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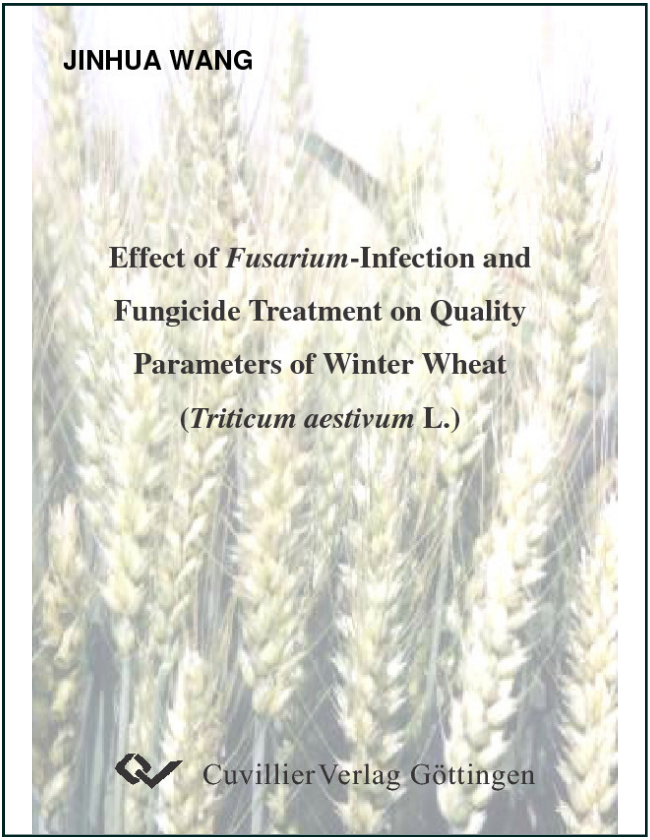
Effect of Fusarium-Infection and Fungicide Treatment on Quality Parameters of Winter Wheat (Triticum aestivum L.)



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1 Introduction

Fusarium head blight (FHB) caused by *F. graminearum* (Schwabe) (teleomorph: Gibberella zeae (Schw.) Petch) and/or *F. culmorum* (Wm. G. Smith) Sacc. (perfect state unknown) leads firstly to the premature fading of individual spikelets and later of the whole ears. As results of the fungal contamination, serious yield losses and degradation of wheat quality occur (Meyer et al. 1986). McMullen et al. (1997) estimated economic losses as a result of the 1993 FHB epidemic in the Northern Great Plains of North America at approximately one billion U.S. Dollars. In European cereal cultivation areas the reduction of 10 to 30 % in crop yield has been calculated (Bottalico 1998). The primary causal organisms in Europe are *F. culmorum* and *F. graminearum.* From the present knowledge, *F. graminearum* is prevalent in warmer regions, whilst *F. culmorum* predominates in the cooler climates of north-western Europe (Parry et al. 1995). Other important *Fusarium* species isolated from FHB damaged wheat ears in Austria are *F. avenaceum* (Corda ex Fr.) Sacc. and *F. poae* (Peck) Wolenw. (Parry et al. 1995). FHB occurs in the most wheat-growing areas of the world. Furthermore, the outbreak of this disease is often accompanied by mycotoxin contamination such as the very stable trichothecene deoxynivalenol (DON), which is degraded neither during storage, milling and the processing nor during treatments at high temperatures (Rotter 1996, Ehling et al. 1997, Eriksen and Alexander 1998).

The attention of FHB impacts on the wheat processing parameters has been increased since the 1980th. Bechtel et al. (1985) characterized *F. graminearum* as an aggressive invader destroying starch granules, storage proteins, and cell walls. *F. graminearum* infection could cause significant changes in carbohydrate, lipid, and protein composition (Boyacioglu and Hettiarachchy 1995), and further serious change the storage protein quality and dough properties. Dexter et al. (1996) found that *Fusarium*-infected Canadian red hard spring wheat from the 1994 Manitoba harvest exhibited weak dough properties and unsatisfactory bread quality. In 1997, they further observed that FHB reduced gluten functionality (strength) of durum wheat (Dexter et al. 1997). Moreover, Meyer et al. (1986) reported that wheat infected by *F. culmorum* exhibited inferior baking quality because of degradation of wheat gluten proteins. Pawelzik et al. (1998) found a high correlation between the *Fusarium*-infection level and the activity of the fungal enzymes such as amylase, chitinase, cellulase, glucanase, xylase and protease. Furthermore, the impact of nitrogen fertilization on the development of Fusarium head blight (FHB) in wheat was also investigated (Lemmens et al. 2004). It was concluded that independent of the kind of fertilizers, increasing nitrogen input led to a significant increase of disease intensity.

However, few literatures focused on the relationship between quality degradation and property of the fungal protease. The quality and technological properties of wheat flour are strongly dependent on both structure and quantity of gluten protein (Wieser 2000).

Generally, the investigations on the effects of FHB on wheat technological properties have been mainly concentrated on the change of storage protein quality. In this context, changes caused by *Fusarium*-infection in the expansion of dough during the baking phase are mainly attributed to changes in protein quantity and quality. Only few literatures focused on the impacts of FHB on polysaccharides such as starch and pentosans of wheat and the relationships between the quality decrease and the character of fungal amylase. Actually, on a purely quantitative basis, the role of wheat starch in the unique breadmaking ability of wheat flour should not be underestimated. Starch has several distinct functions in the breadmaking process. It sets a balance with gluten protein, lipids, water and other compounds during the formation of dough, thereby contributing to the optimal viscoelastic properties of the dough (Kasarda 1989). It is the substrate for the amylases that produce fermentable sugars for yeast fermentation and it severs as a reservoir for water absorption during the baking process (Klingler 1995). Water-extractable pentosans may absorb up to nine times of their own weight of water and water-inextractable pentosans up to five times of their own weight (Pomeranz 1985). The high water binding capacity of pentosans obtained not less attention by the research of wheat flour properties (Hoseney and Faubion 1981, Bettge and Morris 2000).

The use of chemicals in disease management, although a relatively old practice, is still important in ensuring and increasing food production worldwide. Many publications have stated that disease control accounts for most of the yield increase associated with the application of fungicides, particularly those using Azoles and Strobilurine products (Delp and Klopping 1968, Doltsini 1981, Stähle et al. 1998, Wolber 1999, Obst et al. 2000). The research of Weinert and Wolf (1999) showed that treatment with fungicide caused a reduction of FHB up to 80 % in wheat, when the fungicides were applied during the flowering stage. Gerhard (2001) studied the influence of Strobilurine fungicides on the physiological processes during yield formation in winter wheat cultivars. His results indicated that the application of Strobilurine fungicides not only prevented the side-effect of fungal disease, but also induced an

increase in assimilation intensity; in addition, transpiration was optimised and the plants showed improved water use efficiency as well as prolonged green leaf life. Other fungicides didn't show these effects.
The influence of fungicide treatment on wheat quality has often been determined in relation to factors such as the environment (temperature, rainfall), fertilisers or agricultural practices (Cox et al. 1989, Puppala et al. 1998, Kelley 2001, Khalil et al. 2002a and b, Varga et al. 2003). In contrast, only a few studies have considered the effect of chemical fungicides on the processing qualities of wheat.

The falling number method was originally developed by Hagberg (Hagberg, 1960) for sprout-damaged grain. It is also considered as a simple, fast, economical, sensitive and reliable test for the determination of the α-amylase activity and the statement of germ damages in cereals. Today, the falling number (FN) represents one of the most important quality characters of grain products and is worldwide used as a general index in the grain trade for wheat, durum, rye and barley (Perten 1967, AACC Method 56-81b 1995, ICC 1999).
Several studies indicated that a number of factors could affect FN. Entrapped air bubbles and non-homogeneous heating can affect the reading of FN (Möttönen 1978). Changing the water bath temperature had also an effect on the FN value (Brümmer 1984). Lorenz and Wolt (1981) observed the influence of cultivating altitude on FN values of hard wheat flours from 18 varieties. The AACC method was therefore revised in 1982 to incorporate an altitude correction. Chang et al. (1999) explored the dynamics of wheat starch hydrolysis and the changes in the rheological properties of wheat starch samples during FN determination.
In general, most studies were limited by measurement condition underlying the FN procedure. Therefore, there is a need to predict the influence of growing conditions on FN and relationships between grain composition and FN. Nitrogen fertilization could both increase and decrease FN of wheat, rye and triticale (Tabl and Kiss 1983, Gooding et al. 1986, Gruber and Flamme 1989, Stewart and Dyke 1993, Kettlewell and Cashman 1997). However, this influence was lower than the effect of the variety and the climatic conditions, which resulted from the analysis of twenty-year data (Gooding et al. 1997). A higher FN is linked with a higher temperature in the summer (Smith and Gooding 1999). Clarke (1984) did not find the influence of the harvest date on FN, although a delay of sowing date increased the FN (Ekeberg 1994). Tanacs et al. (1999) found that an extreme low or high FN of wheat was avoided by fungicide and herbicide treatments. The change of FN related closely with the difference in

genome and cytoplasma (Oettler and Mares 1994). A serious infection by *F. culmorum* in wheat ears decreased the FN (Pawelzik et al. 1998).
From earlier work (Meyer et al. 1986, Dexter et al. 1996), it is also recognized that FN is only insufficiently suitable to indicate *Fusarium*-infection, which is associated with a higher α-amylase activity. So far, only little effort was made to systematically examine factors influencing FN.

The main objectives of this study, therefore, are
1. to explore the effects of *Fusarium*-infection on gluten proteins and polysaccharides and to characterize the properties of protease and α-amylase from *F. culmorum*. The results should contribute to the explanation why and in which extend *Fusarium* spp. influences the wheat processing quality.

2. to asses the influence of several commercial fungicides and two growth regulators on flour quality, dough properties and breadmaking parameters, applied to three winter wheat cultivars grown in central Germany.

3. to explore how individual external factors such as cultivars, locations, fertilization and fungal attack affect FN. Attempt is also made to determine relationship between the FN value and internal factors, such as starch and pentosan content, the concentration of soluble and insoluble dietary fibre influencing the viscosity of gelatinized flour suspension, thousand-kernel weight (TKW), ash and crude protein content, as well as the α-amylase activity. Additionally, three α-amylase tests are compared to evaluate, if they are really suitable for the determination of enzyme activity.

2 Materials and methods

2.1 Plant materials

Wheat (*Triticum aestivum* L.) of cultivar *Hanseat* (quality class A according to the German "Bundessortenamt"; Federal Office for Plant Variety) was commercially grown and harvested in 1999 at the field station Reinshof, Göttingen, Germany. In order to obtain an infection of wheat plants with *F. culmorum*, the wheat spikes were sprayed artificially with a conidiospore suspension (2.5 x 105 spores ml^{-1}) of *F. culmorum* at the beginning of flowering. According to the infection degree, expressed as *Fusarium*-Protein-Equivalent concentration (FPE in $\mu g\ g^{-1}$) determined with Double Antibody Sandwich Enzyme Linked ImmunoSorbent Assay (DAS – ELISA) (Al-Kubrusli 2000), twenty-seven samples from different field plots were divided into three infection groups: light: FPE <12 $\mu g\ g^{-1}$; moderate: 12 $\mu g\ g^{-1}$< FPE <20 $\mu g\ g^{-1}$ and serious: FPE > 20 $\mu g\ g^{-1}$.

To assess the effects of the fungicide treatment on wheat, six different combinations with six commercial fungicides and two growth regulators (see 4.3, Tables 1-2) were performed in a randomly block design (2m x 10m plots) with four replications in years 2001-2002 at the field station Reinshof, Goettingen, Germany.

The fungicides were applied in order to control the foliar and ear diseases of wheat. The growth regulator was used to strengthen and shorten the wheat plant stems. "Untreated" represents the sample without any fungicide or regulator treatment and was designed as the control. These treatments were used for three commercially grown winter wheat cultivars in parallel trials.

Three of the most commonly grown wheat cultivars were selected based on their different baking qualities (quality classification of the cultivars according to the German "Bundessortenamt": cv. *Bussard* with quality class E (wheat with excellent baking properties), cv. *Flair* with quality class B (wheat for baking purposes), and cv. *Contur* with quality class C (wheat for other uses, except baking). All three cultivars were treated with a seed coating, Arena C. Eighteen grain samples from different treatment groups were obtained.

In 1999 grains of eight most commonly grown wheat cultivars with different baking qualities were selected to assess the influencing factors of FN in according to the classification of German "Bundessortenamt" as follow: cvs. *Pegassos*, *Cardos* and *Hanseat* with quality class A (wheat with good baking quality), cvs. *Semper*, *Pajero*, *Dekan* and *Dream* with quality