Disinfection of Seed Surfaces with Sodium Hypochlorite

D. B. Sauer and R. Burroughs

U.S. Department of Agriculture, Agricultural Research Service, U.S. Grain Marketing Research Laboratory, Manhattan, KS 66502, and Department of Grain Science and Industry, Kansas State University, Manhattan 66506.

Contribution 80-409-J, Kansas Agricultural Experiment Station.

We thank C. M. Christensen and John Tuite for collaborative plating of samples and for helpful comments and L. M. Seitz for advice throughout the study.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

Accepted for publication 24 February 1986 (submitted for electronic processing).

ABSTRACT

Sauer, D. B., and Burroughs, R. 1986. Disinfection of seed surfaces with sodium hypochlorite. Phytopathology 76: 745-749.

Surface disinfection for the purpose of determining the presence of internal fungi in grains or seeds gave variable and sometimes erroneous results. *Aspergillus glaucus* grew from as many as 100% of wheat kernels that were free of viable internal fungi but that had been heavily surface-inoculated before being treated with sodium hypochlorite (NaOCl) and plated on agar. Spores of *Aspergillus* spp. were killed almost instantaneously by 1–5% solution of NaOCl, so the problem appeared to be lack of contact between spores and NaOCl because of air bubbles, cracks, surface hairs, debris, etc., on seed surfaces. Rinsing seeds in wetting agents or detergents before treating with NaOCl did not improve effectiveness, but rinsing in ethanol before NaOCl was effective in reducing surface contamination, especially with wheat. One brand of NaOCl with lower pH and lower total alkalinity than others was the most effective surface disinfectant. Reducing the pH of NaOCl solutions from pH 11–12 to about pH 8 increased effectiveness, but such solutions were unstable. Destruction of spores on seed surfaces depends on the kind and condition of seeds; the amount of surface contamination; the brand, pH, and concentration of NaOCl; and exposure time. Some literature reports on growth of storage fungi in grain may be inaccurate because of failure to eliminate surface contaminants.

Additional key words: corn, surface sterilization.

In the assessment of fungal invasion, seeds are surface-disinfected and placed on an agar medium so that the fungi inside the seeds can grow out and be identified. The procedure varies, but shaking the seeds for 1 min in a 1–5% sodium hypochlorite (NaOCl) solution, then rinsing them once or twice with sterile water, is typical. The purpose is to destroy or remove surface fungi and bacteria without killing internal organisms.

Several reports have indicated that surface disinfection was incomplete when large numbers of spores, particularly those of *Aspergillus* species, were present. Halloin (5) shook cotton (*Gossypium hirsutum* L.) seeds for 2 min in a suspension of spores of *A. flavus* Link and *Rhizopus arrhizus* Fischer, then air-dried the seeds. Apparent invasion exceeded 40% after surface disinfection for 5 min in 1% NaOCl, leading Halloin (5) to suggest that infection occurred as the seeds dried. Harmon and Pfleger (6) substituted a histological procedure for plating methods when they found that NaOCl treatment did not kill spores of the *A. glaucus* group (13) inoculated onto seeds. Christensen and Mirocha (2) washed rough rice (*Oryza sativa* L.) with 1% NaOCl for 1 min immediately after heavy inoculation with spores of *A. parasiticus* Speare and recovered *A. parasiticus* from 57% of the kernels.

Failure of NaOCl to eliminate surface contamination has led to erroneous conclusions about minimum conditions required for growth of the *A. flavus* group. *A. flavus* was shown to invade grain only at moisture contents in equilibrium with 83–85% relative humidity (RH) or higher (above 16% moisture content in starchy cereal grains) (2,12). Boller and Schroeder (1) concluded, however, that *A. parasiticus* invaded rough rice that was heavily inoculated and stored for 7 days at 75 and 80% RH with moisture contents of about 13.5 and 14.5%, respectively. Lillehoj et al (11) stated that *A. flavus* infected and produced aflatoxin in dry fractions of heavily inoculated blends of wet and dry corn that had a mean moisture level of 14% or less. Although invasion by *A. flavus* is usually accompanied by rapid decrease in germinability (12), Lillehoj et al (11) found no reduction in germination during 2 mo of storage when *A. flavus* was recovered from more than 70% of the kernels. Counts were made after kernels were surface-disinfected for 1 min with 1% NaOCl and rinsed twice with sterile water.

Among samples submitted to our laboratory, corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), soybeans (*Glycine max* (L.) Merr.), and rough rice inoculated with *A. glaucus* appeared to have 34–98% invasion after storage at moisture contents we thought too low for growth of *A. glaucus*. At about the same time, corn we had inoculated with dry spores of *A. flavus* (10^5 spores per gram) was disinfected and plated immediately. Three subsamples had counts of 57, 46, and 11% *A. flavus*. These anomalous results occurred after a change in brand of NaOCl used for surface disinfection and led us to examine our surface-disinfection methods. We report a summary of experiments to identify the problems of inconsistent seed surface sterilization and to find a reliable technique for routine determination of internal invasion in corn and wheat kernels.

MATERIALS AND METHODS

The investigations described were conducted on seed lots with natural surface contamination and on seed lots surface-inoculated with fungi. The seed lots were representative of samples encountered in postharvest studies of fungal invasion on cereal grains. Lot A was corn harvested with a combine at 22% moisture content, screened to remove dockage, and ambient-air-dried in a bin. Lot B was corn harvested as described, held in trucks 4–24 hr, dried in a high-temperature drier, and subjected to repeated elevator handling. Lot C was commercial hard red winter wheat inoculated lightly with *A. glaucus* and turned 10 min in a revolving drum mixer. Lot D was white wheat from test plots in western Washington. Lot E was hand-picked, air-dried, hand-shelled corn. Lot F was U.S. no. 2 corn from an export terminal.

Cultures of *A. glaucus* and *A. flavus* were grown on sterilized wheat. Grain for surface-disinfection studies was contaminated

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 1986.

either by atomizing a suspension of spores in distilled water with Tween 20 (two drops per 500 ml) onto grain spread in a single layer on trays (wet method) or by tumbling wheat kernels supporting heavy conidial growth with uninfected grain in plastic bags (dry method). Spore concentration on inoculated grains was determined by a standard dilution count method.

To compare our standard plating procedure with those of other workers, five samples (100–150 g) were mailed to each of two university laboratories engaged in postharvest research on cereal grains with the request that they determine the numbers and kinds of infecting fungi. Samples had 13.5% moisture content. Two samples were relatively clean noninoculated corn from storage bins, one was corn we contaminated with *A. glaucus* by the dry method, and one was wheat similarly contaminated. The fifth was wheat lot C given to us to analyze and had been inoculated with *A. glaucus*.

In our disinfection method, we used uniform quantities of each grain measured by volume: a 50-ml beaker of corn (about 120 kernels) and a teaspoon of wheat (about 200 kernels). The seeds were placed in petri dishes (100 × 15 mm), covered with the disinfecting solution, shaken vigorously for 1 min, drained, covered with sterile distilled water, shaken for 10–20 sec, and drained. One hundred seeds were plated immediately after treatment. Properties of the disinfecting solutions and the effects of prewashes and of numerous supplements to the method are detailed in the text and tables. Each treatment was duplicated, and those of particular interest were repeated several times. Results reported are mean values.

Corn was aseptically plated on malt agar with 4% NaCl and 200 mg/L Tergitol NP-10 (Sigma Chemical Co., St. Louis, MO) (MS4T), and wheat was plated on the same medium but with 8% NaCl (MS8T) unless otherwise stated. Only visually intact seeds were plated; seeds were plated germ-side-up. Plates were incubated 5–7 days at 25 C.

Two nationally marketed brands of household bleach, designated N-1 (Clorox) and N-2 (Purex), and two supermarket brands, S-1 and S-2, were used. Throughout this report, concentrations of bleach are expressed as percent NaOCl. Labels indicated that all contained 5.25% NaOCl, except for N-2, which contained 6%. Adjustments of pH were made with 2 N or 3 N HCl.

RESULTS

Samples evaluated by three laboratories. When the three laboratories surface-disinfected and plated subsamples of the two lots of dry wheat by their own routine procedures, all three reported high percentages of infection by *A. glaucus* (samples 4 and 5, Table 1). The preinoculation counts of *A. glaucus* were < 5% for sample 4 and zero for sample 5. More consistent results were obtained with the corn samples, although counts of *A. flavus* and *A. niger* for sample 3 varied.

Predisinfection treatments and agitation. When we examined kernels of wheat or corn placed in a flask containing water, bubbles that were not easily dislodged by agitation formed on the seed surfaces. Applying a partial vacuum to wheat kernels suspended in water, NaOCl, or detergent solutions resulted in smaller bubbles. No bubbles formed when seeds were immersed in ethanol. When the ethanol was drained off and 2% NaOCl added, few bubbles formed; as agitation continued, bubbles continuously formed on and dislodged from the seeds. A 1:1 mixture of ethanol and 2% NaOCl produced many tiny bubbles that did not dislodge. These observations suggested that air bubbles prevented NaOCl from contacting spores.

We added NaOCl in different concentrations to spore suspensions and after various time intervals removed the NaOCl by pouring the suspension into a vacuum-filtering apparatus and flushing thoroughly with sterile water. Plating the filter membranes on agar showed that NaOCl in concentrations > 1% killed all spores within the fastest treatment time (about 5 sec) we could impose.

Agitating seeds with disinfectant, using a magnetic stirrer or sonic bath, produced no better surface disinfection than did the standard method, so we tried shaking seeds for 20–30 sec in various surfactant solutions before surface-disinfection treatment with 2% NaOCl. Wheat (lot D) inoculated by the dry method with about 10^6 spores of *A. glaucus* per gram was pretreated with 0.02 or 0.1% Tween 20 or with Sparkleen detergent (Fisher Scientific Co., St. Louis, MO), then treated with 2% NaOCl (brand S-1) and plated. Kernels with *A. glaucus* ranged from 76 to 98% with the pretreatments compared with 86% with no pretreatment. A similar lack of improvement was found when inoculated corn (lot A) was pretreated with 1% solutions of Sparkleen, Tween 20, sodium dodecyl sulfate, and Triton X-100, then treated with 2% NaOCl (brand N-1). Counts of *A. glaucus* were 83–94% with the pretreatments and 63% without. Pretreatment for 10 sec with 85 or 100% ethanol, however, reduced *A. glaucus* counts to about 35% in the corn and 10% in the wheat. Ethanol pretreatment without NaOCl treatment resulted in *A. glaucus* growing from 100% of the

TABLE. 1. Kernels of corn and wheat from which *Aspergillus* spp. grew when tested by three laboratories, each using its standard procedure

Sample	Grain	Lot[a]	*A. glaucus* inoculum (spores/g)	Lab	Kernels with *Aspergillus* spp. (%)		
					A. flavus	*A. glaucus*	*A. niger*
1	Corn	A	None	1[b]	2	0	0
				2[c]	1	1	1
				3[d]	4	2	3
2	Corn	B	None	1	10	0	10
				2	4	0	11
				3	13	0	28
3	Corn	A	5×10^3	1	2	6	0
				2	0	3	5
				3	0	7	0
4	Wheat	C	Unknown	1	0	40	0
				2[e]	4	86	0
				3	0	98	0
5	Wheat	D	4×10^5	1	0	100	0
				2[e]	0	73	4
				3	0	86	0

[a] Lot A was corn harvested with a combine at 22% moisture content, screened to remove dockage, and ambient-air-dried in a bin. Lot B was corn harvested as above, held in trucks 4–24 hr, dried in a high-temperature drier, and subjected to repeated elevator handling. Lot C was commercial hard red winter wheat inoculated lightly with *A. glaucus* and turned 10 min in a revolving drum mixer. Lot D was white wheat from test plots in western Washington.

[b] Treated with 2% NaOCl containing about 10% ethanol, rinsed, and plated on tomato juice agar with 6% NaCl.

[c] Treated with 5% NaOCl, rinsed twice, and plated on potato-dextrose agar with 100 mg/L Tergitol NP10 plus 30 mg/L chlortetracycline and on malt agar with 6% NaCl.

[d] Treated with 2% NaOCl (pH 11.7), rinsed twice, and plated on malt agar with 4% NaCl and 200 mg/L Tergitol NP10.

[e] Treated with 1% NaOCl, rinsed twice, and plated on tomato juice agar with 6% NaOCl.

kernels. In these and other tests, the ethanol pretreatment did not appear to reduce the counts of *Alternaria*, which occurs as a subepidermal infection in a high percentage of wheat kernels (7). In corn, however, ethanol pretreatment reduced the counts of *Fusarium, Cephalosporium,* and *Penicillium*.

Source, concentration, and pH of NaOCl solutions. Household bleaches are equilibrium mixtures of NaOCl and water with NaOH and $Ca(OH)_2$ added as stabilizers and Na_2CO_3 as a buffer.

$$NaOCl + H_2O \rightleftarrows HOCl + NaOH$$

The concentration of hypochlorous acid (HOCl), the principal agent in chlorine disinfection, is pH-dependent. In solutions above pH 10, hypochlorite exists almost entirely as OCl^-. As pH decreases below 10, concentration of HOCl increases until at pH 7.4, half of the hypochlorite exists as free acid. At pH 5, nearly all is present as HOCl. Below pH 5, the system contains mainly HOCl and dissolved chlorine (14).

We found that the pH of bleach in freshly opened containers did not vary by more than 0.2 units within brands. N-1 had the lowest pH, and according to its manufacturer, it contains much less NaOH than do other brands.

Of three brands of NaOCl we tested to disinfect corn inoculated with *A. glaucus*, undiluted N-1 was the most effective; efficacy was not improved by lowering the pH to increase HOCl concentration (Table 2). Diluting the bleach solutions lowered pH slightly but decreased their effectiveness. When pH was adjusted with acid to 8 or below, a 2% solution of N-1 was as effective as the undiluted solution. The various treatments listed in Table 2 had minimal effect on the counts of other fungi, although *Fusarium* and *Cephalosporium* decreased slightly as the pH of the NaOCl solution was lowered.

Acidified hypochlorite solutions were not stable; solutions of about pH 8 were effective after 40 min but not after 4 hr. We buffered sodium hypochlorite solutions at pH 8.5 with 3% sodium bicarbonate (w/w) (14), but the buffered solutions were no more effective in removing inoculum of *A. glaucus* than the acidified hypochlorite solutions. We also measured the pH of hypochlorite solutions after treating about 100 kernels of clean, seed-grade corn inoculated by the wet method with 6×10^4 spores of *A. glaucus* per gram. The pH of undiluted N-1 changed from 11.3 to 10.0; that of 2% N-1, from 10.6 to 9.6; and that of 2% S-2, from 11.7 to 11.3.

Attempts to improve efficiency of surface disinfection. We tested various combinations of pretreatments, NaOCl concentrations and pH, agitation methods, and contact time between seeds and disinfectant. In corn and wheat inoculated with *A. flavus* (10^6 spores per gram, dry method), surface disinfection was incomplete although numbers of wheat kernels contaminated with *A. flavus* were reduced to 3% with either an acidified 5% solution of NaOCl or with a 30-sec ethanol pretreatment followed by 2% NaOCl (Table 3). Ethanol pretreatment and a pretreatment with hypochlorite somewhat improved surface disinfection of corn, but none of the methods produced the correct value of 0% *A. flavus*.

In one experiment, corn kernels (lot B) inoculated with *A. glaucus* were put into a wire basket and submerged for 30 sec in 2% N-1 swirling at high speed in a blender. Kernels were pretreated again for 30 sec with solutions of one of the following: absolute ethanol, 1% sodium triphosphate, 0.5% sodium dodecyl sulfate, 1% sodium carbonate, or 0.5% ammonium hydroxide, then treated 1 min in 2% N-1. The NH_4OH pretreatment left only 3% of seeds with *A. glaucus* compared with 36–50% for the other treatments. Kernels with cracks in the pericarp accounted for more than 30% of the *A. glaucus* growth.

Ammonium hydroxide was further tested as a pretreatment and as 1:1 mixtures with hypochlorite at several concentrations in surface-disinfection solutions. Combinations of NH_4OH and NaOCl produce hydrazine (N_2H_4) and nitrogen gas (3), and numerous minute bubbles were formed in the solution and on the grain surfaces. A 1:1 mixture of 0.1% NH_4OH and 2% N-1 destroyed spores of *A. glaucus* on all but 2–3% of corn seeds (lot E) inoculated with 6×10^4 viable spores per gram (about 20,000 per kernel). Undiluted bleach left 6% contaminated and a 2% N-1 solution left 70% contaminated. Ammonium hydroxide as a pretreatment for acidified 2% or for 5.25% N-1 did not surface-disinfect wheat lot C or wheat heavily inoculated with *Penicillium citrinum* Thom. Neither NH_4OH nor N_2H_4 was effective alone as a surface-disinfecting agent.

The effect of length of exposure to undiluted N-1 was checked on corn inoculated with 6×10^4 spores per gram of *A. glaucus*. Both 30- and 60-sec treatments were equally effective in destroying surface spores and produced similar counts of preharvest or field fungi. A 90-sec treatment reduced *A. glaucus* from 5 to 1% but also reduced field fungi counts, particularly *Cephalosporium*. When a corn sample was treated for 60 sec with undiluted N-1 and rinsed once, then allowed to stand in a covered petri dish for 5 min before plating, the percentage of kernels yielding field fungi was also reduced.

No surface-disinfection method was found that gave a reliable and repeatable "zero" for wheat, corn, or rough rice known to have

TABLE 2. Effects of concentration, pH, and source of sodium hypochlorite (NaOCl) on apparent fungal invasion of corn (lot A) inoculated with 6×10^4 spores of *Aspergillus glaucus* per gram

NaOCl[a] treatment			Kernels with *A. glaucus*[d]
Concentration (%)	pH[b]	Source[c]	(%)
6.0	12.2	N-2	51
5.0	12.0	S-2	45
	11.2	N-1	8
	7.5	N-1	8
2.0	11.7	S-2	64
	10.6	N-1	40
	9.0	N-1	38
	8.0	N-1	10
	6.9	N-1	9
0.2	9.8	N-1	85
	9.0	N-1	93
	8.0	N-1	89
	7.1	N-1	64

[a] One minute with shaking, one sterile distilled water rinse.
[b] Less than 10 indicates pH adjusted with HCl.
[c] N-1 and N-2 = nationally advertised brands; S-2 = supermarket brand.
[d] Means of two or more replicates of 100 kernels each. Before inoculation, corn had 0–2% infection by *A. glaucus*.

TABLE 3. Examples of method to surface-disinfect corn and wheat inoculated with about 10^6 spores of *Aspergillus flavus* per gram

Surface-disinfection procedure			Kernels with *A. flavus*
	NaOCl treatment[a]		
Pretreatment	%	pH[b]	(%)
Corn[c] (about 300,000 spores/kernel)			
None	2	10.6	85
100% Ethanol, two quick rinses	2	10.6	32
None	2	8.2	66
2% NaOCl, pH 8.2, 30 sec	2	8.2	51
100% ethanol, two quick rinses	2	8.2	27
None	5	11.2	32
Running water, 30 sec	5	11.2	38
None	5	8.2	23
5% NaOCl, pH 11.2, 30 sec	5	11.2	15
Wheat[c] (about 30,000 spores/kernel)			
None	2	10.6	15
80% Ethanol, two quick rinses	2	10.6	10
None	2	8.2	17
100% Ethanol, 30 sec	2	10.6	3
None	5	11.2	13
None	5	8.2	3

[a] One minute with shaking, one sterile distilled water rinse. NaOCl brand N-1.
[b] Less than 10 indicates pH adjusted with HCl.
[c] Corn lot E, wheat lot D. Neither was infected with *A. flavus*.

no internal infection with storage fungi but heavily inoculated with *A. glaucus, A. flavus, P. citrinum,* or *P. brevi-compactum* Dierckx.

Selecting a surface-disinfection procedure. The decision as to the most suitable method for surface-disinfecting any given sample so that fungal invasion can be evaluated might be based on data like those in Table 4. Results for lot A were all quite similar, so all methods could be considered satisfactory. With lot B, 2% solutions of S-1 or S-2 gave relatively high counts of *A. flavus* and *A. niger*. Disinfection achieved by 2% concentration of any bleach with pH 8.2–8.5, by 2% N-1 (pH 10.6), and by undiluted bleaches indicated a uniformly low invasion by *A. flavus*. Pretreating corn with ethanol generally lowered the counts of *Fusarium moniliforme* Sheldon, *Cephalosporium*, and *Penicillium*.

Samples F-1 and F-2 were representative of 22 export corn samples. The grain was dusty, many kernels were heat-cracked or checked, and it graded no. 2. Surface disinfection with undiluted N-1 gave uniform and repeatable fungi counts for the samples. Even so, about 40% of the *A. glaucus*, 30% of the *Penicillium* spp., and 17% of the *A. flavus* were growing from breaks in the seed surfaces. We could not determine whether such growth represented invasion or contamination.

DISCUSSION

Most studies of the disinfecting activity of chlorine and other halogens have concerned organisms occurring in water supplies (8). Surface disinfection of seeds involves higher disinfectant concentration and shorter exposure time than used for water treatment. The effectiveness of HOCl as a disinfecting agent stems from the small size of the molecule and its electrical neutrality, which allow ready penetration of cells.

Organism concentration is a limiting factor in disinfection. High numbers of spores in a given volume may create an unsatisfied demand for active chemical, and clumping may present barriers to penetration as has been demonstrated with amoebic cysts (15). Our results with heavily contaminated seeds indicate that factors other than availability of chemical can limit the destruction of surface spores. When the concentration of NaOCl is high, incomplete kill of spores is more likely a result of lack of contact between spores and disinfectant. Contact is improved by vigorous agitation to dislodge air bubbles on grain surfaces.

The effectiveness of surface disinfection depends on the pH, formulation, and concentration of the hypochlorite solution (Tables 2 and 4). We found undiluted N-1 best for corn. Results were not satisfactory with other bleach solutions unless the pH was lowered to 8.0–8.5. We attributed the effectiveness of N-1 to its low NaOH content, allowing the equilibrium to shift quickly toward HOCl, and to its low calcium ion concentration. Inorganic calcium ion may impede halogen disinfection (9). The chlorine in a 2% solution of NaOCl should be adequate for surface-disinfecting corn kernels; however, chlorine compounds are potent oxidizers, and their disinfecting capacities can be reduced in reactions with organic and inorganic compounds (10). Many materials are present on corn kernels and in grain dust that may reduce the efficiency of 2% N-1. Wheat samples were disinfected adequately by 2% N-1, especially in conjunction with an ethanol pretreatment, unless heavy inoculum was applied. In such cases, no disinfecting agent destroyed all the surface-contaminating spores.

Spores trapped in cracks may be protected from contact with the disinfectant. *A. glaucus* was observed to grow from cracks in the pericarp of corn kernels even when the most effective surface disinfectant was used. Differences in the characteristics of seed surfaces probably accounted for ethanol pretreatment being more effective on wheat than on corn. For some samples, 95 or 100% ethanol was more effective than 70 or 80% ethanol; for others, the reverse was true. Ethanol lowered counts of some presumably internal field fungi in corn kernels, perhaps by increasing the penetrability of the kernel or attachment tissues by the hypochlorite solution.

Eckhoff et al (4) reported that corn samples containing a few seeds with heavily sporulating colonies of *Penicillium* produced high and erratic counts of *Penicillium* when disinfected with 5% NaOCl, rinsed in sterile water, and plated. They could eliminate the high counts by pretreating the corn with 95% ethanol or by eliminating the water rinse. They believed the water rinse was spreading spores that were not contacted by NaOCl from the heavily contaminated seeds to the clean seeds.

On corn, but not on wheat, dilute NH_4OH, as a pretreatment or combined with NaOCl, assisted removal of surface-contaminating

TABLE 4. Apparent fungal invasion in corn samples after surface disinfection with sodium hypochlorite (NaOCl) solutions

Corn lot	NaOCl treatment[a]			Kernels with fungi (%)					
	Concentration (%)	pH[b]	Source[c]	*F. moniliforme*	*Cephalosporium*	*Penicillium*	*A. flavus*	*A. glaucus*	*A. niger*
A	2	11.7	S-1	26	35	3	8	0	5
	2	11.7	S-2	22	36	5	4	0	3
	2	10.6	N-1	15	39	3	2	0	2
	2	8.2	N-1	22	24	6	3	0	1
	2	8.2	S-1	20	27	5	3	0	3
	5	11.2	N-1	17	34	4	0	0	0
	2[d]	11.7	S-1	19	10	1	0	0	0
B	2	11.7	S-1	17	20	5	23	0	20
	2	11.7	S-2	24	28	3	13	0	28
	2	10.6	N-1	22	20	1	9	0	13
	2	8.5	S-2	19	26	3	4	0	9
	2	8.5	N-1	18	24	0	5	0	13
	5	11.2	N-1	16	19	4	3	0	7
	2[d]	11.7	S-2	6	13	0	3	0	13
F-1	1	10.4	N-1	14	4	23	13	21	1
	2	10.6	N-1	8	13	17	2	31	0
	5	11.9	S-1	17	12	27	5	34	0
	5	11.2	N-1	7	8	8	2	17	1
F-2	1	10.4	N-1	21	6	20	8	21	1
	2	11.7	S-1	18	7	27	9	35	0
	5	11.9	S-1	14	15	14	4	25	0
	5	11.2	N-1	4	3	10	2	17	0

[a] One minute with shaking, one sterile distilled water rinse.
[b] Less than 10 indicates pH adjusted with HCl.
[c] S-1 and S-2 = supermarket brands; N-1 = nationally advertised brand.
[d] Two quick rinses with absolute ethanol before NaOCl wash.

spores of *Aspergillus*. However, its use was judged unsuitable for routine seed disinfection because of toxicity of N_2H_4 and NH_3.

When seeds are inoculated in conjunction with storage experiments, the inoculum should be removable by surface disinfection. Excessive mechanical mixing of seeds and spores may force spores into surface cracks, making destruction difficult. Heavy inoculum may even contain detectable fungal metabolites and lead to the erroneous conclusion that the seeds contain these metabolites. When plating tests indicate that fungi have invaded under conditions known to be unsuitable for fungal growth, the investigator should verify the invasion by visual examination of the germ, germination tests, or appropriate chemical determinations.

LITERATURE CITED

1. Boller, R. A., and Schroeder, H. W. 1974. Influence of relative humidity on production of aflatoxin in rice by *Aspergillus parasiticus*. Phytopathology 64:17-21.
2. Christensen, C. M., and Mirocha, C. J. 1976. Relation of relative humidity to the invasion of rough rice by *Aspergillus parasiticus*. Phytopathology 66:204-205.
3. Cotton, F. A., and Wilkinson, G. 1966. Advanced Inorganic Chemistry. Wiley Interscience, New York. 1,136 pp.
4. Eckhoff, S. R., Tuite, J. F., Foster, G. H., Kirleis, A. W., and Okos, M. R. 1983. Microbial growth inhibition by SO_2 or SO_2 plus NH_3 treatments during slow drying of corn. Cereal Chem. 60:185-188.
5. Halloin, J. M. 1975. Post harvest infection of cottonseed by *Rhizopus arrhizus, Aspergillus niger,* and *Aspergillus flavus*. Phytopathology 65:1229-1232.
6. Harmon, G. E., and Pfleger, F. L. 1974. Pathogenicity and infection sites of *Aspergillus* species in stored seeds. Phytopathology 64:1339-1344.
7. Hyde, M. B., and Galleymore, H. B. 1951. The subepidermal fungi of cereal grains. II. The nature, identity and origin of the mycelium on wheat. Ann. Appl. Biol. 38:348-356.
8. Kruse, C. W., Hsu, Y. C., Griffiths, A. C., and Stringer, R. 1970. Halogen action on bacteria, viruses and protozoa. Pages 113-136 in: Proceedings of the National Speciality Conference on Disinfection. American Society of Civil Engineers, New York.
9. Kuzminski, L. N., Feng, T. H., and Liu, C. C. 1970. Effect of inorganics on disinfection by halogens. Pages 85-111 in: Proceedings of the National Speciality Conference on Disinfection. American Society of Civil Engineers, New York.
10. Laubusch, E. J. 1971. Chlorination and other disinfection processes. Pages 267-292 in: Water Quality and Treatment. 3rd ed. American Water Works Association, Inc. McGraw-Hill, St. Louis, MO.
11. Lillehoj, E. B., Fennell, D. I., and Hesseltine, C. W. 1976. *Aspergillus flavus* infection and aflatoxin production in mixtures of high-moisture and dry maize. J. Stored Prod. Res. 12:11-18.
12. Lopez, L. C., and Christensen, C. M. 1967. Effect of moisture content and temperature on invasion of stored corn by *Aspergillus flavus*. Phytopathology 57:588-590.
13. Raper, K. B., and Fennell, D. I. 1965. The Genus *Aspergillus*. Williams & Wilkins, Baltimore, MD. 686 pp.
14. Ridge, B. P., and Little, A. H. 1942. The control of pH in hypochlorite solutions. Parts I and II. J. Text. Inst., Trans. 33:T33-74.
15. Stringer, R., and Kruse, C. W. 1970. Amoebic cysticidal properties of halogens in water. Pages 319-338 in: Proceedings of the National Speciality Conference on Disinfection. American Society of Civil Engineers, New York.