

## Antifungal Activity of Volatile Fatty Acids on Grains

D. C. HERTING and E. E. DRURY, Health and Nutrition Research Division,  
Tennessee Eastman Company Research Laboratories, Division of Eastman  
Kodak Company, Rochester, New York 14603

### ABSTRACT

Relative activities were determined for volatile fatty acid formulations as antifungal agents for grains. The testing procedures comprised storage of treated or untreated grain with various moisture contents for 1 week or more at 30°C. and at least 70% r.h. Examinations for fungi included both gross and microscopic observations following several plating procedures. Formic, acetic, propionic, butyric, and isobutyric acids were effective fungicides; isobutyric acid showed the highest activity. Most binary and ternary mixtures of the acids were synergistic in their fungicidal activity. The acid formulations were effective antifungal agents on corn, grain sorghum, wheat, oats, barley, and soybeans. The level of a formulation required for protection increased as the moisture content of the grain increased. Protection was often conferred for periods of a year or more.

When starchy grains with a moisture content over 13% are stored for long periods, fungal damage is frequent (1). The trend toward harvesting grains with high moisture and the desirability of utilizing such grains for their enhanced nutritional value in ruminant feeds have increased the probability of fungal growth. Grains rendered unusable by fungal damage mean serious financial losses. Alternatively,

incorporation of moldy grain into feeds may result in suboptimal performance of the animal or the consumption of mycotoxins at levels which cause chronic or acute toxicity (2,3).

Considerable interest has developed during the last five years on the preservation of high-moisture grains by the use of volatile fatty acids (VFA). Although the antifungal properties of these acids have been known for many years (4) and a number of recent articles have alluded to their preservation of moist grain, published reports verifying such activity are few. Cameron reported in 1945 (5) that the addition of butyric acid to the water used to condition grain samples effectively suppressed mold growth. Several years later, workers at the Southern Regional Research Laboratory (5) evaluated a large number of compounds for fungistatic activity on flaxseed. Among the compounds that were equal or superior to their standard, 2-chloroethanol, were acetic, propionic, butyric, and valeric acids. The general consensus, however, appeared to be that chemical treatment was not commercially feasible for prolonging the storage of high-moisture grains (5,6).

This field of study lay generally dormant until the late 1960's when British Petroleum Ltd. announced the usefulness of propionic acid for the long-term preservation of high-moisture grain under practical conditions (7). Since then, both the use of VFA for improving the storage life of high-moisture grains and the enhanced nutrient value of such grains have been reported; but only a patent granted to British Petroleum Ltd. (8) on the use of formic, acetic, and/or propionic acids and publications by Jones (9), Sauer et al. (10), and Christensen (11) have provided any direct data on the efficacy of VFA as fungicides for high-moisture grains.

The objective of this work was to determine the relative activity of individual or mixed VFA as antifungal agents for grain.

## MATERIALS AND METHODS

### Grains

Grains tested were corn, grain sorghum, wheat, oats, and barley. Representative tests were also carried out on soybeans. These materials were obtained locally except for grain sorghum (Arizona) and had never been treated with any fungicidal compound. Moisture contents were determined by the Official Grain Standards (12).

### Volatile Fatty Acids

Formic, acetic, propionic, butyric, and isobutyric acids were obtained either from Eastman Organic Chemicals, Rochester, N.Y., or from the Acids Division, Tennessee Eastman Company, Kingsport, Tenn. The formic acid contained a minimum of 97% acid. The other acids contained a minimum of 99.5% acid.

### Small Beaker Test

A tared 1-gal. bottle was three-fourths filled with grain and weighed. Distilled water was added to raise the moisture content to the desired level and the top was screwed on tightly. The grain was mixed thoroughly and then stored at 4°C. for at least 2 days. During this equilibration period, the bottle was shaken at least once a day (13). The moisture content was checked prior to use of the grain.

Grain samples of 10 g. were placed in 30-ml. beakers. The acids were added by a

micropipet at levels of 0.05, 0.10, and increments of 0.10 up to 1% (v./w.) concentration. Immediately after the acid was added, a piece of 6-mil-thick polyethylene was fastened securely over the top of the beaker (13). The samples were shaken and then incubated at 30°C. and 77% r.h. for 5 days (14). Untreated controls were also run.

After incubation, each sample was examined with a dissecting microscope (magnification 10X). The level of acid at which there was no observable mold growth was used as a reference point to determine the levels to be tested in the bottle test.

#### **Bottle Test**

Polyethylene wash bottles (Nalgene, Dynalab 2402, 1,000 ml. capacity) were filled with 500 g. of grain, and water was added to adjust the moisture content. During equilibration for 2 days at 4°C., the bottles were shaken three times daily (13). A composite sample of a few grains from each bottle was checked for moisture content before the test.

Each acid was tested initially within a range that included as the midpoint the level at which there was no visible mold growth in the beaker test. Each experiment included a bottle to which no acid was added. Because it was impractical to weigh the volatile acids, they were added by volume from a syringe over the surface of the grain. Each bottle was immediately capped and shaken vigorously. The caps were replaced by Bunsen valves as the bottles were transferred to a room maintained at 30°C. (14). The dispenser tube on each bottle was connected to a supply of air which was bubbled through distilled water (15) at about 0.3 liters per hr. The positive pressure ensures adequate oxygen and a high relative humidity for fungal growth.

#### **Evaluation Procedures**

A battery of procedures was used to avoid reliance on a single method. After incubation for 7 days, the contents of each bottle were examined visually and with a dissecting microscope. Duplicate samples (about 20 to 30 kernels each) from the top, middle, and bottom of each bottle were plated on 10% salt malt agar (16) (Difco 0024 with 10% sodium chloride added) and incubated 48 hr. at 30°C. and greater than 70% r.h. (These conditions were favorable for the storage fungi, although some species grow slowly.) The remaining contents of each bottle were mixed thoroughly. Twenty random kernels were placed in 20 ml. of distilled water in a 125-ml. Erlenmeyer flask. The flask was closed with a cotton plug and shaken 4 hr. at about 25°C. Spore counts were obtained on a few drops of water from the flask by the use of a hemacytometer to count the four corners and the middle square (as for a red cell count). This method did not differentiate between living and dead spores but served as a guide to determine appropriate dilution in sterile water for subsequent streaking on 10% salt malt agar.

The plates and the Erlenmeyer flask containing the kernels and water were incubated at 30°C. and 70% (or higher) r.h. After 48 hr., both the plated kernels and the plated spore dilutions were examined grossly for mold growth. The Erlenmeyer flask was swirled vigorously, and samples of the water were placed on microscope slides. Five hundred spores were counted under high-power (430X) magnification and the percent germination was determined (17).

From an evaluation of these tests, the level of acid was determined at which

TABLE I. EVALUATION OF ANTIFUNGAL ACTIVITY OF VOLATILE FATTY ACIDS BY VARIOUS CRITERIA IN DUPLICATE ASSAYS ON CORN WITH 20% MOISTURE

VFA	Level of Addition g./100 g.	Gross Examination <sup>a</sup>		Mold Growth on Plated Kernels, % <sup>b</sup>		Spore Germination, %			
		A	B	A	B	By plating <sup>c</sup>		By microscope <sup>d</sup>	
						A	B	A	B
Acetic	0.87	+	0	20	10	1	2	68	23
	0.96	+	+	10	0	2	0.9	14	54
	1.05 <sup>e</sup>	0	0	0	2	0.1	0.6	0	2
Propionic	0.66	++	+	1	30	14	1	50	41
	0.74	+	0	10	1	0.6	0.3	5	2
	0.83 <sup>e</sup>	0	0	0	0	0.4	0.6	0.6	0
Butyric	0.64	++	0	30	10	3	1	0.8	7
	0.72 <sup>e</sup>	0	0	0.5	0	0.2	0.3	0	1
	0.80	0	0	0	0	1	0.9	0	0
Isobutyric	0.55	++	+	40	30	9	0.3	75	0
	0.63 <sup>e</sup>	0	0	1	2	1	0.9	0	0
	0.71	0	0	0	0	0.1	0.9	0	0.5

<sup>a</sup>Heavy, light, and no mold growth indicated by ++, +, and 0, respectively.

<sup>b</sup>Percentage of kernels that were moldy.

<sup>c</sup>Percentage of mold colonies calculated from the ratio of the number of colonies from the plated spore solution of the test elevator to the number of colonies from the plated spore solution of the control elevator. Spore counts on the control were in the range of  $4 \times 10^7$  per kernel.

<sup>d</sup>Calculated as a ratio of the number of germinated spores per 500 counted in the test solution to the number of germinated spores per 500 counted in the control solution and then converted to percent.

<sup>e</sup>Considered to be the effective fungicidal level (EFL).

inhibition of storage fungi was essentially complete (97 to 100%). If inhibition did not occur, higher levels of acid were tested until the fungicidal level was established.

## RESULTS

Data obtained in sequential bottle tests of the individual VFA are illustrated in Table I. The same lot of corn was reconstituted to 20% moisture for each test. These results show good replication of the effective fungicidal level (EFL) for each acid even though results for the "prefungicidal" level may vary considerably. The EFL is not intended to be a precise level but is the minimum *among the levels tested* that was fungicidal. In practice, storage fungi, field fungi, yeasts, and bacteria were all inhibited.

Average results obtained for the individual VFA during a 2-year period and typical results for binary and ternary mixtures are given in Table II. Isobutyric was the most active single acid. The values for relative activity illustrate the surprising observation that, with the exception of several mixtures containing acetic acid, VFA are synergistic in their fungicidal activity on high-moisture corn. Although these results were obtained with corn reconstituted to 20% moisture, other experiments with freshly harvested high-moisture (28%) corn indicated that antifungal activities of various VFA formulations are analogous to those on corn reconstituted to 28% moisture.

TABLE II. MINIMUM FUNGICIDAL LEVELS OF SINGLE OR MIXED VOLATILE FATTY ACIDS ON CORN WITH 20% MOISTURE

VFA	EFL, g./100 g.		Relative Activity
	Predicted <sup>a</sup>	Observed	
Formic	...	0.64	...
Acetic	...	1.06 <sup>b</sup>	...
Propionic <sup>c</sup>	...	0.80 <sup>b</sup>	...
Butyric	...	0.71 <sup>b</sup>	...
Isobutyric	...	0.56 <sup>b</sup>	...
Acetic:propionic (1:1)	0.93	0.88	106
Acetic:propionic <sup>d</sup> (3:2)	0.96	0.80	120
Acetic:butyric (1:1)	0.88	0.61 <sup>b</sup>	144
Acetic:isobutyric (1:1)	0.81	0.78 <sup>b</sup>	104
Propionic:butyric (1:1)	0.76	0.43	177
Propionic:isobutyric (1:1)	0.68	0.33	206
Butyric:isobutyric (1:1)	0.64	0.41	156
Formic:acetic:propionic <sup>e</sup> (3:3:4)	0.83	0.47	177
Acetic:propionic:butyric (1:1:1)	0.86	0.52	165
Acetic:propionic:isobutyric (1:1:1)	0.81	0.52	156
Acetic:butyric:isobutyric (1:1:1)	0.78	0.60	130
Propionic:butyric:isobutyric (1:1:1)	0.69	0.34	203

<sup>a</sup>Based on the sum of expected contributions by the individual components.

<sup>b</sup>Average of two or more values. All others were single values.

<sup>c</sup>Comparable to Ortho-Guard (Chevron Chemical Co.) and Propcorn (BP Chemicals International, Ltd.).

<sup>d</sup>Comparable to ChemStor (Celanese Corp.) in 1971.

<sup>e</sup>Example showing fungistatic activity in reference 8.

To determine the formulations with maximum activity, we took propionic, butyric, and isobutyric acids in pairs, mixed them in various proportions, and tested them on corn with a 20% moisture content. Regression analyses of the data yielded optimum EFL at 0.30% for 40:60 propionic:isobutyric acids, 0.35% for 50:50 propionic:butyric acids, and 0.40% for 45:55 butyric:isobutyric acids. All binary mixtures of these three VFA were synergistic in their antifungal activity. Similarly, regression analysis of the data obtained for 22 ternary mixtures with various proportions of propionic, butyric, and isobutyric acids indicated that the optimum EFL of 0.30% was achieved for the mixture comprising 37.5% propionic, 25.0% butyric, and 37.5% isobutyric acids. All ternary mixtures tested were synergistic in their antifungal activity.

The VFA are also fungicides for other agricultural substrates. Table III shows results obtained on grain sorghum, wheat, oats, barley, and soybeans with single VFA and/or selected binary and ternary mixtures. Some comparative data were obtained at moisture contents of both 20 and 24%. The substrate *per se* does not appear to increase or decrease the EFL consistently.

Fungi grow faster and sporulate more abundantly as the moisture content of the substrate increases (6). As a correlate, the level of VFA required for fungicidal activity also can be anticipated to increase as the moisture content increases. This is indicated for several grains in Table III and is also shown in Fig. 1 for selected fungicidal formulations on corn. The relationships were linear within the range of 17 to 31% moisture content, although the slopes varied somewhat. Data obtained

TABLE III. ANTIFUNGAL ACTIVITY OF VFA ON VARIOUS GRAINS AND ON SOYBEANS

VFA	EFL, g./100 g.					
	Corn	Grain sorghum	Wheat	Oats	Barley	Soybeans
	20% Moisture content					
Acetic	1.06	...	0.99	...	...	1.15
Propionic	0.80	0.71	0.68	0.61	...	0.89
Butyric	0.71	0.52	0.58	0.58	...	0.77
Isobutyric	0.56	0.51	0.50	0.50	...	0.76
Propionic:butyric (1:1)	0.43	...	0.43	...	...	...
Propionic:isobutyric (1:1)	0.33	0.61	0.42	0.51	...	0.51
Butyric:isobutyric (1:1)	0.41	...	0.41	...	...	...
Acetic:propionic: isobutyric (1:1:1)	0.52	...	0.60	...	...	...
Propionic:butyric: isobutyric (37.5:25.0:37.5)	...	0.52	0.44	0.51	...	0.42
	24% Moisture content					
Propionic	0.96	0.97	1.18	1.18	1.16	...
Isobutyric:propionic (3:2)	0.60	0.85	0.82	0.70	0.88	...
Isobutyric:propionic (9:1)	0.78	0.90	0.97	0.89	1.11	...

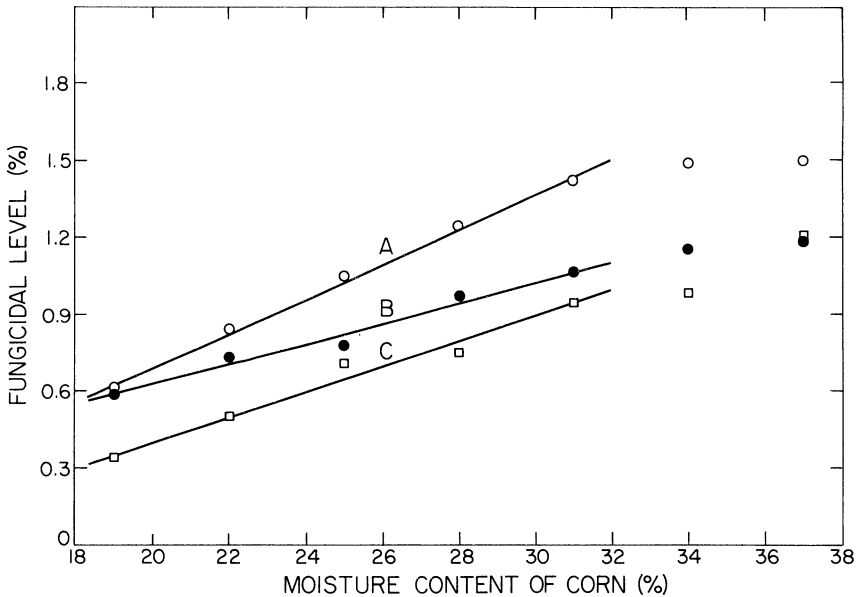
on samples prepared to contain either 34 or 37% moisture were not included in the regression analyses because the required amounts of water were not completely absorbed by the corn during the reconstitution process.

Data on the effectiveness of VFA on grains during long-term storage are given in Table IV. Among the representative formulations evaluated, protection against mold for at least 1 year was generally provided either by the EFL determined in the 1-week test or by approximately 115% of this level.

Because the storage conditions used in our tests did not allow the natural recontamination of grain which results from constant exposure to air-borne spores, each sample of acid-treated grain that showed no viable spores after 52 weeks was inoculated with an actively growing culture of *Aspergillus flavus* (Link) (American Type Culture Collection, No. 1003). After at least 4 additional weeks of storage, no mold was observed in any of these samples; and six of eight grain samples containing 20% moisture showed no viable spores 1 year after inoculation.

## DISCUSSION

The conditions chosen for the testing procedure were relatively rigorous. No effort was made to obtain "clean" grain and, in fact, several samples of soybeans and wheat already contained visible mold. Surface disinfection was not employed. The test conditions of 30°C. and 70 to 90% r.h. are optimum for the growth of storage fungi. The positive air pressure in the polyethylene bottle ensured adequate oxygen for maintaining the fungi in a high-relative-humidity atmosphere. The intention of the test procedures was to optimize growth of the fungi under conditions to some extent simulating the aerated storage of grain. At the same time, we recognize that these procedures do not provide continuous exposure to air-borne spores and that the conditions are not analogous to situations where grain is simply piled without aeration. In addition, the aeration in our system might increase the apparent EFL by increasing volatilization and loss of compounds being tested.



**Fig. 1.** Effect of moisture content of corn on the acid level required for fungicidal activity. Regression equations: A, propionic acid,  $EFL = -0.65 + 0.067(\% \text{ moisture})$ ; B, isobutyric:propionic acids (90:10),  $EFL = -0.18 + 0.040(\% \text{ moisture})$ ; C, isobutyric:propionic acids (60:40),  $EFL = -0.58 + 0.049(\% \text{ moisture})$ .

The antifungal activity of some of the VFA is known (4). Our findings confirm this general knowledge and extend the limited information published about antifungal activity in grain (5,8-11). Isobutyric acid appears to be unique among the  $C_1$  through  $C_4$  VFA. Based on laboratory observations with selected mold species (18,19), antifungal activity for isobutyric acid might be anticipated; but the finding that it was better for grain than formic, acetic, propionic, or butyric acids was unexpected. Equally surprising was the synergism observed for most mixtures of VFA. Although one report (8) alluded to this, the data did not hint at the appreciable effect that we found.

The mechanism of the observed fungicidal effects is obscure. Undissociated molecules of acidic substances can penetrate into cells much more readily than the corresponding ions (4), and the preservative effect appears to be due to the concentration of undissociated acids (20). The EFL may indicate the point at which a sufficiently high concentration of the undissociated acid has been achieved. Reasons for the enhanced activity shown by mixtures of various acids are unknown.

The significance of mycotoxins in agricultural commodities and animal feeds as a potential health hazard is known widely (2,3). Although we have not analyzed for mycotoxins and have not typed fungal populations consistently, our studies have shown that the VFA are active against the major mycotoxin-producing fungi. This antifungal activity was demonstrated by the presence of *A. flavus* in control samples and by its absence in adequately treated samples. The VFA were also fungicidal in other experiments in which grain was inoculated specifically with actively growing cultures of two storage fungi, *A. flavus* (Link) and *Penicillium*

TABLE IV. ANTIFUNGAL ACTIVITY OF VFA ON GRAINS DURING LONG-TERM STORAGE

Grain	Initial Moisture Content %	VFA	Level of addition g./100 g.	First Year		Second Year <sup>a</sup>	
				Period of protection weeks	Moisture content <sup>b</sup> %	Period of protection weeks	Moisture content <sup>c</sup> %
Corn	20 <sup>d</sup>	None	0	<1	21 <sup>e</sup>	...	... <sup>f</sup>
		Acetic	1.08	52	22	52	20
		Propionic	0.86	52	19	52	19
		Butyric	0.74	27	...	...	...
		Isobutyric	0.49	8	...	...	...
		Isobutyric	0.57	20	...	...	...
		Isobutyric	0.65	52	17	40	...
		Propionic:isobutyric (1:1)	0.42	5	...	...	...
		Propionic:isobutyric (1:1)	0.50	27	...	...	...
		Propionic:butyric (1:1)	0.42	52	18	52	18
		Propionic:butyric (1:1)	0.50	52	19	52	24
Wheat	20 <sup>d</sup>	None	0	<1	22 <sup>e</sup>	...	22 <sup>e</sup>
		Propionic	0.89	52	18	52	19
		Butyric	0.78	52	18	52	20
		Isobutyric	0.51	52	17	51	...
		Isobutyric	0.60	38	...	...	...
Corn	28 <sup>g</sup>	None	0	<1	31 <sup>e</sup>	...	...
		Isobutyric:propionic (3:2)	0.68	4	...	...	...
		Isobutyric:propionic (3:2)	0.77	52	24	>4	...
		Isobutyric:propionic:butyric (37.5:37.5:25.0)	0.68	5	...	...	...
		Isobutyric:propionic:butyric (37.5:37.5:25.0)	0.77	52	31	>4	...

<sup>a</sup>Samples showing no mold after 1 year were inoculated with *A. flavus*.

<sup>b</sup>After 52 weeks.

<sup>c</sup>After 104 weeks.

<sup>d</sup>Reconstituted.

<sup>e</sup>Controls kept even though they were moldy.

<sup>f</sup>Not feasible to obtain representative sample.

<sup>g</sup>Freshly harvested.



*rubrum* (Stoll), and one field fungus, *Fusarium sporotrichioides* (Sherbakoff). Further, results on freshly harvested corn with 28% moisture (Table IV) suggest by inference that all fungi are controlled. By inhibiting mold growth, VFA prevent formation of mycotoxins but have no known effect on existing mycotoxins.

A large number of feeding trials (e.g., 21-23) have been carried out with feeds containing these acids, except for isobutyric. The results have been consistently acceptable. In many cases, the primary intent was to compare the feeding value of high-moisture grain with that of dry grain; the addition of an organic acid was simply a device to preserve the grain long enough to complete the feeding trial. No problems with palatability or feed rejection were reported unless the level of acid in the feed was 6% (22) or more (21), and levels as high as 6% of the concentrate in the rations of dairy cows had no adverse effect on milk production or fat test (24). Significant increases in feed efficiency have been reported in most tests, but this may have been due more to the higher digestible energy in the high-moisture corn than to the presence of organic acid *per se*. The contribution of VFA to the nutritive value of the feed is real but small.

Improved harvesting systems, such as the picker-sheller, permit less dependence on weather, enable increased crop yields, and leave a higher quality forage in the field. These factors coupled with the increased nutritive value of high-moisture grain make the harvesting of high-moisture grain a desirable agricultural practice if the grain can be protected against fungal deterioration. Our results confirm and extend observations of others demonstrating that VFA are useful fungicides for preserving high-moisture grain for long periods of time in existing storage facilities. Several formulations are registered with the Environmental Protection Agency. The drawbacks of treating grain with VFA do not appear serious for the farmer-feeder. Odor is transient in nature and corrosion of metallic surfaces can be minimized with epoxy or asphaltic coatings or by lining with nontoxic plastic sheeting. Adequate precautions must be taken in the application of the VFA to the grain. Grain treated in such a fashion will not germinate and cannot be moved in commercial channels at present. On balance, however, protection of grains against fungal deterioration by treatment with VFA appears to be a desirable alternative to drying and to anaerobic sealed storage.

#### Literature Cited

1. CHRISTENSEN, C. M., and KAUFMANN, H. H. Grain storage. The role of fungi in quality loss; chap. 1 and 3. University of Minnesota Press: Minneapolis, Minn. (1969).
2. CHRISTENSEN, C. M., and KAUFMANN, H. H. Grain storage. The role of fungi in quality loss; chap. 6. University of Minnesota Press: Minneapolis, Minn. (1969).
3. WOGAN, G. N., ed. Mycotoxins in foodstuffs. The MIT Press: Cambridge, Mass. (1965).
4. BYRDE, R. J. W. In: Fungicides, ed. by D. C. Torgeson; vol. II, p. 543. Academic Press: New York (1969).
5. Cited by MILNER, M., and GEDDES, W. F. In: Storage of cereal grains and their products, ed. by J. A. Anderson and A. W. Alcock; chap. IV. Amer. Ass. Cereal Chem.: St. Paul, Minn. (1954).
6. SEMENIUK, G. In: Storage of cereal grains and their products, ed. by J. A. Anderson and A. W. Alcock; chap. III. Amer. Ass. Cereal Chem.: St. Paul, Minn. (1954).
7. ANONYMOUS. To market propionic acid for moist grain protection. Feedstuffs, p. 1 (Sept. 7, 1968).
8. HUITSON, J. J., and WATTS, P. W. J. (to BP Chemicals, Ltd.). U.S. Patent 3,595,665 (1971).

9. JONES, G. M. Preservation of high moisture corn with volatile fatty acids. *Can. J. Anim. Sci.* 50: 739 (1970).
10. SAUER, D. B., BURROUGHS, R., and SIMON, J. A. Control of grain storage fungi with propionic and acetic acids. *Phytopathology* 62: 787 (1972).
11. CHRISTENSEN, C. M. Tests with propionic and acetic acids as grain preservatives. *Feedstuffs*, p. 37 (Feb. 26, 1973).
12. ZELENY, L. In: *Seeds. Yearbook of agriculture*, ed. by A. Stefferud; p. 444. U.S. Dep. Agr.: Washington, D.C. (1961).
13. CHRISTENSEN, C. M. Influence of small differences in moisture content upon the invasion of hard red winter wheat by *Aspergillus restrictus* and *A. repens*. *Cereal Chem.* 40: 385 (1963).
14. CHRISTENSEN, C. M., and KAUFMANN, H. H. Grain storage. The role of fungi in quality loss; chap. 2. University of Minnesota Press: Minneapolis, Minn. (1969).
15. YANG, H., and LUCAS, G. B. Effects of N<sub>2</sub>-O<sub>2</sub> and CO<sub>2</sub>-O<sub>2</sub> tensions on growth of fungi isolated from damaged flue-cured tobacco. *Appl. Microbiol.* 19: 271 (1970).
16. CHRISTENSEN, C. M. A note on invasion of durum wheat by storage fungi. *Cereal Chem.* 44: 100 (1967).
17. GATTANI, M. L. The agar plate spore germination method for testing fungicides. *Phytopathology* 44: 113 (1954).
18. YOKOTSUKA, T. Flavoring substances in soy sauce. XIII. The fungistatic substances in brewed soy sauce and the increase of the fungistatic nature by heating the soy sauce. *J. Agr. Chem. Soc. Japan* 28: 114 (1954). [See *Chem. Abstr.* 49: 15164a (1955).]
19. ASANTE, G. S., and NEAL, A. L. Characterization of fungistatic substances produced by a *Bacillus* antagonistic to *Ceratocystis ulmi*. *Phytopathology* 54: 819 (1964).
20. WOODFORD, R., and ADAMS, E. Sorbic acid. *Amer. Perfum. Cosmet.* 85(3): 25 (1970).
21. BOWLAND, J. P., YOUNG, B. A., and MILLIGAN, L. P. Influence of dietary volatile fatty acid mixtures on performance and on fat composition of growing pigs. *Can. J. Anim. Sci.* 51: 89 (1971).
22. MARION, P. T., RIGGS, J. K., ARNOLD, J. L., and DRIEDGER, A. Moist grain rations with VFA for beef cattle. *J. Anim. Sci.* 34: 909 (1972).
23. LARSEN, H. J., JORGENSEN, N. A., BARRINGTON, G. P., and NIEDERMEIER, R. P. Effect of organic acids on preservation and acceptability of high moisture corn. *J. Dairy Sci.* 55: 685 (1972).
24. JONES, G. M. Volatile fatty acids in concentrate rations for lactating dairy cows. *J. Dairy Sci.* 54: 1142 (1971).

[Received March 21, 1973. Accepted June 18, 1973]