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**Review**

# Use of 1% Peroxyacetic Acid Sanitizer in an Air-Mixing Wash Basin to Remove Bacterial Pathogens from Seeds

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**Abstract**

To achieve the production of pathogen-free sprouts, there must be appropriate mixing of liquid sanitizer with the seeds to assure contact. Commercial treatments by irradiation or ozone gas of *Salmonella* spp. artificially inoculated seeds were compared, and these resulted in a 1 log reduction after all treatments. Use of peroxyacetic acid (1%) sanitizer on *Salmonella* spp. or *Escherichia coli* O157:H7 inoculated alfalfa seeds consistently resulted in a greater than 1 log reduction. In addition, during these studies debris was noted after the seeds were removed. Based on this observation, an air-mixing wash basin was developed for commercial use. Validation was done by commercial growers using 1% peroxyacetic acid sanitizer to wash seeds in the air-mixing basin, followed by sprouting the seeds. No positive or false-positive pathogen results were reported after the required testing of the sprout water (run-off during sprouting). Use of 1% peroxyacetic acid sanitizer in the air-mixing wash basin does provide the sprout grower an effective means of sanitizing sprout seeds.

## Introduction

In the mid and late 1990s, there were clusters of foodborne outbreaks attributed to the consumption of contaminated raw alfalfa sprouts both nationally and internationally (Mahon *et al.*, 1997; NACMCF, 1999). The seeds were considered the main source of the contamination, and sprout growers started sanitizing the seeds with calcium hypochlorite based on the U.S. Food and Drug Administration's recommendation (NACMCF, 1999). The production of fresh sprouts that are free of *Salmonella* or *Escherichia coli* O157:H7 pathogens is difficult because of the problems with the sanitizing protocol using calcium hypochlorite, which still does not assure pathogen-free sprouts. This was demonstrated when a multi-state outbreak occurred because of *Salmonella*-associated alfalfa sprouts grown from calcium hypochlorite–sanitized seeds (Proctor *et al.*, 2001; Winthrop *et al.*, 2003).

There are a number of disinfection regimes using various sanitizer protocols reported in the literature that attempted to obtain a 5 log pathogen reduction on artificially inoculated alfalfa seeds (Montville and Schaffner, 2004). Montville and Schaffner (2004) analyzed a wide collection of published data on seed sanitization to identify those factors that influence the efficacy of the procedures. Based on their analysis, they concluded that the seed sanitizer contact time and temperature had little effect on log microbial reductions. They also concluded that when there was sufficient published data on the same chemical treatment, the results were quite variable.

Since no single reported sanitizing treatment was shown to be effective, combination treatments were studied. Scouten and Beuchat (2002) studied the use of heat (55°C) in combination with various sanitizers and ultrasound to reduce *Salmonella* and *E. coli* O157:H7 on alfalfa seeds. They reported that sonication did enhance the reduction, but did not eliminate the pathogens (Scouten and Beuchat, 2002). Bari *et al.* (2003) treated three seed varieties with dry heat, chemicals with and without sonication, and irradiation in an attempt to reduce the population of *E. coli* O157:H7. Dry heat (50°C for 1 hour) combined with irradiation at a dose of 2.0 kGy eliminated this pathogen completely from alfalfa and mung bean seeds, whereas a dose of 2.5 kGy was needed to completely eliminate the pathogen from radish seeds. The combination of dry heat and irradiation did not decrease the percent germination of alfalfa seeds or the length of the alfalfa sprouts, but did decrease the radish and mung bean sprout lengths. However, they did not report on the combination of irradiation followed by chemical sanitization.

In the report on the safety of sprouted seeds (NACMCF, 1999), a chlorine disinfection wash was recommended. This treatment was shown to be ineffective when a multistate outbreak of *Salmonella* occurred (Proctor *et al.*, 2001). Lisle *et al.*

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(1998) reported that when *E. coli* O157:H7 was starved, as on a dry seed coat, the effect of starvation caused this microbe to become chlorine resistant. In addition, when an alfalfa seed is hydrated, it excretes materials that react with the chlorine sanitizer and inactivates it (Rajkowski and Rice, 2004).

A review of the published data on seed sanitization also showed that there is variability in the procedure for mixing of the seed and sanitizer (USFDA, 2005). Charkowski *et al.* (2001) reported that alfalfa seed lots can contain seeds with wrinkled coats and that these seeds would be more difficult to sanitize. Rajkowski (2009) demonstrated that when seeds with wrinkled or cracked coats are inoculated, the bacteria can become trapped under the coat during the process. Those bacteria are difficult to reach to inactivate by a sanitizer. Using small sample sizes (5 or 10 g), researchers reported increased reduction when the sample/sanitizer was mixed by sonication (Scouten and Beuchat, 2002; Bari *et al.*, 2003). However, in the sprouting industry a commercial sack may contain 35 to 50 lbs (16 to 23 kg) of seeds that must be sanitized at once, and thorough mixing is difficult without damaging the seeds. Because of this, sonication is not a viable method for seed sanitation on this scale.

To examine the efficacy of various possible procedures, we preformed three different laboratory studies and a commercial validation study. In Study I, we had *Salmonella* spp.–inoculated alfalfa seeds treated at two commercial irradiation facilities (gamma or electron beam) and at a gaseous ozone facility to determine log reduction and percent injury. In Study II, an alternative sanitizer, peroxyacetic acid, at 1%, 2%, and 3% concentrations was also used to determine its efficacy in combination with the ozone pretreatment. In Study III, the efficacy of the peroxyacetic acid sanitizer to reduce *Salmonella* spp. or *E. coli* O157:H7 counts on artificially inoculated alfalfa seeds was determined. Study IV, based on laboratory observations, was performed with an industrial air-driven washer that was specifically developed to thoroughly wash commercial amounts (35–50 lbs) of seeds, and the efficacy of the washer was verified using the 1% peroxyacetic acid sanitizer concentration.

## Materials and Methods

### *Irradiation of alfalfa seeds*

Sufficient alfalfa seeds from a single lot were obtained and used throughout this study to assure that the same quality of seeds was used in the first three studies. The background microflora was removed using ionizing radiation so that pure cultures of either *Salmonella* or *E. coli* O157:H7 could be retrieved using both nonselective and selective agars.

Two 5-pound (ca. 4.5 kg) cloth bags of alfalfa seeds (Caudill Seed, Louisville, KY) were irradiated to 25 kGy using a self-contained $^{137}$Cs gamma radiation source (Lockheed Georgia, Marrietta, GA) with a dose rate of ca. 0.98 kGy/minute to remove the background microflora. The dose rate was established with alanine transfer dosimeters (National Institute of Standards and Technology, Gaithersburg, MD), and the actual dose was verified by reading the dosimeter alanine pellets on an EPR analyzer (EMN 104 EPR; Bruker, Rheinstelle, Germany). The sample temperature was maintained at 20°C by injecting the gas phase of liquid nitrogen into the irradiation chamber. After irradiation the seeds 500 g were weight out and placed in mesh-lined polyurethane stomacher bags (Seward Model 400; Lab Source, Chicago, IL). The mesh lining was detached from the outer bag, and was heat sealed using an impulse sealer (Model 210-12E; Clamco, Cleveland, OH). The outer bag was also heat sealed. All sterile seeds were kept at room temperature until artificially inoculated.

### *Cultures*

Seven produce-related isolates (*Salmonella enterica* Anatum F4317, *S. enterica* Stanley H0558, *S. enterica* Newport H1275, *S. enterica* Infantis F4319, and *Escherichia coli* O157:H7 strains F4546, SEA 13B88, and C7929) were obtained from the Agricultural Research Service, U.S. Department of Agriculture (Wyndmoor, PA) culture collection.

The purity and identification of each isolate was verified by Gram stain and reaction of the Gram Negative Identification (GNI) card of the Vitek AMS Automicrobic System (bioMérieux Vitek, Hazelwood, MO). All cultures were maintained on tryptic soy agar slants (TSA; Becton-Dickinson, Sparks, MD) at 4°C. Working cultures were prepared in tryptic soy broth (TSB; Becton-Dickinson) and kept at 4°C.

The day before the seed inoculation, each isolate was cultured separately by inoculating 0.1 mL of the working culture into 100 mL of TSB in a 250 mL flask and then agitating at 150 rpm on a rotary shaker for 18 hours at 37 ± 1°C. The four *Salmonella* or the three *E. coli* cultures were combined in equal volumes to make the separate *Salmonell*a or *E. coli* inoculum cocktails with a total volume of 500 mL, and the cocktail cell concentration was determined. The cocktail was serially diluted in 0.1% peptone water (PW; Becton-Dickinson), spiral plated on tryptic soy agar (TSA; Becton-Dickinson), and incubated at 37°C for 24 hours before all visible colonies were counted by hand, not by machine. The cell concentrations for both the *Salmonella* and *E. coli* O157:H7 cocktails were $10^8$–$10^9$ colony-forming unit (CFU)/mL, and these were used to obtain the high inoculated seed samples. In addition to using the *Salmonella* or *E. coli* O157:H7 cocktails with a combined cell concentration of $10^8$–$10^9$ CFU/mL, cocktails of each pathogen were made by diluting the combined cells in sterile distilled water to achieve a final concentration of $10^5$–$10^6$ CFU/mL, which was used to obtain the low inoculated seed samples.

### *Inoculation*

All alfalfa seeds used in the studies (Studies I, II, and III) were inoculated using the same protocol using either the high or low cell concentration. The sealed mesh stomacher bag containing ca. 500 g of irradiated seeds was aseptically removed from the polyurethane bag (covering). The seed to inoculum cocktail ratio was 1 g/1 mL. One mesh bag containing the seeds was immersed in the cocktail (500 mL) and kneaded for 1 minute to assure that all seeds came into contact with the liquid. The seeds were dried to obtain a 6.8% moisture (Rajkowski, 2009). The inocula cocktail was used only once, and after each inoculation the cocktail was discarded.

### *Study I*

Split sample preparation for commercial processing. After the inoculated seeds were dried, they were placed in sterile glass jars and mixed before being weighed out in 25 g portions. The inoculated seeds for the commercial irradiation process were placed in small stomacher bags (BagPage®, approximately 9×18 cm; Interscience Laboratory, Weymouth, MA) and heat sealed using an impulse sealer. The seeds for

the commercial ozone process were placed in sterile cheesecloth sacks, tied off, and then placed in individual plastic baggies for shipping. Five inoculated seed packets were prepared for each of the processors for a total of 15 packs. In addition, 30 packets of un-inoculated seeds were prepared in the same way as the inoculated seed packets. Five un-inoculated seed packs were processed along with the five inoculated seed packs and served as processing controls. Five additional control seed packets (un-inoculated) were shipped, but were not processed, and served as environmental controls.

Commercial processing. The split samples, containing the 15 packets (5 inoculated seeds and 10 controls), were sent for gamma irradiation (Sterigenics, Schaumburg, IL), e-beam irradiation (Isomedix Contract Sterilization, Libertyville, IL), or gaseous ozone processing in a commercial vacuum environmental chamber (Tahoe Food Technology, Sparks, NV). In all cases, the five inoculated and five control samples (cheesecloth sacks removed from shipping plastic baggie) were placed inside commercial sacks (50 lbs) of alfalfa seeds that were taped sealed. The sacks were then placed in the middle of a pallet containing other sacks of alfalfa seeds and processed according to specified conditions for a 1 kGy irradiation dose or a 24-hour ozone treatment. After processing, the 10 processed samples were removed and returned with the environmental controls for microbial analysis.

After processing at the three facilities, seed samples were taken from a different sack that did not come in contact with the inoculated seed packets. These seeds were germinated to determine the yield ratio. The entire pallet of the commercial sacks, including the sacks that contained the inoculated and control samples, was discarded.

Microbial analysis of commercially processed split samples. Before opening the returned irradiated processed seeds in stomacher bag packets, the outside of the bag was disinfected with 70% ethanol. After air-drying, the seed packets were opened and the seeds were placed in filter-lined stomacher bags. For the ozone-treated seeds in the cloth sacks, a separate razor blade was used to cut open each packet, and the seeds were placed in the filter-lined stomacher bags. All seed samples were diluted 1:10 with buffered PW (BPW; Becton-Dickinson) and stomached for 2 minutes. All samples were serially diluted in 0.1% PW and spiral plated in duplicate onto both TSA and xylose lysine thiosulfate Tergitol 4 (XLT-4; Becton-Dickinson) agars. The plates were incubated at 37°C for 24 hours before being hand counted. The 1:10 dilution was used in the enrichment step of the three-tube most probable number (MPN) determination for *Salmonella* with Rappaport-Vassiliadis broth (Becton-Dickinson) as selective enrichment. The Rappaport-Vassiliadis broth was streaked on XLT-4. The MPN samples were all incubated at 37°C for 18–24 hours.

After reviewing the results of the split sample recovery, it was decided to continue working with ozone-treated seeds, since processing and transportation costs were considered lower for commercial application.

### *Study II*

Sample preparation for commercial ozone treatment. Another set of high *Salmonella*-inoculated alfalfa seeds (500 g) was obtained, processed at the commercial gaseous ozone facility (Tahoe Food Technology), and used to determine the sanitizer effect (hurdle effect). The inoculated seeds (150 g/sample) were placed in a brown paper bag and two commercial polypropylene mesh bags (Caudill Seed). Before the ozone processing, one of the commercial plastic mesh bags was opened to assure adequate ozone gas penetration and retied to keep the seeds inside the bag (no seeds were removed). The remainder of the inoculated seeds served as the control.

Microbial analysis of commercial ozone-treated seeds. Recovery of *Salmonella* was done on the ozone-treated seeds. From the different bags, 10 g samples were taken in duplicate before being diluted 1:10 with BPW and stomached for 2 minutes. All samples were serially diluted in 0.1% PW, spiral plated in duplicate onto both TSA and XLT-4 agars, and incubated at 37°C for 24 hours before being hand counted.

Sanitizer treatment on ozone-treated seeds. Two 10 g of seed samples were used for each sanitizing treatment. Commercial ozone-treated and un-treated *Salmonella*-inoculated alfalfa seeds were washed with 20 mL of sterile tap water or 20 mL of 20,000 ppm of calcium hypochlorite (Caudill Seed) or 20 mL of 1%, 2%, or 3% peroxyacetic acid sanitizer (Tsunami 100®; Ecolab, St. Paul, MN) according to the Food and Drug Administration–recommended protocol (Rajkowski *et al.*, 2003). The free chlorine level of the hypochlorite solution was determined after dilution with purified water using a commercial test kit (Accuvac–Model AV; Hack, Loveland, CO). A peracetic/peroxide test kit #311 (Ecolab) was used to verify the peroxyacetic acid level. All sanitizer solutions were made fresh for each trial.

The Food and Drug Administration–recommended procedure of 2 mL sanitizer/g of alfalfa seeds was used for the washing studies. After the sanitizer was added, the seeds were mixed continuously by bubbling filtered air into the mixture during the contact times of 5, 10, 15, or 20 minutes. All washing studies were done at room temperature (21–23°C).

Cell recovery. After the specified contact time, the sanitizer was decanted and equal amounts (20 mL) of either BPW or D/E neutralizing broth (Becton-Dickinson) were added to inactivate the sanitizer, before the seeds were transferred to stomacher bags. After the 2-minute stomaching, the samples were serially diluted in 0.1% PW and spiral plated on both TSA and XLT-4 agars. All plates were incubated at 37°C for 24 hours before being hand counted.

### *Study III*

Determination of sanitizer efficacy for reducing *Salmonella* and *E. coli* on alfalfa seeds. Samples (500 g) of both high and low inoculated *Salmonella* seeds were prepared as above. The high and low inoculated *E. coli* seeds were also prepared following the procedure described above.

*E. coli*–inoculated seed samples and *Salmonella*-inoculated seed samples (serving as control) were tested to determine the efficacy of the peroxyacetic acid sanitizer on reducing the cell concentration. The sanitizer protocol (10 g seeds/20 mL sanitizer) was the same as described above for Study II. Recovery of the cells for this study was done only on TSA agar for both microorganisms.

### *Study IV*

Commercial validation of sanitizer procedure using air-washer. Two sprout growers, one in the northeast and one in the northwest, agreed to verify the efficacy of using the 1% sanitizer concentration in a timed (15–20 minutes) commercially available washing apparatus (exact specification proprietary). The washing container consists of a plastic bin (polypropylene) measuring $2\times2\times2$ ft ($61\times61\times61$ cm) with an external pump delivering filtered air (Fig. 1). On the bottom of the bin, pipes were fitted to deliver air into the liquid sanitizer by an air pump (Fig. 3). The seeds were placed in a wire basket held in place by chains to allow for easy lifting of the basket (Fig. 2). The washing apparatus can hold between 35 and 70 lbs of alfalfa seeds in the wire mesh basket. Mixing of the seeds and the 1% peroxyacetic acid sanitizer is achieved by vigorously bubbling air into the washer (Fig. 3). The sanitizer concentration was monitored by the peracetic/peroxide test kit #311 (Ecolab). After the 15–20 minutes of washing, the alfalfa seeds were sprouted according to the grower's protocol (proprietary). After 24 hours of sprouting, the run-off water from the sprouting equipment (known as sprout water) was aseptically collected and sent to independent laboratories for *Salmonella* and *E. coli* O157:H7 testing according to the Food and Drug Administration requirements.

### *Statistical analysis*

The log reductions were analyzed (by ANOVA; SAS Institute, Cary, NC, 2004) to determine if there was any significant ($p \leq 0.05$) difference among the three peroxyacetic acid concentrations and the calcium hypochlorite wash.

## Results and Discussion

### *Study I*

Split sample challenge studies were conducted with only *Salmonella* artificially inoculated seeds. Alfalfa seeds from the same inoculated batch were treated at two commercial irradiation facilities and a gaseous ozone facility to compare the pathogen reduction after treatment. The dosimeter readings, provided by the processor, for gamma irradiation were 1.22 kGy minimum and 2.37 kGy maximum at room temperature. The processor's dosimeter reading for the e-beam irradiation was 1.6–1.7 kGy at room temperature. The ozone processing conditions were 25°C chamber temperature, 20 psi ozone pressure, 100% relative humidity, and 100% ozone in chamber.

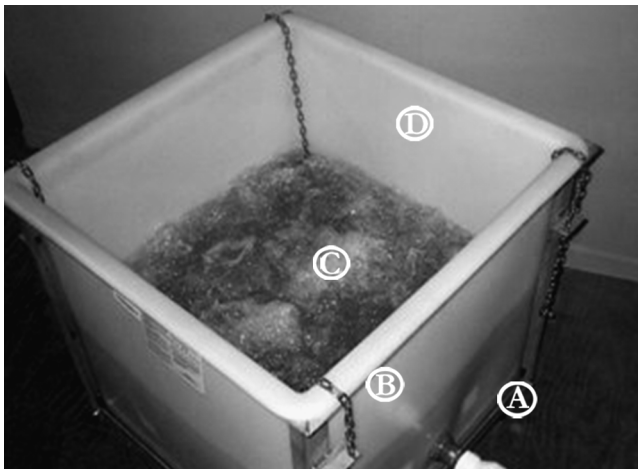


**FIG. 2.** Washing basin in action. (**A**) Drain. (**B**) Basket support chain. (**C**) Air mixing of sanitizer liquid and seeds. (**D**) Polyproplene container.

All the processed control and the environmental control samples (un-inoculated sterile seeds) had no growth when plated on TSA or XLT-4 agars, indicating no cross contamination in shipping or handling at the plants.

The log reduction was calculated from the difference of the control and irradiated, processed, *Salmonella*-inoculated seeds. The *Salmonella* count from the control alfalfa seeds at the time of recovery for the split samples was $6.1 \pm 0.01$CFU/g. After the irradiation process the recovery of *Salmonella* on TSA and XLT-4 ($n = 10$) was $4.9 \pm 0.02$ and $4.5 \pm 0.02$ CFU/g, respectively. The one log reduction of *Salmonella* on TSA, based on the control that remained in the laboratory, is consistent with the reported D-irradiation value of 0.97 kGy for *Salmonella* on alfalfa or broccoli seeds (Rajkowski *et al.*, 2003; Thayer *et al.*, 2003). The maximum dose reported by the both irradiation

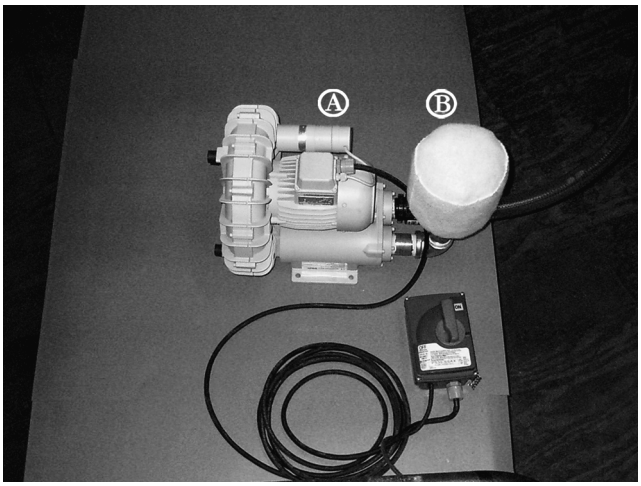


**FIG. 1.** Air pump delivering filtered air to wash basin. (**A**) Air pump. (**B**) Filter.


**FIG. 3.** Washing container. (**A**) Air delivery system. (**B**) Soil/debris after one washing of seeds.

facilities was close to 2 kGy. Rajkowski and Thayer (2001) reported that a dose >2 kGy decreased the yield ratio for production of alfalfa sprouts and that the irradiation treatments as reported by the processors would not have affected the yield. This was confirmed when the alfalfa seeds of the gamma and e-beam processes were sprouted for the yield ratio and were acceptable, even with the gamma irradiation maximum dose of 2.37 kGy. There was no statistical difference ($p < 0.05$) in yield ratio or log reduction between the two irradiation processes.

The % injury was determined by the difference between the counts on TSA and XLT-4. The average % injury of *Salmonella* was calculated to be <10%. This was confirmed by using the MPN selective enrichment procedure. There was no increase in recovery of *Salmonella*. With just a one log reduction and a possible <10% injury, no further work was continued using irradiation on *Salmonella*-inoculated alfalfa seeds.

The recovery of *Salmonella* from the artificially inoculated alfalfa seeds after the gaseous ozone treatment was $4.6 \pm 0.02$ and $4.5 \pm 0.02$ CFU/g on TSA and XLT-4, respectively. This is a 1.5 log based on the TSA recovery plates. A % injury was calculated to be 11%. When the results of the irradiation and ozone process samples were compared, there was no statistical significance ($p > 0.05$). The yield ratio of the ozone-treated alfalfa seeds from that pallet load was acceptable. Based on this preliminary study, work was continued on ozone-treated seeds to determine if further reduction (hurdle effect) would occur using the chemical sanitizers on the injured bacteria.

### Study II

A second batch of high *Salmonella*-inoculated alfalfa seeds was prepared. The seeds with a similar bacterial high *Salmonella* residual level were shipped in typical commercial sack material (paper or plastic mesh) for ozone treatment. Based on an initial count of log 5.9 CFU/g, there was an observed 1.5 log reduction of *Salmonella* after the ozone treatment, which was the same as observed in Study I. There was no statistical difference ($p \leq 0.05$) in reduction due to the different sack material or opening of the one plastic mesh sack.

The results of the ozone process followed by a peroxyacetic acid and calcium hypochlorite sanitizing treatment are presented in Table 1. The reported additional log and % reduction is based on the recovery from the water wash sample. We observed that the water wash and the calcium hypochlorite sanitizer did not achieve any further reduction other than the initial reduction after the ozone treatment. However, when the ozone-injured *Salmonella* was treated with the peroxyacetic acid sanitizer, an additional >1.5 log reduction was observed. This would bring the total additive reduction (ozone plus sanitizer) to >3 logs. Increasing sanitizer concentration from 1% to 2% or 3% showed no further reduction, and it was concluded that the 1% concentration of peroxyacetic acid would be used for the commercial validation study.

The 20,000 ppm calcium hypochlorite solution was obtained using tap water. After treatment we did not observe any log reduction. The ineffectiveness of this sanitizing treatment was confirmed in other reports (Proctor *et al.*, 2001; Montville and Schaffner, 2004).

### Study III

A sanitizer study was conducted using *E. coli* artificially inoculated seeds in addition to a third prepared sample of *Salmonella*-inoculated seeds to determine if similar reduction occurred for *E. coli*. None of the seed samples in this study had any pretreatment before being sanitized. We did not observe any statistical difference ($p \leq 0.05$) in recovery when the seeds were washed for 10, 15, or 20 minutes. We confirmed Montville and Schaffner's (2004) observation that contact time did not affect the reduction.

There was a >1 log reduction in both the *Salmonella* and *E. coli* O157:H7 levels when treated with the 1% peroxyacetic acid ($n = 8$ for each % peroxyacetic acid concentration used to sanitize the *E. coli*–contaminated or *Salmonella*-contaminated seeds) with no statistical significant reduction ($p \leq 0.05$) for the 2% or 3% concentrations. When the low *E. coli* or *Salmonella* inoculums seeds ($10^{5-6}$ CFU/g) were sanitized using 1% peroxyacetic acid, >1 log reduction was observed. The log reduction after the sanitizing treatment with the 1% peroxyacetic acid in this study was similar to the log reduction reported in Table 1 for the ozone-processed seeds. Based on these data it was concluded that there was only an additive affect by combining an ozone process with the sanitizing treatment. The 1% peroxyacetic acid worked equally efficiently in reducing *E. coli* by one log.

### Study IV

During the multiple repeats of the previous laboratory studies, timed air mixing was used to assure constant contact of the seeds and sanitizer. In all of the repeats, dirt or debris was always observed remaining in the bottom of the beakers. The adequacy of the mixing of the sanitizer with large amounts of seeds (30–50 lbs) is a critical issue, and thorough mixing is not easily achieved by hand. A large air-driven washing apparatus was commercially developed, and the 1% peroxyacetic acid concentration was used by commercial sprout growers to validate the efficacy of the washer. To mix the seeds without doing any damage to the germ, air circulation appeared to be the least deleterious. A simple washing container fitted with a wire mesh

Table 1. Log and % Reduction of *Salmonella* from Ozone-Treated Artificially Inoculated Alfalfa Seeds Sanitized with Peroxyacetic Acid or $Ca(ClO)_2$ at Room Temperature for 20 Minutes

| | | *Log CFU/g peroxyacetic acid* | | | *$Ca(ClO)_2$* |
|---|---|---|---|---|---|
| *Sample*[a] | *Water* | *1%* | *2%* | *3%* | *20,000 ppm* |
| Ozonated in paper sack[b] | $0 \pm 0$ (0%) | $1.52 \pm 0$ (49%) | $1.47 \pm 0.15$ (47%) | $1.60 \pm 0.02$ (48%) | 0 (0%) |
| Ozonated in open plastic sack[b] | $0 \pm 0$ (0%) | $1.74 \pm 0.64$ (49%) | $1.57 \pm 0.38$ (44%) | $1.83 \pm 0.17$ (52%) | 0 (0%) |
| Ozonated in closed plastic sack[b] | $0 \pm 0$ (0%) | $1.67 \pm 0.38$ (47%) | $1.95 \pm 0.42$ (55%) | $1.99 \pm 0.24$ (56%) | 0 (0%) |

[a]Untreated, 5.9 CFU/g; ozone treated, $3.3 \pm 0.2$ CFU/g.
[b]$n = 8$.

basket holding the seeds and suspended over an air circulation system using an air pump (Figs. 1 and 3) was developed. The air circulated the 1% peroxyacetic acid sanitizer with the seeds for the required time of 15–20 minutes. (Fig. 2). When the basket containing the seeds was removed, the sprout growers noticed and documented the residual dirt/debris remaining on the bottom of the washing basin (Fig. 3). During the 2 years when the sprout growers used this seed washing apparatus with the 1% peroxyacetic acid sanitizer, they did not report any of positives for *Salmonella* or *E. coli* O157:H7, as determined by the commercial laboratories testing of the 24-hour sprout water.

## Conclusion

The results of the laboratory testing and commercial verification showed that the use of the 1% peroxyacetic acid sanitizer achieved a consistent reduction of *Salmonella* or *E. coli* O157:H7 on artificially contaminated alfalfa seeds. The use of the commercial basin with vigorous air mixing and 1% peroxyacetic acid sanitizer did achieve a consistent pathogen-negative record for the sprout growers over a 2-year period.

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## Disclosure Statement

No competing financial interests exist.

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