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Effect of *Fusarium graminearum* and *F. poae* infection on Barley and Malt Quality

Objectives of this research were to investigate barley and malt quality as impacted by *Fusarium graminearum* and *F. poae* infection. *F. graminearum* is the primary pathogen responsible for the recent Fusarium Head Blight epidemics in the upper Midwestern USA, while *F. poae* is involved to a lesser extent. Inoculation and seed production in the greenhouse were utilized as a means of reducing the interfering effects of other microflora. Harvested seed from control and infected plants was micromalted, and the malt quality of control and inoculated samples analyzed. Large amounts of deoxynivalenol,15-acetyl deoxynivalenol and zearalenone were present on samples inoculated with *F. graminearum*, while only small amounts of nivalenol were detected on the *F. poae* infected samples. The most noticeable effects of infection on barley quality were reduction in kernel plumpness and germination. In general the effects of *F. graminearum* on both barley and malt quality were more pronounced than those of *F. poae*. Reduction in kernel plumpness somewhat confounds the interpretation of malt quality results. However, infection with *Fusarium* did appear to have very pronounced effects on increasing wort soluble nitrogen, free amino nitrogen and wort color. These observations suggested increased proteolysis in the infected material. This is particularly true if one considers that up to 40% of kernels in the infected samples did not germinate, and would thus be expected to display reduced protease activity.

BC 11 Barley

(Descriptors: Barley, fungal deseases, microflora, malt quality.

Deskriptoren: Gerste, Pilzkrankheiten, Mikroflora, Malzqualität).

1 Introduction

Fusarium Head Blight (FHB) can be a significant problem for barley and wheat growers in areas of Asia, Europe and North America, and since 1993, production in the upper Midwestern region of the United States and southern Manitoba in Canada has been devastated by this disease (24, 41). The economic impact of FHB to growers and the malting and brewing industries can be substantial as grain yields and quality often suffer. Moreover, the presence of mycotoxins produced by the *Fusarium* pathogens may render the grain unsuitable for malting and brewing (36).

The impact of FHB infection on malting and brewing has been widely studied as *Fusarium* and its associated mycotoxins have the potential to affect malting and malt quality (4,15 – 18, 25, 40), brewing performance and beer composition (4, 8, 18, 19, 23, 38, 40, 45) and beer flavor (4). Beer gushing and the presence of mycotoxins also may be associated with the use of *Fusarium* infected grain and both topics have been recently reviewed (9, 37). In spite of this extensive study, the precise mechanisms by which *Fusarium* affects barley and malt quality is still a matter of speculation. FHB infection probably affects quality in both direct and indirect manners. Direct effects upon grain quality may be due to the growth and ramification of the fungus throughout the kernel, and the production of metabolites which may alter grain composition or metabolism, or render it unfit for human or animal consumption. Indirect effects relate to reductions in yield that are associated with infection. Mycelia of *Fusarium* may impair the transport of nutrients to the developing kernels (28), and associated yield reductions in barley may result from a decrease in 1000 kernel weight. Pronounced differences in barley kernel composition and in barley malt and wort quality have been reported to result from changes in kernel fill (12). This follows, as with a reduction in kernel fill, kernels are thinner and there tends to be a decrease in the proportion of endosperm to husk.

Studies on the impact of FHB on grain quality generally have followed one of three approaches. These include studies which have employed uninfected plants that were inoculated with *Fusarium* in the field (18), studies in which barley has been inoculated during malting (4, 17, 30, 40), and finally those in which grain or isolated tissues have been spiked with specific *Fusarium* metabolites (13, 16, 27, 32). Field-infected grain best reflects what would be encountered in commercial practice. However, field-infected grain (naturally or artificially infected) typically hosts a range of other fungi and bacteria. Moreover, non-infected control material can be difficult to obtain from the same environment. Inoculation of grain with *Fusarium* during processing (e.g. malting) also suffers from some deficiencies, in that growth and ramification of the fungus may not adequately reflect that occurring under field conditions. Spiking experiments suffer from similar limitations, in that the metabolite is typically deposited only the on the surface of the grain, and may be lost during steeping or germination.

During kernel development, fungi and bacteria may become established in the pericarp and under the lemma and palea (10).

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Growth of *Fusarium* into moderate to heavily infected wheat and barley kernels may be extensive, as confirmed with scanning electron microscopy (SEM) (7, 26, 33). Extensive degradation of both endosperm protein and starch has been observed. This degradation is presumably caused by fungal enzymes, although little has been reported on the identification or characterization of enzymes from infected grain. *Sloey* and *Prentice* (40) hypothesized that increased amyloysis and proteolysis observed in *Fusarium*-infected barley might be due to endogenous barley enzymes synthesized in response to fungal gibberellins. Synthesis of cereal enzymes during germination of infected grain might also be impacted by the presence of various mycotoxins, some of which are known to be potent inhibitors of protein synthesis. For example, spiking experiments in which toxins have been applied to barley prior to malting, or during steeping or germination, have demonstrated diacetoxyscirpenol (DAS), deoxynivalenol (DON) (32), and T-2 toxin (16,27) to impact α-amylase synthesis in germinating barley.

The environment of germination in malting provides an ideal environment for the growth of many field fungi, and growth of *Fusarium* during malting has been reported by a number of authors (4, 15, 20, 36, 44). Cross-contamination or secondary infection of uninfected kernels also appears to occur during malting (20). The presence of *Fusarium* under the lemma and palea of green malts has been observed (36), and in some cases, it is likely that the fungus continues to ramify throughout the kernel as germination precedes. Production of mycotoxins and the factor(s) which cause beer gushing can parallel *Fusarium* growth during germination (34, 35).

Aside from the presence of mycotoxins, there are a number of problems associated with *Fusarium*-infected grain. Infection can result in a reduction of kernel size and weight (2). As the volume of endosperm/total kernel is reduced in smaller kernels, thin or shrunken kernels that result from infection might be expected to have higher relative levels of protein, and to yield less malt extract when mashed. A second problem sometimes associated with FHB infection is reduced germination. *Haikara* (18) reported a large decline in germinative capacity of barley that was inoculated in the field with *F. avenaceum* and *F. poae.* Kernels that do not germinate during malting will not produce the normal complement of hydrolytic enzymes, and the endosperm will remain largely unmodified. A significant number of non-germinative kernels will reduce total malt extract, and may contribute to other problems such as poor run-off.

The most pronounced changes in malt quality associated with *Fusarium* infection involve abnormally high protein modification (4). *Sloey* and *Prentice* (40) inoculated barley with a number of *Fusarium* cultures during steeping, and found resultant malts to exhibit wort nitrogen levels 2 – 31% higher than those of the controls. In a similar study, where barley was inoculated at steeping with cultures of *F. graminearum* and *F. moniliforme*, the Kolbach index was found to increase (111% vs. control) as a result of infection (17). Levels of free amino nitrogen (FAN) were observed to increase 11 – 51% as a result of field infection with *F. avenaceum* and *F. poae* (18). An increase in wort color has generally been observed with an increase in protein modification that results from infection, although it has also been suggested that pigments from the fungus itself may contribute to increased wort color (40). Strong evidence for the involvement of fungal enzymes, or stimulation of endogenous barley enzymes comes from observation of increased levels of α-amylase (17, 18, 40), and decreased wort viscosity (17). *Narziß* et al. (26) observed increased glucanase, as well as protease activity in *Fusarium* infected wheat and barley malts, and *Dickson* (10) reported that levels of water soluble carbohydrate, nitrogenous materials, and the level of free fatty acids increased with blight severity in barley. Results on the impact of *Fusarium* infection on diastatic power and extract have been mixed, with both decreases and increases being observed. In general, the changes in these parameters have not been as pronounced as for soluble protein. No direct report on amyloysis associated with *Fusarium* infection has been made, but *Sloey* and *Prentice* (40) observed that inoculation with one *Fusarium* culture increased levels of wort maltose and decreased dextrins.

The objectives of this study were to investigate barley and malt quality as impacted by *F. graminearum* and *F. poae*. Inoculation of plants in the greenhouse was utilized so as to reduce interference from other microorganisms and to provide non-infected control material from an identical growth environment. *F. graminearum* is the primary pathogen responsible for the recent FHB epidemics in the upper Midwestern USA (31). *F. poae* also has been implicated in causing FHB of barley in this region, but to a very limited extent.

2 Experimental

2.1 Barley and Inoculation with *F. graminearum* and *F. poae*

The six-rowed barley cultivar *Robust,* used throughout this study, was obtained from Great Western Malting (Vancouver, Washington, USA). The Robust seed was increased in Montana in 1995 and was determined at harvest to be free from *Fusarium* infection. Isolates of *F. graminearum* (KB-172) and *F. poae* (KB-654) were from the Department of Plant Pathology at North Dakota State University. The *Fusarium* isolates were grown on half-strength Potato Dextrose Agar (PDA), which was prepared by diluting full strength PDA 1:1 with 1.5% water agar (Difco, Detroit, MI USA). The pH was adjusted to 5.5 with lactic acid after sterilization. Inoculum was prepared from *Fusarium* cultures grown on half strength PDA for 14 – 21 days. Culture plates were flooded with sterile distilled water and scraped with a rubber spatula to harvest spores. The resulting conidial suspension was filtered through 4 layers of cheesecloth to remove mycelial fragments. Spore concentrations of 1,500 – 2,500 macroconidia/ml of *F. graminearum*, and 10,000 – 20,000 microconidia/ml of *F. poae* were obtained by use of a hemacytometer. Previous experiments had shown these respective inoculum levels to be optimal for obtaining a high level of infection on barley. However, after the first inoculation treatment, the level of infection was too high and severe. Thus, the concentration in the second inoculation was reduced.

Seeds of the barley cultivar *Robust* were planted in 15 cm square plastic pots containing a potting mix of 3 parts peat moss and 1 part perlite. The plants were grown in the greenhouse at 22 – 25°C with supplemental lighting (2000 – 2500 μmol photons m^2sec^{-1}) provided by sodium vapor lamps for 16 hrs/day. Barley plants were inoculated with the *Fusarium* species at the late milk to early dough stages of development. Using an atomizer (Model 15, DeVilbiss Inc., Somerset, Pennsylvania, USA) pressured (60 kPa) by air pump, 1.5 – 2 ml of the spore suspension was sprayed on each spike. After inoculation, the plants were placed in mist chambers with 100% RH at 25°C for 36 hrs, initially under 18 hrs of light (100 – 350 photons m^2sec^{-1}) followed by 18 hrs of darkness. Control plants were sprayed with distilled water. The plants were then returned to the greenhouse benches. The amount of disease on each plant was assessed 7 – 14 days after inoculation. Disease severity was calculated as the number of infected kernels divided by the total number of kernels in the spike.

2.2 Micro-Malting

Time required to reach 45% steep-out moisture was determined by pilot-steeping a 10 g sample as previously described (22). Individual samples (150 g) were then steeped in 1.5 l water in beakers (grain to water ratio 1:10). Temperature was maintained at 16°C by placing the beakers in a refrigerated water bath. Steep water was drained every 10 hrs and each drain cycle was followed by a two hr air-rest. During immersion the steeps were aerated with compressed air for 6 min/hr. Total steep times ranged from 30 to 50 hrs. Following steep-out, the samples were germinated in beakers at 16°C and 100% relative humidity for 5 days. Samples were turned daily by hand to prevent matting, and sample weight was adjusted (to 45% moisture) with distilled water. Kilning was conducted in a forced-air laboratory kiln as previously described (22). Total kiln time was 24 hrs, during which temperatures were ramped from 49°C to 85°C. Rootlets were removed from the kilned malt prior to analysis. Malting loss was calculated as previously described (22).

2.3 Determination of *Fusarium* Infected Kernels

A sub-sample of 100 seeds was randomly selected from each sample and surface sterilized with 0.5% sodium hypochlorite (60 sec). The samples were rinsed with sterile distilled water (3x) and plated on the half strength PDA agar. Results were reported as the percent of kernels infected with either *F. graminearum* or *F. poae*.

2.4 Measurement of Mycotoxins

Analysis for the presence of 17 mycotoxins was performed as described previously (36). Five grams of ground barley were extracted with 40 ml acetonitrile:water (84:16) for one hour on a horizontal shaker. Six ml aliquots were then clarified with an alumina:C-18 column (1.5 g, 1:1). Two ml aliquots from the eluent were evaporated. One ml was derivatized with trimethylsilyl-inositol (TMSI) for 15 trichothecenes and the second ml was derivatized with bis(Trimethylsilyl)-trifluoroacetamide (BSTFA) for 2 estrogens (zearalenol and zearalenone). Samples were analyzed on GC-MS (HP 5890, Wilmington, Delaware, USA; Incos 50, Finnigan Corp., San Jose, California, USA) with a 30-m x 0.25-mm (i.d.) fused silica capillary column with a 0.25-µm coating.

2.5 Measurement of Ergosterol

Ergosterol levels of barley or malt were determined according to the modified HPLC method of *Jambunathan* et al. (21) as previously described (36).

2.6 Determination of Barley Quality Parameters

Barley 1000 kernel weight, kernel assortment and germinative energy were determined according to standard methods of the American Society of Brewing Chemists (ASBC) (3). Barley moisture was determined by near infrared reflectance with a Infratec1226 Grain Analyzer (Foss Tecator, Höganäs, Sweden). Kernel brightness was determined according to a modification of the ASBC method Barley-8 (3) using a Pacific Scientific Gardner/Neotec, XL-800 series Colorimeter (Pacific Scientific, Bethesda, Maryland, USA). Brightness is reported as the L-value, with higher numbers indicating greater kernel brightness. Barley β-glucan levels were determined with a Megazyme (Wicklow, Ireland) kit according to the instructions of the manufacturer. Barley protein was determined according to the Kjeldahl method (Method 46-11) of the American Association of Cereal Chemists (1) using the catalyst described by *Williams* (46). Barley samples were assayed for amyloglucosidase (AMG) using a p-nitrophenyl-β-maltoside substrate obtained from Megazyme (Wicklow, Ireland). Ground samples were extracted with 25mM sodium acetate buffer (pH 4.7) for 1 hr.

2.7 Rapid Visco-Analyzer (RVA)

The Rapid Visco-Analyzer continuously measures the viscosity of a heated flour slurry. Lower viscosity is an indication of the presence of α-amylase, which will reduce the viscosity due to degradation of the starch. The method described by *Bason* et al. (5) was followed using 4.0 g of sample ground in a UDY mill (Boulder, Colorado, USA) and mixed with 25 ml distilled water. The mixture was placed in a heating block controlled at 95°C. An initial high speed stirring (900 rpm) for 10 sec. was followed by a constant stirring at 160 rpm. The stirring number (SN) is the viscosity at 3 min.

2.8 Determination of Malt Quality Parameters

All malt quality parameters were determined according to standard or modified ASBC (3) methods as previously described (22), with the exception of protein, α-amylase, β-amylase, and (1→3), (1→4)-β-D-glucans (β-glucans). Total malt protein and β-glucan was determined as described for barley. Beta-glucan loss (%) represents the loss in the absolute amount of β-glucan (total g) that occurred during malting of each sample. Alpha- and β-amylase were determined with kits purchased from Megazyme (Wicklow, Ireland) according to the instructions of the manufacturer. These assays employed p-nitrophenyl maltoheptaoside (PNPG7) and p-nitrophenyl maltopentoside (PNPG5), respectively as substrates. Malt samples utilized for enzyme analyses were freeze-dried and not kilned. Beta- and α-amylase activities are expressed in Betamyl- and Ceralpha units/original barley kernel respectively. Activity measured in units/g was converted to units/kernel by multiplying by the kernel weight in the case of barley. In the case of malt, activities (units/g) were first corrected for malt loss.

2.9 Data Analysis

The experiment was designed to use two different sowing dates as replicates. Plants for each replicate were randomly divided into three sets. The first set served as a control while the other two were inoculated with the *Fusarium* isolates. Disease severity in the first replicate was much higher than expected. Subsequently, the concentration of inoculum for the second replicate was reduced. Due to the differences in inoculum levels, it is not appropriate to treat the results from each sowing date as replicates for the calculation of tests of significance. Consequently only the trends observed between the two sowing dates are discussed.

3 Results and Discussion

3.1 Inoculation

Mycoflora assays indicated that 67% and 76% of kernels were infected with *F. graminearum* and 60% and 84% of kernels were infected with *F. poae* following the first and second treatments, respectively (Table 1). *Fusarium* appeared to be present on the outer layer of the kernel, as well as under the husk in the form of viable spores and mycelium. Previous research in the field and greenhouse showed that *F. graminearum* is more virulent than *F. poae* (31). The same appeared to be true in the current study, as *F. graminearum* yielded infection levels similar to those obtained

Table 1 Infection Rates and Levels (mg/g) of Mycotoxins and Ergosterol in *Fusarium* Infected Barley Samples and Resultant Malts

	Control		*Fusarium poae* Infected		*Fusarium graminearum* Infected	
	Distilled Water		19,000 conidia/ml	10,000 conidia/ml	2,500 conidia/ml	1,500 conidia/ml
Infection Rate (% infected kernels)	0	0	60	84	67	76
(µg/g)						
DON						
Barley	Nd	Nd	0.6	Nd	64.3	29.5
Malt	Nd	Nd	Nd	Nd	7.8	1.7
15-Acetyl DON						
Barley	Nd	Nd	Nd	Nd	10.2	4.4
Malt	Nd	Nd	Nd	Nd	0.9	Nd
Nivalenol						
Barley	Nd	Nd	1.8	0.6	Nd	Nd
Malt	Nd	Nd	Nd	Nd	Nd	Nd
Zearalenone						
Barley	Nd	Nd	Nd	Nd	139	Nd
Malt	Nd	Nd	Nd	Nd	Nd	Nd
Ergosterol						
Barley	2.3	<0.1	6.6	5.7	22.8	4.4
Malt	2.8	<0.1	6.8	9.4	22.7	7.6

Nd; not detected

with *F. poae*, but with much lower rates of inoculation. Although inoculation levels were reduced for the second sowing date, infection levels were actually somewhat higher. This is indicative of the effectiveness of the inoculation method, and probably also of slight variations in greenhouse conditions between the two sowing dates. The dates of sowing were about 4 months apart.

Mycotoxins produced by *F. graminearum* and *F. poae* are shown in Table 1. Large amounts of deoxynivalenol (DON), 15-acetyl DON (15-ADON) and zearalenone were detected on the *F. graminearum* infected samples, while trace levels of DON and nivalenol (NIV) were detected on the *F. poae* infected samples. These results are consistent with those previously reported by *Salas* et al. (31), in that the presence of DON and 15-ADON are suggestive of infection with *F. graminearum*, while the presence of NIV is more indicative of *F. poae.* The levels of DON measured on the *F. graminearum* inoculated samples were at least 3-fold higher than what has been typically been observed with natural infection in the field (34). Again, this is indicative of the effectiveness of the greenhouse inoculation and of the severity of the resultant infection.

Ergosterol is the predominant sterol component of nearly all fungi, and does not occur in plants. *Seitz* and coworkers (39) reported that ergosterol measurement was suitable for detecting *Alternaria* and *Fusarium* growth in grain, even when the fungi were no longer viable. As such, it was used in this study as a measure of *Fusarium* biomass. The levels of ergosterol observed in the greenhouse infected grain were of the same order of magnitude as we have previously observed with naturally infected material (34, 36).

3.2 Barley Quality

Infection with *F. graminearum* and *F. poae* had a large impact upon barley quality parameters at all inoculum concentrations employed in this study (Table 2). The most pronounced effects were on kernel plumpness and germination. Inoculation with *F. poae* reduced germination 8 – 14% relative to the controls, while the reduction with *F. graminearum* was 32 – 42%. *Haikara* (18) observed similar declines in germination with barley which had been inoculated in the field with *F. poae* and *F. avenaceum.* Although the specific cause(s) cannot be identified from the results of this study, reduced germination may be due to a number of factors associated with *Fusarium* infection. Prentice (29) reported that *F. moniliforme* may inhibit germination by inhibiting utilization of oxygen by the seed. Some mycotoxins including zearalenone (ZEN) (11) are also known to reduce seed germination. High levels of both DON and ZEN were observed in the *F. graminearum* infected material in this study (Table 1). Finally, fungal infection of cereal grains is often concentrated around the embryo (4), and it would seem probable that this colonization might damage the viability of the embryo.

Reduction in kernel size is often associated with *Fusarium* infection, especially when the infection occurs at the early stage of kernel development (42). In the current study, kernel plumpness was reduced 10 – 67% (relative to the controls) as a result of infection (Table 2). This magnitude of reduction is not surprising since the inoculations were made at the late milk to early dough stage of kernel development. Reduction in kernel plumpness was closely paralleled by a decrease in 1000 kernel weight, and increase in the number of kernels passing through a 2 mm sieve (thin kernels). Overall, the effects of *F. graminearum* appeared to be much more pronounced than those of *F. poae.* Increases in grain protein also were observed to result from infection. This undoubtedly is related to the decrease in kernel size. Smaller kernels have a higher ratio of embryo/husk, and thus a higher relative percentage of protein. Mold biomass itself may make a small contribution of overall protein. However, *Prentice* and *Sloey* (30) reported that the contribution of nitrogen from fungi used to inoculate barley was generally negligible when total wort nitrogen figures were evaluated.

Table 2 The Effect of Inoculation with *Fusarium graminearum* and *F. poae* on the Physical and Chemical Properties of Barley

Treatment	Control Barley Distilled Water		*Fusarium poae* Infected Barley 19,000 conidia/ml	10,000 conidia/ml	*Fusarium graminearum* Infected Barley 2,500 conidia/ml	1,500 conidia/ml
Kernel Plumpness (%)†	91	76	82	39	37	25
Thin Kernels (%)‡	<1	2	1	9	16	15
1000 Kernel Weight (g)	33.9	32.0	30.7	25	22.4	23.7
Kernel Brightness (L-value)	59	60	55	55	51	53
Germinative Energy (%)	100	100	92	86	58	68
Protein (%)	14.3	15.7	14.7	17.4	13.3	16.7
Stirring Number (cP at 3 min.)	738	976	328	387	284	266

† % kernels retained on a 2.4 mm sieve=plump
‡ % kernels passing through a 2.0 mm sieve=thin

The impact of barley microflora on weathering or darkening has long been recognized (14) and maltsters, by experience, have long avoided the use of stained or weathered barley. Kernel brightness was visibly reduced by greenhouse infection with both *F. graminearum* and *F. poae* (Table 2). However, the effects were not nearly as pronounced as those observed in the field, where a host of bacteria and fungi can contribute to discoloration under wet conditions. Measurement of kernel brightness may have been slightly affected by the reduced kernel size, which changes the sample packing characteristics and the size of interstices in the colorimeter cell.

Perhaps the most interesting change in barley quality observed to result from infection was the decrease in paste viscosity observed with the Rapid Visco-Analyzer (Fig. 1). Infection with both *F. graminearum* and *F. poae* caused a dramatic decrease in peak viscosity and viscosity after 3.0 min (stirring number) when compared to the non-infected control. The profiles seen with the infected samples are somewhat characteristic of those of sprouted grain (5). These results strongly suggest extensive damage to the barley starch during infection and colonization, and/ or the presence of amylolytic enzymes from the *Fusarium.* It is very unlikely that kernel size could have caused the observed difference. A small amount of amylolytic activity was detected in the infected barley by assay with PNPG7. As expected no α-amylase was detected in the sound non-infected control grain. Discussion of these results relative to malt quality will continue in the following section.

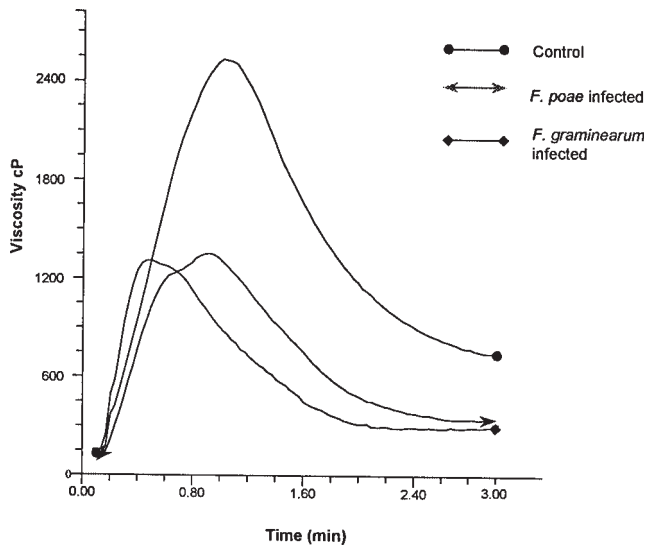

Fig.1 Rapid Visco-Analyzer (RVA) pasting curves for control barley and barley infected with *Fusarium graminearum* and *F. poae.* Stirring number is the viscosity at 3.0 min.

3.3 Malt Quality

Numerous studies have shown that *Fusarium* present on barley can continue to grow during the malting process (15, 20, 44), and in some cases, may produce mycotoxins during germination (36). As such, damage to the kernel can be caused by the initial infection in the field, or by subsequent growth and colonization during malting. However, in the current study, it appeared that very little to no growth of *F. graminearum* or *F. poae* occurred during malting. This is evidenced by the near static levels of ergosterol from barley to finished malt (Table 1). The levels of ergosterol on malt also were much lower than those reported by *Schwarz* et al. (34). Further evidence for limited fungal growth during malting comes from the results of mycotoxin analyses (Table 1). Levels of DON, ZEN, 15-acetyl-deoxynivalenol (15-ADON) and nivalenol (NIV) all declined dramatically following malting. This observation is consistent with their solublization and loss during the steep. Had significant growth of either organism been observed during malting, some synthesis of additional mycotoxin may have been expected (36), and final levels on malt may have been greater. The reason for lack of growth during malting is not readily apparent. Although a decrease in mycotoxin production is associated with a decline in *Fusarium* viability (6), the samples in the current study were malted less than 4 weeks following harvest of the second replicate. Results of *Vaag* and *Pederson* (44) suggest that growth of *Fusarium* (change in *Fusarium* antigen levels) during the germination-phase of malting can be quite variable.

Table 3 Quality Parameters of Malts Prepared from *Fusarium* Infected Barley Samples

Treatment	Control Malt Distilled Water		*Fusarium poae* Infected Malt 19,000 conidia/ml	10,000 conidia/ml	*Fusarium graminearum* Infected Malt 2,500 conidia/ml	1,500 conidia/ml
Malting loss (%)	11.8	13.9	13.7	15.2	16.9	16.8
Extract (fine grind %)	79.2	79.6	78.6	77.2	75.0	75.7
Fine-Coarse Grind Extract Difference (%)	1.4	2.2	1.3	1.7	2.2	1.3
Wort Color (SRM)	3.4	2.6	4.2	5.7	7.7	6.6
Wort Viscosity (cP)	1.38	1.39	1.32	1.30	1.30	1.33
Malt Protein (%)	14.5	16.5	14.8	17.8	13.7	17.5
Soluble Protein (% malt)	7.1	7.3	7.5	9.2	7.5	9.1
Kolbach Index	49.5	44.0	50.5	51.5	55.0	52.0
Free Amino Nitrogen (mg/L)	136	147	161	191	152	169

Given the lack of evidence for growth of *F. graminearum* or *F. poae* during malting in this study, it might be assumed that declines in malt quality can be directly attributed to damage caused by the initial infection, or to metabolites produced at this time. Infection with both *F. graminearum* or *F. poae* was observed to cause prodigious changes in malt quality (Table 3 and 4). While some of these alterations might be expected as a result of decreased kernel size and weight associated with infection, close consideration of the data strongly suggests the direct involvement of *Fusarium*.

As observed by other researchers (17, 18, 40), the most striking changes in malt quality to result from *Fusarium* infection relate to soluble protein. In the current study, infection with *Fusarium* was observed to increase soluble protein, Kolbach index and levels of FAN (Table 3). In extreme cases, infection resulted in increases in these parameters up to 30% above the controls. A concomitant increase in proteolytic activity is strongly suggested, and the *Fusarium* fungus itself would appear to be the most likely source. This follows as both *F. graminearum* and *F. poae* infected samples displayed reduced germination. The bulk of proteolytic enzymes present in green malt are synthesized during germination, and sound barley displays very little enzyme activity. Damage to the endosperm protein matrix and starch granules in *Fusarium* infected grain has been observed by SEM (7, 26), and *Narziß* and coworkers (26) have reported elevated protease levels in *Fusarium* infected barley malts. On the other hand, *Prentice* and *Sloey* (30, 40) speculated that changes in malt quality parameters may have been due to the presence of endogenous fungal gibberellins. This

Table 4 Levels of α-Amylase, β-Amylase, and β-Glucans in *Fusarium* Infected Barley Samples and Resultant Malts

Samples	Control Distilled Water		*Fusarium poae* Infected 19,000 conidia/ml	10,000 conidia/ml	*Fusarium graminearum* Infected 2,500 conidia/ml	1,500 conidia/ml
α-Amylase (units/kernel)†						
Barley	Nd	Nd	0.03	0.03	0.02	0.02
Malt	13.9	16	13.6	17.3	10.7	14.9
β-Amylase (units/kernel)†						
Barley	79	69	48	49	39	43
Malt	48	53	52	46	37	47
β-Glucan (%)						
Barley	4.7	5.3	3.7	4.1	2.8	3.9
Malt	1.5	1.4	1.1	1.0	0.7	1.1
β-Glucan Loss (%)‡	73	78	74	80	80	77

Nd: not detected

†Malts for analysis of α- and β-amylase were freeze-dried following germination.

‡β-Glucan loss is the loss in absolute amount (g) of β-glucan following 5 days of germination.

is of interest as synthesis of several malt enzymes is known to be promoted by GA (gibberellic acid), and species of *Gibberella* and *Fusarium* are well known producers of gibberellins. *Tuomi* et al (43) evaluated 40 fungi isolated from Finnish barley for GA, ABA (abscisic acid) and indole-3-acetic acid production (IAA). They found no ABA or GA produced by any the five (unidentified) *Fusarium* species evaluated, and further concluded that the maximum amounts GA and ABA produced by several species of *Aspergillus, Penicillium* and *Alternaria* would not make a significant contribution to the overall hormonal pool of the grains.

The increased FAN values may have been responsible for the higher wort colors observed with the infected samples (Table 3), as at least a portion of wort color is due to amino-carbonyl reactions between amino acids and reducing sugars. It also has been suggested that increased color could be attributable to pigments produced by *Fusarium* (40). Additional evidence for the presence and importance of *Fusarium* enzymes can be seen in the results for malting loss, malt extract, α-amylase, and β-glucan (Tables 3 and 4). Infection with *F. graminearum* or *F. poae* was observed to increase malt loss in all cases. This is surprising in that relatively high levels of non-germinative kernels would not suffer respiration or rootlet loss, or be expected to modify. As such, malting losses should have decreased somewhat in proportion to the percentage of non-germinative kernels, although thinner kernels will contribute to a greater loss. It would appear that fungal enzymes are in a sense contributing to the modification of the kernel, and that this contribution was enough to offset the absence of enzymatic activity from the non-germinative grains. The same argument would hold for malt extract, where *Fusarium* infected samples yield extract levels only 0.6-4.2 units below the controls, and fine coarse-grind extract differences that were slightly greater than those of the control. Fungal enzymes may have contributed to the modification of the barley endosperm.

The reduction in peak viscosity and SN in the RVA (Fig. 1), observed to result from *Fusarium* infection, are strongly suggestive of starch damage and /or the presence of fungal amylase(s). Assay of the barley samples for α-amylase with the PNPG7 substrate did detect the presence of very small amounts of enzyme in infected materials, which was absent in the controls (Table 4). Nevertheless, the activity detected in infected barley was still <0.2% of that seen in the control malts. This observation might be explained in a number of manners. First, it could simply be that the SN measurement is extremely sensitive to α-amylase. Second, infection during kernel development may have resulted in actual changes in starch properties. Finally, it seemed plausible that *Fusarium* was producing amylolytic activity that was not readily measured with the PNPG7 substrate. Some fungi, such as *Aspergillus,* are known to produce amyloglucosidase (AMG). AMG would not readily attack PNPG7. However, assay of infected and control barley with pNP-β-maltoside did not reveal the presence of AMG.

In this study α-amylase activity is expressed as units/kernel, in order to partially compensate for the confounding reduction in kernel size that was caused by *Fusarium* infection, as thin samples will generally display a much higher level of α-amylase when activity is expressed as units/g. When the results are examined on a per kernel basis, there appeared to be very little difference in α-amylase activity between the control and infected malt samples (Table 4). As the non-germinative kernels in the *Fusarium* infected samples would not be expected to produce α-amylase, the activity observed in the infected malts would appear to be, at least in part, directly attributable to the *Fusarium* fungus.

Cysteine was included in the β-amylase extraction buffer, so the assay is a measure of total (free+bound) activity. In most cases there was a slight reduction in activity from barley to malt, and this loss is most likely attributable to freeze-drying of the green malts. Beta-amylase activity was highest in the control barley samples. In malt, levels in the *F. graminearum* infected samples were slightly lower than the controls and *F.* poae infected material. The reason and potential significance of this observation are not readily apparent.

Results of this study also seem to suggest that *Fusarium* produces an enzyme(s) which is capable of degrading β-glucan in barley endosperm cell walls. This seems plausible, as production of extracellular cell-wall degrading enzymes would seem prerequisite for successful colonization of the grain. Approximately 75 – 80% of β-glucan present in the infected barley was degraded following germination, compared to 73 – 78% in the control samples (Table 4). Endogenous β-glucanases are synthesized in barley only during germination, and thus degradation of β-glucan would not have been expected in non-germinative kernels, and the overall wort viscosity of the infected samples should have been higher (Table 3). Decreases in wort viscosity resulting from fungal infection also have been reported by *Gjertsen* et al. (17). *Narziß* et al (26), however, reported that even though *Fusarium* infected malt samples displayed elevated β-glucanase activity, the impact upon wort viscosity was minimal.

4 Conclusions

Although the effects of *Fusarium* on barley and malt quality have previously been evaluated, the use of greenhouse grown and inoculated seed in this study was unique in that it allowed most variables to be carefully controlled. Principal among these were the ability to obtain control and infected materials from identical growth environments, and to greatly limit contamination of the grain with other microflora. While the *Fusarium* infection levels of barley used in this study far exceed that normally encountered in commercial practice, it is assumed that the results might be extrapolated to what is observed with field infections of lesser severity.

These observations clearly suggest the importance of fungal enzymes produced during infection and colonization, although the possibility of hormones cannot be completely ruled out based on current evidence. Future research in this area should address the question of what levels of infection may be tolerable for production of quality malt, as the complete avoidance of *Fusarium* infected grain may not be practical in the case of widespread epidemics. In addition, it may be of interest to evaluate specific changes in wort composition that might be attributable to *Fusarium* infection. Changes in amino acid or fermentable sugar/ dextrin profile, that might result from the presence of (fungal) enzymes with a different action-pattern or chemical properties could lead to changes in processing performance. Preliminary research in our laboratory has suggested that while FAN is greatly increased as a result of *Fusarium* infection, the actual amino acid profile is not appreciably altered (P. Schwarz, unpublished data).

Acknowledgments

The authors wish to thanks Busch Agricultural Resources, Inc. (St Louis, Missouri) for partial support of this research, Dr Uwe Scholz, Plant Pathology, NDSU, for assistance with manuscript preparation, Dr Richard Horsley, Plant Sciences, NDSU, for assistance with data analysis, and Dr Howard Casper, Veterinary and Microbiological Sciences, NDSU, for mycotoxin analysis

5 Zusammenfassung

Schwarz, P. B., Schwarz, J. G., Zhou, A. , Prom, L. K., und Steffenson, B. J.: Einfluss von *Fusarium graminearum* und *F. poae* Infektionen auf die Qualität der Gerste und Brauqualität — Monatsschrift für Brauwissenschaft 54, No 3/4, 55 – 63, 2001

BC 11 Gerste

Es wird der Einfluss von *Fusarium graminearum* und *F. poae* Infektionen auf die Qualität der Gerste und Brauqualität untersucht. *F. graminearum* ist der Hauptkrankheitserreger der Ährenfusariose im Mittleren Westen der USA, während *F. poae* von geringerer Bedeutung ist. Die Inokulation und Abreife der Pflanzen unter Gewächshausbedingungen verringerte die Kontamination durch andere Mikroorganismen. Die Samen der Kontrolle und der inokulierten Behandlungen wurden vermalzt und deren Malzqualität bestimmt. Hohe Konzentrationen an Desoxynivalenol, 15-Azethyl-Desoxynivalenol und Zearalenone wurden in mit *F. graminearum* inokulierten Körnern gefunden. Dagegen wurden nur geringe Mengen von Nivalenol durch *F. poae* produziert. Am stärksten wurden die Qualitätsparameter Korngröße und Keimfähigkeit reduziert. Insgesamt verursachte *F. graminearum* eine deutlichere Verminderung der Korn- und Brauqualität als *F. poae*. Die Reduktion der Korngröße durch die Pathogene erschwert die Interpretation der Ergebnisse der Brauqualität. Allerdings scheint der *Fusarium*-Befall den Anteil des löslichen Stickstoffes, freien Aminostickstoffes und der Würzefarbe zu erhöhen. Dies gilt insbesondere, da gegenüber der Kontrolle 40% weniger Körner keimten und deren Vermälzung eine verringerte Protease-Aktivität haben würde.

Schwarz, P. B., Schwarz, J. G., Zhou, A. , Prom, L. K., et Steffenson, B. J.: Effets de contaminations de *Fusarium graminearum* et *F. poae* sur la qualité de l'orge et du malt — Monatsschrift für Brauwissenschaft 54, No 3/4, 55 – 63, 2001

BC 11 Orge

On a examiné l'influence de contaminations de Fusarium graminearum et F. poae sur la qualité de l'orge et la qualité brassicole. Le F. graminearum est le principal contaminant de maladie de fusariose d'épis au centre ouest des USA. Le F. poae a une influence moins importante. L'inoculation et la maturation des plantes sous serre diminuent la contamination par d'autres microorganismes. Les semences témoin et celles inoculées ont été maltées et la qualité du malt a été évaluée. On a trouvé des concentrations élevées en désoxynivalénol, 15-acétyl-désoxynivalénol et zéaralénone sur des grains inoculés par F. graminearum. Toutefois le F. poae produisait de faibles quantités de nivalénol. Les paramètres de qualité les plus affectés étaient la taille des grains et la capacité de germination qui se trouvaient réduits. Dans l'ensemble, le F. graminearum provoquait une diminution plus significative de la qualité du grain et de la qualité brassicole que F. poae. La réduction de la taille du grain par des pathogènes rend plus difficile l'interprétation de la qualité brassicole.
Toutefois la contamination du Fusarium augmente le taux d'azote soluble, l'azote aminé libre et la couleur du moût. Ce fait est accentué par une réduction de 40 % de grains germés comparé au témoin. Le maltage des grains contaminés donnait du malt avec une activité protéasique plus faible.

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(Manuskripteingang: 23. November 2000)