (1993) The course of the SDS and Zeleny sedimentation tests for gluten quality and related phenomena studies using the light microscope *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* 196: 122-125.

Edwards, S. G. (2004) Influence of agricultural practices on *Fusarium* infection of cereals and subsequent contamination of grain by trichothecene mycotoxins *Toxicology Letters* 153: 29-35.

Edwards, S. G. and N. P. Godley (2010) Reduction of *Fusarium* head blight and deoxynivalenol in wheat with early fungicide applications of prothioconazole *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment* 27: 629-635.

Edwards, S. G., S. R. Pirgozliev, M. C. Hare and P. Jenkinson (2001) Quantification of trichothecene-producing *Fusarium* species in harvested grain by competitive PCR to determine efficacies of fungicides against *Fusarium* head blight of winter wheat *Applied and Environmental Microbiology* 67: 1575-1580.

Eggert, K., H. M. Rawel and E. Pawelzik (2011) *In vitro* degradation of wheat gluten fractions by *Fusarium graminearum* proteases *European Food Research and Technology* in press.

Eggert, K., H. Wieser and E. Pawelzik (2010) The influence of *Fusarium* infection and growing location on the quantitative protein composition of (part I) emmer (*Triticum dicoccum*) *European Food Research and Technology* 230: 837-847.

Elbanna, A. A., P. Y. Lau and P. M. Scott (1983) Fate of mycotoxins during processing of foodstuffs 2. Deoxynivalenol (vomitoxin) during making of Egyptian bread *Journal of Food Protection* 46: 484-486.

EMAN (2011) Deoxynivalenol. Basic Factsheet. Naturally Occurring Deoxynivalenol and Related Compounds. Retrieved 03/07/2011, from http://services.leatherheadfood.com/mycotoxins/display.asp?factsheetid=9&noback=yes&strFiltera=deoxynivalenol.

Eriksen, G. S. and H. Pettersson (2004) Toxicological evaluation of trichothecenes in animal feed *Animal Feed Science and Technology* 114: 205-239.

FAO (2011) Dimesions of need - An atlas for food and agriculture. Staple foods: What do people eat? Retrieved 01/03/2011, from http://www.fao.org/docrep/u8480e/u8480e07.htm.

Finney, K. F. (1954) Physical, chemical, and baking properties of flours milled from scabby wheat In: *17th Ann. Rep. Hard Winter Wheat Quality Lab.* USDA/ARS 1953. crop. Kansas Agric. Exp. Stn., Manhattan.

Finney, K. F. and M. A. Barmore (1948) Loaf volume and protein content of hard winter and spring wheats *Cereal Chemistry* 25: 291-312.

Finney, K. F., J. W. Meyer, F. M. Smith and H. C. Fryer (1957) Effects of foliar spraying of Pawnee wheat with urea solutions on yield, protein content, and protein quality *Agronomy Journal* 49: 341-347.

Foroud, N. A. and F. Eudes (2009) Trichothecenes in Cereal Grains International *Journal of Molecular Sciences* 10: 147-173.

Fuertes-Mendizabal, T., A. Aizpurua, M. B. Gonzalez-Moro and J. M. Estavillo (2010) Improving wheat breadmaking quality by splitting the N fertilizer rate *European Journal of Agronomy* 33: 52-61.

Gang, G., T. Miedaner, U. Schuhmacher, M. Schollenberger and H. H. Geiger (1998) Deoxynivalenol and nivalenol production by *Fusarium culmorum* isolates differing in aggressiveness toward winter rye *Phytopathology* 88: 879-884.

Gartner, B. H., M. Munich, G. Kleijer and F. Mascher (2008) Characterisation of kernel resistance against *Fusarium* infection in spring wheat by baking quality and mycotoxin assessments *European Journal of Plant Pathology* 120: 61-68.

Gauer, L. E., C. A. Grant, D. T. Gehl and L. D. Bailey (1992) Effects of nitrogen fertilization on grain protein content, nitrogen uptake, and nitrogen use efficiency of six spring wheat (*Triticum aestivum* L.) cultivars, in relation to estimated moisture supply *Canadian Journal of Plant Science* 72: 235-241.

Gervais, L., F. Dedryver, J. Y. Morlais, V. Bodusseau, S. Negre, M. Bilous, C. Groos and M. Trottet (2003) Mapping of quantitative trait loci for field resistance to *Fusarium* head blight in an European winter wheat *Theoretical and Applied Genetics* 106: 961-970.

Gilbert, J. and A. Tekauz (2000) Review: Recent developments in research on *Fusarium* head blight of wheat in Canada *Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie* 22: 1-8.

GMF (2004) Brotvielfalt: Kleiner Führer durch die deutschen Brot- und Brötchenlande. Retrieved 02/03/2011, from http://www.gmf-info.de/frames.htm.

Gödecke, R. (2010). Einflussfaktoren der Mykotoxinbildung durch Ährenbefall mit *Fusarium* spp. in verschiedenen Winterweizenfruchtfolgen (in German). PhD thesis. Göttingen.

Gödecke, R. and A. von Tiedemann (2011) Effect of the previous crop on *Fusarium* spectrum and mycotoxin contamination in grains of winter wheat *Zuckerindustrie* 136: 172-178.

Goesaert, H., K. Brijs, W. S. Veraverbeke, C. M. Courtin, K. Gebruers and J. A. Delcour (2005) Wheat flour constituents: How they impact bread quality, and how to impact their functionality *Trends in Food Science & Technology* 16: 16-30.

Gosman, N. E. (2001). Analysis of the genetic basis of resistance to *Fusarium culmorum* in wheat. PhD thesis. University of East Anglia, UK.

Goswami, R. and H. Kistler (2004) Heading for disaster: *Fusarium graminearum* on cereal crops *Molecular plant pathology* 5: 515-525.

Grant, C. A., K. R. Brown, G. J. Racz and L. D. Bailey (2001) Influence of source, timing and placement of nitrogen on grain yield and nitrogen removal of durum wheat under reduced- and conventional-tillage management *Canadian Journal of Plant Science* 81: 17-27.

Grausgruber, H., M. Oberforster, M. Werteker, P. Ruckenbauer and J. Vollmann (2000) Stability of quality traits in Austrian-grown winter wheats *Field Crops Research* 66: 257-267.

Groth, J. V., E. A. Ozmon and R. H. Busch (1999) Repeatability and relationship of incidence and severity measures of scab of wheat caused by *Fusarium graminearum* in inoculated nurseries *Plant Disease* 83(11): 1033-1038.

Hagberg, S. (1960) A rapid method for determining alpha-amylase activity *Cereal Chemistry* 37: 218-222.

Hallmann, J., A. Quadt-Hallmann and A. Von Tiedemann (2007). Phytomedizin. Grundwissen Bachelor. Stuttgart, GE.

Hamilton, R. M. G. and H. L. Trenholm (1984) Observations on the Chemical and Nutritive Content of White Winter and Spring Wheats Contaminated with Deoxynivalenol (Vomitoxin) *Animal Feed Science and Technology* 11: 293-300.

Hart, L. P. and W. E. Braselton (1983) Distribution of vomitoxin in dry milled fractions of wheat infected with *Gibberella zeae Journal of Agricultural and Food Chemistry* 31: 657-659.

Hartl, L. (2010) Die Bedeutung des Proteingehaltes von Backweizen aus Sicht der Landwirtschaft *Getreidetechnologie* 64: 111-114.

Hermann, W., W. Aufhammer, E. Kübler and H.-P. Kaul (1999) Auswirkungen von Ährenbefall mit *Fusarium graminearum* auf die Kornqualität von Winterweizen, Wintertriticale und Winterroggen *Pflanzenbauwissenschaften* 3: 82-87.

Hilton, A. J., P. Jenkinson, T. W. Hollins and D. W. Parry (1999) Relationship between cultivar height and severity of *Fusarium* ear blight in wheat *Plant Pathology* 48: 202-208.

Homdork, S., H. Fehrmann and R. Beck (2000) Influence of Different Storage Conditions on the Mycotoxin Production and Quality of *Fusarium*-Infected Wheat Grain *Journal of Phytopathology* 148: 7-15.

Horberg, H. M. (2002) Patterns of splash dispersed conidia of *Fusarium poae* and *Fusarium culmorum European Journal of Plant Pathology* 108: 73-80.

Huebner, F. R., J. Kaczkowski and J. A. Bietz (1990) Quantitative variation of wheat proteins from grain at different stages of maturity and from different spike locations *Cereal Chemistry* 67: 464-470.

Huff, W. E. and W. M. Hagler (1985) Density segregation of corn and wheat naturally contaminated with aflatoxin, deoxynivalenol and zearalenone *Journal of Food Protection* 48: 416-420.

ICC (Ed.) (1999). Standard Methods of the International Association for Cereal Science and Technology (ICC). Vienna, ICC.

Ichinoe, M., H. Kurata, Y. Sugiura and Y. Ueno (1983) Chemotaxonomy of *Gibberella zeae* with special reference to production of trichothecenes and zearalenone *Applied and Environmental Microbiology* 46: 1364-1369.

Jones, B. L. (2005) Endoproteases of barley and malt *Journal of Cereal Science* 42: 139-156.

Jones, B. L., D. Fontanini, M. Jarvinen and A. Pekkarinen (1998) Simplified endoproteinase assays using gelatin or azogelatin *Analytical Biochemistry* 263: 214-220.

Jones, R. K. and C. J. Mirocha (1999) Quality parameters in small grains from Minnesota affected by *Fusarium* head blight *Plant Disease* 83: 506-511.

Jovicevic, B. (1972) Influence of *Fusarium graminearum* chw. on biological and technological characteristics of wheat grain (in Croatian) *Zastita Bilja* 23: 305-309.

Kang, Z. and H. Buchenauer (2000b) Ultrastructural and cytochemical studies on cellulose, xylan and pectin degradation in wheat spikes infected by *Fusarium culmorum Journal of Phytopathology-Phytopathologische Zeitschrift* 148: 263-275.

Kang, Z. S. and H. Buchenauer (2000) Cytology and ultrastructure of the infection of wheat spikes by *Fusarium culmorum Mycological Research* 104: 1083-1093.

Karpenstein-Machan, M. and C. Weber (2010) Energiepflanzenanbau für Biogasanlagen *Naturschutz und Landschaftsplanung* 42: 312-320.

Kieffer, R., H. D. Belitz, M. Zweier, R. Ipfelkofer and G. Fischbeck (1993) A 10-g Micro-version of the Rapid-Mix-Test *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung* 197: 134-136.

Kieffer, R., J.-J. Kim and H. D. Belitz (1981) Zugversuche mit Weizenkleber im Mikromaßstab *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung* 172: 190-192.

Kieffer, R., H. Wieser, M. H. Henderson and A. Graveland (1998) Correlations of the breadmaking performance of wheat flour with rheological measurements on a micro-scale *Journal of Cereal Science* 27: 53-60.

Klix, M. B., M. Beyer and J.-A. Verreet (2008) Effects of cultivar, agronomic practices, geographic location, and meteorological conditions on the composition of selected *Fusarium species* on wheat heads *Canadian Journal of Plant Pathology* 30: 46-57.

Klotzel, M., U. Lauber and H. U. Humpf (2006) A new solid phase extraction clean-up method for the determination of 12 type A and B trichothecenes in cereals and cereal-based food by LC-MS/MS *Molecular Nutrition & Food Research* 50: 261-269.

Koch, H. J., C. Pringas and B. Maerlaender (2006) Evaluation of environmental and management effects on *Fusarium* head blight infection and deoxynivalenol concentration in the grain of winter wheat *European Journal of Agronomy* 24: 357-366.

Koh, A., K. Nishimura and R. Urade (2010) Relationship between endogenous protein disulfide isomerase family proteins and glutenin macropolymer *Journal of Agricultural and Food Chemistry* 58: 12970-12975.

Kolb, F. L., G. H. Bai, G. J. Muehlbauer, J. A. Anderson, K. P. Smith and G. Fedak (2001) Host plant resistance genes for *Fusarium* head blight: Mapping and manipulation with molecular markers *Crop Science* 41: 611-619.

Kushiro, M. (2008) Effects of Milling and Cooking Processes on the Deoxynivalenol Content in Wheat International *Journal of Molecular Sciences* 9: 2127-2145.

Lacey, J., G. L. Bateman and C. J. Mirocha (1999) Effects of infection time and moisture on development of ear blight and deoxynivalenol production by *Fusarium* spp. in wheat *Annals of Applied Biology* 134: 277-283.

Lancova, K., J. Hajslova, M. Kostelanska, J. Kohoutkova, J. Nedelnik, H. Moravcova and M. Vanova (2008) Fate of trichothecene mycotoxins during the processing: Milling and baking *Food Additives and Contaminants* 25: 650-659.

Lemmens, M., K. Haim, H. Lew and P. Ruckenbauer (2004) The effect of nitrogen fertilization on *Fusarium* head blight development and deoxynivalenol contamination in wheat *Journal of Phytopathology* 152: 1-8.

Lidell, C. M. (2003) Systematics of *Fusarium* species and allies associated with *Fusarium* head blight. In: *Fusarium head blight of wheat and barley* (Ed.) K. J. Leonard and W. R. Bushnell. St. Paul, Minnesota, USA, APS Press: 35-43.

Lienemann, K., E. C. Oerke and H. W. Dehne (2001) Variation in the spectrum of *Fusarium* species on winter wheat *Mycotoxin Research* 17: 5-9.

Lindhauer, M. (2009) Brotweizenqualität: Aktueller Status und Perspektiven. In: *Schriftenreihe des Instituts für Pflanzenbau und -züchtung der CAU Kiel: Norddeutsches Weizenforum 2009* (Ed.) H. Kage and F. Hollmann. Kiel, Germany, Instituts für Pflanzenbau und -züchtung der CAU Kiel: 139-145.

Littel, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger and O. Schabenberger (2006) SAS for Mixed Models. Cary, NC, USA, SAS Institue Inc.

Liu, W. Z., W. Langseth, H. Skinnes, O. N. Elen and L. Sundheim (1997) Comparison of visual head blight ratings, seed infection levels, and deoxynivalenol production for assessment of resistance in cereals inoculated with *Fusarium culmorum European Journal of Plant Pathology* 103: 589-595.

Logrieco, A., G. Mulè, A. Moretti and A. Bottalico (2002) Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe *European Journal of Plant Pathology* 108: 597-609.

Magan, N., R. Hope, A. Colleate and E. S. Baxter (2002) Relationship between growth and mycotoxin production by *Fusarium* species, biocides and environment *European Journal of Plant Pathology* 108: 685-690.

Martin, R. A., J. A. Macleod and C. Caldwell (1991) Influence of productions inputs on incidence of infection by *Fusarium* species on cereal seed *Plant Disease* 75: 784-788.

Matthaus, K., S. Danicke, W. Vahjen, O. Simon, J. Wang, H. Valenta, K. Meyer, A. Strumpf, H. Ziesenib and G. Flachowsky (2004) Progression of mycotoxin and nutrient concentrations in wheat after inoculation with *Fusarium culmorum Archives of Animal Nutrition* 58: 19-35.

Matthies, A. and H. Buchenauer (2000) Effect of tebuconazole (Folicur (R)) and prochloraz (Sportak (R)) treatments on *Fusarium* head scab development, yield and deoxynivalenol (DON) content in grains of wheat following artificial inoculation with *Fusarium culmorum Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection* 107: 33-52.

McMullen, M., R. Jones and D. Gallenberg (1997) Scab of wheat and barley: A re-emerging disease of devastating impact *Plant Disease* 81: 1340-1348.

Meier, A., B. Birzele, E. C. Oerke and H. W. Dehne (2000) Impact of growth conditions on the occurrence of *Fusarium* spp. and the mycotoxin content of wheat *Mycotoxin Research* 16: 12-15.

Mesterhazy, A. (1995) Types and components of resistance to *Fusarium* head blight of wheat *Plant Breeding* 114: 377-386.

Mesterhazy, A. (2003). Breeding for for *Fusarium* head blight resistance in Europe. In: *Fusarium head blight of wheat and barely*. (Ed.) K. J. Leonard and W. R. Bushnell. St. Paul, Minnesota, USA, APS Press: 363-381

Meyer, D., D. Weipert and H. Mielke (1986) Beeinflussung der Qualität von Weizen durch den Befall mit *Fusarium culmorum* (in German) *Getreide Mehl Brot* 40: 35-39.

Miedaner, T. (1997) Breeding wheat and rye for resistance to *Fusarium* diseases *Plant Breeding* 116: 201-220.

Miedaner, T., C. Reinbrecht, U. Lauber, M. Schollenberger and H. H. Geiger (2001) Effects of genotype and genotype-enviromment interaction on deoxynivalenol accumulation and resistance to *Fusarium* head blight in rye, triticale, and wheat *Plant Breeding* 120: 97-105.

Miedaner, T., C. Reinbrecht and A. G. Schilling (2000) Association among aggressiveness, fungal colonization, and mycotoxin production of 26 isolates of *Fusarium graminearum* in winter rye head blight *Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection* 107: 124-134.

Miller, J. D. and P. G. Arnison (1986) Degradation of deoxynivalenol by suspension cultures of the resistant wheat cultivar Frontana *Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie* 8: 147-150.

Miller, J. D., R. Greenhalgh, Y. Z. Wang and M. Lu (1991) Trichothecene chemotypes of 3 *Fusarium* species *Mycologia* 83: 121-130.

Miller, J. D., J. C. Young and D. R. Sampson (1985) Deoxynivalenol and *Fusarium* head blight resistance in spring cereals *Phytopathologische Zeitschrift-Journal of Phytopathology* 113: 359-367.

Mladenov, N., N. Przulj, N. Hristov, V. Djuric and M. Milovanovic (2001) Cultivar-by-environment interactions for wheat quality traits in semiarid conditions *Cereal Chemistry* 78: 363-367.

Murphy, P. A., S. Hendrich, C. Landgren and C. M. Bryant (2006) Food mycotoxins: An update *Journal of Food Science* 71: R51-R65.

Nganje, W. E., D. A. Bangsund, F. L. Leistritz, W. W. Wilson and N. M. Tiapo (2004) Regional economic impacts of *Fusarium* Head Blight in wheat and barley *Review of Agricultural Economics* 26: 332-347.

Nicholson, P., E. Chandler, R. C. Draeger, N. E. Gosman, D. R. Simpson, M. Thomsett and A. H. Wilson (2003) Molecular tools to study epidemiology and toxicology of *Fusarium* head blight of cereals *European Journal of Plant Pathology* 109: 691-703.

Nightingale, M. J., B. A. Marchylo, R. M. Clear, J. E. Dexter and K. R. Preston (1999) *Fusarium* head blight: Effect of fungal proteases on wheat storage proteins *Cereal Chemistry* 76: 150-158.

Nowicki, T. W., D. G. Gaba, J. E. Dexter, R. R. Matsuo and R. M. Clear (1988) Retention of *Fusarium* mycotoxin deoxynivalenol in wheat during processing and cooking of spaghetti and noodles *Journal of Cereal Science* 8: 189-202.

Obst, A., J. Lepschy, R. Beck, G. Bauer and A. Bechtel (2000) The risk of toxins by *Fusarium graminearum* in wheat — interactions between weather and agronomic factors *Mycotoxin Research* 16: 16-20.

Oldenburg, E., A. Bramm and H. Valenta (2007a) Influence of nitrogen fertilization on deoxynivalenol contamination of winter wheat - Experimental field trails and evaluation of analytical methods *Mycotoxin Research* 23: 7-12.

Oldenburg, E., J. Brunotte and J. Weinert (2007b) Strategies to reduce DON contamination of wheat with different soil tillage and variety systems *Mycotoxin Research* 23: 73-77.

Osborne, L. E. and J. M. Stein (2007) Epidemiology of *Fusarium* head blight on small-grain cereals *International Journal of Food Microbiology* 119: 103-108.

Osborne, T. B. (1924). The vegetable proteins. London, UK, Longsman, Breen & Co.

Parry, D. W., P. Jenkinson and L. McLeod (1995) *Fusarium* ear blight (scab) in small grain cereals - a review *Plant Pathology* 44: 207-238.

Parry, D. W., T. R. Pettitt, P. Jenkinson and A. K. Lees (1994). The cereal *Fusarium* complex In: Ecology of Plant Pathogens (Ed.) J. P. Blakeman and B. Williamson. Wallingford, UK, CAB International: 301-320.

Paul, P. A., S. M. El-Allaf, P. E. Lipps and L. V. Madden (2005a) Relationships between incidence and severity of *Fusarium* head blight on winter wheat in Ohio *Phytopathology* 95: 1049-1060.

Paul, P. A., P. E. Lipps, D. E. Hershman, M. P. McMullen, M. A. Draper and L. V. Madden (2007) A quantitative review of tebuconazole effect on *Fusarium* head blight and deoxynivalenol content in wheat *Phytopathology* 97: 211-220.

Paul, P. A., P. E. Lipps and L. V. Madden (2005b) Relationship between visual estimates of *Fusarium* head blight intensity and deoxynivalenol accumulation in harvested wheat grain: A meta-analysis *Phytopathology* 95: 1225-1236.

Pawelzik, E., H. H. Permady, J. Weinert and G. A. Wolf (1998) Untersuchungen zum Einfluß einer Fusarien-Kontamination auf ausgewählte Qualitätsmerkmale von Weizen *Getreide Mehl Brot* 52: 264-266.

Pekkarinen, A., L. Mannonen, B. L. Jones and M. L. Niku-Paavola (2000) Production of proteases by *Fusarium* species grown on barley grains and in media containing cereal proteins *Journal of Cereal Science* 31: 253-261.

Pekkarinen, A. I., C. Longstaff and B. L. Jones (2007) Kinetics of the inhibition of *Fusarium* serine proteinases by barley (*Hordeum vulgare* L.) inhibitors *Journal of Agricultural and Food Chemistry* 55: 2736-2742.

Pekkarinen, A. L. and B. L. Jones (2002) Trypsin-like proteinase proteases produced by *Fusarium culmorum* grown on grain proteins *Journal of Agricultural and Food Chemistry* 50: 3849-3855.

Perkowski, J., I. Kiecana, U. Schumacher, H. M. Muller, J. Chelkowski and P. Golinski (1997) Head infection and accumulation of **Fusarium** toxins in kernels of 12 barley genotypes inoculated with *Fusarium graminearum* isolates of two chemotypes *European Journal of Plant Pathology* 103: 85-90.

Pestka, J. J. (2010) Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance *Archives of Toxicology* 84: 663-679.

Pirgozliev, S. R., S. G. Edwards, M. C. Hare and P. Jenkinson (2002) Effect of dose rate of azoxystrobin and metconazole on the development of *Fusarium* head blight and the accumulation of deoxynivalenol (DON) in wheat grain *European Journal of Plant Pathology* 108: 469-478.

Pomeranz, Y. (1988). Chemical composition of kernel structures In: Wheat - Chemistry and Technology. (Ed.) Y. Pomeranz. St. Paul, Minnesota, USA, AACC: 97-158.

Ponts, N., L. Couedelo, L. Pinson-Gadais, M.-N. Verdal-Bonnin, C. Barreau and F. Richard-Forget (2009) *Fusarium* response to oxidative stress by H_2O_2 is trichothecene chemotype-dependent *FEMS Microbiology Letters* 293: 255-262.

Posner, E. S. and A. N. Hibbs (Ed.) (2005) Wheat Flour Milling. St. Paul, Minnesota, U.S.A, American Association of Cereal Chemists: 163-171

Prange, A., B. Birzele, J. Kramer, A. Meier, H. Modrow and P. Kohler (2005) *Fusarium*-inoculated wheat: deoxynivalenol contents and baking quality in relation to infection time *Food Control* 16: 739-745.

Preston, K. R., P. R. March and K. H. Tipples (1982) An assessment of the SDS-sedimentation test for the prediction of Canadian bread wheat quality *Canadian Journal of Plant Science* 62: 545-553.

Raiffeisen (2011) Qualitätskriterien von Weizen Retrieved 08/08/2011, from http://www.raiffeisen.com/pflanzen/ackermanager/weizen_html#Qualitätskriterien von Weizen

Randall, P. J., K. Spencer and J. R. Freney (1981) Sulfur and nitrogen fertilizer effects on wheat. I. Concentrations of sulfur and nitrogen and the nitrogen to sulfur ratio in grain, in relation to the yield response *Australian Journal of Agricultural Research* 32: 203-212.

Reverberi, M., A. Ricelli, S. Zjalic, A. A. Fabbri and C. Fanelli (2010) Natural functions of mycotoxins and control of their biosynthesis in fungi *Applied Microbiology and Biotechnology* 87: 899-911.

Ribichich, K. F., S. E. Lopez and A. C. Vegetti (2000) Histopathological spikelet changes produced by *Fusarium graminearum* in susceptible and resistant wheat cultivars *Plant Disease* 84: 794-802.

Rintelen, J. (2000) Ist das starke Auftreten von *Giberella zeae* (*Fusarium graminearum*) an Getreideähren auf die Zunahme des Maisanbau zurückzuführen? In: *Schriftenreihe der Bayerischen Landesanstalt für Bodenkultur und Pflanzenanbau, Risiken durch den Ährenparasiten Fusarium graminearum - Ergebnisse eines LBP-Forschungsverbundes* (Ed.) Bayerische Landesanstalt für Bodenkultur und Pflanzenbau. Freising und München: 11-14.

Roberts, T. H. and J. Hejgaard (2008) Serpins in plants and green algae *Functional Integrative Genomics* 8: 1-27.

Samar, M. M., M. S. Neira, S. L. Resnik and A. Pacin (2001) Effect of fermentation on naturally occurring deoxynivalenol (DON) in Argentinean bread processing technology *Food Additives and Contaminants* 18: 1004-1010.

Saric, M., R. Sekulic and D. Petric (1980) Influence of *Fusarium* attack on wheat quality (in Croatian) *Zito Hieb* 7: 3-14.

SBA and BMELV (2010) Anbau, Ertrag und Ernte der Feldfrüchte Retrieved 01/03/2011, from http://berichte.bmelv-statistik.de/SJT-3072200-0000.pdf.

SBA and BMELV (div.) Statistisches Jahrbuch über Ernährung. Landwirtschaft und Forsten verschiedener Jahrgänge Retrieved 01/03/ 2011, from http://www.bmelv-statistik.de/index.php?id=139.

Schaafsma, A. W., L. Tamburic-Ilinic, J. D. Miller and D. C. Hooker (2001) Agronomic considerations for reducing deoxynivalenol in wheat grain *Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie* 23: 279-285.

Schade-Schütze, A., J. Muthomi, E. C. Oerke and H. W. Dehne (2000) Yield reduction and mycotoxin contamination of wheat due to *Fusarium culmorum Mycotoxin Research* 16: 34-37.

Schelle, J. (2010). Einfluss von *Fusarium*-Befall auf Gluteneigenschaften im Weizenmehl (in German). Bachelor thesis. Göttingen, Germany.

Schlüter, K., U. Kropf and P. Karlovsky (2006) Untersuchungen zur systemischen Infektion von *Fusarium culmorum* an Winterweizen in Schleswig-Holstein *Gesunde Pflanzen* 58: 107-116.

Schroeder, H. W. and J. J. Christensen (1963) Factors affecting resistance of wheat to scab caused by *Giberella zea Phytopathology* 53: 831-838.

Scott, P. M., S. R. Kanhere, P. Y. Lau, J. E. Dexter and R. Greenhalgh (1983) Effects of experimental flour milling and breadbaking on retention of deoxynivalenol (vomitoxin) in hard red spring wheat *Cereal Chemistry* 60: 421-424.

Seibel, W. (Ed.) (2005). Warenkunde Getreide - Inhaltsstoffe, Analytik, Reinigung, Trocknung, Lagerung, Vermarktung, Verarbeitung. Bergen/Dumme, Deutschland, Agrimedia GmbH: 61-173

Seibel, W. P., K.; Schrader, B. (1985) Analytische, teigrheologische und backtechnische Studien zur Optimierung des Weizen-Backversuches, Rapid-Mix-Test. I. Entwicklung des optimierten Rapid-Mix-Testes (RMT) *Getreide, Mehl und Brot* 39: 174-178.

Seitz, L. M. and D. B. Bechtel (1985) Chemical, physical, and microscopical studies of scab infected hard red winter wheat *Journal of Agricultural and Food Chemistry* 33: 373-377.

Seitz, L. M., W. D. Eustace, H. E. Mohr, M. D. Shogren and W. T. Yamazaki (1986) Cleaning, Milling, and Baking Tests with Hard Red Winter Wheat Containing Deoxynivalenol *Cereal Chemistry* 63: 146-150.

Seitz, L. M., W. T. Yamazaki, R. L. Clements, H. E. Mohr and L. Andrews (1985) Distribution of deoxynivalenol in soft mill streams *Cereal Chemistry* 62: 467-469.

Seling, S. (2010) Die Bedeutung des Proteingehaltes von Backweizen aus Sicht der Wissenschaft *Getreidetechnologie* 64: 103-110.

Shewry, P. R. (2009) Wheat *Journal of Experimental Botany* 60: 1537-1553.

Shewry, P. R. and N. G. Halford (2002) Cereal seed storage proteins: structures, properties and role in grain utilization *Journal of Experimental Botany* 53: 947-958.

Shewry, P. R., N. G. Halford, P. S. Belton and A. S. Tatham (2002) The structure and properties of gluten: an elastic protein from wheat grain *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 357: 133-142.

Shi, C. and L.-L. Xu (2009) Characters of cysteine endopeptidases in wheat endosperm during seed germination and subsequent seedling growth *Journal of Integrative Plant Biology* 51: 52-57.

Shotwell, O. L., G. A. Bennett, R. D. Stubblefield, G. M. Shannon, W. F. Kwolek and R. D. Plattner (1985) Deoxynivalenol in hard red winter wheat - Relationship between toxin levels and factors that could be used in grading *Journal of the Association of Official Analytical Chemists* 68: 954-957.

Simmonds, P. M. (1968). Wheat seed discolorations and blemishes. In: *Technical Bulletin No. 1.* (Ed.) Research Branch, Agriculture Canada, Saskatoon, Saskatchewan,Canada: 94.

Simpson, D. R., G. E. Weston, J. A. Turner, P. Jennings and P. Nicholson (2001) Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain *European Journal of Plant Pathology* 107: 421-431.

Siuda, R., A. Grabowski, L. Lenc, M. Ralcewicz and E. Spychaj-Fabisiak (2010) Influence of the degree of fusariosis on technological traits of wheat grain *International Journal of Food Science and Technology* 45: 2596-2604.

Smewing, J. (1995). The measurement of dough and gluten extensibility using the SMS/Kieffer rig and the TA.XT2 Texture Analyser. Surrey, UK, Stable Micro Systems Ltd.

Smith, G. P. and M. J. Gooding (1999) Models of wheat grain quality considering climate, cultivar and nitrogen effects *Agricultural and Forest Meteorology* 94: 159-170.

Snijders, C. H. A. (1990) Genetic variation for resistance to *Fusarium* head blight in bread wheat *Euphytica* 50: 171-179.

Snijders, C. H. A. (2004) Resistance in wheat to *Fusarium* infection and trichothecene formation *Toxicology Letters* 153: 37-46.

Snijders, C. H. A. and C. F. Krechting (1992) Inhibition of deoxynivalenol translocation and fungal colonization in *Fusarium* head blight resistant wheat *Canadian Journal of Botany-Revue Canadienne De Botanique* 70: 1570-1576.

Stack, R. W. (2000) Return of an old problem: *Fusarium* head blight of small grains Retrieved 05/03/ 2011, from http://www.plantmanagementnetwork.org/pub/php/review/fhb/.

Stack, R. W. (2003). History of *Fusarium* head blight with emphasis on North America. In: Fusarium head blight of wheat and barley (Ed.) K. J. Leonard and W. R. Bushnell. St. Paul, MI, USA, APS Press: 1-34.

Sugita-Konishi, Y., B. J. Park, K. Kobayashi-Hattori, T. Tanaka, T. Chonan, K. Yoshikawa and S. Kumagai (2006) Effect of cooking process on the deoxynivalenol content and its subsequent cytotoxicity in wheat products *Bioscience Biotechnology and Biochemistry* 70: 1764-1768.

Sugiura, Y., Y. Watanabe, T. Tanaka, S. Yamamoto and Y. Ueno (1990) Occurrence of *Gibberella zeae* strains that produce both nivalenol and deoxynivalenol *Applied and Environmental Microbiology* 56: 3047-3051.

Sweeney, M. and A. Dobson (1998) Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* species *International Journal of Food Microbiology* 43: 141-158.

Szentpetery, Z., C. Kleinheincz, G. Szollosi and M. Jolankai (2005) Effect of nitrogen top-dressing on winter wheat yield, quantity and quality *Acta Alimentaria* 34: 177-185.

Tanaka, T., A. Hasegawa, S. Yamamoto, Y. Matsuki and Y. Ueno (1986) Residues of *Fusarium* mycotoxins, nivalenol, deoxynivalenol and zearalenone, in wheat and processed food after milling and baking *Journal of the Food Hygienic Society of Japan* 27: 653-655.

Teich, A. H. (1987) Less wheat scab with urea than with ammonium nitrate fertilizers *Cereal Research Communications* 15: 35-38.

Terzi, V., C. Morcia, P. Faccioli, N. Faccini, V. Rossi, M. Cigolini, M. Corbellini, D. Scudellari and G. Delogu (2007) *Fusarium* DNA traceability along the bread production chain *International Journal of Food Science and Technology* 42: 1390-1396.

Tkachuk, R., J. E. Dexter, K. H. Tipples and T. W. Nowicki (1991) Removal by specific gravity table of tombstone kernels and associated trichothecenes from wheat infected with *Fusarium* head blight *Cereal Chemistry* 68(4): 428-431.

Toth, B., A. Mesterhazy, Z. Horvath, T. Bartok, M. Varga and J. Varga (2005) Genetic variability of central European isolates of the *Fusarium graminearum* species complex *European Journal of Plant Pathology* 113: 35-45.

Triboi, E., A. Abad, A. Michelena, J. Lloveras, J. L. Ollier and C. Daniel (2000) Environmental effects on the quality of two wheat genotypes: 1. quantitative and qualitative variation of storage proteins *European Journal of Agronomy* 13: 47-64.

Triboi, E., P. Martre and A. M. Triboi-Blondel (2003) Environmentally-induced changes in protein composition in developing grains of wheat are related to changes in total protein content *Journal of Experimental Botany* 54: 1731-1742.

Trigo-Stockli, D. M., C. W. Deyoe, R. F. Satumbaga and J. R. Pedersen (1996) Distribution of deoxynivalenol and zearalenone in milled fractions of wheat *Cereal Chemistry* 73: 388-391.

Tuite, J., G. Shaner and R. J. Everson (1990) Wheat scab in soft red winter wheat in Indiana in 1986 and its relation to some quality measurements *Plant Disease* 74: 959-962.

Ueno, Y. (1983). Trichothecenes-Chemical, biological and toxicological aspects In: *General toxicology I* (Ed.) Elsevier Science Publishing Company. New York, NY, USA: 135-146.

Vendl, O., F. Berthiller, C. Crews and R. Krska (2009) Simultaneous determination of deoxynivalenol, zearalenone, and their major masked metabolites in cereal-based food by LC-MS-MS *Analytical and Bioanalytical Chemistry* 395: 1347-1354.

Waalwijk, C., P. Kastelein, I. de Vries, Z. Kerényi, T. van der Lee, T. Hesselink, J. Köhl and G. Kema (2003) Major Changes in *Fusarium* spp. in Wheat in the Netherlands *European Journal of Plant Pathology* 109: 743-754.

Wang, J. (2004). Effect of *Fusarium*-infection and fungicide treatment on quality parameters of winter wheat. PhD thesis. Göttingen, Cullivier Verlag.

Wang, J. H., E. Pawelzik, J. Weinert and G. A. Wolf (2005a) Impact of *Fusarium culmorum* on the polysaccharides of wheat flour *Journal of Agricultural and Food Chemistry* 53: 5818-5823.

Wang, J. H., E. Pawelzik, J. Weinert, Q. Zhao and G. A. Wolf (2008) Factors influencing falling number in winter wheat *European Food Research and Technology* 226: 1365-1371.

Wang, J. H., H. Wieser, E. Pawelzik, J. Weinert, A. J. Keutgen and G. A. Wolf (2005b) Impact of the fungal protease produced by *Fusarium culmorum* on the protein quality and breadmaking properties of winter wheat *European Food Research and Technology* 220(5-6): 552-559.

Wanjiru, W. M., Z. S. Kang and H. Buchenauer (2002) Importance of cell wall degrading enzymes produced by *Fusarium graminearum* during infection of wheat heads *European Journal of Plant Pathology* 108: 803-810.

Webb, C. and G. W. Owens (2003) Milling and flour quality In: *Bread making: Improving quality* S. P. Cauvain. Cambridge, UK, Woodhead Publishing Limited: 200-218.

Weegels, P. L., R. J. Hamer and J. D. Schofield (1996) Critical review: functional properties of wheat glutenin *Journal of Cereal Science* 23: 1-18.

Wieser, H. (2000) Simple determination of gluten protein types in wheat flour by turbidimetry *Cereal Chemistry* 77: 48-52.

Wieser, H. (2007) Chemistry of gluten proteins *Food Microbiology* 24: 115-119.

Wieser, H., S. Antes and W. Seilmeier (1998) Quantitative determination of gluten protein types in wheat flour by reversed-phase high-performance liquid chromatography *Cereal Chemistry* 75: 644-650.

Wieser, H. and R. Kieffer (2001) Correlations of the amount of gluten protein types to the technological properties of wheat flours determined on a micro-scale *Journal of Cereal Science* 34: 19-27.

Wieser, H. and W. Seilmeier (1998) The influence of nitrogen fertilisation on quantities and proportions of different protein types in wheat flour *Journal of the Science of Food and Agriculture* 76: 49-55.

Wieser, H., W. Seilmeier and H. D. Belitz (1994) Quantitative determination of gliadin subgroups from different wheat cultivars *Journal of Cereal Science* 19: 149-155.

Wieser, H. and G. Zimmermann (2000) Importance of amounts and proportions of high molecular weight subunits of glutenin for wheat quality *European Food Research and Technology* 210: 324-330.

Wilcoxson, R. D., R. H. Busch and E. A. Ozmon (1992) *Fusarium* head blight resistance in spring wheat cultivars *Plant Disease* 76: 658-661.

Williams, R. M., L. O'Brien, H. A. Eagles, V. A. Solah and V. Jayasena (2008) The influences of genotype, environment, and genotype x environment interaction on wheat quality Australian *Journal of Agricultural Research* 59: 95-111.

Windels, C. E. (2000) Economic and social impacts of *Fusarium* head blight: Changing farms and rural communities in the Northern Great Plains *Phytopathology* 90: 17-21.

WSG (2003) Klimabericht für Göttingen Retrieved 10/03/2011, from http://www.wetterstation-goettingen.de/klimabericht.htm.

WSG (2010) Wetter, Klima, Datenarchiv Retrieved 09/03/2011, from www.wetterstation-goettingen.de

Wu, Y. X. and A. von Tiedemann (2001) Physiological effects of azoxystrobin and epoxiconazole on senescence and the oxidative status of wheat *Pesticide Biochemistry and Physiology* 71: 1-10.

Xu, X. M. (2003) Effects of environmental conditions on the development of *Fusarium* ear blight *European Journal of Plant Pathology* 109: 683-689.

Xu, X. M., W. Monger, A. Ritieni and P. Nicholson (2007) Effect of temperature and duration of wetness during initial infection periods on disease development, fungal biomass and mycotoxin concentrations on wheat inoculated with single, or combinations of, *Fusarium* species *Plant Pathology* 56: 943-956.

Xu, X. M. and P. Nicholson (2009) Community Ecology of Fungal Pathogens Causing Wheat Head Blight *Annual Review of Phytopathology* 47: 83-103.

Xu, X. M., P. Nicholson, M. A. Thomsett, D. Simpson, B. M. Cooke, F. M. Doohan, J. Brennan, S. Monaghan, A. Moretti, G. Mule, L. Homok, E. Beki, J. Tatnell, A. Ritieni and S. G. Edwards (2008a) Relationship between the fungal complex causing *Fusarium* head blight of wheat and environmental conditions *Phytopathology* 98: 69-78.

Xu, X. M., D. Parry, P. Nicholson, M. Thomsett, D. Simpson, S. Edwards, B. Cooke, F. Doohan, S. Monaghan, A. Moretti, G. Tocco, G. Mule, L. Hornok, E. Béki, J. Tatnell and A. Ritieni (2008b) Within-field variability of *Fusarium* head blight pathogens and their associated mycotoxins *European Journal of Plant Pathology* 120: 21-34.

Xu, X. M., D. W. Parry, P. Nicholson, M. A. Thomsett, D. Simpson, S. G. Edwards, B. M. Cooke, F. M. Doohan, J. M. Brennan, A. Moretti, G. Tocco, G. Mule, L. Hornok, G. Giczey and J. Tatnell (2005) Predominance and association of pathogenic fungi causing *Fusarium* ear blight in wheat in four European countries *European Journal of Plant Pathology* 112: 143-154.

Yi, C. L., H. P. Kaul, E. Kubler and W. Aufhammer (2002) Populations of *Fusarium graminearum* on crop residues as affected by incorporation depth, nitrogen and fungicide application *Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection* 109: 252-263.

Young, J. C., R. G. Fulcher, J. H. Hayhoe, P. M. Scott and J. E. Dexter (1984) Effect of milling and baking on deoxynivalenol (vomitoxin) content of Eastern Canadian wheats *Journal of Agricultural and Food Chemistry* 32: 659-664.

Zandstra, J. and R. Squire (2007). Assessing harvest losses; evaluating potential damage to sugarbees caused by harvesting and piling equipment using and impact recording device In: *Final*

Project Report, CORD IV Project Ontario Sugarbeet Growers Association. University of Guelph. Ridgetown, ON, USA.

Zecevic, V., D. Knezevic, J. Boskovic and M. Madic (2009) Effect of genotype and environment on wheat quality *Genetika-Belgrade* 41: 247-253.

Zhang, Y. J., P. S. Fan, X. Zhang, C. J. Chen and M. G. Zhou (2009) Quantification of *Fusarium graminearum* in Harvested Grain by Real-Time Polymerase Chain Reaction to Assess Efficacies of Fungicides on *Fusarium* Head Blight, Deoxynivalenol Contamination, and Yield of Winter Wheat *Phytopathology* 99: 95-100.

Zhao, F. J., M. J. Hawkesford and S. P. McGrath (1999) Sulphur assimilation and effects on yield and quality of wheat *Journal of Cereal Science* 30(1): 1-17.

Zhou, B., Y. Li, J. Gillespie, G. Q. He, R. Horsley and P. Schwarz (2007) Doehlert matrix design for optimization of the determination of bound deoxynivalenol in barley grain with trifluoroacetic acid (TFA) *Journal of Agricultural and Food Chemistry* 55: 10141-10149.

Zinedine, A., J. M. Soriano, J. C. Molto and J. Manes (2007) Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin *Food and Chemical Toxicology* 45: 1-18.

Zorb, C., C. Grover, D. Steinfurth and K. H. Muhling (2010) Quantitative proteome analysis of wheat gluten as influenced by N and S nutrition *Plant and Soil* 327: 225-234.

Zorb, C., D. Steinfurth, S. Seling, G. Langenkamper, P. Koehler, H. Wieser, M. G. Lindhauer and K. H. Muhling (2009) Quantitative Protein Composition and Baking Quality of Winter Wheat as Affected by Late Sulfur Fertilization *Journal of Agricultural and Food Chemistry* 57: 3877-3885.

Zwatz, B. (1975) Ährenfusariosen des Weizens - Ertrags- und Qualitätsminderung *Pflanzenarzt* 28: 48-50.

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