

Fate of Dwarf Bunt Fungus Teliospores During Milling of Wheat into Flour

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ABSTRACT

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Wheat contaminated with teliospores of *Tilletia controversa* Kühn (TCK) was mixed with uncontaminated wheat and processed through the Kansas State University pilot mill. Two 50-bu lots of the contaminated mixture were cleaned, tempered, and milled. Approximately 500 samples of wheat, cleanings, and mill fractions were collected and examined for the presence of intact and broken TCK teliospores. Whole wheat samples (50 g) were washed, sieved through a 60- μ m nylon sieve, and pelleted by centrifugation. Contents of the pellet were examined microscopically for the presence of TCK spores. The procedure was modified as needed to

accommodate cleanings and mill fractions. Levels of spore contamination in whole wheat samples decreased at each step during the handling process, and large numbers of spores were found in materials that were sieved or aspirated from the grain. Very few spores were found in bran, germ, and shorts; none were detected in red dog or straight-grade flour. The results showed that a high percentage of spores can be removed from wheat by mechanical cleaning but that it is not feasible to remove all of them.

The People's Republic of China has restrictions on imports of U.S. wheat that might contain teliospores of the dwarf bunt fungus, *Tilletia controversa* Kühn (TCK). This fungus infects fall-sown wheat through tiller initials and becomes systemic, subsequently replacing grain endosperm tissue with a mass of teliospores enclosed by the maternal ovarian wall. This fragile structure, called a sorus or smut ball, ruptures and releases dust-like spores during harvesting and grain handling (Hoffman 1982).

In the United States, dwarf bunt occurs in a small percentage of the winter wheat acreage and primarily in specific areas of the Pacific Northwest (PNW) and intermountain areas of Utah and Colorado, with only a few isolated historical reports in the northeast. Except for a few areas of southern Idaho and northeastern Utah, the disease generally occurs sporadically and at low levels (Purdy et al 1963, Hoffmann 1982, Trione 1982, Goates 1995) because the required environmental conditions conducive for infection do not occur every year. Control measures in the United States are typically restricted to the regions that are historically conducive to the disease and include use of resistant wheat cultivars (Goates 1995) and fungicide-treated seed wheat (Sitton et al 1993, Keener et al 1995). There are no phytosanitary restrictions on movement of wheat contaminated with TCK across state boundaries in the United States because the risk of disease spread and TCK infection is not considered to be of agronomic significance.

Trade problems occur when countries place phytosanitary restrictions on trace amounts of teliospores in U.S. grain exports (Purdy et al 1963, King et al 1991). As TCK-contaminated wheat moves through marketing channels to export elevators, it becomes mixed with uncontaminated wheat and results in detectable amounts of TCK teliospores in export shipments. In 1973, China placed an import embargo on all U.S. wheat originating from PNW ports.

The majority of TCK research has focused on the biology and epidemiology of the fungus and disease (Trione and Krygier 1977,

Grey et al 1986, Hess and Trione 1986, Stockwell and Trione 1986, Trione and Hall 1986, Rattan et al 1995, Mathre 1996). Though that research was relevant to understanding disease control and yield loss, little attention has been given to the potential risk associated with the importation of contaminated wheat destined to be milled into flour.

The major areas of concern are the dust and cleanings removed from grain before milling and the milling by-products, especially the bran. Bran, dust, and cleanings are commonly combined for use in animal feeds and are considered by the Chinese as a possible source of TCK dispersion through loss during transport or by the spread of contaminated manure on fields. This study was conducted to determine the fate of TCK spores in a typical mill during the conversion of contaminated wheat into flour.

MATERIALS AND METHODS

General

An initial spore count of 50–400 teliospores per 50 g of wheat was considered important for an adequate assessment of spore distribution across the various milling operations (*unpublished data*). No commercial lots of wheat could be identified with those levels of contamination at the time of the experiment. To achieve the desired level of spore concentration, a small sample of heavily bunted white wheat from the 1993 Idaho harvest containing both intact sori (smut balls) and free teliospores was mixed and blended with uncontaminated hard red winter (HRW) wheat.

The wheat was cleaned, tempered, and milled into flour at the Kansas State University (KSU) pilot-scale flour mill under normal operating conditions. The mill is a small commercial mill whose operation and flow diagram have been previously described (Gwirtz et al 1996). Wheat and milling fractions are transported pneumatically, and the mill has a cloth-filter dust-control system that returns the filter-collected flour to a sifter and then back into the flour stream. Wheat from the same lot of HRW wheat without TCK spores was used to bring the mill to initial equilibrium and for clean-out after each run of contaminated wheat. No significant cross-contamination of TCK spores between runs was observed. Milling fractions, dust, and cleanings were analyzed at the U.S. Grain Marketing and Production Research Center for the presence of TCK spores. The experiment was conducted once in July and repeated in August 1996. Milling by-products were pelleted (Wandra et al 1996) in the KSU feed mill to determine the effects of pelletization on TCK spores. Extrusion was also conducted on milling by-products to determine whether spores could be inactivated.

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Wheat Preparation

Heavily bunted white wheat (approximately 200 g) containing about 1.24×10^6 teliospores/g of wheat was premixed with 1 bu of clean HRW wheat in a MacLellan mixer. No attempt was made to remove or break up sori present in the highly contaminated white wheat sample. This premix was then added through a small vibratory feeder into a 9.1-m screw conveyor carrying 50 bu (approximately 1.5 tons) of clean HRW wheat and distributed into three bins. Further blending was accomplished by simultaneously drawing wheat from the three bins and placing it into a mill-mix bin. The wheat was transferred back to the three bins, and the mixing process was repeated. Wheat for milling was drawn from the mill-mix bin, weighed, and cleaned. Foreign material was removed in the receiving separator, which consisted of screens and an aspirator. The wheat was then passed sequentially through a gravity selector, a cylinder separator, an entoleter-scourer-aspirator (ESA), and a final aspirator. After it was cleaned, the wheat was tempered by transferring it by pneumatic lift to the tempering conveyor, where water was added. It was then transferred by screw conveyor into two stainless steel tempering bins, where it was held overnight at room temperature. Uncontaminated, clean control wheat was cleaned and tempered in a similar fashion and stored in separate tempering bins. Samples were taken at various times and points during the mixing and cleaning process (Table I).

Milling

The next day, wheat was drawn from the tempering bins simultaneously for additional mixing, cleaned in another ESA, and then transferred to the prebreak rolls of the mill. Clean wheat (approximately 1,500 lb) was milled for 1 hr followed by a 1-hr run of the contaminated test wheat, a 1-hr run of clean wheat, a 1-hr run of test wheat, and finally a 1-hr run of clean wheat. No significant contamination was found in any of the clean wheats processed through the mill; only a few spores were detected occasionally in the clean wheat. The entire process was repeated 1 week later with another 50-bu mixture of HRW wheats. Since the filters are changed infrequently, they could provide a source of contamination when they are replaced.

Sampling

Three 50-g subsamples of the heavily bunted white wheat were assayed for spores. After 200 g of contaminated wheat was thoroughly mixed with 1 bu of clean wheat, three 500-g samples were removed from the MacLellan mixer and subjected to the standard analysis procedure for TCK spores (see below).

TABLE I
Sampling Points for Wheat and Other Materials

Material	Sample Size (g)
Initial contaminated white wheat	3 × 50
Premix (1 bu) of contaminated and clean wheat	3 × 500
Wheat coming from mill-mix bin before cleaning	3 × 500
Cleanings removed by receiving separator	All
Cleanings removed by gravity selector	All
Cleanings removed by cylinder separator	All
Cleanings removed by entoleter-scourer-aspirator	All
Cleanings removed by final aspirator	All
Wheat being transferred to temper conveyor	3 × 500
Wheat coming out of tempering bins	3 × 500
Cleanings removed by entoleter-scourer-aspirator	All
Samples collected from each 1-hr run	
Wheat coming out of entoleter on way to mill rolls	3 × 500
Germ sampled every 10 min during milling	6 × 150
Bran sampled every 10 min during milling	6 × 100
Shorts sampled every 10 min during milling	6 × 150
Red dog sampled every 10 min during milling	6 × 150
Flour (straight grade) sampled every 10 min, at agitator	6 × 200
Dust collected by cyclone during milling	All

Three 500-g wheat samples were collected as the wheat left the mill-mix bin before cleaning. Other samples were taken as the wheat moved through the cleaning house and mill (Table I). Slide gates or access doors were installed for removal of samples at each sampling point. The three control wheat runs were sampled the same way as the contaminated wheat and analyzed for TCK spores. The samples of milling streams, dust, and cleanings were subsampled and examined by light microscopy (LM) for TCK. At least three subsamples of each sample were analyzed.

Spore Assays

Spores were isolated from samples of whole wheat, bran, and dust by standard centrifuge seed-wash protocols of the International Seed Testing Association with minor modifications (Pirson 1978). The procedure involves washing, sieving, centrifuging, and resuspending pelleted spore and extraneous material and then examining it by LM. Identification of TCK spores was not a problem in these tests because of the absence or near absence of similar-appearing spores of other species. The high numbers of spores that were added to the wheat made the possibility of an occasional non-TCK smut spore an insignificant issue. A hemacytometer and standard dilutions were used to assess the spore concentration of heavily contaminated white wheat, since spore numbers were too great to be counted by the standard LM method.

Whole wheat samples were analyzed by placing a 50-g subsample in a 500-ml Erlenmeyer flask with 100 ml of water containing two drops of Tween 20. The flask was shaken for 5 min on an orbital shaker at 400 rpm. The contents were then poured onto a 60- μ m nylon mesh sieve, and the water was collected in a beaker. After the screen was rinsed with 10 ml of water, the contents of the beaker were poured into three centrifuge tubes and centrifuged at $600 \times g$ for 5 min. The supernatant was poured off, and the pellet was collected with a disposable Pasteur pipette and transferred to a disposable 1.5-ml microcentrifuge tube and centrifuged to form a pellet. The pellet was resuspended in water as needed, transferred as drops to microscope slides, covered with glass coverslips, and examined by LM at 400 \times . The entire area under the cover glass was examined on each slide, and the intact and broken spores were counted. Broken spores were counted only if 50% or more of the spore was present. Teliospores were examined at 1,000 \times as necessary to confirm that they were morphologically TCK.

Cleanings, dust, and milling fractions were analyzed by using 1- to 10-g subsamples of material. Since many starch granules were present in these samples and starch and other grain components made it nearly impossible to accurately find and count spores, the starch and storage proteins were enzymatically digested. The subsample was extracted by the standard isolation method, and the pellet was digested with malt extract prepared from freshly ground commercial two-row barley malt. Malt extract was prepared by placing 30 g of ground malt in 60 ml of water and incubating the mixture at 37°C for 30 min. Water (40 ml) was added, and the mixture was incubated at 50°C for 30 min and then at 70°C for an additional 30 min. The mixture was then filtered to yield 67 ml of extract, which was stored frozen in 10-ml aliquots. This crude enzyme preparation was thawed and used for 30 min each at 50 and 70°C with agitation every 10 min to digest starch and proteins present in the samples. After digestion, the remaining material and spores were centrifuged, washed, and prepared for LM examination. The enzyme treatment did not have any visible effect on the spores.

Pelletized and extruded samples were first soaked in water to facilitate suspension and then extracted and examined for intact and broken TCK spores.

Millfeed Processing

Milling by-products, including dust and screenings, were pelletized by standard procedures at the KSU feed mill (Wandra et al

1996). Pellets were examined microscopically for the presence of intact and broken TCK spores. Millfeed material was also passed through a single-screw extruder, and the extruded material was examined for intact and broken spores.

Germination

Viability tests of teliospores isolated from the extruded millfeed were conducted according to standard germination protocols (Goates and Peterson 1997). Teliospores were surface sterilized, placed into petri plates containing 2% water agar with 100 mg each of streptomycin sulfate and ampicillin per liter, and incubated for 8–10 weeks at 5°C with exposure to 12 hr of light per day. Untreated bran, artificially contaminated with teliospores, was processed as a positive control.

RESULTS AND DISCUSSION

Variability in spore counts is a limiting factor in any study of this type. Variability was a problem particularly in the August replication of the experiment in which the individual spore counts in the mill-mix wheat were not proportional to the sum of the cleanings and mill fractions (Tables II and III). Although these results did not contradict the overall conclusions reached in this study, the data resulted in a negative number for losses after cleaning (Table III). Similarly, there was a negative loss following tempering and cleaning in the ESA for the July mill run (Table III). Much of this extreme variability stemmed from the presence of smut balls (sori). We purposely did not remove the sori from the white wheat used to create the mill-mix samples because sori occur naturally in commercial shipments leaving the PNW on their way to export terminals and we wanted to mimic a commercial lot of wheat. Variation in teliospore count was common when contaminated wheat was sampled close to the harvest source but was not observed in export samples. Studies showed that TCK teliospores were fairly uniform in number and distribution among multiple whole-ship and ship-hold composite samples (*unpublished data*). This uniformity was caused by the continuous handling, mixing, and compositing of wheat in transit to the export terminal, resulting in the rupture of sori and the dilution and dispersion of individual teliospores throughout the grain mass.

Since most export lots of PNW wheat had spore counts too low to be used in a study such as this one, we were forced to produce our own contaminated wheat. Because of the limited amount of grain handling, sori were not completely broken apart and spores were not evenly dispersed. When a sorus or portion of a sorus was included in a sample that was examined for TCK spores, the isolation procedure broke up the spore mass and released huge quantities of spores, resulting in large variations in spore counts

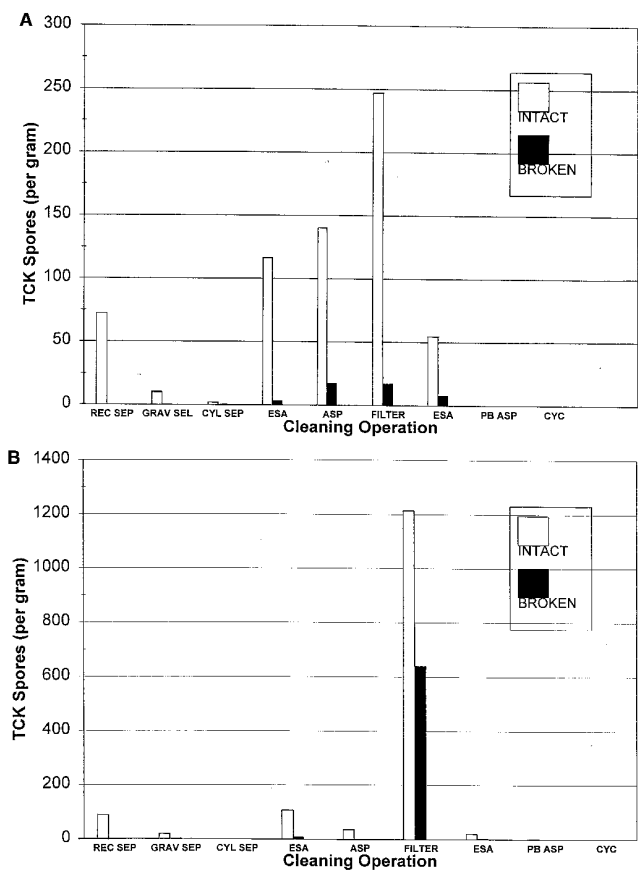


Fig. 1. *Tilletia controversa* Kühn (TCK) spores isolated from cleanings from the July (A) and August (B) milling runs. REC SEP = receiving separator; GRAV SEL = gravity selector; CYL SEP = cylinder separator; ESA = entoleter-scourer-aspirator; ASP = final aspirator; FILTER = filter; PB ASP = prebreak aspirator; and CYC = cyclone.

TABLE II
Tilletia controversa Spore Content in Wheat and Milling Fractions

Description	July					August				
	Count Range for Each Rep	Samples Analyzed (no.)	Sample Size (g)	Reps Analyzed (no.)	Geometric Average	Count Range for Each Rep	Samples Analyzed (no.)	Sample Size (g)	Reps Analyzed (no.)	Geometric Average
Wheat from mill-mix bin	124–978	3	50	9	301	5–296	3	50	15	34
Cleanings from receiving separator	606–811	1	10	3	72	26–3,330	1	10	5	89
Cleanings from gravity separator	52–149	1	10	3	9.4	129–272	1	10	3	19
Cleanings from cylinder separator	7–22	1	10	3	1.4	0–4	1	10	3	0.1
Cleanings from ESA ^a	262–6338	1	10	3	117	743–1,617	1	10	5	106
Cleanings from final aspirator	46–236	1	10	6	140	41–2,173	1	10	7	35
Wheat to temper conveyor	3–326	4	50	12	44	4–200	3	50	11	26
Cleanings from house filter	69–802	1	1	6	247	1,090–1,393	1	1	3	1215
Wheat from tempering bins	0–46	12	50	40	6.9	0–61	12	50	36	8.1
Cleanings from ESA	0–639	4	1	14	54	0–467	6	1	23	20
Wheat, entoleter to mill	0–47	6	50	24	1.8	0–27	6	50	19	0.8
Dust from prebreak aspirator	0	6	0.2	21	0	0–11	6	0.2	18	1.2
Germ	0–2	7	1	23	0.1	0–2	6	1	18	0.3
Bran	0–1	6	1	18	0	0–3	8	1	24	0.2
Shorts	0–3	6	1	20	0.1	0–3	6	1	18	0.4
Red dog	0	6	1	18	0	0	6	1	18	0
Flour (straight grade)	0	6	0.2	18	0	0	6	0.2	18	0
Dust from cyclone (milling)	0	2	0.2	6	0	0	2	0.2	6	0

^a Entoleter-scourer-aspirator.

for whole wheat samples. For example, wheat taken as it left the mill-mix bin during the August run contained 5 to 296 spores in a set of 15 50-g samples. In a similar situation, a single wheat lot heavily infected with Karnal bunt of wheat (*T. indica* Kühn) was sampled 43 times, and counts ranged from 1 to 3,256 teliospores/50 g (mean = 493; SD = 821) (*unpublished results*). Therefore, it is not surprising that individual spore counts do not add up to the total number of spores added to the wheat.

The extreme variability also created problems in data analysis. Simple arithmetic averages resulted in standard deviations that were far greater than the means. Therefore, geometric averages were used, and a count range for each replicate was reported. Geometric averages were calculated for each treatment by the following method: 1 (one) was added to the number of broken or intact spores of each replicate (to avoid taking the natural logarithm of zero), the natural logarithm of that number was taken, the natural logarithms of the replicates for that treatment were summed, the sum was divided by the number of replicates in the treatment (to obtain the mean), the exponent of the sum was taken, and 1 (one) was subtracted from the exponent to obtain the geometric mean. The range of spore counts per replicate, sample size, number of samples analyzed, number of replicates for the TCK-contaminated samples, and the geometric average are given in Table II.

There are two other sources of possible variation for which there was no control. The number of spores that were broken during processing could not be accurately determined because only if >50% of a broken spore was present was it counted during the counting regimen. Another source of variation was the cleaning house filter, a room-sized structure that contained large numbers of filter socks from which only a small fraction of the dust could be removed. These socks may have held large numbers of spores that could not be quantified.

The results of this study demonstrate that cleaning and milling significantly reduce the number of TCK teliospores present in wheat. In both the July and August mill runs, the majority of initially intact teliospores either fractured or were removed during the cleaning stages. The house filter contained the highest concentration of spores (Fig. 1 and Table II). The receiving separator, ESA, and final aspirator also removed large numbers of spores, while relatively few teliospores were found in the material removed by the gravity selector and cylinder separator (Fig. 1 and

Table II). Tempering further reduced spore levels in the wheat (see below). The cleaning and premilling processes reduced the spore counts from preclean levels of 301 and 34 spores/50 g of wheat to 1.8 and 0.8 spores/50 g of wheat as it entered the mill for the July

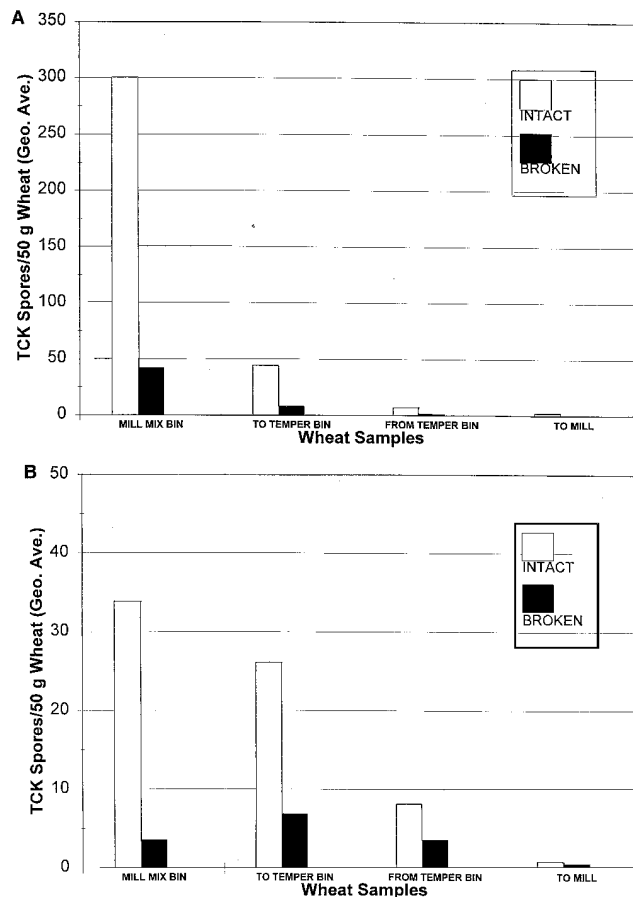


Fig. 2. *Tilletia controversa* Kühn (TCK) spores isolated from wheat samples during July (A) and August (B) milling tests.

TABLE III
Mass Balance for Wheat and *Tilletia controversa* (TCK) Teliospores

Milling and Cleaning Process	July Mill Run			August Mill Run		
	Wheat/ Component Mass (g)	Spores per gram	Total Number of Spores	Wheat/ Component Mass (g)	Spores per gram	Total Number of Spores
Wheat mill-mix	1,390,909	8.058	11,207,945	1,390,909	1.414	1,966,745
All cleanings	(21,363)	87.572	1,870,788	(29,869)	88.234	2,635,485
House filter	(576)	364	209,664	(864)	1,221.666	1,055,520
Teliospore loss (gain)	7,016,993	(2,964,740)
Wheat to tempering	1,368,970	...	2,110,500	1,360,176	...	1,240,480
Teliospore loss (gain)	1,812,065	813,385
Wheat from tempering	1,368,970	0.218	298,435	1,360,176	0.314	427,095
Entoleter-scourer-aspirator	1,078	207.214	223,377	1,465	60.478	88,600
Teliospore loss (gain)	(34,373)	281,429
Wheat prior to mill	1,367,892	0.08	109,431	1,358,711	0.04	57,066
Prebreak aspirator	5,980	0.00	0	2,098	3.89	8,157
Wheat in mill	1,361,912	...	109,431	1,356,613	...	48,909
Germ	27,238	0.17	4,739	25,132	0.78	19,502
Bran	163,429	0.06	9,152	150,794	0.33	50,365
Shorts	204,287	0.25	51,072	188,492	0.56	104,802
Red dog	13,619	0.00	0	12,566	0.00	0
Flour	948,299	0.00	0	871,587	0.00	0
Cyclone dust	5,040	0.00	0	8,000	0.00	0
Teliospore loss (gain)	44,468	(125,760)
Total teliospores in cleanings and mill-feed	2,368,792	3,962,431
TCK spores in cleanings and mill-feed, %	21.1	201.5

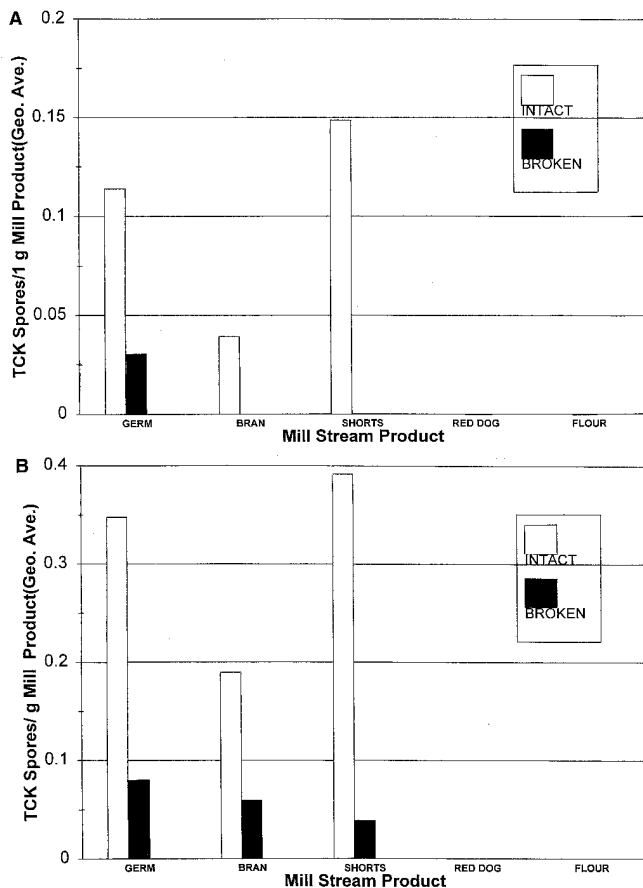


Fig. 3. *Tilletia controversa* Kühn (TCK) spores present in the July (A) and August (B) mill fractions.

and August runs, respectively (Fig. 2 and Table II). Broken spores were found mainly in material removed by the final aspirator, house filter, and premill ESA for the July run, while most of the broken spores were found in the house filter for the August run. The July run showed a much greater decrease in the number of spores found during the pretemper cleaning than the August run. This may be the result of a higher initial number of spores for the July run, which would have also included smut balls. Broken spores were found mainly on the wheat from the mill-mix and the wheat going to the tempering process.

The tempering process had a dramatic effect on the number of spores recoverable from the wheat; this number was reduced by 84 and 69% for the July and August milling runs, respectively (Fig. 2 and Table II). The reason for these decreases is undetermined. It is most unlikely that spores were retained in the smooth-sided, stainless steel tempering bins. For spores to have been destroyed, they would have had to have been damaged during prior handling and, upon contact with tempering water, broken apart. This should have resulted in a large number of broken spores in the wheat coming from the tempering bin, unless the spores broke into small fragments, but this was not observed (Fig. 2 and Table II). Another explanation may be that the wetting action of the tempering process caused an adherence of the spores to the wheat surface that could not be disrupted by our spore-isolation procedures. This seemed unlikely, since both detergent and vigorous agitation were used during isolation. Microscopic examination of the surface of the wheat was not efficacious because the spore counts were too low. A third possibility was that the pneumatic transport system used to move the grain removed spores and deposited them with the dust collected from the cyclone separator. This scenario is also unlikely, however, since

spores were not found in dust from the cyclone separator. We do not yet have an explanation for this repeatable phenomenon.

Spore counts on the wheat were further reduced when the tempered grain was passed through a final ESA just before going to the prebreak rolls of the mill. The wheat going to the mill contained only 1.8 and 0.8 spores/50 g for July and August runs, respectively, reduced from counts of 301 and 34 prior to cleaning. The prebreak aspirator removed no spores from wheat during the July run and only a few during the August run (Tables II and III). Very few spores were recovered in the various mill fractions, and no spores were detected in mill fractions coming from the internal parts of the grain, principally red dog, wheat flour, and dust (Fig. 3 and Table II). A few spores were found on germ, bran, and shorts fractions. These results indicate that TCK is principally a surface contaminant on wheat grain (except for teliospores in sori).

TCK cleaning and milling data can also be analyzed by calculating the mass balance for wheat and TCK spores for July and August milling runs (Table III). The mass balance shows where in the cleaning and milling operations losses (and apparent gains) occurred. The largest loss occurred during the cleaning operation for the July run, in which more than 7 million spores were unaccounted for. The apparent gain of nearly 3 million spores during cleaning for the August run clearly shows how sori can cause variable results. The July milling data showed that 21.1% of the total spores present in the mill-mix were concentrated in the final mill feed stream if all nonflour fractions and cleanings were combined (Table III). Because of the skewed data, probably caused by the presence of sori, the cleaning phase of the August study yielded more spores than were detected in the mill-mix and resulted in a spore recovery of more than 200%.

Only 2.7 and 4.4% of recoverable teliospores for the July and August milling runs, respectively, were found in the germ, bran, and shorts fractions. Therefore, the cleanings accounted for 97.3 and 95.6% of the spores in the July and August runs, respectively. Samples of the mill-feed fractions comprising germ, bran, shorts, and red dog were pelleted or extruded. TCK spores in pelleted or extruded material could not be isolated. A sample of mill-feed was then heavily spiked with TCK spores and extruded. Intact spores were easy to find in that extruded sample, but they failed to germinate. The high temperatures and pressures in the extrusion process probably killed the spores.

This information is important to any consideration of risk-management steps to lower the introduction of a potential pathogen. Since it is a common practice to incorporate the cleanings into a final mill-feed product, a method such as extrusion could be used to inactivate a pathogen such as TCK. Alternatively, the cleanings could be heat treated prior to incorporation in the mill-feed, thus greatly reducing the risk of pathogen introduction (Bonde et al 1997). This study shows that large quantities of TCK spores can be effectively removed from contaminated wheat during cleaning and milling and can be inactivated by currently available processing regimens.

LITERATURE CITED

- Bonde, M. R., Peterson, G. L., Smilanick, J. L., and Schaad, N. S. 1997. Karnal bunt of wheat. *Plant Dis.* 81:1370-1377.
- Goates, B. J. 1995. Dwarf bunt and common smut. Pages 12-25 in: *Bunts and Smut Diseases of Wheat: Concepts and Methods of Disease Management*. R. D. Wilcoxson and E. E. Sarri, eds. CIMMYT, Mexico City.
- Goates, B. J., and Peterson, G. L. 1997. Relationship between levels of soil-borne and seed-borne *Tilletia controversa* and the incidence of dwarf bunt of wheat. (Abstr.) *Phytopathology* 87:S34.
- Grey, W. E., Mathre, D. E., Hoffmann, J. A., Powelson, R. L., and Fernández, J. A. 1986. Importance of seedborne *Tilletia controversa* for infection of winter wheat and its relationship to international commerce. *Plant Dis.* 70:122-125.
- Gwirtz, J. A., Eustace, W. D., and Curran, S. P. 1996. Effect of kernel

- size and test weight on the Kansas State University (KSU) pilot mill. Assoc. Oper. Millers Bull. June:6731-6741.
- Hess, W. M., and Trione, E. J. 1986. Use of electron microscopy to characterize teliospores of *Tilletia caries* and *T. controversa*. Plant Dis. 70:458-460.
- Hoffman, J. A. 1982. Bunt of wheat. Plant Dis. 66:979-986.
- Keener, T. K., Stougaard, R. N., and Mathre, D. E. 1995. Effect of winter wheat cultivar and difenoconazole seed treatment on dwarf bunt. Plant Dis. 79:601-604.
- King, A. D., Jr., Ruch, D. L., and Goodman, N. 1991. TCK bunt spores and export wheat. Cereal Foods World 36:509-512.
- Mathre, D. E. 1996. Dwarf bunt: Politics, identification, and biology. Annu. Rev. Phytopathol. 34:67-85.
- Pirson, H. 1978. A simple method for the detection of bunt and covered smut spores as contaminants on cereal seeds. Pages 54-57 in: Working Group on Temperate Climate Cereals. Rep. Int. Workshop Seed Pathol., 16th. Int. Seed Testing Assoc.: Zurich.
- Purdy, L. H., Kendrick, E. L., Hoffman, J. A., and Holton, C. S. 1963. Dwarf bunt of wheat. Annu. Rev. Microbiol. 17:199-222.
- Rattan, G. S., Aujla, S. S., and Randhawa, H. S. 1995. Morphology of the teliospores of some smut fungi—An electron microscopy. Cereal Res. Commun. 23:445-451.
- Sitton, J. W., Line, R. F., Waldher, J. T., and Goates, B. J. 1993. Difenoconazole seed treatment for control of dwarf bunt of winter wheat. Plant Dis. 77:1148-1151.
- Stockwell, V. O., and Trione, E. J. 1986. Distinguishing teliospores of *Tilletia controversa* from those of *T. caries* by fluorescence microscopy. Plant Dis. 70:924-926.
- Trione, E. J. 1982. Dwarf bunt of wheat and its importance in international wheat trade. Plant Dis. 66:1083-1088.
- Trione, E. J., and Hall, M. J. 1986. Dwarf bunt of wheat in China: Potential sites from satellite studies. Agron. J. 78:148-150.
- Trione, E. J., and Krygier, B. B. 1977. New tests to distinguish teliospores of *Tilletia controversa*, the dwarf bunt fungus, from spores of other *Tilletia* species. Phytopathology 67:1166-1172.
- Wandra, K. J., Hancock, J. D., Behnke, K. C., Hines, R. H., and Kennedy, G. A. 1996. Grinding, pelleting affects nutritional value of cereal grains, diets for swine. Feedstuffs 68:13-17.

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