that their UV chromophores were very similar.

It has been previously shown that roquefortine (Figure 3, roquefortine A (Figure 2, **1a**), and roquefortine B (Figure 2, **1c**) were produced by certain strains of *Penicillium roqueforti* and have been found naturally occurring in varieties of blue cheese (Scott et al., 1976).

The alkaloids fumigaclavine A and B produced by *Aspergillus fumigatus* Fres. were shown to be stereoisomers of roquefortines A and B, respectively. These alkaloids differ only in the stereochemistry at positions 8 and 9 (Arnoux et al., 1978).

The alkaloids were produced on both the solid and the liquid (beer) media; however, yields on the solid media were observed to be higher under the cultural conditions imposed. The physical environmental conditions under which the *P. crustosum* grew in the can of beer are not known and, therefore, could not be reproduced to see what toxic secondary metabolites, if any, were produced under these conditions. Since samples of the contaminated beer were not available for analysis, it is not known if these alkaloids were present in the beer and involved in the apparent intoxication. However, it has been demonstrated that this *P. crustosum* isolate has the capability of producing two different classes of alkaloids in laboratory culture.

ACKNOWLEDGMENT

We thank G. E. Barden for isolating the fungi from mycelial material and Dr. K. B. Raper for identifying the *P. crustosum* isolate.

Registry No. **1a**, 58800-19-4; **1b**, 569-26-6; **1c**, 58800-20-7; roquefortine, 58735-64-1.

LITERATURE CITED

Arnoux, B.; Merrien, M. A.; Pascard, C.; Polonsky, J.; Scott, P. M. *J. Chem. Res., Synop.* **1978**, 210.

Bach, N. J.; Boaz, H. E.; Kornfeld, E. C.; Chang, C.-J.; Floss, H. G.; Hagaman, E. W.; Wenkert, E. *J. Org. Chem.* **1974**, *39*, 1274.

Cole, R. J.; Cox, R. H. In "Handbook of Toxic Fungal Metabolites"; Academic Press: New York, 1981; Chapter 10, pp 527–568.

Kirksey, J. W.; Cole, R. J. *Mycopathol. Mycol. Appl.* **1974**, *54*, 291.

Ohmomo, S.; Sato, T.; Utagawa, T.; Abe, M. *Agric. Biol. Chem.* **1975a**, *39*, 1333.

Ohmomo, S.; Sato, T.; Utagawa, T.; Abe, M. *J. Agric. Chem. Soc. Jpn.* **1975b**, *49*, 53.

Scott, P.; Merrien, M. A.; Polonsky, J. *Experientia* **1976**, *32*, 140.

Scott, P. M.; Polonsky, J.; Merrien, M. A. *J. Agric. Food Chem.* **1979**, *27*, 201.

Spilsbury, J. F.; Wilkinson, S. *J. Chem. Soc.* **1961**, 2085.

Richard J. Cole*[1]
Joe W. Dorner[1]
Richard H. Cox[2]
Lawrence W. Raymond[3]

[1]U.S. Department of Agriculture
National Peanut Research Laboratory
Dawson, Georgia 31742
[2]Philip Morris USA
Research Center
Richmond, Virginia 23261
[3]Llewellyn Park
West Orange, New Jersey 07052

Received for review September 7, 1982. Accepted December 12, 1982. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or Philip Morris USA and does not imply its approval to the exclusion of other products that may also be suitable.

Distribution of Vomitoxin in Dry Milled Fractions of Wheat Infected with *Gibberella zeae*

Two samples of winter wheat, one from Canada and the other from the United States, naturally infected with *Gibberella zeae*, were dry milled and the separate fractions analyzed for the presence of the mycotoxin vomitoxin (3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one). Initial concentrations of vomitoxin in both samples of the whole wheat was 2 μg/g of grain. Vomitoxin was distributed throughout all fractions of the milled U.S. and Canadian grain. After being milled, 65% of the vomitoxin was in straight grade flour fractions and the rest in the bran, red dogs, shorts, and low-grade fractions in the U.S. sample. Although there were differences in the concentration of vomitoxin between the different fractions of the milled U.S. wheat, when the fractions were grouped into (1) bran, red dogs, and shorts, (2) straight-grade flour, and (3) low-grade flour, there did not appear to be a concentration of vomitoxin in any one fraction. The final concentration of vomitoxin in each of the groupings was 5.2, 4.5, and 4.0 μg/g, respectively. The total amount of vomitoxin extracted from the milled U.S. sample was more than 2 times greater than that predicted from the initial weight of the whole wheat and may have resulted from fungal growth during the week long tempering to raise the moisture content of the wheat.

Vomitoxin (3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one), is a naturally occurring cytotoxic trichothecene responsible for emesis and feed refusal in swine (Mirocha et al., 1976; Forsyth et al., 1977; Vesonder et al., 1973) and is commonly associated with cereal grains infected by *Fusarium roseum* f. sp. *graminearum* (Schw.) Synder and Hansen (conidial state of *Gibberella zeae* Schwabe).

Vomitoxin is not known to be mutagenic or carcinogenic. Its LD_{50} of 40–70 mg/kg of body weight in rats is higher than other trichothecenes such as T-2, HT-2, or diacetoxyscirpenol (Yoshizawa and Morooka, 1977; Sato and Ueno, 1977). Because vomitoxin is difficult to produce in large quantities there have been no studies on the long-term effect of feeding sublethal doses to animals. However, sublethal doses of T-2 and diacetoxyscirpenol have been shown to suppress the immune response of test animals (Rosenstein et al., 1979, 1981). In 1980, because of the high incidence of vomitoxin in Canadian wheat, the Canadian Government suggested a tolerance level of 300 ng of vomitoxin/g of wheat intended for human consumption and recommended a zero tolerance for wheat intended for use in infant food. Concentrations of vomitoxin in the Can-

0021-8561/83/1431-0657$01.50/0

adian winter wheat crop of 1980 ranged between 0.01 and 4.3 μg of vomitoxin/g of grain (Scott et al., 1981). The FDA recently issued a level of concern for vomitoxin in U.S. grains of 2 μg/g of grain in whole grain and 1 μg/g of finished product.

Several studies on the distribution in corn of zearalenone, another mycotoxin produced by *G. zeae* and often found in the same grain samples as vomitoxin, indicated that zearelenone was distributed throughout the different fractions of dry milled corn (Bennett et al., 1976). The distribution of vomitoxin and zearalenone in wheat is not known. However, since wheat is primarily used for human consumption in bread, cookies, infant food, and cereal, the distribution of vomitoxin in the wheat may be an important consideration in use of the different milled components of wheat. The purpose of this study was, therefore, to determine the fate of vomitoxin in naturally contaminated wheat from the United States and Canada during the milling process.

METHODS AND MATERIALS

Two samples of *Fusarium*-infected wheat were obtained: a red winter wheat variety Hart from the United States (Dr. Richard Stucky, University of Kentucky) and a white winter wheat from Canada (Dr. Roy Greenhalgh, Agriculture Canada). The U.S. sample was dry milled by Dr. Robert Clements, Soft Wheat Quality Laboratory, OARDC, Wooster, OH 44691, into 19 fractions consisting of bran, red dogs, shorts, and straight-grade flour. The sample was classified as moderately to severely shriveled. The moisture content of the wheat was raised from 12% to 15.3% moisture by tempering for 1 week prior to milling, making the weight of the wheat 4670 g. The fiber content of each fraction (percent ash) was also determined by the Soft Wheat Quality Laboratory. The Canadian sample consisted of subsamples of whole wheat milled into eight fractions, in addition to a separate sample of the whole wheat. Each sample weighed approximately 250 g.

Vomitoxin was determined in each milled fraction by methods similar to those outlined by Scott (1982). Two 50-g subsamples were extracted twice by blending in a Waring blender for 5 min with 250 mL of methanol–water (60:40). The methanol was removed under reduced pressure in a rotary evaporator at 50 °C. Thirty milliliters of saturated NaCl solution was added to the remaining aqueous fraction and solids were allowed to settle out overnight. The solution was then extracted 3 times with ethyl acetate (100 mL each time). The ethyl acetate fractions were passed through anhydrous sodium sulfate and the preparation was evaporated to dryness under reduced pressure. The residue was dissolved in 1–2 mL of methanol and spotted directly on Whatman LHP–thin layer chromatography plates. Samples difficult to read on TLC plates because of interfering compounds were passed through a Bio-Beads S-X3 200–400 mesh (Bio-Rad Laboratories, Richmond, CA) column (1.5 × 45 cm). Methanol (100%) was used as the column solvent at a flow rate of 21 mL/h. Vomitoxin was eluded in the 40–55-mL fractions. The fractions were respotted on TLC plates and developed in chloroform–methanol–water (90:10:2).

Fifty-gram samples of clean corn and white wheat were spiked with 200 μg of vomitoxin to determine the efficiency of recovery. Extractions and cleanup were made as described above. Serial dilutions in methanol were made to determine the lowest concentrations of vomitoxin visible on TLC plates when compared to standards.

Vomitoxin was visible on the plates after spraying with 50% H_2SO_4 in methanol and heating on a hot plate for 5 min. Concentrations were determined with vomitoxin standards of known concentration of a vomitoxin preparation from the Myco-Lab (Chesterfield, MO 63017). Absence of a spot at the R_f value of the standard specifies a concentration of less than 150 ng/g. Confirmation of vomitoxin in randomly selected samples was made by selected ion monitoring (Mirocha et al., 1976) of Me_3Si derivatives on a GC–MS spectrometer (Finnigan Model 3200 GC–MS) interfaced to a Riber SADR data acquisition and control system. Vomitoxin should be handled with caution, and contact with skin should be avoided.

Table I. Levels of Vomitoxin from Spiked Corn and White Wheat[a]

sample	concentration μg/g	concentration total	concentration %
corn	3.5	175	87.5
white wheat	3.4	170	85.0

[a] 50 g of each grain was spiked with 200 μg of vomitoxin. Each concentration is the average of two extractions. Analyses were made on Whatman LHP-TLC plates, and concentrations were determined with vomitoxin standards of known concentration.

RESULTS

The efficiency of the vomitoxin extraction procedure for spiked samples of corn and white winter wheat is shown in Table I. The lowest concentration of vomitoxin visible on the TLC plates was 0.05 μg of vomitoxin/g of grain. However, a concentration of 150 ng/g was determined to be a more reliable estimate which did not require cleanup through an SX-3 column. Zearalenone, if present in a sample, can be extracted by the procedures described here but is identified by viewing the TLC plates under shortwave ultraviolet light before spraying with H_2SO_4 (Hart et al., 1982). Zearalenone, however, was not detected in any sample or fraction of wheat used in this study.

Vomitoxin was found throughout the milled fractions of the Canadian and U.S. wheat (Tables II and III). The U.S. wheat was divided into bran, red dogs, shorts, and straight flour grade wheat. All straight flour grade fractions must pass through a 40-mesh screen and are primarily the heart of the endosperm. The shorts, red dogs, and low-grade fractions of the straight-grade flour were high in fiber content (ash percent) and designated for animal feed. Red dogs and shorts are derived from the inner layer of bran and the outer layers of the endosperm. Brans, shorts, and red dogs comprised 29.6% of the total wheat (1353 g), straight-grade flour less the low-grade fractions 65.1% (2973 g), and low-grade fractions 5.3% (238 g). The total weight of the wheat in all fractions was 4564 g (97.7% of the original wheat weight). Although the amount of vomitoxin recovered was high in some individual fractions (Table II), the total vomitoxin recovered in major groupings of (1) bran, red dogs, and shorts, (2) straight flour grade, and (3) low-grade fractions was 5.2 μg/g of wheat (29.6%), 4.5 μg (62.6%), and 4.0 μg (4.6%), respectively. Because the Canadian fractions were subsamples of milled wheat the percent recovery in each fraction could not be determined, but the red dogs and bran contained the highest concentrations of vomitoxin (Table III).

The total amount of vomitoxin recovered in the fractions was 4.7 μg/g of wheat as compared to 2 μg/g of wheat in the whole wheat.

DISCUSSION

The results presented herein show that wheat intended for human consumption can contain vomitoxin and clearly indicate that vomitoxin was distributed through all major

Table II. Levels of Vomitoxin from a Whole and Dry Milled Sample of U.S. Red Winter Wheat

sample	μg/g[a]	ash, %
whole wheat	2.0	1.7
bran	4.5	6.0
1st shorts	11.5	4.9
2nd shorts	<0.1	4.2
red dog	<0.1	2.7
straight flour grade		
1st break	<0.1	0.4
2nd break	4.0	0.3
3rd break	1.5	0.4
4th break	2.5	0.4
5th break	1.0	0.6
6th break	4.5	
1st reductions	10.0	0.3
2nd reductions	2.0	0.3
3rd reductions	1.0	0.4
4th reductions	2.0	0.7
5th reductions	<0.1	0.9
sizings	3.5	1.0
1st low grade	2.5	1.5
2nd low grade	9.0	ND[b]
3rd low grade	5.0	ND

[a] Concentrations were determined on Whatman LHP-TLC plates with vomitoxin standards of known concentration. Breaks, reductions, and sizings refers to flour fractions of different qualities and size. Final flour products are a mixture of these fractions. Low-grade fractions are contaminated with small amounts of seed coat and bran. [b] Not determined.

Table III. Levels of Vomitoxin from a Whole and Dry Milled Sample of Canadian White Winter Wheat

sample	μg/g[a]
whole wheat[b]	2.0
bran[b]	0.8
shorts[b]	0.8
red dogs[b]	2.5
1st milling[b]	0.9
2nd milling[b]	0.9
bread 1[c]	0.7
bread 2[c]	0.4
bread 3[c]	0.8

[a] Concentrations were determined on Whatman LHP-TLC plates with vomitoxin standards of known concentrations. [b] Average from two separate subsamples. [c] Average from four separate subsamples.

fractions of the wheat. While some individual fractions had a higher concentration of vomitoxin, there did not appear to be a concentration affect within major fractions. The recovery of vomitoxin may be more efficient in milled fractions of wheat. However, the greater amount of vomitoxin recovered in the milled wheat compared to the whole wheat may also have resulted from the week long tempering of the wheat to raise the moisture from 12% to 15.3%. Tempering is a process of adding a specific amount of water to wheat to raise the moisture content to a desired level and then incubating to achieve uniform moisture. Wheat is milled at 15% moisture because the bran will mill into larger sizes and not contaminate the flour. There was some potential for fungal growth during this time. Commercially, tempering takes from a few hours to 24 h and fungal growth should not be a problem.

The research results are similar to those found in milled corn contaminated with zearalenone (Bennett et al., 1976). Actual concentrations of vomitoxin in processed food products would depend on the extent of mixing of contaminated and clean grain and on the concentration of the toxins in contaminated samples. Although it is not yet known what affect food processing has on the trichothecenes, their apparent stability to heat and chemical treatments (Bamburg, 1976) indicates they probably can survive cooking and baking to a large degree.

Currently there are no regulations in the United States concerning vomitoxin in wheat, but a more conservative approach is being taken by the Canadian government which has set tolerance levels of 300 ng/g for wheat intended for human consumption and zero tolerance for wheat used in the preparation of infant food. The two approaches by these respective governments and the results presented here showing vomitoxin in milled wheat indicate a clear need for further research on the carry-over properties of vomitoxin in food products, as well as long-term studies on its toxicity.

Registry No. Vomitoxin, 51481-10-8.

LITERATURE CITED

Bamburg, J. R. *Adv. Chem. Ser.* **1976**, *No. 149*, 144–162.
Bennett, G. A.; Peplinski, A. J.; Brekke, O. L.; Jackson, L. K.; Wichser, W. R. *Cereal Chem.* **1976**, *53*, 299.
Forsyth, D. M.; Yoshizawa, T.; Morooka, N.; Tuite, J. *Appl. Environ. Microbiol.* **1977**, *34*, 547.
Hart, L. P.; Braselton, W. E.; Stebbins, T. C. *Plant Dis.* **1982**, *66*, 1133.
Mirocha, C. J.; Pathre, S. V.; Schauerhamer, B.; Christensen, C. M. *Appl. Environ. Microbiol.* **1976**, *32*, 553.
Rosenstein, Y.; Kretschmer, R. R.; Lafarge-Frayssinet, C. *Immunology* **1981**, *44*, 555.
Rosenstein, Y.; Lafarge-Frayssinet, C.; Lespinats, G.; Loisillier, F.; Lafont, P.; Frayssinet, C. *Immunology* **1979**, *36*, 111.
Sato, N.; Ueno, Y. "Mycotoxins in human and animal health"; Pathotox Publishers: Park Forest South, IL, 1977; pp 295–307.
Scott, P. M. *J. Assoc. Off. Anal. Chem.* **1982**, *65*, 876.
Scott, P. M.; Lau, P.; Kanhere, S. R. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 1364.
Vesonder, R. F.; Cieglar, A.; Jensen, A. H. *Appl. Microbiol.* **1973**, *26*, 1008.
Yoshizawa, T.; Morooka, N. "Mycotoxins in human and animal health"; Pathotox Publishers: Park Forest South, IL, 1977; pp 309–321.

Lynn P. Hart*[1]
William E. Braselton, Jr.[2]
[1]Department of Botany and Plant Pathology and the Pesticide Research Center
Michigan State University
East Lansing, Michigan 48824
[2]Department of Pharmacology and Toxicology
Michigan State University
East Lansing, Michigan 48824

Received for review September 27, 1982. Revised manuscript received December 23, 1982. Accepted February 15, 1983. Michigan Agricultural Experiment Station Journal Article No. 10597.