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Effect of Fungicide Treatment on the Quality of Wheat Flour and Breadmaking

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Fungicides are applied to crop plants to ensure disease protection and improve growth. To assess the effects of five commercial foliar and spike fungicides in four different combinations on wheat (*Triticum aestivum* L.), various quality parameters and flour processing properties, including baking quality, were determined. Three commonly used wheat cultivars with different quality classes (E, B, and C) were tested. Falling number, crude protein content, water absorption ability, protease activity, viscosity, and the free amino acid content were mainly lower in the fungicide-treated grains than in the untreated grains. None of the fungicides caused any significant changes in the wet gluten content, dough properties, the mono- and oligosaccharide content, or the breadmaking quality. In general, the commercial fungicide treatments did not cause any statistically significant differences between the treated and the untreated samples with respect to the quality parameters analyzed, although there were indeed significant differences between the three cultivars themselves.

KEYWORDS: Wheat; fungicide treatment; grain protein; dough properties; baking quality

INTRODUCTION

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 (*1*−*5*). The research of Weinert and Wolf (*6*) showed that treatment with fungicide caused a reduction in head blight of up to 80% in wheat, when the fungicides were applied during the flowering stage. Gerhard (*7*) 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 optimized, and the plants showed improved water use efficiency as well as prolonged green leaf life. Other fungicides did not show these effects (*7*).

The influence of fungicide on wheat quality has often been determined in relation to factors such as the environment (temperature, rainfall), fertilizers, or agricultural practices (*8*−*13*). In contrast, only a few studies have considered the effect of chemical fungicides on the processing qualities of wheat (*14*, *15*). This paper describes some responses of flour quality, dough properties, and breadmaking parameters to five commercial fungicides used in four combinations and two growth regulators in three winter wheat cultivars cultivated in the wheat-growing areas of central Germany.

MATERIALS AND METHODS

Field Trial Design. Six different treatments with five commercial fungicides and two growth regulators (**Tables 1** and **2**) were performed in a random block design (2 m × 10 m plots) with four replications from 2001 to 2002 at the field station Reinshof, University of Göttingen, Germany. The fungicides were applied in order to control the foliar and ear diseases of wheat. The growth regulators were used to strengthen and shorten the wheat plant stems. "Untreated" represents the sample without any fungicide or regulator treatment, and it 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 as they had different baking qualities (quality classification of the cultivars according to the German "Bundessortenamt" [Federal Office for Plant Variety], *16*): Bussard with quality class E (wheat with excellent baking properties), Flair with quality class B (wheat for baking purposes), and Contur with quality class C (wheat for other uses, except baking). All three cultivars were treated with a seed coating, arena C.

Grain Sample. Eighteen samples from different treatment groups were harvested in 2002 and were ground using a falling number mill (Laboratory Mill 120, Perten, Germany) containing a 0.8 mm sieve. The whole kernel flour was stored at 4 °C and was used in all of the subsequent experiments except for breadmaking. For the latter process, flour with a mineral content of between 0.49 and 0.58 mg 100 g^{-1} DM was prepared with a Bühler milling automat (Bühler, Switzerland).

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Table 1. Description of the Five Commercially Produced Fungicides Used in the Trials

fungicide	active ingredient	class
A	azoxystrobin	strobilurine
B	kresoxim-methyl, epoxiconazol, fenpropimorph	strobilurine, azole, morpholine
C	picoxystrobin	strobilurine
D	propiconazol, tebuconazol, fenpropidin	azole propidine
E	cyprodinil	anilinopyrimidine

Table 2. Field Trial Design 2001 (for a Description of the Fungicides A–E, See **Table 1**)[a]

	date of application			
	April 12/13	April 30	May 1	May 31
	growth regulator		fungicide	
treatment	BBCH 29/30[b]	BBCH 31[b]	BBCH 32[b]	BBCH 51/55[b]
untreated	no treatment			
1	CCC 1.0	Moddus 0.4		
2	CCC 1.0	Moddus 0.4		A 0.6 + D 0.6
3	CCC 1.0	Moddus 0.4	E 0.7	A 0.6 + D 0.6
4	CCC 1.0	Moddus 0.4	E 0.7 + B 0.5	A 0.6 + D 0.6
5	CCC 1.0	Moddus 0.4	E 0.7 + C 0.6	A 0.6 + D 0.6

[a] CCC, chlorocholine chloride; Moddus, microemulsion with trinexapac-ethyl. Within columns, values show the dosage of fungicide and growth regulator in L ha^{-1}. [b] BBCH, development stage of wheat according to the Federal Biological Research Centre for Agriculture and Forestry (BBA), Bundessortenamt (Federal Office for Plant Variety), and the chemical industry. BBCH 31, 32: principle growth stage 3, stem elongation; 31, first node at least 1 cm above tillering node; 32, node 2 at least 2 cm above node 1. BBCH 51/55: principle growth stage 5, inflorescence emergence, heading; 51, beginning of heading, tip from inflorescence emerged from sheath; 55, middle of heading, half of inflorescence emerged.

Quality Parameters. Hagberg falling number and wet gluten content were determined according to the standard procedures described in the International Association of Cereal Science and Technology (ICC) no. 107 and no. 106, respectively (*17*). The crude protein content ($N \times 5.7$) was determined by the DUMAS method using the microprocessor-controlled CNS-2000 analyzer from LECO Analyses System (LECO Instrumente GmbH, Germany) (*18*). The sedimentation value was determined using the GB/T 15685-1995 method developed in China by Wang (*19*). This is a modification of the 1983 American Association of Cereal Chemists (AACC) method, in which lactic acid in 2% sodium dodecyl sulfate (SDS) [weight to volume (w/v)] is used to precipitate gluten protein for 30 min.

Physical Dough Tests. Dough extensibility tests and the dough's resistance to extension were carried out according to the method described by Kieffer (*20*) with the TA.XT2 Texture Analyzer (SMS/Stable Micro Systems Ltd., Great Britain). Valorigrams were obtained with a valorigraph Type QA-203 (METEFEM, Hungary), similar to the Brabender farinograph, using the modified ICC standard procedure no. 115 (*17*). Fifty grams of whole kernel flour based on 15% moisture content was used to measure the water absorption after mixing for 3 min and to produce dough with a maximum consistency of 500 VU (valorigram unit). The consistency is the resistance, measured as torque, expressed in specific apparatus units (VU), of a sample of dough mixed in the valorigraph at a constant speed. The degree of dough softening was determined after 12 min of mixing.

Analytical Tests. Extracellular amylase and protease activities were determined by using the enzyme test developed by Wirth and Wolf (*21*). To assess the changes in gluten protein composition, the proteins were extracted stepwise from the flour samples according to the method described by Gorinstein et al. (*22*). The proteins were extracted with a suitable solvent, and the sequence of solvents was used as follow: The total albumins and globulins were twice extracted with 0.5 M sodium chloride. To obtain the total alcohol soluble gliadin (GLI) fraction, first, the rinse rest was shaken with 70% [volume to volume (v/v)] 2-propanol at room temperature for 45 min and centrifuged for 10 min with a speed of 10000*g*, and then, the new rinse rest was shaken with 70% (v/v) 2-propanol containing 4% (v/v) 2-mercaptoethanol (2-ME) and centrifuged under the same conditions. The supernatants from these two steps were combined and freeze-dried. Afterward, the rinse rest was used to obtain the total glutenin (GLU) fractions. First, the rinse rest was twice shaken with 0.125 M sodium borate buffer, pH 8.9, containing 3% (v/v) 2-ME. The supernatant was freeze-dried to obtain the low molecular weight GLU subunits (LMW-GLU). Second, the new produced rinse rest was twice shaken with 0.125 M sodium borate buffer, pH 9.4, containing 0.5% (w/v) SDS. The supernatant was freeze-dried to obtain the high molecular weight GLU subunits (HMW-GLU). A sample of flour (10 g) was placed in the relevant solvent with a solvent/sample ratio of 10:1 (v/w).

The freeze-dried GLIs and two types of GLU with different molecular weights were then identified by SDS polyacrylamide gel electrophoresis (PAGE) using the Biometra Mini-Power Pack 040-100 and PP 2000 with glass plates (6.6 cm × 7.7 cm) (Biometra, Germany). The content distribution of GLI, total GLU, HMW-GLU, and LMW-GLU were measured by turbidimetry (*23*).

The AccQ·Tag method was used to analyze the free amino acid composition of the isolated proteins in the cultivars Bussard and Flair (*24*). The extraction of the free amino acids from the flour samples was conducted according to the modified method described by Herbert et al. (*25*). The derivatization reaction was carried out according to Cohen and Michaud (*26*). The free amino acid derivatives were separated by reversed phase high-performance liquid chromatography (RP-HPLC) and were determined by their fluorescence at 395 nm. The following HPLC system (Waters, Germany) was used for the analysis of the samples: two HPLC double piston pumps 510; autosampler 717 plus; programmable scanning fluorescence detector 474; pump control module HPLC for Millennium; Thermostat column compartment; inline degasser; Vertex per-column (5 mm × 4 mm, Spherimage 80 ODS2, 5 μL); and a Vertex separation column (150 mm × 46 mm, Spherimage 80 ODS2, 5 μm). An acetonitrile/water solution (60/40; v/v) and acetate buffer (pH 4.70) were used as gradient eluents. The separation was achieved at a flow rate of 1 mL min^{-1} and a temperature of 33 °C. The detection limit was 2 pM with the exception of cysteine (1 pM).

The water extract viscosity was measured according to the method described by Dusel et al. (*27*). Mono- and oligosaccharides were extracted from the flour samples using 80% ethanol (*28*) and were then also measured by RP-HPLC, although the HPLC system differed to that used for the amino acids; i.e., degasser WellChrom K-5004 (Knauer, Germany); pump WellChrom MaxiStar K-1000; autosampler 2157 (Pharmacia LKB, Uppsala, Sweden); Thermostat columns Jetstream 2 (Knauer, Germany); refractive index detector 198.00 (Knauer, Germany); integrator Mega 2 (Shimadzu, Japan); per-column LiChroCART 4-4 (LiChrosorb 100 NH2, 5 μm; Merck, Germany); and separation column LiChroCART 250-4 (LiChrospher 100 NH2, 5 μm; Merck). An acetonitrile/water solution (80/20, v/v) was used as the eluent. The separation of the mono- and oligosaccharides was achieved at a flow rate of 1 mL min^{-1} and a temperature of 20 °C. The detection limit was 250 ppm.

Baking Experiments. The flour samples were baked using the rapid mix test (RMT), and the loaf volumes were determined by rapeseed displacement (*29*).

Statistical Analysis. The calculation of the mean values and standard deviations was performed using Microsoft Excel 2000. The statistical analyses were undertaken using the All Pairwise Multiple Comparison Procedures (one way analysis of variance, Tukey's test) of the SigmaStat 2.03 program. Significant differences are marked in the corresponding tables and figures (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). With the Pearson product moment correlation analysis, the relationship between mono- and oligosaccharide content and viscosity was examined.

RESULTS AND DISCUSSION

Thousand Kernel Weight (TKW). An increase in the TKW was found in all three wheat cultivars after the application of the fungicides, while those cultivars treated with growth

Table 3. Change in TKW in Wheat after Fungicide and Growth Regulator Treatments as Compared to Untreated Samples (in %)[a]

	cultivar		
treatment	Bussard	Flair	Contur
untreated (g)	44.2[a]	43.9[a]	41.3[a]
1	–2.1[b]	–2.5[b]	0.0[a]
2	3.2[c]	7.0[c]	14.0[b]
3	4.4[d]	4.0[d]	15.0[b]
4	4.0[d]	9.0[e]	16.0[b]
5	3.4[cd]	6.0[f]	13.0[c]

[a] Within columns, values followed by the same letter are not significantly different ($p < 0.05$).

regulator alone exhibited either no change in TKW or only a slight decrease (**Table 3**). As compared with the untreated samples, the average increase in TKW was 3.75% for the cultivar Bussard, which was less than that found in either Flair or Contur (6.5 and 15.25%, respectively).

Kelley (*30*) reported that a foliar fungicide application could be beneficial in protecting grain yield and test weight potential of both hard and soft winter wheat cultivars, when conditions are favorable for foliar fungal epidemics. This author also concluded that foliar fungicides had a greater impact on test weight than the planting date in most of the years of his experiment. As the absolute test weights were highest for all three cultivars with foliar fungicide, these results suggest that the fungicides have a positive effect on the TKW.

Indirect Quality Parameters of Wheat Flour. Table 4 shows that none of the fungicide applications had any significant impact on the quality parameters falling number, crude protein content, sedimentation value, and water absorption within the cultivars themselves. This result for crude protein agrees with the results of Kelley (*30*), who showed that foliar fungicide had no beneficial effects on grain protein, even though the upper leaf canopy was noticably greener for 3 weeks directly after application. Gerhard (*7*) also did not find any increase in crude protein content after treatment with a strobilurine fungicide.

In comparison to the present results with respect to falling number, Dimmock and Gooding (*31*) found that fungicide treatment appeared to reduce the Hagberg falling number; however, this effect depended on the cultivar. In agreement with this latter observation, significant differences were observed for all four indirect quality parameters tested between the three cultivars in this investigation.

Wet Gluten Content. After wheat flour is mixed with water, the storage proteins form a rubbery mass—the gluten that is formed from the interaction of GLU and GLI (*32*). Gluten is of primary importance for the technological quality of wheat and durum. In the present investigation, the fungicide treatments

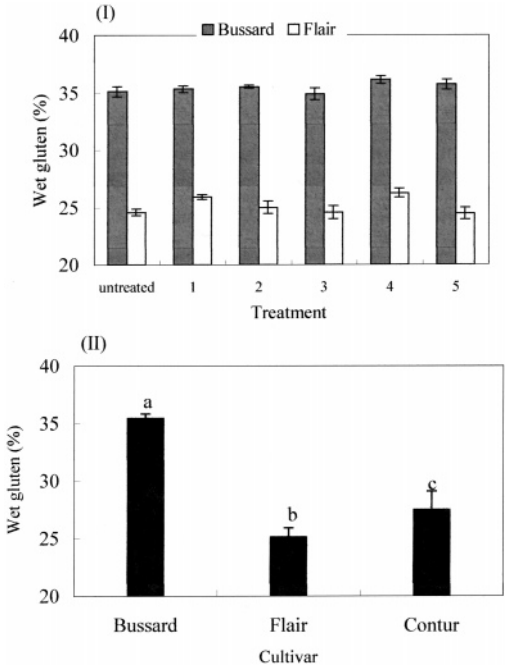

Figure 1. Wheat wet gluten content. (**I**) Effect of treatments with fungicides and growth regulators on the wet gluten content of the cultivars Bussard and Flair. (**II**) The mean values of all treatments for each of the three cultivars ($n = 6$; $p < 0.05$, bars with the same letter are not significantly different).

did not significantly affect the wet gluten content ($P = 0.191 > 0.05$) in either of the cultivars Bussard or Flair, although there was a slight increase in all of the treatments apart from treatment 3, where there was a decrease (**Figure 1I**). In comparison, there were statistically significant differences between the three cultivars **(Figure 1II)**.

Dough Rheological Properties. As shown in **Table 5**, the cultivars differed significantly in their dough property parameters, except for dough resistance. In comparison with Flair and Contur, Bussard had a much higher average dough extensibility, valorigram number, and energy as was expected from its quality classification. No significant differences could be determined in the rheological properties between fungicide treatments.

Analytical Results for Wheat Flour. α-*Amylase and Protease Activity.* α-Amylase and protease activity are closely related to flour's starch and protein properties, and these enzymes are essential factors for determining bread quality (*33*, *34*). The α-amylase activity in the kernels after the fungicide treatments increased but not significantly (**Figure 2II**), while the protease activity only changed very slightly. In comparison, a significant difference was found for α-amylase activity among

Table 4. Changes in Indirect Quality Parameters of Wheat Flour after Fungicide and Growth Regulator Treatments in Comparison to Untreated Samples[a]

	falling number (s)			crude protein (% DM)			sedimentation value (ml)			water absorption (%)*			
treatment	Bussard	Flair	Contur	Bussard	Flair	Contur	Bussard	Flair	Contur	Bussard	Flair	Contur	
untreated	425	365	392	12.1	10.6	10.7	69.9	44.0	45.7	67.0	65.2	62.0	NS
1	13	–24	11	–0.3	0.1	0.2	2.8	2.7	–5.5	–0.4	0	–0.2	NS
2	–4	–27	–6	–0.5	–0.1	0.0	–1.3	2.0	–3.5	–0.6	–0.8	–0.2	NS
3	3	–47	–39	–0.7	–0.4	0.3	2.3	0.4	–4.5	–1.0	–1.0	–0.2	NS
4	–56	–28	–21	0.5	–0.4	0.1	0.4	0.8	–4.5	–1.4	–1.2	–0.2	NS
5	–53	–69	–23	0.8	–0.3	0.0	–0.3	–0.3	–5.4	–3.8	2.0	–0.6	NS
	a	b	a	a	b	b	a	b	c	a	a	b	

[a] Within the last row, the same letter means not significantly different ($p < 0.05$); last column; NS, not significant within treatments ($p > 0.05$). *On the basis of 15% moisture according to ICC 115 (*15*).

Table 5. Summary of Selected *F* Tests ($p < 0.05$) on the Effects of the Fungicide and Growth Regulator Treatments on the Wheat Dough Properties[a]

	dough properties						extensiograph			treatments (comparison to untreated sample)				
cultivar	D (min)	S (min)	R (min)	S (VU)	E (VU)	VN	E° (mm)	R° (g)	EN (cm^2)	1	2	3	4	5
Bussard	2.7[a]	0.5[a]	3.2[ab]	182[a]	158[ac]	3.8[a]	61.7[a]	16.4[a]	8.3[a]	NS	NS	NS	NS	NS
Flair	2.3[b]	1.2[b]	3.5[a]	165[b]	139[a]	2.6[ab]	39.7[b]	15.4[a]	4.9[b]	NS	NS	NS	NS	NS
Contur	2.0[b]	1.1[bc]	2.9[b]	196[a]	167[bc]	2.2[b]	36.4[b]	16.6[a]	4.6[b]	NS	NS	NS	NS	NS

[a] Results are the mean values of all treatments. D = development time; S = stability; R = resistance; S = softening degree; E = elasticity; E° = extensibility; R° = dough resistance in extensiograph; VN = valorigram number; and EN = energy. Within columns, values followed by the same letter are not significantly different ($p < 0.05$); NS, not significant ($p > 0.05$).

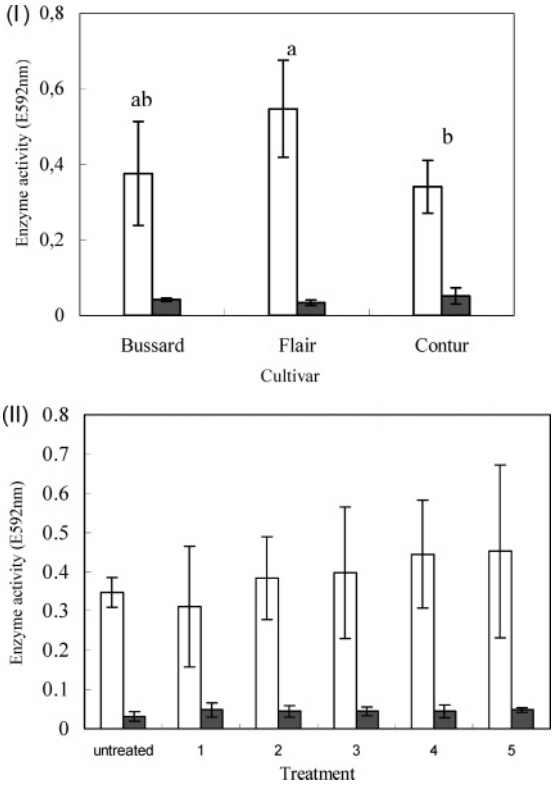

Figure 2. α-Amylase (open bars) and protease activity (closed bars) of wheat flour. (**I**) Enzyme activity responses of wheat cultivars to fungicide and growth regulator treatments ($n = 6$, averages values; $p < 0.05$, bars with the same letter are not significantly different). (**II**) The influence of fungicide treatments on the wheat flour enzymes (averages of the three different cultivars, $n = 3$).

the cultivars ($p < 0.05$) (**Figure 2I**). The cultivar Flair had the highest α-amylase activity averaging 0.55 extinction units at 592 nm, whereas in Bussard and Contur the activity was in the range of 0.29−0.37 extinction units. The fact that α-amylase activity is inversely proportional to falling number (*35*) was confirmed by the results of the present study (compare **Table 4**).

Free Amino Acid Content. All fungicide applications caused considerable changes in the free amino acid content of the wheat kernels in Bussard and Flair (**Table 6**). The total free amino acid content ranged from 767 to 1686 $\mu g\ g^{-1}$ DM. The main amino acids in the untreated samples in both Bussard and Flair were aspartic acid, glutamic acid, glycine, alanine, and proline. For the cultivar Flair, after treatments 1−3 and 5, the content of all amino acids, except for cysteine in treatment 2 and methionine in treatment 3, increased by between 9 and 71%, while after treatment 4 the content of all amino acids decreased by between 1 and 32% with the exception of serine, which increased by about 10%. In contrast, the cultivar Bussard contained fewer free amino acids than Flair and the fungicide treatments caused a decrease in many of the free amino acids in comparison to the untreated samples. The relative decreases in total amino acid were in the range of 8 and 30%. These changes in the amino acids were not reflected in either the crude protein content (**Table 4**) or the protease activity (**Figure 2**) in either of these cultivars. Again, these results indicate a cultivar-dependent response to fungicide treatment.

Flavor-enhancing and browning occur when reducing sugars and amino acids react at high temperatures during the Maillard reaction (*36*). It is questionable whether the change in amino acid content would actually have any serious influence on the breadmaking process itself, because the intensity of the Maillard reaction is also dependent on the amount of reducing sugar present in the flour.

Gluten Protein Fractionation Analysis by SDS−PAGE. The electrophoregrams shown in **Figure 3** indicate that no treatment-associated changes occurred in the GLI subunit with a molecular mass between 29 and 45 kDa in any of the three cultivars. Denser bands of two GLU type molecules were found at positions 66 and 97−116 kDa for cultivars Flair and Bussard after treatments 4 and 5. This suggests that the fungicides used in these treatments could cause a change in the amount of HMW-GLU subunits present. In contrast, the intensity of the GLU bands in the cultivar Contur was weaker than in the other two cultivars, which correlates with the poor baking quality of this cultivar.

Effects on GLI, Total GLU, HMW-GLU, and LMW-GLU. The cultivar Flair was used as an example for all three cultivars in the investigation of the response of the gluten proteins to the fungicide treatments. The results are shown in **Figure 4** and are compared to untreated samples of the cultivars Bussard and Contur. In general, a decrease in the concentrations of GLI, total GLU, HMW-GLU, and LMW-GLU after fungicide treatment was observed. There were significant differences between treatment 4 and untreated with respect to GLI, between treatment 5 and untreated with respect to total GLU, and between both treatments 4 and 5 and untreated with respect to HMW-GLU. In comparison, neither of these fungicide treatments caused any significant change in LMW-GLU. Till now, possible interactions between fungicides and metabolic processes in the plant during protein synthesis are not clear and need further investigations.

The decrease in GLI could also cause a reduction in the viscosity of the dough, whereas the decrease in total GLU and its subunits would imply not only a lowering of the strength and elasticity of the dough but also a potentially reduced loaf volume (*32*, *37*). The presented results of loaf volume in Flair treatments 4 and 5 confirm these findings (see **Table 8**).

Table 6. Effect of Fungicide and Growth Regulator Treatment on Free Amino Acid Concentration in Bussard and Flair

	cultivar											
	Bussard						Flair					
		relative change to untreated (%)						relative change to untreated (%)				
amino acid	U (μg g^{-1} DM)	1	2	3	4	5	U (μg g^{-1} DM)	1	2	3	4	5
aspartic acid	253.4	–9	–19	–25	–15	–38	345.9	12	28	12	–19	9
serine	54.1	–37	–23	–44	–17	–42	38.6	10	71	10	10	31
glutamic acid	163.2	–22	–9	–15	2	–21	231.3	12	42	20	–6	24
glycine	248.4	–57	–45	–39	–3	–44	176.9	18	65	19	–1	31
arginine	104.8	–28	–14	–14	–27	–30	101.2	14	36	17	–11	15
threonine	17.7	–32	–22	–39	–41	–40	17.5	18	39	22	–8	23
alanine	62.8	–18	–7	–13	–11	–32	76.6	11	44	30	–4	32
proline	26.2	2	–12	–12	–12	–4	55.3	17	24	12	–15	14
cysteine	32.7	70	–14	–3	19	23	33.0	32	–14	0	–25	41
tyrosine	23.0	9	–23	–15	–23	–10	20.8	39	24	11	–32	20
valine	26.0	6	–1	–7	–6	–15	35.7	19	25	17	–19	15
methionine	5.3	66	13	11	–3	47	6.0	33	12	–2	–28	40
lysine	31.6	21	–9	1	2	–15	27.0	24	20	16	–19	31
isoleucine	13.9	25	16	5	–12	–15	22.2	24	30	21	–13	11
leucine	16.0	–4	–4	–5	–1	–20	16.5	48	52	40	–1	37
phenylalanine	17.7	–25	–16	–19	–10	–17	15.9	60	64	46	–6	37
total	1096.8[a]	–21[b]	–21[b]	–22[b]	–8[b]	–30[b]	1221[a]	16[b]	38[b]	17[b]	–11[b]	20[b]

[a] Sum of the individual amino acids. [b] Relative change of the sum of all determined free amino acids to the untreated sample; U, untreated; 1–5, fungicide and growth regulator treatments (see **Table 1**).

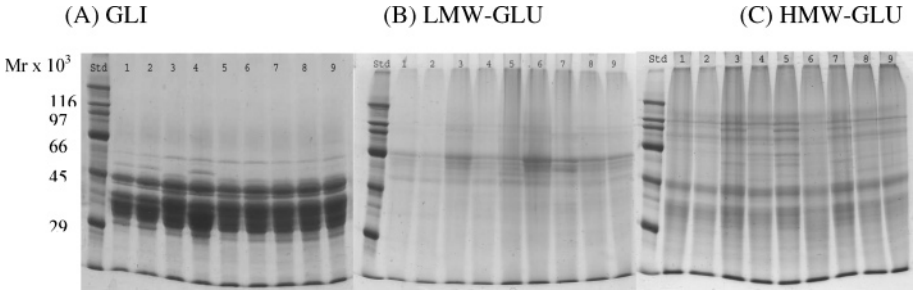

Figure 3. Effect of fungicide treatments on the composition of gluten protein determined by SDS–PAGE of wheat flour. Lane std, marker proteins; lanes 1–3, cultivar Flair—untreated, treatments 4 and 5; lanes 4–6, cultivar Bussard—untreated, treatments 4 and 5; and lanes 7–9, cultivar Contur—untreated, treatments 4 and 5.

Table 7. Effect of Fungicide and Growth Regulator Treatments on the Sucrose Content (% DM) in Wheat Flour[a]

	cultivar			
treatment	Bussard	Flair	Contur	average
untreated	0.52	0.71	0.68	0.64
1	0.46	0.80	0.67	0.64
2	0.50	0.70	0.70	0.63
3	0.54	0.92	0.70	0.72
4	0.48	0.76	0.69	0.64
5	0.53	0.72	0.69	0.64
average	0.50	0.77	0.69	
STD	0.03	0.08	0.01	
	b**	a	c*	

[a] STD, standard deviation. In the last row, values with the same letter are not significantly different (*p < 0.05, **p < 0.001).

The ratios of GLI to HMW-GLU based on their extinction values (corresponding to 250 mg of flour) for treatments 4 and 5 were 1.4 and 2.0, respectively, while that of the untreated sample was 1.7. These changed ratios could cause modifications in the dough resistance (*37*); however, in the present study, a statistically significant difference in dough resistance between the fungicide treatments was not found (see **Table 5**).

Viscosity can be changed by a variation in the amount of soluble nonstarch polysaccharides present in wheat; an increase in soluble nonstarch polysaccharide content is accompanied by an increased viscosity (*27*). After treatments 4 and 5, the viscosity of all three cultivars was significantly affected as compared with the untreated samples. The viscosity in both Bussard and Flair was significantly decreased after treatment 4. Treatment 5, on the other hand, caused a reduction in viscosity in only Bussard, whereas this treatment was associated with an increased viscosity in Contur (**Figure 5**). These results suggest that these two fungicide combinations promote the synthesis of soluble nonstarch polysaccharides or assist in the release of some nonstarch polysaccharide splitting enzymes, such as cellulase or xylase, during the physiological processes associated with yield formation. Nevertheless, there is a need for a further verification of this theory as the generally higher amount of α-amylase present after these two treatments (see **Figure 2)** could also have caused the reduction in viscosity (*38*). Indeed, higher concentrations of soluble nonstarch polysaccharides, such as pentosans, could contribute to the water-holding capacity of the flour during breadmaking (*36*), which is of importance for the bread texture.

Mono- and Oligosaccharide Content. Only the sucrose content is shown in **Table 7** as the fructose, glucose, galactose, arabinose, xylose, maltose, and raffinose contents of the samples were lower than the detection limit of the method used. There were no significant differences between the untreated and the treated samples in each cultivar, with the exception of treatment 3 in Flair, where a distinct increase in sucrose content was

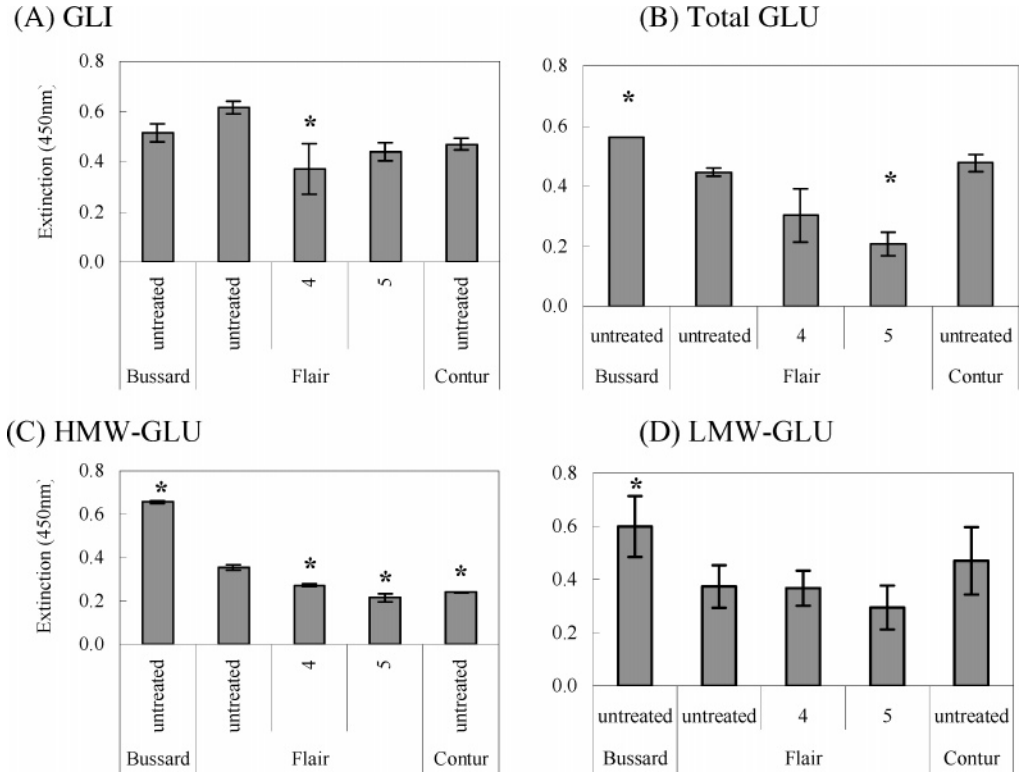

Figure 4. Effect of fungicide treatments on GLU protein subunits in cultivars Bussard, Flair, and Contur (*significant difference to the untreated Flair samples, $p < 0.05$).

Table 8. Results of Breadmaking by the RMT of Flour from Bussard and Flair Comparing the Untreated Samples to the Fungicide Treatments 4 and 5

	cultivar					
	Bussard			Flair		
treatment	untreated	4	5	untreated	4	5
falling number (s)	545[a]	570[b]	520[c]	510[a]	415[b]	400[c]
water absorption (mL 100 g^{-1} flour[a])	56.7	58.8	59	53.5	53.7	53.9
flour yield (g)	157.4	158.8	158.8	154.4	154.6	154.3
dough surface	normal	normal	normal	normal	normal	normal
dough elasticity	normal	normal	normal	strong	strong	strong
small bread weight (g)	128.6	128.1	128.5	126.6	126.2	127.1
loaf volume (cm^3 100 g^{-1} flour[a])	637	660	656	522	494	488
crispness	satisfactory	good	good	unsatisfactory	unsatisfactory	unsatisfactory
browning	normal	normal	normal	normal	normal	normal
crumb structure	uneven	uneven	uneven	uneven	even	even
crumb elasticity	good	good	good	good	good	good

[a] On the basis of 14% moisture according to Schrader (*27*). Within rows for the same cultivar, values followed by the same letter are not significantly different ($p < 0.05$).

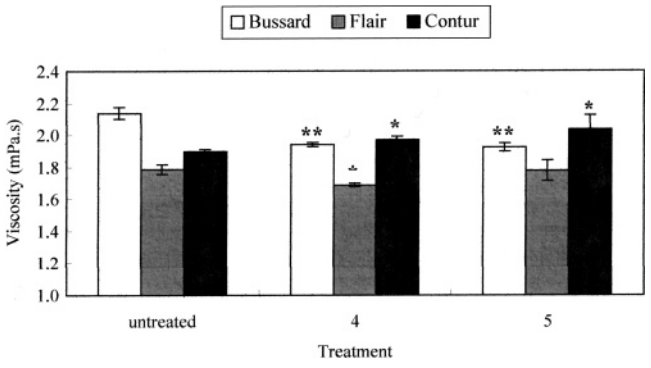

Figure 5. Effect of fungicide treatments on the viscosity of wheat flour comparing treatments 4 and 5 with the untreated samples in each cultivar (*$p < 0.05$; **$p < 0.001$).

observed. Again, a statistically significant difference between the cultivars was found for this parameter, too. The sucrose content was found to be inversely proportional to viscosity based on the mean values of the untreated samples and the samples after treatments 4 and 5 ($r = -0.612$, $p = 0.03$).

Breadmaking Quality Characteristics. The main effects of the treatment with fungicides on breadmaking with cultivars Bussard and Flair are shown in **Table 8**. As compared to the untreated samples, there were significant differences in falling number and loaf volume of both of these cultivars and in the water absorption capacity of Bussard alone. Both treatments 4 and 5 caused a decrease in loaf volume of Flair by 28 and 34 cm^3 per 100 g flour, respectively, while those of Bussard were increased by 23 and 19 cm^3 per 100 g flour, respectively. This decrease in loaf volume in Flair reflected the reduction in gluten protein content (see **Figures 1** and **4**) as a high bread volume

is related to a higher content of HMW- and LMW-GLU subunits (*32*, *37*).

There was a higher water absorption in Bussard after fungicide treatment in comparison to the untreated samples, whereas no differences were found in Flair. Virtually no changes were observed in either flour yield or small bread weight for Bussard and Flair, nor could any significant influence of fungicide treatment on dough surface, crispness, texture, browning, crumb structure, and crumb elasticity of the bread be detected in either of these cultivars.

In conclusion, the application of fungicides ensured both disease protection and an improved growth of the crop plants. In the cases of the three tested wheat cultivars of different quality classes, falling number, crude protein content, sedimentation value, and water absorption ability were generally lower in the fungicide-treated grains than in the untreated grains, or they did not change significantly. None of the fungicides caused any significant changes in the wet gluten content or dough properties. The effects observed concerning the breadmaking quality were partly cultivar-dependent. In general, the commercial fungicide treatments did not cause any statistically significant differences between the treated and the untreated samples with respect to the quality parameters analyzed, although there were indeed significant differences between the three cultivars themselves.

ABBREVATIONS USED

AACC, American Association of Cereal Chemists; ICC, the International Association of Cereal Science and Technology; w/v, weight to volume; v/v, volume to volume.

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LITERATURE CITED

(1) Delp, C. J.; Klopping, H. L. Performance attributes of a new fungicide and mite ovicide candidate. *Plant Dis. Rep*. **1968**, *52*, 95−99.

(2) Doltsini, S. Einfluss der systemischen fungizide nuarimol und imazahlil auf den stoffwechsel von weizenpflanzen. Ph.D. Thesis, Hohenheim, Germany, 1981.

(3) Stähle, A.; Oerke, E. C.; Dehne, H. W. Auftreten und Bekämpfungsmöglichkeiten von *Fusarium* spp. und Mykotoxingehalte in winterweizen 1997. In *Deutsche Pflanzenschutztagung*; Wolfrudolf, L., Ed.; Halle/Saale: Germany, 1998; Vol. 51, pp 30−34.

(4) Wolber, D. M. Ein Vergleich Praxisüblicher Bekämpfungsstrategien gegen Pilzkrankheiten in Winterweizen in Norddeutschland. Dissertation, Göttingen, Germany, 1999.

(5) Obst, A.; Lepschy, J.; Beck, R.; Bauer, G.; Bechtel, A. The risk of toxins by *Fusarium graminearum* in wheat interactions between weather and agronomic factors. Proceedings of the 22nd Mycotoxin Workshop, 5−7 June 2000, Bonn, Germany. *Mycotoxin Res.* **2000**, *16A*, 16−20.

(6) Weinert, J.; Wolf, G. A. Erfahrungen zum Auftreten von Ährenfusariosen und zur Bekämpfungsstrategie im Weizen. *Fusarium Symposium*; University of Hohenheim: Germany, 1999; pp 18−19.

(7) Gerhard, M. Der Einfluss Strobilurinhaltiger Fungizide auf Physiologische Abläufe der Ertragsbildung an Winterweizensorten. Dissertation, München, Germany, 2001.

(8) Cox, W. J.; Bergstrom, G. C.; Reid, W. S.; Sorrels, M. E.; Otis, D. J. Fungicide and nitrogen effects on winter wheat under low foliar disease severity. *Crop Sci.* **1989**, *29*, 164−170.

(9) Puppala, V.; Herrman, T. J.; Bockus, W. W.; Loughin, T. M. Quality response of twelve hard red winter wheat cultivars to foliar disease across four locations in central Kansas. *Cereal Chem*. **1998**, *75*, 94−99.

(10) Kelley, K. W. Planting date and foliar fungicide effects on yield components and grain traits of winter wheat. *Agron. J.* **2001**, *93*, 380−389.

(11) Khalil, I. H.; Carver, B. F.; Krenzer, E. G.; MacKown, C. T.; Horn, G. W. Genetic trends in winter wheat yield and test weight under dual-purpose and grain only management systems. *Crop Sci.* **2002a**, *42*, 710−715.

(12) Khalil, I. H.; Carver, B. F.; Krenzer, E. G.; MacKown, C. T.; Horn, G. W.; Rayas-Duarte, P. Genetic trends in winter wheat grain quality with dual-purpose and grain only management systems. *Crop Sci.* **2002b**, *42*, 1112−1116.

(13) Varga, B.; Svečnjak, Z.; Jurković, Z.; Kovačević, J.; Jukić, Ž. Wheat grain and flour quality as affected by cropping intensity. *Food Technol. Biotechnol.* **2003**, *41*, 321−329.

(14) Saunders, N.; Salmon, S. *Effects of strobilurin fungicides on the milling quality of breadmaking winter wheat varieties*; HGCA: London, 2000; 32 pp.

(15) Salmon, S. E.; Cook, R. J. Effects of fungicides on the milling and baking quality of wheat. *Aspects Appl. Biol.* **1987**, *15*, 373−384.

(16) Bundessortenamt. *Beschreibende Sortenliste 2002*; Getreide, M., Ed.; Ölfrüchte; Leguminosen (grosskörnig); Hackfrüchte (ausser Kartoffeln); Deutscher Landwirtschaftsverlag GmbH: Hannover, 2002; pp 109−113.

(17) ICC. *Standard Methods of the International Association for Cereal Science and Technology*; ICC: Vienna, 1999.

(18) Sweeney, R. A.; Rexroad, P. R. Comparison of LECO FP-228 "Nitrogen Determinator" with AOAC copper catalyst Kjeldahl method for crude protein. *JAOAC* **1987**, *70*, 1028−1030.

(19) Wang, Z. Physicochemical property, food quality evaluation and technology of wheat and wheat flour. *Quality Analyses of Cereal, Oil Plant Products and Food*; China Light Industry Publishing House: Beijing, 2000; pp 369−371.

(20) Kieffer, R. Direkter Vergleich des Dehnverhaltens von Weizenteig und-kleber durch Mikrozugversuche. *Food Technol. Mag.* **1995**, *3*, 28−31.

(21) Wirth, S. J.; Wolf, G. A. Microplate colourmetric assay for endo-acting cellulase, xylanase, chitinase, 1,3-β-glucanase and amylase extracted from soil horizons. *Soil Biol. Biochem*. **1992**, *24*, 511−519.

(22) Gorinstein, S.; Pawelzik, E.; Delgado-Licon, E.; Haruenkit, R.; Weisz, M.; Trakhtenberg, S. Characterisation of pseudocereal and cereal proteins by protein and amino acid analyses. *J. Sci. Food Agric.* **2002**, *82*, 886−891.

(23) Wieser, H. Simple determination of gluten protein types in wheat flour by turbidimetry. *Cereal Chem.* **2000**, *77*, 48−52.

(24) Anonymous. Millipore Waters Chromatograph, Manual Number WAT052874TP, 1993.

(25) Herbert, P.; Barros, P.; Ratola, N.; Alves, A. HPLC determination of amino acids in musts and port wine using OPA/FMOC derivatives. *J. Food Sci.* **2000**, *65*, 1130−1133.

(26) Cohen, S. A.; Michaud, D. P. Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high-performance liquid chromatography. *Anal. Biochem.* **1993**, *211*, 279−287.

(27) Dusel, G.; Kluge, H.; Glaser, K.; Simon, O.; Hartmann, G.; Lengerken, J.; Jeroch, H. An investigation into the variability of extract viscosity of wheat-relationship with the content of nonstarch-polysaccharide fractions and metabolisable energy for broiler chickens. *Arch. Anim. Nutr*. **1997**, *50*, 121−135.

(28) Wang, Z. Determination of carbohydrate. *Quality Analyses of Cereal, Oil Plant Products and Food*; China Light Industry Publishing House: Beijing, 2000; pp 103−104.

(29) Schrader, B. Analytische, Teigrheologische und Backtechnische Studien zur Optimierung des Weizen-Backversuches, Rapid-Mix-Test. Dissertation, Bonn, Germany, 1985.
(30) Kelley, K. W. Nitrogen and foliar fungicide effects on winter wheat. *J. Prod. Agric.* **1993**, *6*, 53−57.
(31) Dimmock, J. P. R. E.; Gooding, M. J. The effects of fungicides on Hagberg falling number and blackpoint in winter wheat. *Crop Prot*. **2002**, *21*, 475−487.
(32) Antes, S.; Wieser, H. Effects of high and low molecular weight glutenin subunits on rheological dough properties and bread-making quality of wheat. *Cereal Chem*. **2001**, *78*, 157−159.
(33) Maninder, K.; Jorgensen, B. Interrelations of starch and fungal alpha-amylase in breadmaking. *Starch* **1983**, *35*, 419−426.
(34) Pedersen, L.; Kaack, K.; Bergsøe, M. N.; Adler-Nissen, J. Rheological properties of biscuit dough from different cultivars, and relationship to baking characteristics. *J. Cereal Sci*. **2004**, *39*, 37−46.
(35) Perten, H. Application of the falling number method for evaluating alpha-amylase activity. *Cereal Chem.* **1964**, *41*, 127−140.
(36) Belitz, H. D.; Grosch, W.; Schieberle, P. *Lehrbuch der Lebensmittelchemie*; Springer-Verlag: Berlin, Heidelberg, 2001; pp 661, 687−688, and 258−260.
(37) Wieser, H.; Seilmeier, W.; Kieffer, R. Relationship between the amount of gluten protein types and the rheological properties of different wheat cultivars. *Gluten Proteins* **1993**, 141−150.
(38) Matthäus, K.; Dänicke, S.; Vahjen, W.; Simon, O.; Wang, J.; Valenta, H.; Meyer, K.; Strumpf, A.; Ziesenib, H.; Flachowsky, G. Progression of the mycotoxin and nutrient concentration in wheat after inoculation with *Fusarium culmorum*. *Arch. Anim. Nutr*. **2004**, *58*, 19−35.

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