

Monte Carlo Simulation of Pathogen Behavior during the Sprout Production Process

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Received 8 May 2004/Accepted 15 September 2004

Food-borne disease outbreaks linked to the consumption of raw sprouts have become a concern over the past decade. A Monte Carlo simulation model of the sprout production process was created to determine the most-effective points for pathogen control. Published literature was reviewed, and relevant data were compiled. Appropriate statistical distributions were determined and used to create the Monte Carlo model with Analytica software. Factors modeled included initial pathogen concentration and prevalence, seed disinfection effectiveness, and sampling of seeds prior to sprouting, sampling of irrigation water, or sampling of the finished product. Pathogen concentration and uniformity of seed contamination had a large effect on the fraction of contaminated batches predicted by the simulation. The model predicted that sprout sampling and irrigation water sampling at the end of the sprouting process would be more effective in pathogen detection than seed sampling prior to production. Day of sampling and type of sample (sprout or water) taken had a minimal effect on rate of detection. Seed disinfection reduced the proportion of contaminated batches, but in some cases it also reduced the ability to detect the pathogen when it was present, because cell numbers were reduced below the detection limit. Both the amount sampled and the pathogen detection limit were shown to be important variables in determining sampling effectiveness. This simulation can also be used to guide further research and compare the levels of effectiveness of different risk reduction strategies.

Seeds and bean sprouts have become a serious food safety concern during the past decade. Outbreaks of salmonellosis and food-borne illness from pathogenic *Escherichia coli* have been linked with contaminated sprouts. These outbreaks have been most commonly associated with alfalfa sprouts but have also been linked to clover, radish, and mung bean sprouts. One of the earliest reports of illness associated with sprouts was in 1973, when an outbreak of *Bacillus cereus* occurred due to contaminated home sprouting kits (17). Reported outbreaks have increased steadily in the intervening decades (32). Despite increased awareness, outbreaks continue unabated (28, 58).

Sprout production poses unique challenges for food safety microbiologists for a variety of reasons. Seeds for sprouting are harvested from the agricultural environment, and at the time of harvest it may not be known if the seeds are to be used for human consumption or planting. Conditions conducive to seed germination also favor microbial growth: seeds require the regular addition of water and incubation under warm conditions (room temperature). Water is typically added at regular intervals that are as short as hourly intervals to as long as several times per day. The regular addition of irrigation water is required by the rapidly growing sprouts, and this water also serves to facilitate the spread of any pathogens which may be present. Altering the process or treating the seeds to retard or eliminate bacterial growth would also prevent proper sprouting of seeds. Treating the finished product to remove pathogens has also not been found to be practical because of the delicate

nature of sprouts and the intimate association of pathogens with the finished product.

The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) examined the issue in depth in 1999. The NACMCF recommended seed disinfection, using methods demonstrated to produce at least 5-log₁₀ reductions in the numbers of *E. coli* and *Salmonella* organisms, specifically mentioning 20,000 ppm chlorine. They also encouraged implementation of microbiological testing programs (32).

Quantitative microbial risk assessment is a useful tool in organizing available data and determining their implications (6, 11, 57). Our objective in the research presented here was to create a mathematical model to determine the most-effective points for pathogen control in the sprout production process. Our primary focus was placed on realistic intervention techniques, such as those recommended by the NACMCF, including sampling programs and seed disinfection. The effects of agricultural conditions during primary seed production and postsprouting handling were not included in the simulation.

Literature review. Extensive research has been conducted on the microbiology of seed sprouting. Various aspects of the sprouting process studied include, but are not limited to, the microbial quality of raw seeds and sprouts, microbial growth during the sprouting process, and various pre- and postproduction intervention techniques (such as chemical seed and sprout disinfection).

Examination of the microbial quality of raw seeds and sprouts has consistently revealed high microbial counts and pathogen contamination. Prokopowich and Blank (36) sampled alfalfa sprouts, onion sprouts, and a sprout mixture purchased from a retail outlet and found aerobic plate counts ranging from 3 to 9 log₁₀ CFU/g. They isolated *Staphylococcus*

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aureus in 24% of samples. Sprout seeds sampled had aerobic plate counts of 2 to 5 log₁₀ CFU/g, although *Listeria* spp. and *Salmonella* spp. were not detected (36). Liao and Fett found plate counts on average greater than 4 log₁₀ CFU of aerobic organisms/g for both alfalfa and clover seeds (22). In another study examining the microbial quality of sprouting seeds, *Bacillus cereus* was found in 56 of 98 sprouting kits purchased. When seeds from contaminated kits were sprouted, contamination rose to a mean level of 3.72 log₁₀ CFU/g for alfalfa sprouts and 4.53 log₁₀ CFU/g for mung bean sprouts (17). Investigation of seed sprouts in Norway found thermotolerant coliform bacteria in 24% of 300 samples, and *E. coli* was isolated from 8 samples. Separate analysis of sprout irrigation water yielded no positive results for *E. coli* O157 or *Listeria monocytogenes*; however, *Salmonella enterica* serovar Reading was found in three samples on three consecutive days (40).

A large proportion of sprout-related research has focused on the disinfection of seeds prior to sprouting. A thorough analysis of the literature available on seed disinfection prior to sprouting has recently been completed in our lab (31). Despite the concentrated efforts of researchers, no method of disinfection has been able to ensure pathogen-free seeds and sprouts. Even the highly touted 20,000-ppm chlorine treatment has only a median reduction of 2.5 log₁₀ CFU/g (31).

Most research shows that the method of microbial testing of seeds has a minimal effect on the isolation of pathogens. The use of surfactants and increased temperature of soaking has failed to demonstrate increased recoveries of *E. coli* from seeds (59). The method of seed processing (stomaching or blending) proved to have little effect on cell recovery but was more effective than rinsing seeds (59). Inami et al. used three different lots of naturally contaminated alfalfa seed to compare isolations of *Salmonella* by seed shredding and sprouting. Shredding samples yielded 35 positive out of 90 samples, whereas sprouting yielded 40 positive out of 90 samples (20).

Bacterial growth dynamics during sprouting have been studied using naturally and artificially contaminated seeds. Seed germination in water for 16 to 18 h has been found to increase aerobic plate counts on average 1 log₁₀ CFU (36). Growth of *Escherichia coli* O157:H7 on artificially inoculated seeds reached a level of 5 to 6 log₁₀ CFU/g regardless of initial inoculum size after 1 or 2 days (47). When seeds were inoculated with extremely low levels of *S. enterica*, the final concentration was also close to 6 log₁₀ CFU/g (19). Gandhi et al. (16) observed higher final concentrations from inoculated seeds; *S. enterica* serovar Stanley reaches levels over 1 log₁₀ CFU/g higher than those of *E. coli* JM109 after 3 days of sprouting, despite starting at a lower inoculum level. One study used seeds implicated in a salmonellosis outbreak that had low-level contamination prior to sprouting (less than -1 log₁₀ CFU/g) (49). After 48 h of sprouting, levels ranged from 2 to 4 log₁₀ CFU/g. Although this is lower than levels observed with artificially contaminated seeds, the relative amount of growth observed is comparable. Regardless of organism and inoculation technique, all data show that the majority of growth occurs within the first 24 h of sprouting.

Data on the effect of sprouting temperature and irrigation frequency are scarce. Charkowski et al. examined the effects of sprouting temperature and initial inoculum level for both *E. coli* and *Salmonella* on sprouts and in irrigation water. Their

experimental design had increasing inoculum sizes paired with increased temperature such that it was impossible to separate the effect of temperature and inoculum size. For *E. coli*, all sprouts (regardless of sprouting temperature) had final levels of 5 log₁₀ CFU/g, although concentration in the irrigation water was as high as 7 log₁₀ CFU/ml. *Salmonella* reached higher final concentrations with higher sprouting temperatures and when initial inoculum sizes were higher (8).

Many of the aforementioned studies examine microbial populations on sprouts and irrigation water simultaneously, giving insight into the effectiveness of testing irrigation water in lieu of sprouts (19, 47, 49). Fu et al. performed the most thorough comparison of spent irrigation water and sprouts, using both naturally occurring total aerobic mesophiles and inoculated *E. coli* O157:H7. They found, on average 1 log₁₀ CFU/g or ml more on sprouts than in irrigation water for both naturally occurring floras and inoculated *E. coli* O157:H7 (15).

MATERIALS AND METHODS

Scientific and medical indexes were used to locate relevant literature on sprouts. Relevant literature was grouped into two main subject categories: growth of pathogens during sprouting (1, 8, 16, 19, 33, 41, 42, 47, 49) and pathogen detection on seeds or sprouts (8, 15, 20, 21, 23, 36, 45, 48, 55, 59). Epidemiological literature was reviewed to assemble a comprehensive understanding of primary risk factors in the sprouting process (3, 4, 9, 25, 29, 30, 35, 37, 52, 53, 58). Data on processes not currently used in sprouting operations were not included in our model (10, 12, 13, 18, 27, 34, 38, 39, 42-44, 54, 56). Some publications contained limited quantities of data which could not be incorporated into our model.

Data were combined in Excel spreadsheets (Microsoft, Redmond, Wash.) where appropriate, and histograms were created and correlations determined. Graphical data were converted to numerical data using SigmaScan Pro (SPSS, Chicago, Ill.) where necessary. A simulation model was created with Analytica software 2.0 (Lumina Decision Systems, Los Gatos, Calif.). A summary of the simulation variables affecting final pathogen concentration on sprouted seeds are shown in Table 1, and the details are discussed below.

Seed lot characteristics. There are many factors affecting pathogen contamination of seeds that would be completely unique for every seed lot and always unknown (unless every seed in the entire lot were subjected to microbial analysis). In our model, a seed lot is defined as all seeds within a delivery (e.g., thousands of kilograms). A batch is the amount of seeds sprouted at one time in one chamber (generally between 20 and 35 kg), although this is dependent on the type of sprouts and sprouting equipment used. A sample is a portion of seeds taken for analysis prior to production (generally less than 0.5 kg). Other user-defined inputs include pathogen prevalence per sample (the proportion of 25-g samples in the seed lot that contain a pathogen, which can also be thought of as uniformity of contamination) and minimum and maximum pathogen concentrations if a 25-g sample is contaminated. These variables allow the user to determine how varying prevalence and concentration affects simulation outputs like the probability of contaminated batches or of detecting a pathogen if present. The likelihood of having a positive sample was described using the Bernoulli distribution (if the uniform distribution [0 or 1] is less than *P*, then the likelihood is 1 or else 0), where the probability of having any pathogen in a given sample is equal to the prevalence of the pathogen in the lot. Pathogen concentration in positive samples was determined using uniform distribution with user inputs for the minimum and maximum numbers of pathogens per sample. The prevalence and/or concentration of pathogens within a batch (i.e., many samples) were determined by summing the number of pathogens present in an appropriate number of samples. For example, if a sample is 25 g and a batch is 35 kg, the concentration of pathogens in the batch would be determined by summing the results from 1,400 samples. Sample size, batch size, type of sample (preproduction seeds, sprouts, or irrigation water), number of samples taken, and detection limit of sampling were all defined user inputs.

The efficacy of a seed disinfection treatment (log₁₀ reduction by disinfection) was described by the triangular distribution with the minimum, most likely, and maximum values input by the user (31). Bacterial death as determined by this distribution was described using the binomial equation (26). The probability of a single cell surviving disinfection (*P*) was 10^{log₁₀} reduction, where the number of

TABLE 1. Simulation variables affecting final pathogen concentration on sprouted seeds

Test category	Variable	Definition
Initial pathogen concn on seeds	Pathogen prevalence per 25-g sample	User defined
	Probability given sample has pathogen	Bernoulli (proportion of contaminated samples)
	Pathogen concn per 25-g sample	Triangular (minimum, most likely, and maximum)
	Log ₁₀ reduction from seed disinfection	Triangular (minimum, most likely, and maximum)
	Probability of cells surviving disinfection	10 ^{log₁₀ reduction}
	Mean no. of cells surviving	Probability of cell survival × initial cell no.
Pathogen growth during sprouting	SD of cells surviving	Square root of [probability of cell survival × initial cell × no. × (1 - probability of cell survival)]
	No. of doublings after 24 h	Triangular (0, 11, 21)
	No. of doublings after 48 h	Triangular (2, 14, 21)
	No. of doublings after 72 h	Triangular (4, 10, 21)
	No. of doublings after 96 h	Triangular (6, 9, 21)
	Increased no. of doublings at 30°C	No. of doublings × uniform (1.1, 1.75)
Pathogen detection on seeds and sprouts	Increased no. of doublings at 35°C	No. of doublings × uniform (1,2)
	Probability pathogen detected from contaminated seeds	Bernoulli (0.8)
	Difference between concentration in water and on sprouts	Triangular (-0.75, 0.5, 2.25)

cells in the population was n . The number of surviving cells was assumed to be normally distributed, where the mean number of cells to survive disinfection was P times n , and the standard deviation was the square root of n times P times the result of 1 minus P . For example, if the reduction caused by disinfection is 2.5 log₁₀ CFU, then P was 10^{-2.5} or 0.003 and the population size (n) was 10⁴ (or 10,000); the mean number of cells to survive would be 30, and standard deviation was 5.4 cells.

Microbial growth during sprouting. Microbial growth during the sprouting process was described in a sufficiently detailed manner in 10 different publications to include in our analysis (2, 5, 7, 8, 14, 16, 19, 46, 47, 49). Although other publications reported this information, it was not sufficiently detailed or quantitative to allow its inclusion in the simulation. Data were generally presented as a log₁₀ increases over a period of days. These data were converted to numbers of doubling times by using the Gompertz equation (60). When growth during sprouting was determined using an initial inoculum size of greater than 4 log₁₀/g, data were excluded due to decreased potential for growth (due to the proximity to the maximum population density) and the low probability of seeds naturally having such high levels of pathogen contamination (8, 15). All data included (except one observation which was determined to be statistically similar to other data) were obtained from seeds sprouting between 20 and 25°C. Data available were levels of growth of various *Salmonella* serovars, *E. coli*, total aerobic mesophiles, and *B. cereus*. Type of organism had no statistically significant effect on the number of doubling times, despite published reports that *Salmonella enterica* grow more quickly than *E. coli* O157:H7 (8). The expected number of doubling times after 24, 48, 72, and 96 h of sprouting were all described using the triangular distribution, with details shown in Table 1.

A simple multiplicative scaling factor for pathogen growth at higher sprouting temperatures was obtained by comparing growth curves for *E. coli* and *Salmonella* using the Pathogen Modeling Program (51), such that the growth rate was increased at higher temperatures in proportion to the increase predicted by the Pathogen Modeling Program. While different researchers have used different irrigation frequencies and volumes of added water, there were insufficient published data on the effect of irrigation frequency or water volume to include these variables in the simulation. Research is under way in our lab to try to determine the influence of this variable on pathogen prevalence and concentration in irrigation water and on sprouts.

Pathogen detection from seeds and sprouts. Most literature available on the detection of pathogens from seed and sprouts used a low number of experimental replicates. The method of seed processing (stomaching or blending) was deemed insignificant based on published comparisons (59). The probability that a pathogen, if present, would be detected from the seed was calculated using presence and absence data where a large number of samples of both seeds and sprouts from the same lot were tested (20). The number of positive seed samples (40) was divided by the number of positive samples after sprouting (50, assumed to be the true number of positive samples) giving a probability of 0.8. The

detection of a pathogen (if present) on unsprouted seeds was assumed to follow a Bernoulli distribution where P was equal to 0.8.

Four publications have provided data on pathogen concentration from simultaneously sampling sprouts and irrigation water, allowing for an approximation of the difference in concentrations depending on the type of sampling (15, 19, 47, 49). The difference in microbial load on sprouts and irrigation water was calculated by subtracting the log₁₀ count in water from the log₁₀ count on sprouts. A total of 80 observations from the four publications cited above were fit to a triangular distribution with parameters (-0.75, 0.5, and 2.25) (Fig. 1). The log₁₀ difference was converted to a percentage and multiplied by the concentration of pathogens on sprouts to simulate the concentration of pathogens expected to partition into the irrigation water and to distribute evenly in that water.

If final bacterial concentration (either on sprouts or in a sample) was greater than the detection level, a value of 1 (i.e., a positive result) was assigned to that iteration. If the sample or sprouts were pathogen free or if the level was lower

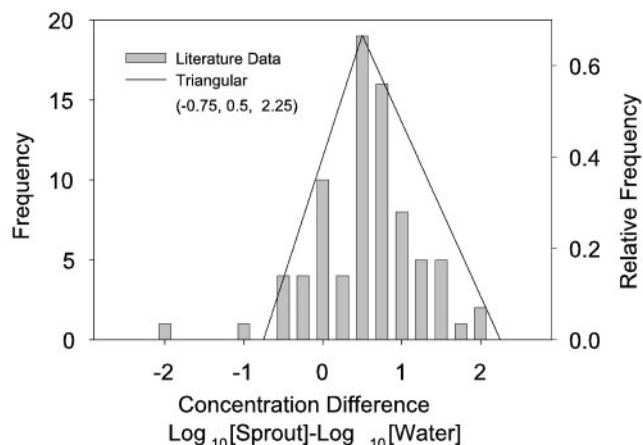


FIG. 1. Pathogen partitioning between sprouts and irrigation water in the sprout production process. The difference in microbial loads on sprouts and in irrigation water was calculated by subtracting log₁₀ numbers of counts per milliliter of water from log₁₀ numbers of counts per gram of sprouts. A total of 80 observations from four publications (15, 19, 47, 49) were fit to a triangular distribution with parameters (-0.75, 0.5, and 2.25).

TABLE 2. Simulation results for the effect of sampling conditions on the detection of contaminated sprouts^a

Sampling	Day of sampling	Disinfection?	% of samples that were positive			% of batches contaminated
			Seeds	Sprouts	Irrigation water	
Preproduction	1	No	0.1	0.1	0.1	77.0
	2		0.1	0.1	0.1	77.0
	3		0.1	0.1	0.1	77.0
	4		0.1	0.1	0.1	77.0
Postproduction	1	No	NA	75.7	71.3	77.0
	2		NA	76.8	74.0	77.0
	3		NA	76.9	75.2	77.0
	4		NA	77.0	76.1	77.0
	1	Yes	NA	1.1	0.8	1.2
	2		NA	1.2	1.0	1.2
	3		NA	1.2	1.1	1.2
	4		NA	1.2	1.1	1.2

^a One out of every 1,000 samples of 25 g was positive at a level that varied uniformly between 1 and 5 CFU/g. Batches of sprouts were made from 36.3 kg of seed, and preproduction samples were composed of 160 subsamples of 25 g each. The pathogen detection limit was 1 log₁₀ CFU/g. ^bA positive sample is a sample where a pathogen was detected. Irrigation water in the preproduction Irrigation water in the preproduction sample is that water used to irrigate only the seed sample (4 kg), while irrigation water in postproduction is that water used to irrigate the entire production batch (36.3 kg) of seeds. A contaminated batch is a batch of sprouts (made from 36.3 kg of seed) containing at least one pathogen CFU.

than the detection limit, a value of 0 was assigned. Simulations were run using Monte Carlo sampling for 1,000 iterations.

RESULTS

Effect of sampling methods. A comparison of sampling methods is shown in Table 2. For all conditions shown in Table 2, seed contamination was uniformly distributed between 1 and 5 CFU/g at a prevalence of 0.001. Batches of sprouts were made from 36.3 kg of seed. The detection limit was set to 1 log₁₀ CFU/g for this example. When sprouts or irrigation water were tested from these samples, sprouting took place at 35°C (the optimal temperature for microbial growth in our simulation). In this example, the preproduction sample was composed of a composite of 160 subsamples that were 25 g each.

Preproduction sampling indicated that only 0.1% of samples were positive when in fact 77% of all batches were contaminated, regardless of day of sampling and type of sample taken. Postproduction sampling was generally quite effective with most (but not all) positive batches being detected. Seed disinfection dramatically lowered the number of positive batches but did not eliminate them. The day of postproduction sampling had a slight effect on the number of positive sprout and irrigation water samples, with samples taken later having a greater tendency to be positive. In all cases, whether seeds were disinfected and regardless of day of sampling, sampling sprouts was slightly more effective than sampling irrigation water.

Effect of seed contamination level and frequency. Pathogen prevalence and concentration on seeds had varied effects on the ubiquity of contaminated batches. Results in Table 3 compare low (1 to 5 CFU/g) and high (100 to 5,000 CFU/g) contamination levels with varying uniformity of contamination (prevalence from 0.0001 to 1, where 0.0001 means that 1 out of 10,000 25-g samples was positive and 1 means all 10,000 samples were positive at some level). In the example presented in Table 3, for preproduction sampling, irrigation water from a

composite sample composed of 50 25-g subsamples was tested after 3 days of sprouting at 35°C. For postproduction sampling, irrigation water from a 36.3-kg (80-lb) batch of sprouts was tested on the third day of sprouting. The detection limit for both pre- and postproduction sampling was set to 2 log₁₀ CFU/g, which is higher than the 1 log₁₀ CFU/g used in the Table 2 example.

When seeds were untreated, microbial load had little effect on the percentage of batches produced with contamination; the proportions of contaminated batches with low and high levels of initial contamination were identical except at a prevalence of 0.001 (74.6% versus 80.9% actually contaminated), where a prevalence of 0.001 means that 1 out of 1,000 25-g samples is positive. Not surprisingly, however, as prevalence increased, the proportion of contaminated batches increased. For untreated seeds at a prevalence greater than 0.001, all batches produced were contaminated regardless of initial contamination level.

When seeds were disinfected prior to sprouting, the initial contamination level had a greater effect on the production of contaminated sprouts. At the lowest prevalence (0.0001, or 1 out 10,000 25-g samples is positive), the difference was smallest: 8% of batches were actually contaminated at high contamination levels (100 to 5,000 CFU/g) versus 0.1% of batches actually contaminated at low contamination levels (1 to 5 CFU/g). The disparity grew with increasing prevalence. Where contamination was uniform on seeds (prevalence, 1), seeds contaminated at a high level produced batches that were 87.7% contaminated, while seeds contaminated at a low level resulted in batches that were 14.7% contaminated.

Effectiveness of seed disinfection. Disinfection of seeds (assuming a triangular log₁₀ CFU reduction with parameters (1, 2.5, and 6.5) prior to sprouting did not guarantee pathogen-free sprouts (Table 3). However, in all cases disinfection reduced the percentage of contaminated batches. Seed disinfection was most effective when contamination was sporadic and

TABLE 3. Simulation results for varying prevalence and contamination level on seeds with and without disinfection, where the detection limit for both pre- and post-production sampling was 2 log₁₀CFU/g

Contamination level, disinfection status	Prevalence (% of positive 25-g samples)	% of samples in which contamination was detected		% of batches contaminated	% of batches reaching the consumer
		Preproduction ^a	Postproduction ^b		
High (100 to 5,000 CFU/sample), without disinfection	0.0001	0	13.7	13.7	0.0
	0.001	0	80.9	80.9	0.0
	0.01	0.4	100.0	100.0	0.0
	0.1	39.6	100.0	100.0	0.0
	1	100.0	100.0	100.0	0.0
Low (1 to 5 CFU/sample), without disinfection	0.0001	0	12.1	13.7	1.6
	0.001	0	66.2	74.6	8.4
	0.01	0.5	98.2	100.0	1.8
	0.1	35.9	99.9	100.0	0.1
	1	100.0	100.0	100.0	0.0
High (100 to 5,000 CFU/sample), with disinfection (triangular log ₁₀ CFU reduction [1, 2.5, 6.5])	0.0001	0	6.4	8.0	1.6
	0.001	0	45.4	49.7	4.3
	0.01	0.2	75.3	79.3	4.0
	0.1	24.1	83.7	85.2	1.5
	1	82.3	87.1	87.7	0.6
Low (1 to 5 CFU/sample), with disinfection (triangular log ₁₀ CFU reduction [1, 2.5, 6.5])	0.0001	0	0.1	0.1	0.0
	0.001	0	1.3	1.7	0.4
	0.01	0	4.2	5.0	0.8
	0.1	0.6	8.9	9.8	0.9
	1	6.3	13.7	14.7	1.0

^a Irrigation water from composite samples (50 subsamples of 25 g each) tested after 3 days of sprouting but before the entire batch of 36.3 kg of seeds was placed into production.

^b Irrigation water from 36.3 kg of the batch tested after 3 days of sprouting.

at low levels; at a prevalence of 0.0001, the percentage of contaminated batches was reduced from 13.7 to 0.1%. Where contamination levels were higher (100 to 5,000 CFU/g), the disinfection step proved less effective in producing pathogen-free sprouts (8% of batches were contaminated when contamination was most sporadic). At a prevalence of 1, the percentage of contaminated batches was reduced from 100 to 14.7%. Where the initial contamination was high and uniform (prevalence, 1), the proportion of contaminated batches was reduced only from 100 to 87.7%.

Effectiveness of preproduction sampling. Table 3 also shows that microbial sampling of a small portion of seeds (in this case, a composite sample composed of 50 25-g subsamples) by sprouting and testing irrigation water prior to sprouting for production was effective only for untreated seeds where pathogen prevalence was uniform (i.e., 1). When seeds were used without disinfection, no contamination was detected prior to sprouting at prevalences of 0.0001 and 0.001, despite 81% of the batches actually being contaminated. As prevalence or uniformity of contamination increased, the efficacy of preproduction sampling increased. At a prevalence of 0.01 (when all batches were in fact contaminated), preproduction sampling was able to detect only 0.4 and 0.5% of the contaminated batches at high and low contamination levels, respectively.

When the results in Table 3 are compared to those in Table 2, it is clear that decreasing the number of subsamples (from 160 to 50) and raising the detection limit (from 1 to 2 log₁₀ CFU/g) had only a slight negative impact on the already low effectiveness of preproduction sampling.

Effectiveness of postproduction sampling. Postproduction sampling was more effective than preproduction sampling (Table 3). At high contamination levels (100 to 5,000 CFU/g) when seeds were untreated, all contaminated batches were detected by sampling irrigation water on the third day of sprouting, regardless of the uniformity of contamination. At lower contamination levels (1 to 5 CFU/g), detection was good, although not perfect. At a prevalence of 0.001, greater than 8% of contaminated batches remained undetected. In all other cases, however, less than 2% of contaminated sprouts went undetected by postproduction sampling. When seeds were disinfected prior to sprouting, post production detection was similarly effective, regardless of initial contamination level. At high contamination levels, fewer than 5% of contaminated batches went undetected in all cases. When initial contamination was low, at most 1% of contaminated batches were undetected. At the lowest contamination level and prevalence, postproduction sampling detected all positive batches (0.1%).

Figure 2 shows the influence of the number of subsamples on the probability of detection of contaminated sprouts where 1 out of every 10 samples of 25 g was positive at a level that varied uniformly between 1 and 5 CFU/g. Batches of sprouts were made from 36.3 kg of seed, preproduction samples were composed of 160 subsamples of 25 g each, and the pathogen detection limit was 1 log₁₀ CFU/g. If the sample is composed of a small number of subsamples (e.g., 1 to 10), the chance of detecting any pathogens present is very low (less than 10%). As the number of subsamples increases, the chance of detecting any pathogens present goes up, such that when 100 subsamples

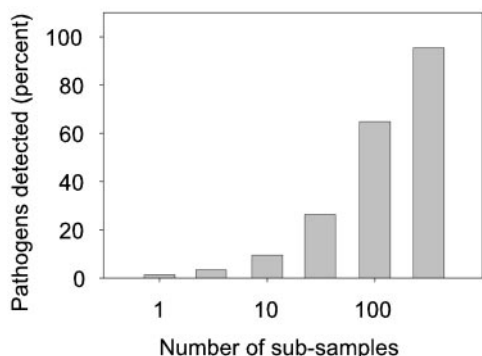


FIG. 2. Influence of the number of subsamples on the probability of detection of contaminated sprouts where (i) 1 out of every 10 samples of 25 g was positive at levels that varied uniformly between 1 and 5 CFU/g, (ii) batches of sprouts were made from 36.3 kg of seed, (iii) preproduction samples were composed of 160 subsamples of 25 g each, and (iv) the pathogen detection limit was 1 log₁₀ CFU/g.

are used, the chance of detecting pathogens is greater than 60%, and when more than 300 subsamples are used, the probability is very close to 100%.

Figure 3 shows the influence of the detection limit on the probability of detection of contaminated sprouts. In this case, 1 out of every 100 samples of 25 g was positive at a level that varied uniformly between 1 and 5 CFU/g. Batches of sprouts were made from 36.3 kg of seed, and preproduction samples were composed of 160 subsamples of 25 g each. When the detection limit is low (i.e., 1 to 10 organisms per ml of water), the probability of detecting any pathogens present is very close to 100%. As the detection limit rises, the chance of detection falls, such that at very high detection limits, like 6 log₁₀ CFU/ml, the chance of detecting any pathogens present is less than 20%.

DISCUSSION

Prevalence and concentration. Epidemiological investigations of sprout outbreaks have provided some insight into uniformity and level of contamination on seeds. As part of a traceback investigation of an *E. coli* O157:H7 outbreak in 1997, 500 g of implicated seeds was tested, and another 500 g was sprouted and tested (from same 50-lb bag). Neither yielded a positive result for *E. coli*, but the 50-lb bag was less than 0.003% of the implicated seed lot (3). These findings support our result that preproduction sampling of seeds is unlikely to yield positive samples, except when contamination is quite uniform throughout the lot.

In contrast to these results, a traceback investigation of a *Salmonella* serovar Kottbus outbreak in 2001 was able to isolate the pathogen from ungerminated seeds, although methods were not reported, so further comparison with our results is not possible (58). Similarly, experiments utilizing naturally contaminated seeds from a *S. enterica* serovar Mbandaka outbreak were able to isolate the pathogen from 67% of 100-g samples by conventional methods and from 72% of samples by molecular methods (50). These results may suggest that natural contamination of seeds in some cases can be fairly uniform.

Testing of three different lots of seeds implicated in three

different outbreaks showed a wide range in pathogen prevalence (20): in seeds from the first outbreak, 10% of 30 samples of 100 g each were positive for *Salmonella*, in seeds from the second outbreak, 33% of 30 samples of 100 g each were positive for *Salmonella*, while in seeds from the last outbreak, 90% of 30 samples of 100 g each were positive for *Salmonella*. The most probable numbers of *Salmonella* organisms present were estimated to be 0.07, 0.36, and 1.8 CFU/100 g, respectively. The sample size here (100 g) is larger than for the calculations shown in Table 2 (25 g), but the pathogen concentrations and prevalences were similar. Rerunning the simulation using the data from the study of Inami et al. (20) produces nearly identical results when no disinfection is used (data not shown). When disinfection is assumed, the total fraction of positive batches simulated from seeds contaminated with the level found in the work of Inami et al. (20) is lower than in Table 2 but is not zero, and the simulated results for the detection of pathogens in irrigation water finds a similar fraction of positive batches (data not shown).

Effect of seed treatment. Our results show that although disinfection was able to reduce the proportion of contaminated batches, in no case did it completely eliminate contaminated batches. This result is also supported by the epidemiological literature. In 1999, a multistate outbreak of *Salmonella* in the Midwest was caused by seeds that had been treated for 15 min with 2% calcium hypochlorite. *Salmonella* was recovered from only 15% of samples collected from implicated grocery stores and restaurants (35). An outbreak of *S. enterica* serovar Typhimurium in Colorado was traced to seeds used by two sprouters. Most practices were comparable in the two operations, except one sprouter soaked seeds in 20,000 ppm chlorine for 20 min prior to sprouting. For every one case attributed to this sprouter, there were four cases from sprouts from untreated seeds (4). Last, a *Salmonella* serovar Kottbus outbreak in 2001 was also linked to seeds that were disinfected. One method of disinfection involved heating seeds, cooling them with well water, and then exposing them to a 2,000 ppm sodium hypochlorite soak for 15 min. Alternatively, seeds were treated with 20,000 ppm chlorine for 15 min, although records could not verify whether the concentration was actually 20,000 ppm (58).

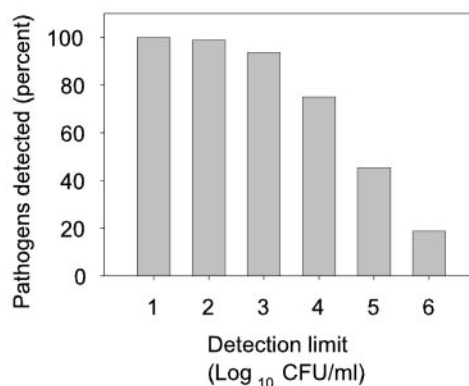


FIG. 3. Influence of detection limit on the probability of detection of contaminated sprouts where (i) 1 out of every 100 samples of 25 g was positive at a level that varied uniformly between 1 and 5 CFU/g, (ii) batches of sprouts were made from 36.3 kg of seed, and (iii) preproduction samples were composed of 160 subsamples of 25 g each.

All these epidemiological findings support our result that disinfected seeds can produce contaminated sprouts, although not all batches of sprouts will contain pathogens.

It should also be noted that the effectiveness of treatment with 20,000 ppm chlorine (modeled by a triangular distribution with a minimum of $1 \log_{10}$ CFU/ml, a mode of $2.5 \log_{10}$ CFU/ml, and a maximum of $6.5 \log_{10}$ CFU/ml, based on our analysis of the literature data [31]), is not consistent with published data on the effectiveness of such a treatment on naturally contaminated seeds. When the simulation parameters were adjusted to try to match the results reported by Stewart et al. (49), the resulting effectiveness showed minimum values around $0 \log_{10}$ CFU/ml (i.e., no log reduction at all) to maximum values of only $1 \log_{10}$ CFU/ml or less.

Sampling. Although research has shown there to be some difference in the levels of efficacy of various sampling methods, our results shows that one of the most important variables in any sampling program may be the number of subsamples taken from a batch to make up the test sample. The detection limit of sampling may also affect the value of sampling results. In our simulation, when the detection limit was lowered to $1 \log_{10}$ CFU/g, postproduction sampling rates improved. Additionally, the example presented above (Fig. 3) shows that methods that are able to detect pathogens only at high levels may be of limited effectiveness (24, 47).

Conclusions. Seed disinfection is a highly variable process, with no method yet proven to completely eliminate pathogens. In our simulation, seed disinfection alone was unable to guarantee safe sprouts. Seed disinfection can reduce the percentage of contaminated batches, particularly when prevalence and concentration are low, but it can also reduce the likelihood of detecting positive batches with postproduction sampling. Because of this finding, we wish to stress that having data on the prevalence and concentration of pathogens in implicated seed lots is imperative to truly evaluate the effectiveness of seed disinfection. Data on the effect of disinfection on naturally contaminated production-sized batches are also needed. While testing seeds prior to production is mostly ineffective, postproduction testing of sprouts or irrigation water appears to have a very high likelihood of detecting contaminated batches of sprouts. We hope that our simulation can be used to guide future research on safe sprout production. The Analytica file described in this research is available online at <http://foodsci.rutgers.edu/schaffner/files.htm>.

ACKNOWLEDGMENT

We thank Bob Sanderson, President of Jonathan Sprouts, Inc., for his passion for the sprout business and for inspiring this research.

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