

INSECT AND FUNGAL RESPONSE TO  
SORBIC ACID TREATED STORED COMMODITIES

by

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Sorbic acid is a conjugated diunsaturated fatty acid produced in the fruit of the mountain ash tree and by the pigment producing cells of some aphids. The structure of sorbic acid corresponds to the saturated fatty acid caproic which is present in butterfat at concentrations of 1-2%. Under non-fasting conditions in mammals sorbic acid is completely oxidized to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  and yields its entire 6.63 cal/g for energy.

In 1945 a U.S. patent was approved for its fungistatic properties. Several years earlier a German patent had been approved for its synthesis method. Germany and Japan are now the largest producers of sorbic acid. England, China and many other countries produce and use the compound in minor amounts. The U.S. is the world's 3rd largest producer.

Following its discovery as a fungistat, it was approved by the U.S. Food and Drug Administration for use in food as GRAS (Generally Regarded As Safe). Approval was at a level (0.3% w/w) near to that which I and others (Burkholder et al. 1973, Baker & Mabie 1973, Boush et al. 1968, Dunkel et al. 1982, Dunkel unpubl.) have since found it to be an effective insecticide. Long term mammalian toxicity studies detected no carcinogenic effect at levels of 10% w/w in diet (Gaunt et al. 1975) --a value 400 x in excess of the level set by the Joint FAO/WHO committee on Food Additives in 1967.

Sorbic acid is now used in cheese, baked goods, medium moisture processed foods, and liquids around the world. Today we are going to explore the possibilities it has as an insect growth regulator in stored grain, pulses and milled products and its potential as a stored grain fungal growth regulator.

First, the insects. Thus far, all storage insect species tested have been found to be sensitive (Figure 1). In addition, data will, within the next few weeks be completed on Prostephanus truncatus, the larger grain borer and Callosobruchus chinensis, one of the bruchids. Positive data is available for their fellow family members, Rhyzopertha dominica and Acanthoscledes obtectus (Dunkel et al. 1982, Dunkel unpubl.). The sensitive list, includes almost all the families, i.e. bran and fine feeding insects such as Cryptolestes, malathion resistant populations of Plodia interpunctella as well as the major internal infestors, Sitotroga cerealella, Sitophilus spp. and R. dominica. On this second list of less generally frequently encountered storage insects we see that fungivores such as Typhaea stercorea are sensitive as well as several Dermestidae including species in the same genus as the Khapra beetle.

As Dr. Fumio Matsumura yesterday encouraged us to ask, "What do we know now regarding insect site of action, developmental time of action and mode of action for this compound?" For Coleoptera and 1 family of Lepidoptera, the Geleciidae, we know that the disruption of development occurs early in embryonic development, prior to blastoderm formation. For Tribolium confusum dye penetration studies have shown that this lethal effect occurs in conjunction with disruption of the waxy layer of the egg shell (Dunkel submitted). These data from over 1000 embryo dissections indicates the critical exposure period is the moment of oviposition and 2 hrs thereafter (Figure 2). This can be traced by comparing the mean % survival at the 0.5 level with and without that 'critical moment.'

O<sub>2</sub> uptake studies indicates physiological death occurred within the first 4 hours after oviposition if oviposition occurred directly in the sorbic acid containing medium. The hypothesized mode of action is that after entry through chorion and waxy layer competitive inhibition of fatty acid synthesis during this accelerated growth phase of the insect occurs.

The mode of action in Pyralidae, namely Cadra cautella, Plodia interpunctella and Anagasta kuehniella may be the same, but different egg coats or chorion composition may protect the embryo and delay the effect until the larval period. The larval period doubled as the % sorbic acid in the diet increases (Figure 3). Those insects that do pupate showed a high % of non-emergence and twisted wings which interfered with mating. All in all the total control effect in a bin with another pyralid, malathion-resistant Plodia is dramatic--data to support this will be shown with our bin experiment discussed later in the paper.

For the remainder of this presentation I will present data from 2 projects--one in storages in Guangdong China and one in bins in Minnesota.

Figure 1. Stored Product Insects of primary and secondary importance.

(● indicates those sensitive to sorbic acid.)

*Alphabetical list of the major insect pests of stored grain*

Scientific Name	Common Name	Family
<i>Acarus siro</i> L.	Grain mite	Acaridae
● <i>Anagasta kuhniella</i> (Zeller)	Mediterranean flour moth	Phycitidae
● <i>Cadra cautella</i> (Walker)	Almond moth	Phycitidae
<i>Cryptolestes ferrugineus</i> (Stephens)	Rusty grain beetle	Cucujidae
● <i>Cryptolestes pusillus</i> (Schönherr)	Flat grain beetle	Cucujidae
<i>Cryptolestes turcicus</i> (Grouv.)	Flour-mill beetle	Cucujidae
<i>Ephesia elutella</i> (Hübner)	Tobacco moth	Phycitidae
● <i>Oryzaephilus surinamensis</i> (L.)	Saw-toothed grain beetle	Cucujidae
<i>Oryzaephilus mercator</i> (Fauv.)	Merchant grain beetle	Cucujidae
● <i>Plodia interpunctella</i> (Hübner)	Indian-meal moth	Phycitidae
● <i>Rhyzopertha dominica</i> (F.)	Lesser grain borer	Bostrichidae
<i>Sitophilus granarius</i> (L.)	Granary weevil	Curculionidae
● <i>Sitophilus oryzae</i> (L.)	Rice weevil	Curculionidae
● <i>Sitophilus zeamais</i> Motschulsky	Maize weevil	Curculionidae
● <i>Sitotroga cerealella</i> (Olivier)	Angoumois grain moth	Gelechiidae
<i>Tenebroides mauritanicus</i> (L.)	Cadelle	Ostomatidae
● <i>Tribolium castaneum</i> (Herbst)	Red flour beetle	Tenebrionidae
● <i>Tribolium confusum</i> Duval	Confused flour beetle	Tenebrionidae
<i>Trogoderma granarium</i> Everts	Khapra beetle	Dermestidae

**Minor Pests Most Frequently Encountered in Stored Grain**

Scientific Name	Common Name	Family
<i>Ahasverus advena</i> (Waltl.)	Foreign grain beetle	Cucujidae
<i>Alphitobius diaperinus</i> (Panzer)	Lesser mealworm	Tenebrionidae
<i>Araecerus fasciculatus</i> (DeGeer)	Coffee-bean weevil	Platystomidae
● <i>Attagenus piceus</i> (Olivier)	Black carpet beetle	Dermestidae
<i>Carpophilus dimidiatus</i> (F.)	Corn sap beetle	Nitidulidae
<i>Carpophilus hemipterus</i> (L.)	Dried fruit beetle	Nitidulidae
<i>Caulophilus oryzae</i> (Gyllenhal)	Broad-nosed grain beetle	Curculionidae
<i>Corcyra cephalonica</i> (Staint.)	Rice moth	Galleriidae
● <i>Cynaues angustus</i> (LeConte)	Larger black flour beetle	Tenebrionidae
<i>Gnathocerus cornutus</i> (F.)	Broad-horned flour beetle	Tenebrionidae
● <i>Lasioderma serricornis</i> (F.)	Cigarette beetle	Anobiidae
<i>Latheticus oryzae</i> (Waterhouse)	Long-headed flour beetle	Tenebrionidae
<i>Liposcelis</i> spp.	Psocids	Psocoptera
<i>Palorus ratzeburgi</i> (Wissmann)	Small-eyed flour beetle	Tenebrionidae
<i>Palorus subdepressus</i> (Wollaston)	Depressed flour beetle	Tenebrionidae
<i>Ptinus claviceps</i> (Panzer)	Brown spider beetle	Ptinidae
<i>Ptinus villiger</i> (Reitter)	Hairy spider beetle	Ptinidae
<i>Prostephanus truncatus</i> (Horn)	Larger grain borer	Bostrichidae
<i>Stegobium paniceum</i> (L.)	Drugstore beetle	Anobiidae
<i>Tenebrio molitor</i> (L.)	Yellow mealworm	Tenebrionidae
<i>Tenebrio obscurus</i> (F.)	Dark mealworm	Tenebrionidae
<i>Tribolium audax</i> (Halstead)	Black flour beetle	Tenebrionidae
● <i>Trogoderma</i> spp.	Grain-feeding dermestids	Dermestidae
● <i>Typhaea stercorea</i> (L.)	Hairy fungus beetle	Mycetophagidae

Figure 2. Embryonic time of death determined by dissection of eggs of *Tribolium confusum* DuVal exposed to sorbic acid for varying periods post-oviposition.

# EMBRYONIC DEVELOPMENT






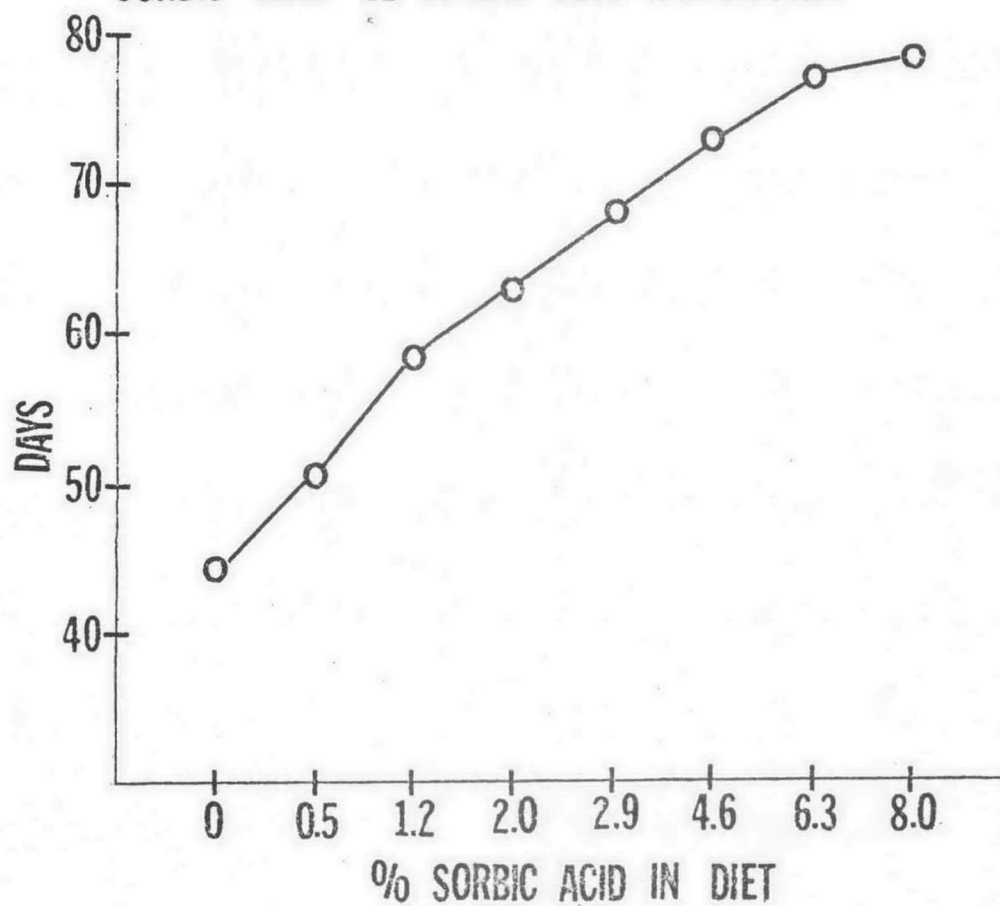
% (W/W) SORBIC ACID	EXPOSURE PERIOD (HR. POST OVIPOSITION)	HIGHEST LEVEL OF DEVELOPMENT (MEAN %)				
						
0.0	0—2					
	2—120	9.2	0.8	6.1	1.8	76.5
0.25	0—120	55.7	0.5	4.3	0	7.5
0.50	0—2	59.8	2.8	9.3	6.3	8.0
	2—120	8.7	3.0	1.7	10.0	74.5
1.00	2—120	89.5	0	0	0	0
4.60	2—120	88.8	0	0	0	0

Figure 3.

AVERAGE LENGTH OF LARVAL PERIOD  
FOR CADRA CAUTELLA PLACED ON  
SORBIC ACID 12-24 HRS POST-OVIPOSITION



In South China winter wheat is harvested in March during the rainy season. Rainfall and humidity are high and mean daily sun hours are 3.2. Most wheat has to be dried by the sun by the production team (or village). Residuals and fumigants are applied only after this 2-3 month drying period (Dunkel et al. 1984). Thus there was a need for both short term insect and fungal protection that could be used in the village and in long term large, central grain storage structures where alternatives were being sought for malathion and phostoxin due to insect resistance problems.

The main insect problems in Chinese stored grain are internal infestors: Sitotroga cerealella, Sitophilus oryzae, Sitophilus zeamais, Rhyzopertha dominica. The most significant aspect of this study is that at levels approved for human consumption in the U.S., sorbic acid suppressed  $\geq 85\%$  of the populations of these obligate internal feeders (Dunkel et al. 1982). With this data from the Sitophilus species we added to our understanding of sorbic acid behavior this hypothesis: that sorbic acid can penetrate the pericarp and migrate through the kernel's endosperm.

With this study in China we also added to our understanding of the fungal suppressent behavior of sorbic acid with high moisture grain which I will discuss in the next main section.

At Minnesota we are 3 months into a year long study of the long term effectiveness of sorbic acid as a bulk grain protectant. 10-100 bu metal bins (modified hog feeders) were built with an acrylic sealant added at both bolt holes and rim junctures. Bins were filled with U.S. no. 2 yellow corn to which the following applications were made:

Aflaban SA - one of 3 sorbic acid formulations produced by Monsanto. 5000 ppm

Fenoxycarb - An IGR carbamate product under development by Maag Chemical Co. 5 ppm

malathion - 10 ppm

d H<sub>2</sub>O - 10 ppm

The latter 3 applications were made by the drip method similar to U.S. farm applications. The aflaban was applied with a granular applicator at the proximal end of the auger. There is quite a difference among these 2 treatments types in order of magnitude of dosage. Sorbic acid it must be remembered, is approved for use in food in the U.S. at this level. Moreover, in the application process the wind dispersed some. Residue analyses indicate a mean of 3000 ppm was left on grain. Sampling occurred as follows:

1. Temperatures at 4 locations/bin, probe trap contents at 3 locations/bin, and Plodia pheromone trap catches are taken weekly.



2. Corn samples were withdrawn by 5' trier and deep cup probe at 0, 2, 7, 30, and 90 days and will be continued at 6 months, 9 months, and 1 year. To obtain the samples, the bin was divided into vertical quadrants and the graduated crossbrace was used to locate randomized coordinate values. 8 such samples/bin were obtained on each sample day. From these % MC (both Motomco and air oven determinations) and germination studies are done as well as damage and residue analyses.

The most significant aspect of this study is that in a real storage situation for at least 3 months, sorbic acid continued to suppress population development of 5 stored grain insects (Figure 4). Three of these species, Tribolium confusum, C. pusillus, S. oryzae were introduced at levels of 1.1 insect/kg. immediately after sampling on day 0. Plodia interpunctella and Typhea stercorea entered the bins as contaminants. These values for the Coleoptera were obtained from modified brass probe traps of Loshiavo modified by Barak. The traps per bin were placed top mid and bottom in the spoutline. Plodia data were obtained in the 10 ft. bins with pheromone sticky traps hung inside cover of bin above grain. Values are the mean cumulative counts from 5 to 11 weeks expressed as % of suppression. For all species control with Aflaban was good (Figure 5, 6). That of malathion was remarkably poor except with S. oryzae. In the case of Typhea, more individuals were obtained in the malathion bins than in the control bins.

The Plodia data reveals an interesting phenomenon (Figure 6). The Aflaban data show a sharp increase after 11 weeks. Lab data indicate sorbic acid can more than double the larval period of Pyralids from 4 to 10 weeks. These data reflect that.

The other noteworthy data is from the germination studies. If sorbic acid is to be used for seed protection, germination is an important property. Data shown here indicate germination is not affected (Figure 7).

Lest we forget, sorbic acid's sole claim to fame up until now has been as a fungistat, and it is this property to which I will address my final remarks. Because our Minnesota binned study was done at 12.5 - 13.0% moisture content, mold inhibition has not been an issue. In China we dealt with the period during drying by the sun in a rainy season when moisture content in winter wheat after harvest may be 22% or above. I will draw upon that data at this time (Figure 8). Visible mold growth was recorded from whole wheat at 22% MC used for the insect studies (Dunkel et al. 1982). Only 5 da after inoculation samples held at 30°C fungal growth were already significantly different in treated and untreated wheat. The fungistatic action continued and after 70 days, the difference was even more marked (Figure 8). These same samples, when surface sterilized and used to inoculate plates of tomato juice agar + 6% NaCl and potato dextrose agar (+ 1% lactic acid) produced the following:

Figure 4. Percent suppression indicated by probe trap catch of insect population after sorbic acid and other treatments on binned whole kernel corn in Minnesota.

	% SUPPRESSION				
	<u>Plodia</u> <u>interpunctella</u>	<u>Tribolium</u> <u>confusum</u>	<u>Cryptolestes</u> <u>pusillus</u>	<u>Sitophilus</u> <u>oryzae</u>	<u>Typhea</u> <u>stercorea</u>
Aflaban-SA	93.2	97.0	83.0	60.2	81.0
Fenoxycarb	91.8	48.2	50.7	45.6	-43.0*
Malathion	39.8	28.3	6.0	57.9	-14.2*

\*% higher than controls



Figure 5. Mean weekly collections of insects in probe traps placed in spoutline of 100 bu. whole kernel corn bins in Minnesota.

a) Tribolium confusum, b) Cryptolestes pusillus, c) Sitophilus oryzae, d) Typhea stercorea.

		Mean # larvae + adults/trap <sup>1</sup> Weeks after inoculation						
a)	Treatment	5	6	7	8	9	10	11
	Aflaban-SA	1.3	3.3	0.6	5.7	1.7	7.3	2.3
	Fenoxycarb	117.3	58.0	62.4	87.7	51.3	6.4	2.7
	Malathion	216.0	133.0	4.1	52.0	66.0	42.0	21.0
	d H <sub>2</sub> O	13.5	38.5	91.5	233.0	310.0	54.5	4.0

		Mean # larvae + adults/trap <sup>1</sup> Weeks after inoculation						
b)	Treatment	5	6	7	8	9	10	11
	Aflaban-SA	0.7	12.3	5.6	15.0	8.3	11.0	1.7
	Fenoxycarb	9.8	36.7	43.0	39.3	29.3	2.0	2.7
	Malathion	10.0	14.0	44.5	42.5	118.5	65.5	15.0
	Control	9.5	32.5	67.0	104.5	98.5	7.5	0.5

<sup>1</sup> 3 reps/bin; 3 aflaban bins; 3 phenoxycarb bins; 2 malathion bins;  
2 control bins.

Figure 5.(cont.) Mean weekly collections of insects in probe traps placed in spoutline of 100 bu. whole kernel corn bins in Minnesota. a) Tribolium confusum, b) Cryptolestes pusillus, c) Sitophilus oryzae, d) Typhea stercorea.

		<u>Mean # larvae + adults/trap<sup>1</sup></u> <u>Weeks after inoculation</u>						
c)	Treatment	5	6	7	8	9	10	11
	Aflaban	1.3	7.7	1.3	1.7	1.7	6.0	3.0
	Fenoxycarb	0	2.3	5.3	1.7	16.7	3.3	1.7
	Malathion	0	0.5	0	7.0	10.5	3.5	2.5
	Control	1.0	0.5	0.5	4.5	26.5	23.5	0.5

		<u>Mean # larvae + adults/trap<sup>1</sup></u> <u>Weeks after inoculation</u>							
d)	Treatment	5	6	7	8	9	10	11	Total
	Aflaban	0.7	15.4	7.3	47.3	8.8	56.6	24.7	160.8
	Fenoxycarb	63.3	71.3	139.6	112.0	738.4	84.4	21.6	1230.6
	Malathion	35.5	28.5	173.5	232.0	236.5	109.5	166.0	981.
	Control	45.0	25.5	79.5	202.5	345.5	161.0	3.5	859.

<sup>1</sup>3 reps/bin; 3 aflaban bins; 3 fenoxycarb bins; 2 malathion bins; 2 control bins.

Figure 6. Mean weekly collection of Plodia interpunctella from pheromone traps hung inside cover of 100 bu. whole kernel corn bins in Minnesota.

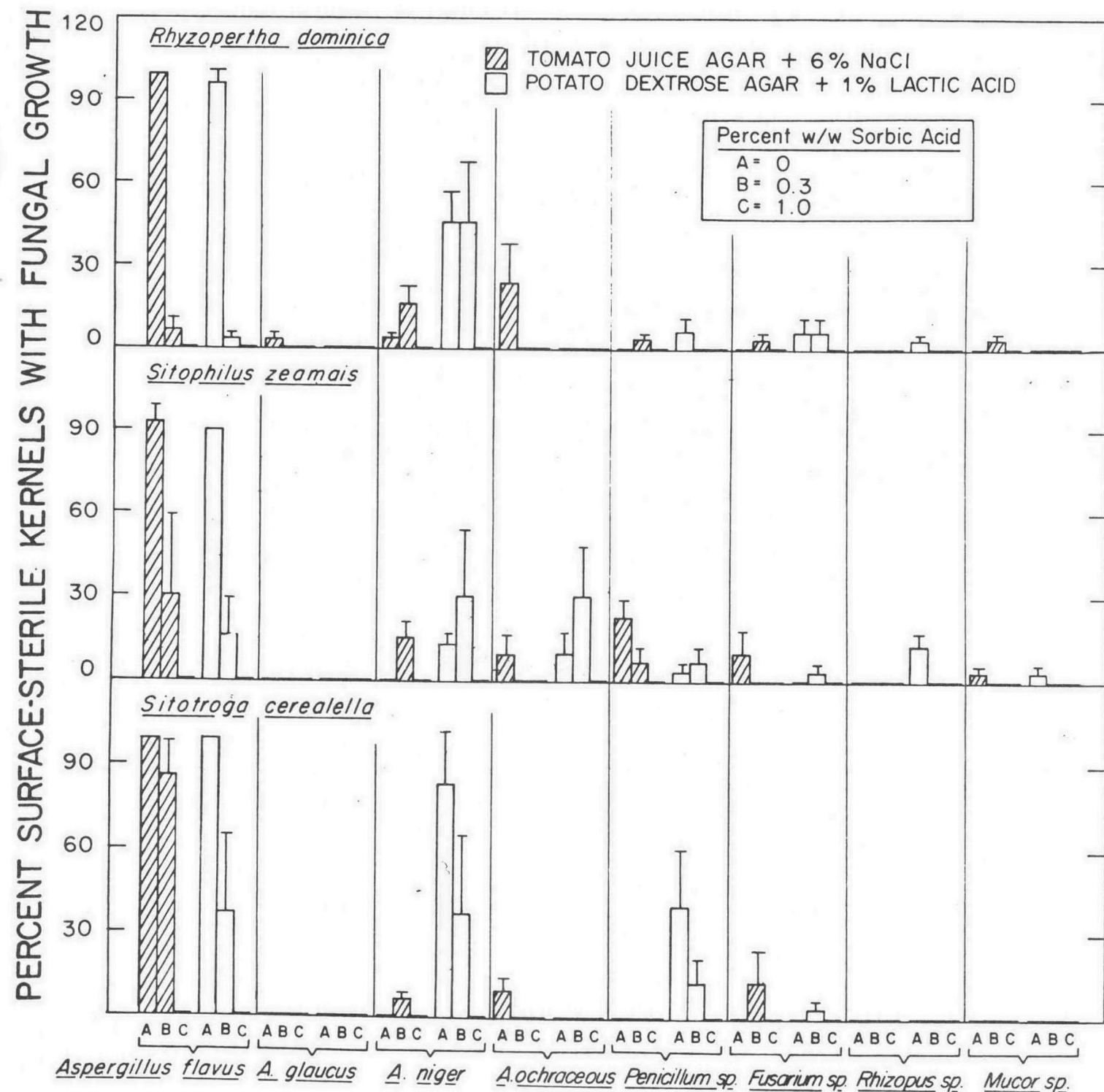
Treatment	# adults/trap <sup>1</sup>						
	Weeks after bin filling						
	5	6	7	8	9	10	11
Aflaban-SA	2.3	6.3	6.3	4.3	3.0	4.0	52.7
Fenoxycarb	9.7	12.7	23.7	19.3	3.7	7.0	19.7
Malathion	26.0	83.5	165.5	142.0	-	118.5	165.0
Control	146.5	110.5	192.5	179.0	-	285.5	249.0

<sup>1</sup> 3 aflaban bins; 3 phenoxycarb bins; 2 malathion bins; 2 control bins.

Figure 7. Mean % normal germination expressed as % of controls in whole kernel corn.

	Day 0	Day 7	Day 30
Aflaban	95.2	120.8	95.2
Malathion	90.8	115.4	97.6

Figure 8. Frequency and abundance of storage fungi grown from surface-sterile whole wheat treated with sorbic acid and insects in the laboratory. From Dunkel et al. 1982.



primarily Aspergillus flavus and Asperillus niger, with less of a frequency of occurrence for other Aspergillus species, Penicillium spp, Fusarium sp; Mucor sp. and Rhizopus sp.

If the Chinese farmers harvest and thresh wheat in the rain, it may come in at over 40%. If the days are not sunny immediately following the decline to 22% moisture content may not occur for ca 5 da. It was hoped that in this critical period sorbic acid would hold the fungal development in the grain and indeed it did, but not with large differences as shown by the mean cumulative % of surface kernels with growth. Sorbic acid's effect is, however, pH dependent which may explain the lack of dramatic effect in the village study.

Dr. Lloyd Bullerman, mycotoxicologist, University of Nebraska, is continuing to look at the usefulness of sorbic acid as a whole grain protectant versus fungi in different pH environments.

In conclusion, sorbic acid can now be considered an effective insect growth inhibitor with a static effect on storage fungi which produce mycotoxins. As such it has much potential for complete protection of high moisture commodities during storage.

Although the individual cost per bu is ca \$.15 this must be compared with the cost per bu of insecticide treatments which may not work as well or be as broad spectrum as sorbic acid plus the cost of application of a fungistat. Therefore, we conclude that sorbic acid can be a cost effective mammalian-safe protectant for stored grain and pulses used for food feed and seed as well as for milled and processed food as both an insect and fungal growth regulator.

#### Acknowledgements

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