Mycotoxin and Odor Formation in Moist Cereal Grain During Granary Storage[1]

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ABSTRACT

Half-bushel parcels of barley, wheat, and oats at 21% moisture content were placed inside bulk stored oats in a farm granary for 20 weeks during summer and autumn in Manitoba to study quality changes. Temperature, moisture, O_2 and CO_2 levels, microfloral incidence and abundance, seed germination, free fatty acids, aflatoxins (B_1, B_2, G_1, and G_2), zearalenone, citrinin, penicillic acid, ochratoxin A, sterigmatocystin, and fungal volatiles were monitored during storage. By four weeks, ochratoxin A had formed at detectable levels in barley and wheat but not in oats; barley contained over five times more ochratoxin A than did wheat after 20 weeks. Strains of *Penicillium verrucosum* var. *cyclopium* were associated with ochratoxin A production. No other mycotoxins were detected. The fungal volatiles increased 10-fold to 15-fold by seven weeks and declined to control levels by 16 weeks. Of the three known fungal odors tentatively identified, amounts of 1-octanol were greater than those of 3-methyl-1-butanol and 3-octanone. Of the three grains, barley showed the least heating, moisture increase, O_2 decrease, CO_2 increase, fat acidity value increase, and germination loss.

Spoilage of stored cereals results from interactions among many biotic and abiotic variables over a period of time (Sinha 1973). Grain that is spoiled and unfit for human or animal consumption can be recognized easily by conspicuous objectionable features, such as heating, caking, discoloration, off-odor and, often, toxicity. During the early stages of deterioration, however, incipient spoilage is usually detectable only by sensitive instruments and techniques. Little is known about mycotoxin production or odor formation in spoiling grain and the role of microfloral ecology in these processes. Information obtained from simultaneous measurement of the variables would be useful in developing an early-warning system for decaying stored grain.

Scott et al (1972) examined 25 samples of deteriorated Canadian grain of unknown storage history associated with human and animal health problems and found ochratoxin A in 18 samples, citrinin in 13 samples, and sterigmatocystin in one sample. In a survey of 848 wheat samples, Shotwell et al (1976) found 11 samples containing ochratoxin A; in another similar study, Shotwell et al (1977) found zearalenone in 19 of 102 wheat samples.

The development of mycotoxins in stored agricultural produce is not well understood. Many studies have sought to determine how mycotoxin levels are affected by single factors, eg, moisture, temperature, mechanical damage, hot spots, time, O_2 and CO_2 levels, nature of substrate, mineral nutrition, chemical treatment, plant stress, invertebrate vectors, fungal infection, fungal strain differences, and interaction of microorganisms (Hesseltine 1976). Interplay among these and other factors in typical storage structures has been shown (Sinha 1973); such interactions would probably affect mycotoxin levels. Using a novel approach, Shotwell et al (1975) mapped the aflatoxin and zearalenone levels around a naturally occurring hot spot in a corn bin in Illinois.

Volatile compounds are produced from both the dormant seed and the associated microflora. Hougen et al (1971) explored the odor profiles of corn, wheat, barley, oats, flax, and rapeseed. Dravnieks and co-workers (Dravnieks and Watson 1973, Dravnieks et al 1973) extensively investigated and classified odor profiles of seed corn. The volatile odor compounds produced by the fungi that commonly infect cereals and oilseeds (*Aspergillus* spp., *Penicillium* spp., and other Fungi Imperfecti) have been investigated (Kaminski et al 1973, 1974, 1975). From their laboratory study of volatiles produced by fungi grown on damp ground wheat, Kaminski et al (1975) determined the olfactory characteristics of different compounds; using mass spectrometry they also identified several main volatile compounds, including 1-octen-3-ol, 3-octanol, 1-octanol, 3-octanone, and 3-methyl-1-butanol. Richard-Mollard et al (1976) followed the development patterns of some of these compounds in corn in airtight storage and suggested that the sequential production of volatiles could be useful as early indicators of microbial spoilage in commercial storage. Our investigation emphasized the ecological and multidisciplinary aspect of deteriorating stored grains in which fungal volatiles may play an important role.

The purpose of this exploratory investigation was to determine the time sequence for the appearance of mycotoxins (if any) and fungal volatiles and of several biotic and abiotic variables associated with their production in damp grain pockets within a western Canadian farm granary.

MATERIALS AND METHODS

Cereal Samples and Chemical Standards

Certified grade No. 2 Canada western red spring wheat (*Triticum aestivum* L. cv. Neepawa), crop year 1978, and certified grade No. 2 Canada barley (*Hordeum vulgare* L. cv. Bonanza), crop year 1978, were obtained from the Manitoba Pool Elevators, Winnipeg, Manitoba. Canada western oats (*Avena sativa* L. cv. Random), crop year 1978, grown near Glenlea, Manitoba, were not graded.

Standards of aflatoxins B_1, B_2, G_1, and G_2, ochratoxin A, zearalenone, penicillic acid, citrinin, and sterigmatocystin were purchased from Supelco Inc., Bellefont, PA. Standards of 3-methyl-1-butanol (98%), 1-octen-3-ol (98%), 3-octanone (99%), and 1-octanol (99%) were obtained from Aldrich Chemical Co., Milwaukee, WI.

Preparation, Implantation, and Sampling of Cereal Parcels

Cereal samples were adjusted to approximately 21% moisture

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content with deionized water and kept for 24 hr at 2.5°C. Half-bushel parcels of barley (10.9 kg), wheat (13.6 kg), and oats (7.7 kg) were packaged in double bags 81.3 cm long and 45.7 cm wide made of polyethylene film 0.08 mm thick.

Each parcel was equipped with a gas sampling tube and thermocouple before implantation in the oat bin. The gas sampling tubes were comprised of a 1.3-m length of 3.5-mm id polyethylene tubing closed with nylon mesh (0.42-mm aperture) at one end and rubber serum stoppers at the other. The mesh-covered end of the tube was located, along with a copper-constantan thermocouple, near the center of each cereal parcel.To ensure an aerobic environment, the bottom half of each bag was perforated 130 times with a 16-gauge needle and the top closed with a cotton plug.

The bags were implanted in a weatherproof plywood granary 3.65 m long, 3.07 m wide, and 2.41 m to roof eaves, located at Glenlea, Manitoba and left there during June-October 1979. The granary contained oats (*Avena sativa* L. cv. Random) with 12.7–13.8% mean moisture content to a depth of 150 cm. The plywood floor of the granary was supported 10 cm above ground level by wooden skids. Each parcel was implanted with the top 15 cm below the surface of the oats and approximately 1 m from the center of the bin.

Samples (25 g) were taken weekly from the parcels for measurement of moisture content and fat acidity values (FAV). Additional samples (425 g) were removed biweekly for determination of ochratoxin A, seed germination, and microfloral presence and abundance.

Measurement of Variables

Temperature of ambient air in the granary was monitored continuously by a recording thermograph with its sensor approximately 10 cm above the center of the oat surface. The temperature of the granary oat bulk was measured using a copper-constantan thermocouple at the center of the granary 0.5 m below the surface of the oats. Temperatures in the implanted cereal parcels were measured using a similar thermocouple placed in the center of each bag.

Moisture contents were determined after oven drying 10-g samples of barley, wheat, and oats for 20, 19, and 22 hr, respectively, at 130°C and were reported on a wet weight basis (ASAE 1975). FAV figures were determined for barley and wheat according to the procedure of the American Association of Cereal Chemists (1962) and, for oats, following the modified procedure of Sinha et al (1979).

The germinability and fungal infection of cereal seeds (25 seeds per sample) were determined by the filter paper method (Wallace and Sinha 1962). An additional microfloral determination was made using filter paper and 7.5% (w/v) NaCl (Mills et al 1978). The total fungal propagule count was made according to the method of Bothast et al (1974). In brief, 11 g of seed were added to 99 ml of sterile 0.1% (w/v) peptone and 10 g of sterile sand, and from this primary dilution (1:10) serial dilutions (without sand) were prepared. One-milliliter amounts of solution from the appropriate dilution were pipetted onto plates of yeast extract agar containing 30 ppm of tetracycline. Plates were incubated at 28°C and counted after three days.

Mycotoxin Screening

Samples (50 g) were taken from cereal bulks at the time of implantation and at eight and 20 weeks afterwards. Samples were ground to pass through a 0.85-mm aperture, extracted, and purified according to Wilson et al (1976). Determination of aflatoxins B_1, B_2, G_1, and G_2, ochratoxin A, penicillic acid, and zearalenone was performed by thin-layer chromatography. For citrinin, the thin-layer solvent system of Hald and Krogh (1973) was used because it produced more compact fluorescent spots for the citrinin standards. To screen for sterigmatocystin in samples taken at 20 weeks, the procedure of Josefsson and Möller (1977) was employed.

Ochratoxin A Analysis

Triplicate samples of the cereals were prepared separately for analysis by the method of the Association of Official Analytical Chemists (1975). Each sample residue represented the extract of 10 g of cereal.

Samples were analyzed using a high-performance liquid chromatographic system that included the following components connected in flow series: a Waters 6000A solvent delivery system; a Rheodyne 7010 injector valve with a 50-μl loop and a Rheodyne 7020 loop filler port; a Whatman guard column (70 mm long and with 2-mm id) packed with 25–38 μm diameter Whatman Co-Pell ODS pellicular packing; a Merck analytical column (250 mm long and with 4.6-mm id) packed with Merck Lichrosorb RP-18 10-μm diameter packing; and a Turner Model 111 fluorimeter fitted with a 10-μl flow cell, a Wratten 46A primary filter, and a Wratten 2A secondary filter. The response was measured using an Isco type 613 potentiometric recorder.

All samples were dissolved in 500 μl of acetonitrile using sonication and were passed through a Millipore fluorocarbon filter having 0.5-μm pores. Using the loop injector, 50-μl aliquots, representing the final extract of 1 g of sample, were analyzed. A standard curve was established by injecting increasing amounts of ochratoxin A standards (16, 50, and 85 ng/μl in acetonitrile) by the technique of partial loop filling. Satisfactory resolution was obtained with a mobile phase of 65% methanol and 35% acetic acid/water (2:45) at a flow rate of 1.7 ml/min. Ochratoxin A was quantitatively determined by the peak-height method. The identity of ochratoxin A was confirmed chemically by formation of its methyl ester and subsequent chromatography (Scott et al 1972).

Gas Chromatography

To measure O_2 and CO_2 levels in the cereal parcels, the method of Singh et al (1977) was employed. Fifty milliliters of intergranular air was drawn from the gas sampling tubes and discarded, and a second 50 ml was taken for O_2 and CO_2 analysis. For O_2 determination, 1 ml of the air sample was injected into a Matheson 8430 gas chromatograph with dual thermal conductivity detectors, He flow rate of 40 ml/min, isothermal oven temperature of 34°C, and a column (183 cm long, 3.18 mm od) of 0.17–0.25 mm diameter Linde molecular sieves, type 5A. For CO_2 determination, identical conditions were employed using a similar column of 0.17–0.29 mm diameter Porapak Q.

To investigate volatile compounds of fungal origin, a modified Varian 2100 gas chromatograph was used. A thermal desorption injection system was constructed, following the specifications of Murray (1977). Traps for the volatiles were constructed of 3.18-mm od stainless steel tube 90 mm long and were packed with 100 ± 2 mg of 0.17–0.25 mm diameter Johns-Manville Chromosorb 105, secured with silanized glass wool and 0.14-mm aperture stainless steel screens at either end. Samples were analyzed by drawing 400 ml of intergranular air from the gas sampling tubes through the traps subsequent desorption of the volatiles into the gas chromatograph.

The traps were tightly wrapped in foil and maintained until analysis at ambient temperature for a maximum period of 5 hr. Each trap was purged with He at 25°C for 1 min at a gas flow rate of 10 ml/min and was then subjected to volatiles desorption under He at 150°C for 5 min. The volatiles were recondensed at the temperature of liquid N_2 for 6 min in a 2-cm precolumn packed with 0.17–0.25 mm diameter silanized glass beads. The volatiles were then swept onto the column upon application of a 160°C brass probe to the precolumn. Dual flame ionization detectors and matched columns of 15% (w/w) Carbowax 20*M* on 0.17–0.25 mm diameter Chromosorb P AW-DMCS, 366 cm long and with 3.18-mm od were used. The following temperature program was used: 100°C for 5 min, an increase of 4 degrees per minute for 10 min, and 140°C for 20 min. Response was measured using a Hewlett-Packard 3380S computing integrator.

Qualitative standards were run by placing 0.4 ml of the standard in a 50-ml flask and sweeping the vapors for 30 sec into a trap with 99.999% N_2 at 60 ml/min.

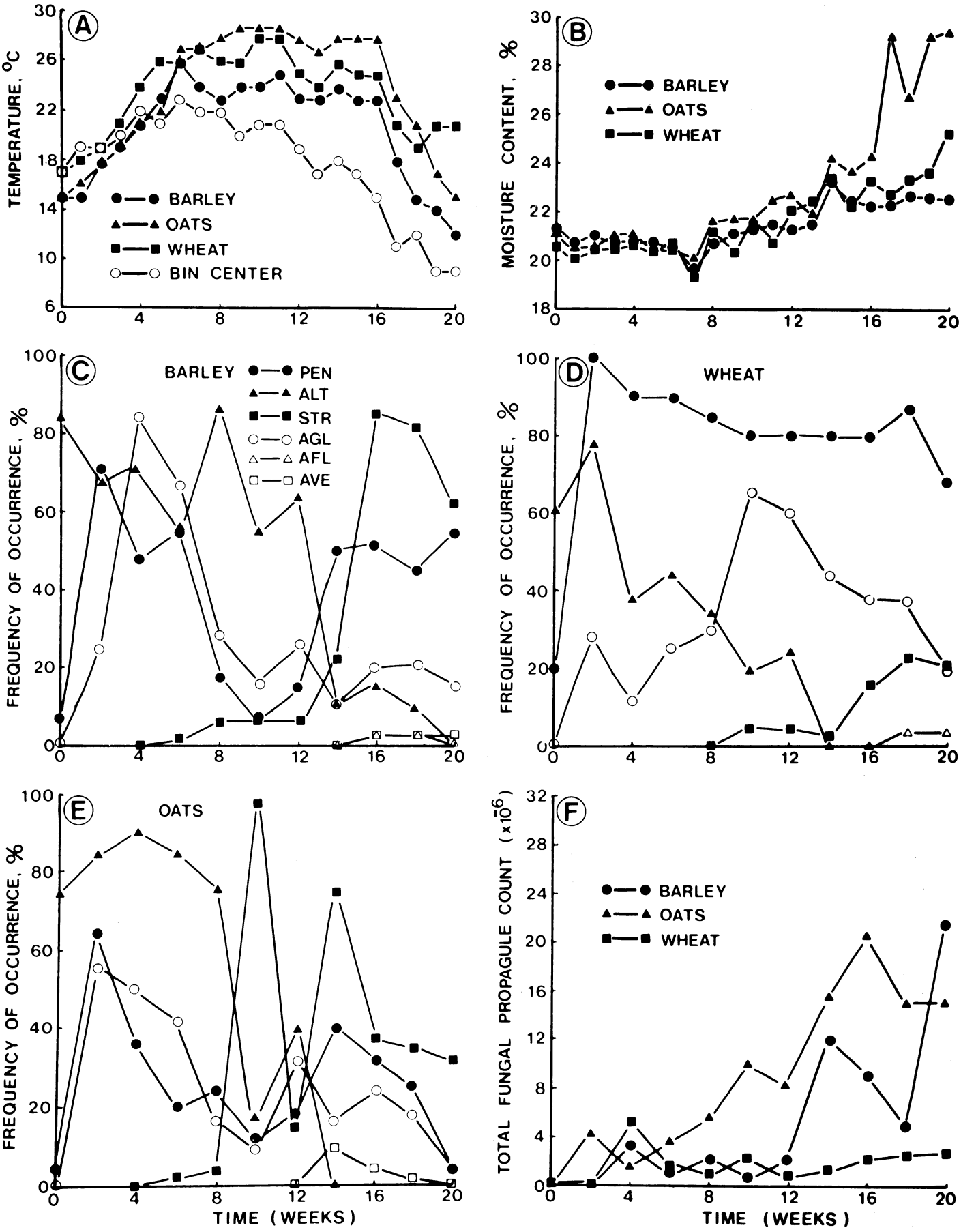
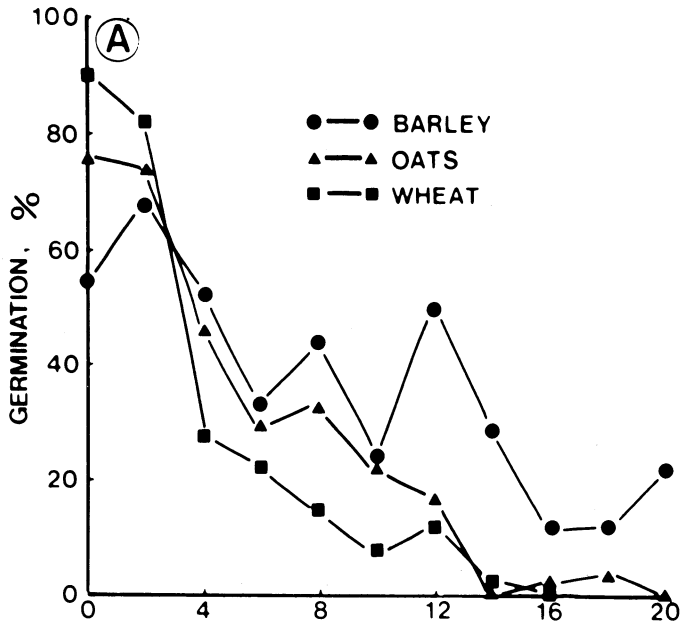
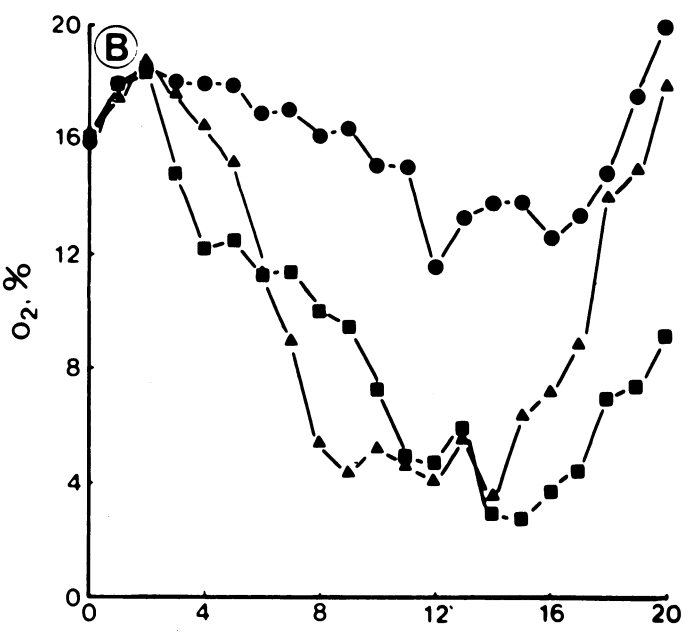
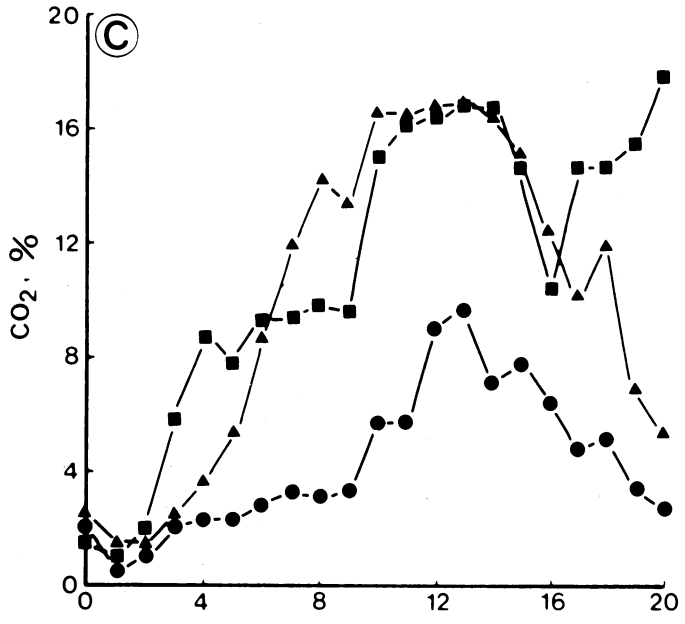
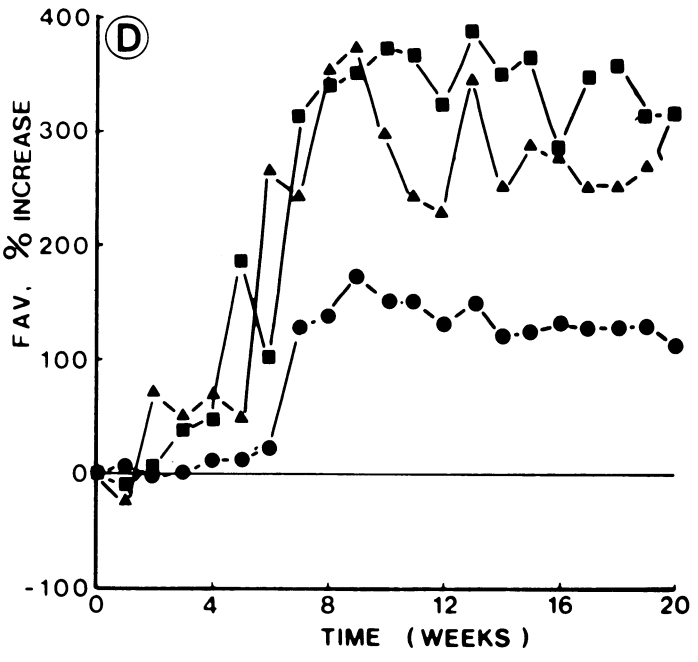


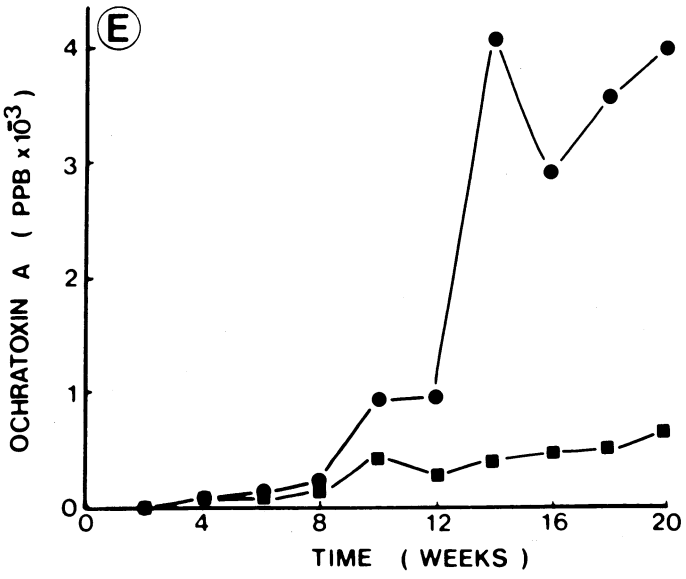
Fig. 1. Changes in biotic and abiotic variables in stored cereals within a Manitoba granary during June-October 1979. **A,** temperature; **B,** moisture; **C–E,** fungal presence; **F,** fungal abundance. PEN = *Penicillium* spp., ALT = *Alternaria alternata,* STR = *Streptomyces* spp., AGL = *Aspergillus glaucus* gr. spp., AFL = *A. flavus,* AVE = *A. versicolor.*











RESULTS AND DISCUSSION

Temperature

The temperature of the ambient air in the granary showed normal seasonal fluctuations. Temperatures at the granary center and within the implanted parcels of barley, wheat, and oats during the 20-week storage period are shown in Fig. 1A. The center temperature rose from an initial 15°C during the warm July weather to a maximum of 21–23°C between four and eight weeks and gradually decreased with the cooler weather of mid-August. The wheat heated first and after five weeks was 5° above the temperature of the surrounding grain mass. By the seventh week, the barley parcel was 2° above, and the wheat and oats 5° above, the temperature of the surrounding grain mass; by the 16th week, this temperature difference reached 8, 10, and 13° in the respective parcels. Beyond the eighth week, the barley heated the least and the oats the most, and this continued until the 18th week.

Fig. 2. Changes in biotic and abiotic variables in stored cereals within a Manitoba granary during June-October 1979. **A,** germination; **B,** oxygen; **C,** carbon dioxide; **D,** fat acidity values; **E,** ochratoxin A.

Moisture Content

Changes in moisture content in the cereal parcels are shown in Fig. 1B. The fluctuating moisture content values gradually increased until the 16th week, after which the differences became more pronounced between oats and two other crops. By the 20th week, barley showed the least and oats the greatest increase in moisture content above initial values.

Microflora Presence and Abundance

The frequency of occurrence of *Alternaria alternata* (Fr.) Keissler, *Penicillium* spp., *Aspergillus glaucus* group species, *Asp. flavus* Link ex. Fr., *Asp. versicolor* (Vuill.) Tiraboschi, and *Streptomyces* spp. are shown for barley, wheat, and oats in Figs. 1C, D, and E, respectively. *Asp. glaucus* group species included *Eurotium amstelodami* Mang., *E. repens* de Bary, *E. rubrum* Konig et al, and *E. chevalieri* Mang. Occurrence of *Alternaria* declined to zero levels by the 20th week in barley and by the 14th week in wheat and oats. *Penicillium*, identified as mainly *P. verrucosum* var. *cyclopium* (Westling) Samson, Stolk, and Hadlock, rose to maximum values in the first two weeks in all grains and continued to predominate throughout the storage period in wheat. Frequency of occurrence of *Penicillium* and *Asp. glaucus* rose and fell together in barley and oats; *Penicillium* recurred in barley after 12 weeks. *Asp. glaucus* increased to maximum values in wheat by the 10th week and declined thereafter.

All crops contained additional *Aspergillus* species during the last eight weeks of storage, with *Asp. flavus* and *Asp. versicolor* attaining maxima of 2% in barley, *Asp. flavus* 4% in wheat, and *Asp. versicolor* 10% in oats. *Streptomyces* reached maximum levels in all grains from the 10th to 18th weeks, reaching a maximum of 98% in oats. Lowest levels of *Streptomyces* were found in wheat. The total fungal propagule count (Fig. 1F) gradually increased to maxima of $2.08–2.16 \times 10^7$ propagules per gram in oats and barley but to only 5.16×10^6 propagules per gram in wheat during the 20 weeks of storage.

TABLE I
Volatiles of Damp Barley in a Manitoba Granary During June-October 1979

Chromatogram Characteristics	Storage Time, days							
	1	49	105	112	119	126	133	140
Number of peaks	11	49	6	12	6	19	10	14
Relative total peak area[a]	0.5	65.6	5.1	0.7	4.9	8.6	2.0	1.0
3-Methyl-1-butanol, %	0	0.26	0.90	0	0	0	0	0
3-Octanone, %	4.52	7.63	0	0	0	2.12	0	0.29
1-Octanol, %	0	65.19	0	0	0	9.39	0	1.39
Number of unknown peaks > 1%	9	12	1	9	2	5	5	10

[a]Total peak area of oats at 49 days = 100.0.

TABLE II
Volatiles of Damp Wheat in a Manitoba Granary During June-October 1979

Chromatogram Characteristics	Storage Time, days							
	1	49	105	112	119	126	133	140
Number of peaks	14	22	16	12	14	16	16	12
Relative total peak area[a]	0.4	56.4	4.6	0.7	1.2	0.8	2.0	4.0
3-Methyl-1-butanol, %	2.52	0.28	0.35	0	0	0	0	0
3-Octanone, %	2.60	8.03	12.23	58.72	10.61	0	12.32	2.35
1-Octanol, %	3.30	81.17	80.39	0	73.22	0	61.64	11.72
Number of unknown peaks > 1%	10	3	2	9	7	12	7	8

[a]Total peak area of oats at 49 days = 100.0.

TABLE III
Volatiles of Damp Oats in a Manitoba Granary During June-October 1979

Chromatogram Characteristics	Storage Time, days							
	1	49	105	112	119	126	133	140
Number of peaks	14	17	5	5	12	18	9	12
Relative total peak area[a]	0.6	100.0	7.3	0.1	3.9	2.3	0.9	1.1
3-Methyl-1-butanol, %	0	0	0	0	0.26	0	0	0
3-Octanone, %	1.35	4.96	99.28	0	3.95	12.16	0	0.74
1-Octanol, %	8.42	51.67	0	0	13.19	40.21	0	0
Number of unknown peaks > 1%	10	3	0	5	2	9	8	8

[a]Total peak area of oats at 49 days = 100.0.

Seed Germination

Seed germination progressively decreased in all grains over 20 weeks (Fig. 2A). This loss was least in the barley, with 22% germination at the 20th week, and greatest in wheat and oats, with negligible germination after 14 weeks and zero germination by 20 weeks.

O_2 and CO_2 Levels

The levels of O_2 and CO_2 in the intergranular air of the cereal parcels are shown in Figs. 2B and C. Of the grains tested, the levels in barley showed the least change compared to initial levels. The decrease in O_2 and increase in CO_2 was most pronounced between the eighth and 16th weeks in all grains. The O_2 and CO_2 percentages approached original values in barley and oats by the 20th week.

FAV

Changes in the FAV of the three grains are shown in Fig. 2D. The greatest FAV increase occurred between the fifth and 10th weeks; afterwards FAV remained relatively unchanged. The FAV increase was least in barley and greatest in wheat and oats. The final FAV for the barley, wheat, and oats were 314, 115, and 319 mg of KOH per 100 g, respectively.

Mycotoxins

Of all mycotoxins analyzed, only ochratoxin A was found. The appearance of *Asp. versicolor* by the 16th week in barley and 14th week in oats indicated additional testing for sterigmatocystin in all samples taken at 20 weeks, but none of this toxin was detected.

Ochratoxin A was found in barley and wheat but not in oats; none was formed until the fourth week in either cereal (Fig. 2E). Ochratoxin A gradually accumulated in wheat to give 700 ppb after 20 weeks. Levels of this toxin began to increase in barley after eight weeks of storage and continued to increase to 3,900 ppb at the end of the experiment. Fluctuations in ochratoxin levels in samples taken at weeks 14 and 16 probably reflect sampling difficulties inherent in mycotoxin analysis. The identity of the toxin in barley and wheat was confirmed by chromatography of the methyl ester derivative.

Fungal Volatiles

In a preliminary study of fungal volatiles, samples of 400 ml of intergranular air from the cereal parcels were surveyed for the presence of known fungal odor components. The expressions "volatiles" and "odors" have separate meanings because volatile compounds are not necessarily odoriferous. Only compounds with demonstrated olfactory characteristics, for instance, 1-octanol, are referred to as "odors."

Analysis of the intergranular air from the cereals for the known fungal odors 1-octen-3-ol, 1-octanol, 3-octanone, and 3-methyl-1-butanol (Kaminski et al 1973, 1974, 1975) revealed the presence of the last three in the samples. These odors were identified by cochromatography with authentic standards. Under our analysis conditions, retention times for 3-methyl-1-butanol, 3-octanone, and 1-octanol were 12.7, 15.2, and 33.6 min, respectively.

Results of the gas chromatographic analyses of volatiles from the cereal parcels are listed in Tables I–III. Four replicates were taken at the beginning and end of the storage period, at one day and 140 days, respectively. Only single samples were taken at the intermediate times to avoid excessive disturbance of the delicate O_2-CO_2 balance.

In every cereal parcel, maximum levels of volatiles were found in samples taken on the 49th day. At this time, 1-octanol was the predominant component; 3-octanone comprised approximately 5–8% of the total volatiles; and 3-methyl-1-butanol was at levels of less than 1% or absent. Abundant 1-octanol probably accounted for the observed "fruity" smell of the intergranular air samples taken for O_2-CO_2 analysis. The highest level of volatiles was produced in the oats, but the greatest number of volatile components at 49 days occurred in the barley.

In all samples, 3-methyl-1-butanol was either absent or comprised less than 1% of the total volatiles. Of the three known fungal odors, 1-octanol predominated whenever present, except in wheat at 112 days, barley at 1 day, and oats at 105 and 140 days.

Samples taken at 105 days and later indicated at least a 10-fold diminution of volatiles. These samples also demonstrated considerable variability in total volatile levels and in composition. This variability may be explained by minor shifting of the end of the gas sampling tube during withdrawal of grain samples and the resulting exposure of the tube end to a different portion of the grain parcel. Compacting of the increasingly moist grain around the end of the tube and channelling of the intergranular air from various parts of the cereal parcel could also account for this variability.

CONCLUSIONS

Ochratoxin A formed in barley and wheat but not in oats at 21% moisture content and 12–29°C in a farm granary. Aflatoxins, zearalenone, citrinin, penicillic acid, and sterigmatocystin were not produced. From the comparison of ochratoxin A levels after 20 weeks, barley appeared to be a better substrate than wheat. The *Penicillium* species isolated were mainly *P. verrucosum* var. *cyclopium*; western Canadian strains of this fungus are therefore probably potent ochratoxin producers. In barley and wheat, the gradual accumulation of ochratoxin A indicated that the toxin was produced under a range of temperatures.

The total fungal volatiles rose 10-fold to 15-fold by the seventh week of storage and declined to near first-day levels by the 16th week. Further sampling at times before and after seven weeks would be necessary to know where the peak occurred and whether the appearance of fungal odors paralleled mycotoxin formation. Of the three known fungal odors, 3-methyl-1-butanol was present in the least amount, and 1-octanol was usually found in the greatest amount. Other major components in the fungal volatiles await identification by mass spectrometry.

The nature of the substrate appeared to be the most important variable in mycotoxin formation in this test. Although most ochratoxin was produced in barley, this crop of the three tested was associated with the least change in the following variables indicating fungal activity; heating, moisture increase, loss of germination, O_2 decrease, CO_2 increase, and FAV increase. Further experiments are necessary to define more precisely the relationship of these variables to mycotoxin formation.

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LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1962. Approved Methods of the AACC. Method 02-01, approved April 1961. The Association: St. Paul, MN.

AMERICAN SOCIETY OF AGRICULTURING ENGINEERS. 1975. Moisture measurement—grain and seeds, method S352. Agricultural Engineers' Yearbook. The Society: St. Joseph, MI.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1975. Official Methods of Analysis (12th ed.). The Association: Washington, DC.

BOTHAST, R. J., ROGERS, R. F., and HESSELTINE, C. W. 1974. Microbiology of corn and dry milled corn products. Cereal Chem. 51:829.

DRAVNIEKS, A., REILICH, H. G., WHITFIELD, J., and WATSON, C. A. 1973. Classification of corn odor by statistical analysis of gas chromatographic patterns of headspace volatiles. J. Food Sci. 38:34.

DRAVNIEKS, A., and WATSON, C. A. 1973. Corn odor classification from low-resolution gas-chromatographic profiles of head space volatiles. J. Food Sci. 38:1024.

HALD, B., and KROGH, P. 1973. Analysis and chemical confirmation of citrinin in barley. J. Assoc. Off. Anal. Chem. 56:1440.

HESSELTINE, C. W. 1976. Conditions leading to mycotoxin contamination of foods and feeds. Page 1 in: Rodricks, J. V., ed. Mycotoxins and Other Fungal Related Food Problems. American Chemical Society: Washington, DC.

HOUGEN, F. J., QUILLIAM, M. A., and CURRAN, W. A. 1971. Headspace vapors from cereal grains. J. Agric. Food Chem. 19:182.

JOSEFSSON, B. G. E., and MÖLLER, T. E. 1977. Screening method for the detection of aflatoxins, ochratoxin, patulin, sterigmatocystin and patulin in cereals. J. Assoc. Off. Anal. Chem. 60:1369.

KAMINSKI, E., STAWICKI, S., and WASOWICZ, E. 1974. Volatile flavor compounds produced by molds of Aspergillus, Penicillium and other Fungi imperfecti. Appl. Microbiol. 27:1001.

KAMINSKI, E., STAWICKI, S., and WASOWICZ, E. 1975. Volatile flavor substances produced by molds on wheat grain. Acta Aliment. Polon. 1:153.

KAMINSKI, E., STAWICKI, S., WASOWICZ, E., and PRZYBYLSKI, R. 1973. Detection of deterioration of grain by gas chromatography. Ann. Technol. Agric. 22:401.

MILLS, J. T., SINHA, R. N., and WALLACE, H. A. H. 1978. Assessment of quality criteria of stored rapeseed—a multivariate study. J. Stored Prod. Res. 14:121.

MURRAY, K. E. 1977. Concentration of headspace, airborne and aqueous volatiles on Chromosorb 105 for examination by gas chromatography and gas chromatography-mass spectrometry. J. Chromatogr. 135:49.

RICHARD-MOLARD, D., CAHAGNIER, B., POISSON, J., and DRAPRON, R. 1976. Evolutions comparées des constituents volatils et de la microflore de maïs stockés sous différentes conditions de température et d'humidité. Ann. Technol. Agric. 25:29.

SCOTT, P. M., VAN WALBEEK, W., KENNEDY, B., and ANYETI, D. 1972. Mycotoxins (Ochratoxin A, citrinin and sterigmatocystin) and toxigenic fungi in grains and other agricultural products. J. Agric. Food Chem. 20:1103.

SHOTWELL, O. L., GOULDEN, M. L., BENNETT, G. A., PLATTNER, R. D., and HESSELTINE, C. W. 1977. Survey of 1975 wheat and soybeans for aflatoxin, zearalenone and ochratoxin. J. Assoc. Off. Anal. Chem. 60:778.

SHOTWELL, O. L., GOULDEN, M. L., BOTHAST, R. J., and HESSELTINE, C. W. 1975. Mycotoxins in hot spots in grains. I. Aflatoxin and zearalenone occurrence in stored corn. Cereal Chem. 52:687.

SHOTWELL, O. L., GOULDEN, M. L., and HESSELTINE, C. W. 1976. Survey of U.S. wheat for ochratoxin and aflatoxin. J. Assoc. Off. Anal. Chem. 59:122.

SINGH, N. B., SINHA, R. N., and WALLACE, H. A. H. 1977. Changes in O_2, CO_2, and microflora of stored wheat induced by weevils. Environ. Entomol. 6:111.

SINHA, R. N. 1973. Interrelations of physical, chemical and biological variables in the deterioration of stored grains. Page 15 in: Sinha, R. N., and Muir, W. E., eds. Grain Storage—Part of a System. AVI: Westport, CT.

SINHA, R. N., WALLACE, H. A. H., MILLS, J. T., and McKENZIE, R. I. H. 1979. Storability of farm-stored hulless oats in Manitoba. Can. J. Plant Sci. 59:949.

WALLACE, H. A. H., and SINHA, R. N. 1962. Fungi associated with hot spots in farm-stored grain. Can. J. Plant Sci. 42:130.

WILSON, D. M., TABOR, W. H., and TRUCKSESS, M. W. 1976. Screening method for the detection of aflatoxin, ochratoxin, zearalenone, penicillic acid and citrinin. J. Assoc. Off. Anal. Chem. 59:125.

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