Antifungal Activity of Volatile Fatty Acids on Grains. II. Effect of Aqueous Dilutions

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ABSTRACT

The effect of dilution with water on antifungal activity was determined for volatile fatty acid formulations added to grains. The volatile fatty acids used were acetic, propionic, butyric, and isobutyric acids. All aqueous dilutions were effective fungicides. Blends containing low levels of water and high levels of acid showed decreased activity which corresponded to the degree of dilution. Blends of acids with 50% or more water were synergistic in their activity, possibly the result of converting acid-acid dimers to acid-water dimers. Aqueous formulations were effective antifungal agents on barley, corn, grain sorghum, oats, and wheat. The level of formulation required for protection increased as the moisture content of the grain increased. Protection was often afforded for periods of greater than 1 year.

Recent trends in agricultural practices are resulting in an increase in the amount of corn being harvested with high moisture content. Such grain is utilized more efficiently than dry grain by ruminants (1,2), but storage of high-moisture grain under aerobic conditions allows fungal growth and results in rapid and extensive deterioration, possibly accompanied by the appearance of mycotoxins (3).

Volatile fatty acids (VFA) have been proposed for use in preserving grain of high moisture content (4). We showed that isobutyric acid is the most effective antifungal agent among the common C_1 through C_4 acids and that, except for several mixtures containing acetic acid, binary or ternary mixtures of these acids are synergistic in their preservative action (5). The objective of this investigation was to determine the activity of aqueous dilutions of VFA as antifungal agents for grain.

MATERIALS AND METHODS

Grains and procedures used for testing and evaluation were described fully (5). In brief, treated or untreated grain with various moisture contents was stored 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 to evaluate both external and internal inoculum.

Aqueous dilutions were made v./v. with distilled water. The degree of maximum dilution possible varies among the acids and their mixtures and depends on solubility of the acids. Most of the aqueous blends tested were 1:1 dilutions. Only in the case of isobutyric acid was slight warming required to effect solution.

RESULTS AND DISCUSSION

Results obtained for aqueous dilutions of the individual VFA and of binary and several ternary mixtures of the acids are given in Table I. Because these formulations were applied to corn reconstituted to 20% moisture, the final moisture content of the corn was slightly over 20%. The aqueous dilutions of VFA were effective fungicides at levels lower than those anticipated from simple dilution of the individual or mixed acids. Although the data are not reported here, a similar conclusion was drawn from comparisons of antifungal activities of aqueous dilutions of VFA with those of anhydrous VFA on freshly harvested corn containing 28% moisture.

The lowered effective fungicidal levels (EFL) of aqueous blends (based on amount of *acid* required) indicated synergism. To determine whether this apparent synergism resulted solely from better distribution of the VFA throughout the grain or possibly from other mechanisms, the EFL were determined for formulations comprised of varying proportions of water and a ternary mixture of VFA (propionic:butyric:isobutyric acids, 50:25:25) on corn containing 20% moisture. Table II shows that the amount of VFA required remained approximately constant with an increase of water in the dilutions until the water content of the formulations exceeded 40%. The amount of VFA required as a fungicide then decreased with an increase in aqueous dilution until an apparent minimum amount of approximately 0.18% VFA (w./w.) was reached and maintained at dilutions of 70% or more.

These results were checked with an alternate procedure utilizing corn with lower moisture contents (16.9 and 18.4%) and, for each moisture content, adding a constant volume of water (sufficient to raise the moisture content of the corn to 20%) containing varying amounts of the ternary VFA mixture. A minimum requirement for 0.17% VFA (w./w.) was confirmed.

TABLE I. MINIMUM FUNGICIDAL LEVELS OF 50% AQUEOUS DILUTIONS OF VOLATILE FATTY ACIDS ON CORN CONTAINING 20% MOISTURE

Volatile Fatty Acids Formulation	Proportions	Effective Fungicidal Level, g/100 g. Predicted[a]	Observed	Relative Activity[a]
Acetic:H_2O	50:50	2.12	1.41	150
Propionic:H_2O	50:50	1.60	0.86[b]	186
Butyric:H_2O	50:50	1.42	0.84	169
Isobutyric:H_2O	50:50	1.12	0.71	158
Acetic:Propionic:H_2O	25:25:50	1.76	0.96	183
Acetic:Propionic:H_2O	30:20:50	1.60	0.88	182
Acetic:Butyric:H_2O	25:25:50	1.22	0.96	127
Acetic:Isobutyric:H_2O	25:25:50	1.56	1.04	150
Propionic:Butyric:H_2O	25:25:50	0.86	0.60	143
Propionic:Isobutyric:H_2O	25:25:50	0.66	0.51	129
Butyric:Isobutyric:H_2O	25:25:50	0.82	0.59	139
Propionic:Butyric:Isobutyric:H_2O	25:12.5:12.5:50	0.68[c]	0.52[b]	131
Propionic:Butyric:Isobutyric:H_2O	15:20:15:50	0.60[d]	0.70	86

[a]Based on simple dilution of the EFL for acid alone (4).

[b]Duplicate values. All others single.

[c]EFL for propionic:butyric:isobutyric acids (50:25:25) = 0.34.

[d]EFL for propionic:butyric:isobutyric acids (30:40:30) = 0.30.

TABLE II. EFFECT OF DEGREE OF AQUEOUS DILUTION ON AMOUNT OF VOLATILE FATTY ACID REQUIRED FOR FUNGICIDAL ACTIVITY IN CORN CONTAINING 20% MOISTURE

Composition of Formulation: Water %	Volatile fatty acid[a] %	Effective Fungicidal Level g./100 g.	Volatile Fatty Acid Required g./100 g.
0	100	0.34	0.34
10	90	0.34	0.31
20	80	0.43	0.34
30	70	0.52	0.36
40	60	0.61	0.37
50	50	0.52	0.26
60	40	0.70	0.28
70	30	0.61	0.18
80	20	0.88	0.18
90	10	1.67	0.17

[a]Ternary mixture of propionic:butyric:isobutyric acids (50:25:25).

Improved distribution of VFA in water due to increased volume does not appear to be the explanation for the increased antifungal activity. The response appears instead to be due to simple dilution at low water-VFA ratios, followed by synergism as the proportion of water in the formulation is increased. The VFA are dimeric in the anhydrous state because of hydrogen bond formation (6,7); however, addition of water disrupts the acid-acid dimeric structure and results in the formation of water-VFA cross dimers. An excess of water could double the number of potentially active molecules. The results of the fungicidal tests are consistent

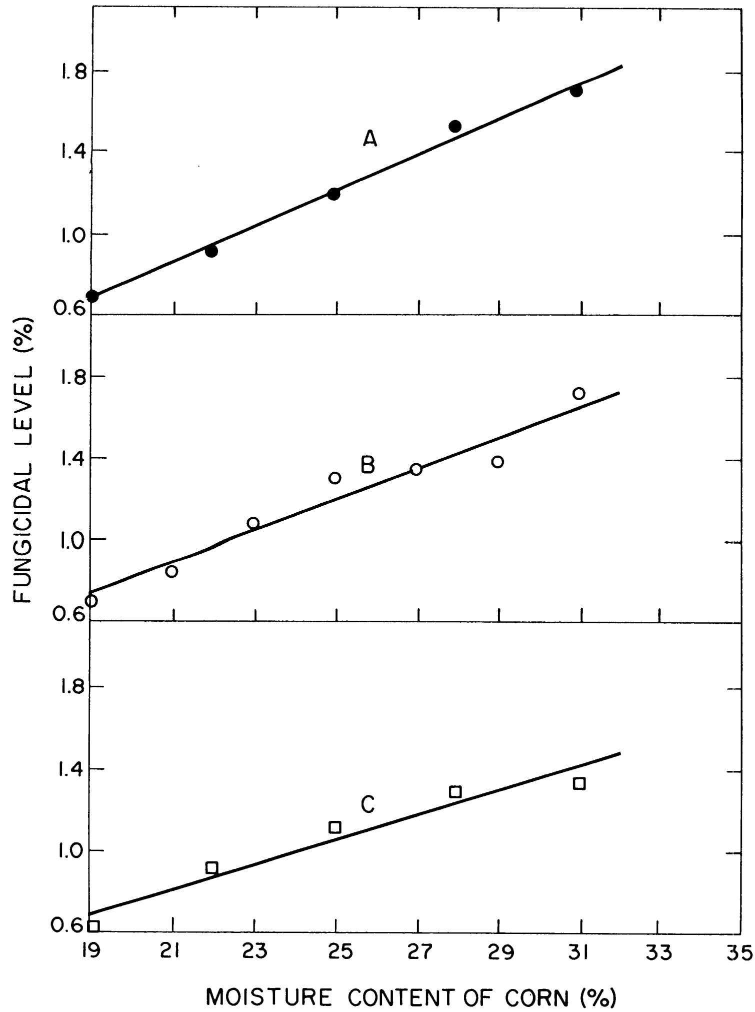

Fig. 1. Effect of moisture content of corn on the level of formulation required for fungicidal activity. Regression equations: A, isobutyric:propionic:H_2O (45:5:50), EFL = 0.087 (% moisture) –0.98; B, propionic:H_2O (50:50), EFL = 0.076 (% moisture) –0.70; C, isobutyric:propionic:H_2O (30:20:50), EFL = 0.061 (% moisture) –0.46.

with this hypothesis because the amount of VFA required to effect antifungal activity when added in aqueous dilutions containing high proportions of water was about half of that required for the anhydrous VFA.

Regression analysis and contour plotting of EFL for 18 single, binary, and ternary VFA formulations added in a constant volume of water to corn of 18.5% moisture content (sufficient to raise the moisture content of the corn to 20%) gave an optimum fungicidal activity of 0.18% (w./w.) VFA with a mixture of propionic:butyric:isobutyric acids (35:20:45) at a VFA-water ratio of 9:91.

TABLE III. FUNGICIDAL ACTIVITY OF 50% AQUEOUS SOLUTIONS OF VOLATILE FATTY ACIDS ON CORN DURING LONG-TERM STORAGE

Initial Moisture Content %	Volatile Fatty Acid Formulation	Level of Addition g./100 g.	First Year: Period of protection weeks	First Year: Moisture content[b] %	Second Year[a]: Period of protection weeks	Second Year[a]: Moisture content[c] %
20[d]	None	0	< 1	21[e]	...	...[f]
	Propionic:H_2O (1:1)	0.94	52	20	52	18
	Butyric:H_2O (1:1)	0.84	52	17	52	16
	Propionic:Isobutyric:H_2O (1:1:2)	0.51	5	...	...	...
	Propionic:Isobutyric:H_2O (1:1:2)	0.59	8	...	...	...
28[g]	None	0	< 1	31	...	...
	Propionic:Butyric:H_2O (1:1:2)	1.38	52	30	>23	...
	Propionic:Butyric:H_2O (1:1:2)	1.48	52	27	>23	...
	Propionic:Isobutyric:H_2O (1:1:2)	1.28	52	27	>23	...
	Propionic:Isobutyric:H_2O (1:1:2)	1.38	52	32	>23	...
	Propionic:Butyric:Isobutyric:H_2O	1.18	7	...	...	...
	(25:12.5:12.5:50)	1.28	52	25	>23	...

[a]Samples showing no mold after 1 year were inoculated with *A. flavus.*

[b]After 52 weeks.

[c]After 104 weeks.

[d]Reconstituted.

[e]Controls kept even though moldy.

[f]Not feasible to obtain representative sample.

[g]Freshly harvested.

Similarly, regression analysis and contour plotting of EFL for 16 single, binary, and ternary VFA formulations added in a constant volume of water to corn of 24.8% moisture content (sufficient to raise the moisture content of the corn to 26%) gave an optimum fungicidal activity of 0.34% (w./w.) VFA with a mixture of propionic:butyric:isobutyric acids (32:15:53) at a VFA-water ratio of 18:82. Composition of the optimum VFA mixture was about the same at both levels of moisture content in the grain.

For studies of other parameters influencing the EFL, 50% aqueous dilutions of various VFA formulations were used to avoid addition of excessive amounts of water to the grain. Figure 1 shows the effect of moisture content of corn on the level of formulation required for fungicidal activity. Data were also obtained on samples prepared to contain either 34 or 37% moisture but were not included in the regression analyses because the required amounts of water were not completely absorbed by the corn during the reconstitution process. Differences in EFL among these aqueous blends were not so great as those noted previously (5) for the anhydrous VFA tested under the same conditions. The three aqueous formulations in Fig. 1 were also tested on barley, grain sorghum, oats, and wheat. Differences in EFL for a given moisture content were minimal among the formulations and among the four small grains. In general, however, the small grains usually required about 20% more formulation than did corn of the same moisture content.

Data on the effectiveness of aqueous dilutions of VFA on grain during long-term storage are given in Table III. Although only a few representative formulations were evaluated, protection against mold growth for at least a year was provided by most of them.

Because storage conditions did not allow recontamination of the grain by constant exposure to airborne spores, each sample of grain that showed no viable spores after 52 weeks of storage was inoculated with an actively growing culture of *Aspergillus flavus* Link (American Type Culture Collection, No. 1003). No mold was observed in the corn with 28% moisture content after an additional 23 weeks of storage or in the corn with 20% moisture content after an additional 52 weeks of storage.

The results reported herein have shown not only that aqueous dilutions of VFA can be used as fungicides for moist grain without loss of effectiveness of the VFA, but also that dilution of VFA formulations with water has an apparent synergistic effect when the proportion of water in the formulations is sufficiently high. Such an effect has not been demonstrated previously. Cameron (8) reported fungicidal activity for butyric acid added to the water used to condition grain samples, and two patents (9,10) on the use of VFA as antifungal compounds in crops and animal feedstuffs mentioned that the VFA may be added in aqueous solution. None of these references described the synergism.

Under practical conditions, the use of aqueous dilutions of VFA with *optimum* fungicidal activity may result in the addition of excessively large quantities of water to the grain. Aqueous blends also may be more corrosive to some types of available application equipment than the anhydrous acid. On the other hand, aqueous blends of VFA formulations containing 50 to 60% water may be an economical approach to supplying antifungal activity and may be particularly advantageous for grains being reconstituted or conditioned for animal feeding.

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