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January 13, 2001

Food & Drug Administration
Dockets Management Branch (HFA - 305)
5630 Fishers Lane, ; m.1061
Rockville MD 20852
USA

Re: Docket # 99D-4488 & 99D-4489

To whom it might concern,

In December 1999, we have submitted already comments regarding the Guidance for the industry "Reducing 'Microbial Food Safety Hazards for Sprouted Seeds".
Our company -with over 25 years experience in developing equipment for the sprouting industry - has been participating actively in the research to find a practical solution to prevent food poisonings caused by of E-coli and Salmonella on sprouted products. We are based in Japan.

During the last year we have collected again valuable results, which we would like to share and we hope that these facts will be also taken into consideration .

A detailed description of the test and the results are enclosed. Some comments:

- The testing was done at a factory in operation, Fuji Natural Foods in Ontario CA.
- Beside the testing, our Automatic Seed Pasteurizing System was in operation and the performance was monitored by following the Guideline for "Sampling Microbial Testing of Spent Irrigation Water During Sprout Production"
- During this tests we were comparing different Heat Treatment Patterns snd the recommended 20'000ppm Calcium Hypo Chlorite Treatment.
- We have tried to simulate a '"worst case scenario" and included many variabjes - such as different ways of inoculation on seed of different origin.
- We strongly believe that these results have highest value, even if we did not achieve the results (in terms of log reduction) we were hoping for. This applies also to the 20'000ppm Calcium Hypo Chlorite treatment, which in fact produced results in all cases far below average.

Seed Treatment Trials on Alfalafa Seed used for Sprouting

At:

Fuji Natural Foods, Ontario CA

Daisey Machinery Co Ltd, Japan
Together with
National Center for Food Safety and Technology (NCFST)
Summit – Argo, Illinois

June 2000

Seed Treatment Trials at Fuji Natural Foods, Ontario

June 2000

NCFST

Daisey Machinery Co Ltd

Seed Treatment Trials at Fuji Natural Foods, Ontario

June2000

NCFST
Daisey Machinery Co Ltd

INTRODUCTION

The purpose of this test series is to

1. Verify the results from lab trials at an actual Factory producing Alfalfa Sprouts,
2. compare the Seed Pasteurizing Method with the recommended 20'000ppm Calcium Hypochlorite and
3. collect data helping to establish practical recommendations for the sprouts grower to successfully disinfect seed prior sprouting.

Outline of inoculation:

Ec K12 was inoculated to two different seed lots of Australian and US origin. K12 has been chosen because the trial were done at an actual factory.

Because the trails were not done on naturally contaminated seeds, two different inoculation methods were chosen.

a) SPRAY INOCULATION simulating mainly surface contamination and

b) SOAK INOCULATION simulating deeper reaching contamination.

In addition the damage rate of the seed was higher than usual, which makes a successful decontamination even more difficult.

Sampling and Testing

- Seed samples to be analyzed were taken immediately after treatment, packed and chilled.
- The plating was done on the same day.
- Irrigation water samples were taken after 47hrs, chilled and tested the same'day.
- For further details please refer to the "NCFST Project Proposal" from May 26, 2000" by Dr Peter Slade on the following page

Other details:

- The test were done between June 5th and June 9th, 2000
- Seeds were taken from lots used for sprouting at Fuji Natural Foods, Ontario CA in early 2000
- Inoculation was done at National Center for Food Safety and Technology (NCFST), Summit-Argo, Illinois.
- The samples were sanitized at Fuji Natural Foods. Heat Treated Seeds were

processed using Daisey Machinery's Automatic Seed Pasteurizing System SPS100, supervised by Daisey Machinery Co Ltd Staff.

- The samples were analyzed by Silliker in Los Angeles
- Samples were grown at Fuji Natural Foods

NCFST Project Proposal
May 26, 2000

Title: *Seed Pasteurizing System – Calcium Hypochlorite Comparison Test*

Sponsor: *National Center for Food Safety and Technology*

Goal: *To assess the effect of a proprietary heat pasteurization process in comparison to treatment with 2% calcium hypochlorite solution on populations of a non-pathogenic, surrogate strain of Escherichia coli with a streptomycin resistance marker (E.coli K12), inoculated into alfalfa seed.*

Rationale: *Exploration of concept feasibility.*

Experimental:

1. *Inoculation of Seeds*

Two lots of seeds (one Australian and one U.S.) will be inoculated at NCFST using two different methods (soaked and sprayed) to give a population of about 10^6/g of seed.

2. *Proprietary Heat Pasteurization Treatment*

Will be performed by NCFST client, at their facility in the LA area. NCFST will advise on cleanup and sanitation of processing environment.

3. *Testing by Independent Test Lab*

The test lab sub-contracted by NCFST will perform all microbiological analyses as follows:

a. *Media*

Brain Heart Infusion Agar (BHIA) with streptomycin (prepared and delivered to test lab by NCFST)
MacConkey Agar (MAC) with streptomycin (prepared and delivered to test lab by NCFST)
Buffered Peptone Water (BPW) diluent

b. Seed Samples (total 28 seed samples)

US seed: 6 x 100g samples of treated soak-inoculated seed (sample # 1-6)
1 x 100g sample untreated soak-inoculated control (sample # 7)

6 x 100g samples of treated spray-inoculated seed (sample # 8-13)
1 x 100g sample untreated spray-inoculated control (sample # 14)

Australian seed: 6 x 100g samples of treated soak-inoculated seed (sample # 15-20)
1 x 100g sample untreated soak-inoculated control (sample # 21)

6 x 100g samples of treated spray-inoculated seed (sample # 22-27)
1 x 100g sample untreated spray-inoculated control (sample # 28)

c. Dilution of seed samples

A $1/10^1$ dilution will be prepared by stomaching 25g of sample with 225 ml BPW for one (1) minute. Further decimal dilutions (to $1/10^3$) will be prepared by adding 1 ml to 9ml of dilution blank.

d. Spread plating

For all preparations of treated seeds the following dilutions will be pipeted and spread on the surface of both BHIA, and MAC:

0.2 ml of $1/10^1$ dilution to five plates each BHIA and MAC
0.1 ml of $1/10^2$ dilution to two plates each BHIA and MAC
0.1 ml of $1/10^3$ dilution to two plates each BHIA and MAC

For controls preparations 0.1 ml of each of the above dilutions will be pipeted and spread on the surface of just two plates both BHIA, and MAC.

e. Sprout Irrigation Water Samples - 48h Sprout Growth (total 28 water samples)

After 48 hours sprout growth, 28 x 100 ml samples of spent irrigation water will be sent to the lab for testing. Decimal dilutions (to $1/10^6$) will be prepared by adding 1 ml to 9ml of dilution blank.

f. Spread plating

For all preparations of spent irrigation water the following dilutions will be pipeted and spread on the surface of MAC only:

0.1 ml of $1/10^4$ dilution to two plates MAC
0.1 ml of $1/10^5$ dilution to two plates MAC
0.1 ml of $1/10^6$ dilution to two plates MAC

g. Incubation and Colony Counts

All inoculated plates will be incubated at 35-37ºC for 24-48h. All colonies on plates will be counted and reported as raw data to NCFST.

Seed Treatment Trials at Fuji Natural Foods, Ontario

June2000

NCFST
Daisey Machinery Co Ltd

OUTLINE OF TEST AND CODING OF SAMPLES

Seed Pasteurizing System / Calcium Hypochlorite Comparison Test

(please refer also to the NCFST Project Proposal)

Seed used: US and Australian

Inoculation: Done at NCFST, using K12

Two inoculation methods: spray and dip

Presoaking: (if applied) 2 Minutes at about 20° C

Sample Size: 2 x 100g (good size to be cultivated in trays), one sample for cultivation, one sample for Micro and other tests.

Treatments:

SEED #	INOCULATION METHOD	TREATMENT	PATTERN
us1	DIP	PRESOAK	80oC X 9 sec
us 2	DIP	PRESOAK	850C X 9 sec
us 3	DIP		80oC X 9 sec
us 4	DIP		83oc X 9 sec
us 5	DIP		850C X 9 sec
US 6	DIP	CHC	20'000ppm
US CO DIP	**DIP**		
US 7	SPRAY	PRESOAK	80oC X 9 sec
US 8	SPRAY	PRESOAK	850C X 9 sec
us 9	SPRAY		80oC X 9 sec
us 10	SPRAY		83oc X 9 sec
us 11	SPRAY		850C X 9 sec
us 12	SPRAY	CHC	20'000ppm
US COSPRAY	**SPRAY**		

AU 1	DIP	PRESOAK	80oC X 9 sec
AU 2	DIP	PRESOAK	850C X 9 sec
AU 3	DIP		80oC X 9 sec
AU 4	DIP		83oc X 9 sec
AU 5	DIP		85oC X 9 sec
AU 6	DIP	CHC	20'000ppm
AU CO DIP	**DIP**		
AU 7	SPRAY	PRESOAK	80oC X 9 sec
AU 8	SPRAY	PRESOAK	850C X 9 sec
AU 9	SPRAY		80oC X 9 sec
AU 10	SPRAY		83oc X 9 sec
AU 11	SPRAY		850C X 9 sec
AU 12	SPRAY	CHC	20'000oom
AU CO SPRAY	**SPRAY**		

CHC = Calcium Hypochlorite

Seed Treatment Trials at Fuji Natural Foods, Ontario

June2000

NCFST

Daisey Machinery Co Ltd

TOTAL SAMPLES:	28
Testing:	by NCFST recommended Lab in Los Angeles (Silliker)
Testing of:	1) Can inoculated organism be recovered after treatment
	2) Testing seed after treatment and irrigation water
	3) General behavior during growing.
Growing:	in trays at Fuji Natural Foods, separated from other product

EFFECT OF VARIOUS SEED TREATMENT ON GERMINATION

The seed has been inoculated in March 2000 at the NCFST and has been stored there until the tests were done in June. There was about 3 month of storage.
It is very common that seed, which has been inoculated[5] and during this process is immersed or sprayed, has a reduced Germination Rate.
There were partly very high numbers of seeds on the bottom of each tray, which would have a made, it very difficult to determine accurately the germination rate.
In a visual inspection the area covered with ungerminated seed on the bottom of the tray was analyzed. Please refer to pictures of each sample.

Seed from the same lots have been used at Fuji Natural Foods over a period of time. During this time the germination and growth has been closely monitored and the germination was “normal” at around 90%+, which is significantly higher than the rate observed with the inoculated seed samples.

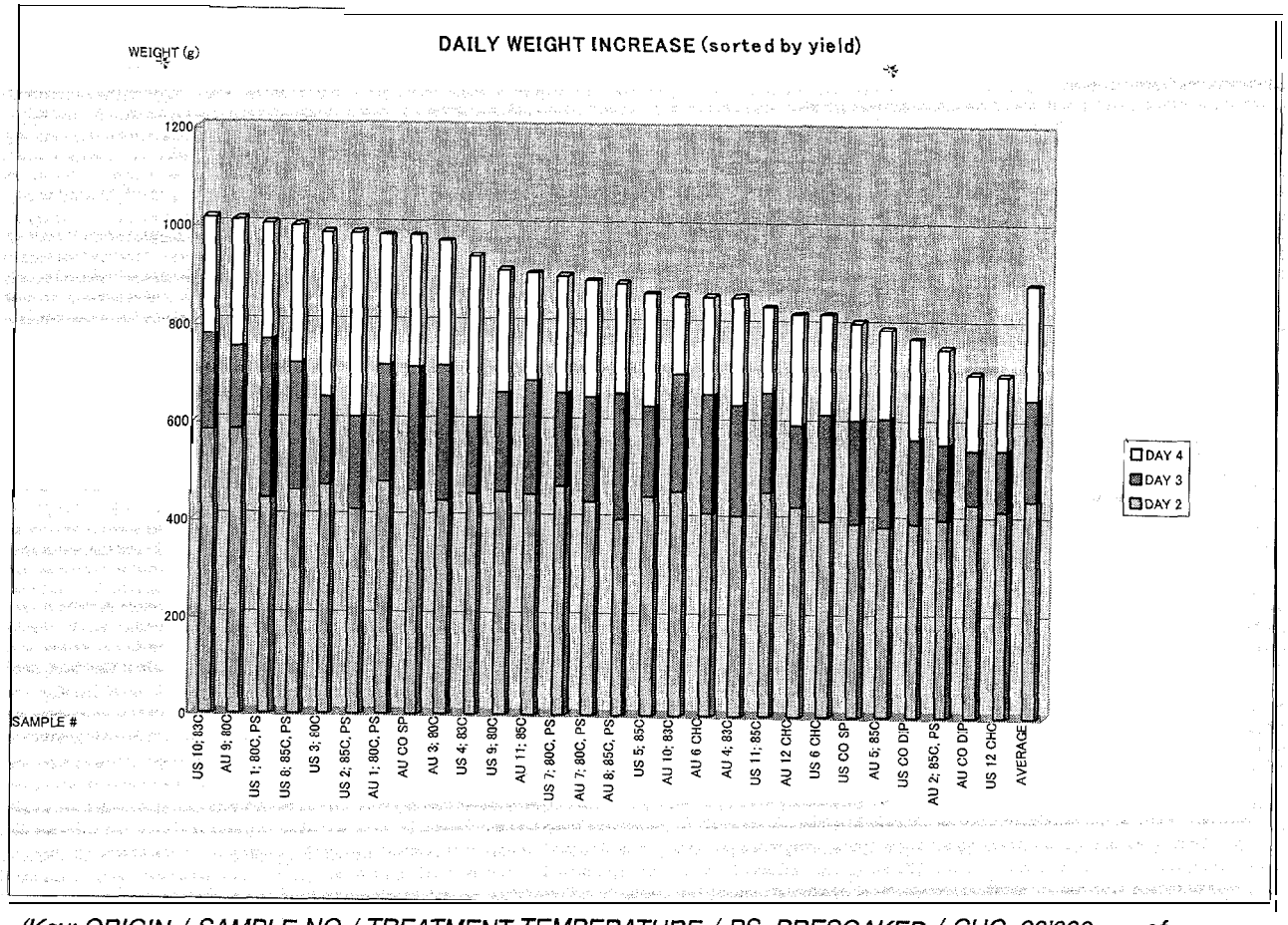
Because the germination rate could not be determined accurately, the growth and yield was analyzed and used to compare the effects of the various treatments. Please refer to the following section.

Seed Treatment Trials at Fuji Natural Foods, Ontario

June2000

NCFST
Daisey Machinery Co Ltd

EFFECT OF VARIOUS SEED TREATMENT ON GROWTH AND YIELD



(Key: ORIGIN / SAMPLE NO / TREATMENT TEMPERATURE / PS=PRESOAKED / CHC=20'000ppm of Calcium Hypochlorite / CO= Control / SP= Spray Inoculated / DIP= Soak Inoculated)

Comments & Analyzes (please refer to the above graph)

Seed of different origin:

Looking at the samples, which yielded, 'more than average, there was almost an equal number of samples of each origin (USA and Australia) above average (8 US Samples & 7 Australian Samples).

Impact of Presoaking on Growth & Yield:

All but one samples which "was presoaked (for 2min) before treatment yielded below

average. Most of the presoaked samples yielded higher than average.

In the early stage (day 2), a reduced growth of the presoaked samples was observed, but the differences disappeared during the following days (day 3 & 4)

impact of Treatment Temperature on Growth & Yield:

There was no sign that lower treatment temperatures resulted in better yields.

Impact of 20'000 Calcium Hypochlorite treatment on Growth & Weld:

All of the four Calcium Hypochlorite (CHC) Samples yielded below average. Slow progress was also observed on day 2 & 3.

Control Samples:

It was quite surprising that the Control Samples (CO) did not grow as healthy compared with the others. 3 out of 4 samples have yielded quite significantly below average.

Others:

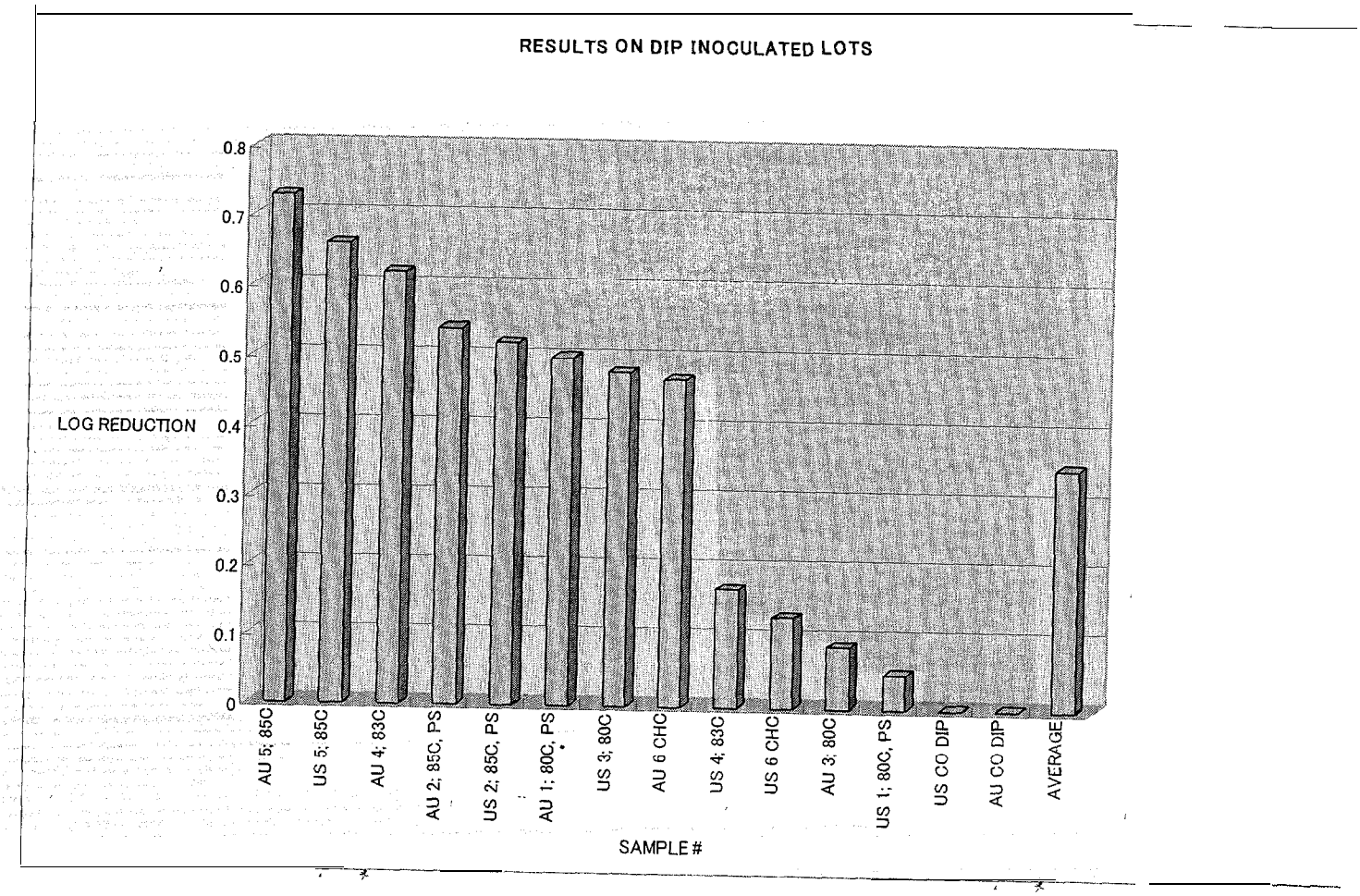
Samples, which were not treated with hot water (Control and Calcium Hypochlorite Samples) didn't grow as healthy and yielded less than average. This seems to indicate that a short hot water treatment (seed pasteurizing) stimulates the growth.

RESULTS OF DIFFERENT TREATMENTS

Please refer also to the attached report from Dr Peter Slade, National Center for Foods Safety and Technology (NCFST Project No. 000602).

SOAK INOCULATED SAMPLES

The Soak Inoculated Samples were most difficult to disinfect, regardless of the origin of the seed. Accordingly the Log Reduction was in average only about 0.4 log, but with quite some differences between the various treatments (please refer to graph below).



(Key: ORIGIN / SAMPLE NO / TREATMENT TEMPERATURE / PS=PRESOAKED / CHC=20'000ppm of Calcium Hypochlorite / CO= Control / SP= Spray Inoculated / DIP= Soak Inoculated)

Comments & Analyzes (please refer to the above graph)

The total in Log Reduction and the differences between the different samples are quite small, but there is a pattern appearing which allows to compare the different treatments.

Seed of different origin:

The results of disinfection appears very similar with both origins of seed.

Impact of Treatment Temperature on Seed Disinfection:

It appears quite clearly that the high temperature treated samples (85 Celsius) showed the best results of disinfection.

impact of Presoaking on Seed Disinfection:

Presoaking resulted in 3 out of 4 cases in higher than average reduction

Impact of 20'000 Calcuim Hypochlorite treatment on Seed Disinfection:

One of the samples treated with 20'000ppm Calcium Hyochlorite resulted in above average disinfection, the second sample quite clearly below.

Others:

It can be concluded that the Seed Pasteurizing is in all the cases more, or at least as effective as the 20'000ppm Calcium Hypochlorite treatment

SPRAY INOCULATED SAMPLES

As expected, the Spray Inoculated seeds samples resulted in a higher log reduction (please refer to the report from Dr Peter Slade, NCFST Project No. 000602).

All samples except the two treated with 20'000ppm Calcium Hypochlorite (US12 & AU12) had <10 cfu / a Survivina Ec K12, resulting in a log reduction >2.

Compared with the heat-treated samples, there was surprisingly a substantial number of survivors and injured survivors on the two samples disinfected by using 20'000ppm Calcium Hypochlorite.

GENERAL COMMENTS

1. Inoculated seed has been used to demonstrate the effectiveness of the various treatments. The samples can be classified as “difficult do disinfect” due to the high damage rate and quite extended storage period after inoculation.
2. The disinfection rate is not very high (in terms of log reduction) on all treatments, with a clear difference between Spray Inoculated (mainly surface contamination), and Soak Inoculated samples.
3. As in other tests, again we were observing that the recommended 20’000ppm of Calcium Hypochlorite Treatment did not result in a high log reduction at all (such as 3 logs or more).
4. Further more in our tests, the Calcium Hypochlorite samples (4 samples of 2x100g each totaling 800g) have been treated in a quantity of liquid suitable to disinfect 25kg (50lb) of seeds. There must have been more free Chlorin available during the entire treatment period than under production conditions.
5. For results and comments about Irrigation Water test please refer to the report from Dr Peter Slade, NCFST Project No. 000602.

Please refer also to the following

ATTACHMENTS:

- NCFST Project Proposal, Title: Seed Pasteurizing System – Calcium Hypochlorite Comparison Test, Sponsor: National Center for Food Safety and Technology
- Report from Dr Peter Slade, National Center for Foods Safety and Technology (NCFST Project No. 000602).
- Raw data from Siliker Laboratories

NCFST Project No. 000602

June 20, 2000

Results: Seed Pasteurizing System – Calcium Hypochlorite Comparison Test

1. U.S. Seed

Sample #	Total Surviving *Ec* K12 (cfu/g)	Injured Surviving *Ec* K12 (cfu/g)	% Injured	% Total Survivors from Initial	Log Reduction
US-1	1536	998	65	90	0.05
US-2	508	376	74	30	0.52
US-3	558	488	87	33	0.48
US-4	1140	850	75	67	0.17
US-5	374	320	86	22	0.66
US-6	1700	0 (?)	0 (?)	73	0.13
US-CO-DIP		N/A	N/A	100 (Initial)	N/A
US-7	<10	<10	N/A	N/A	>2.00
US-8	<10	<10	N/A	N/A	>2.00
US-9	<10	<10	N/A	N/A	>2.00
US-10	<10	<10	N/A	N/A	>2.00
US-11	<10	<10	N/A	N/A	>2.00
US-12	664	130	N/A	N/A	0.27
US-CO SPR	1240	N/A	N/A	100 (Initial)	N/A

2. Australian Seed

Sample #	Total Surviving Ec K12 (cfu/g)	Injured Surviving *Ec* K12 (cfu/g)	% Injured	% Total Survivors from Initial	Log Reduction
AU-1	804	712	88	31	0.50
AU-2	748	672	90	29	0.54
AU-3	2104	1736	83	81	0.09
AU-4	618	460	74	24	0.62
AU-5	478	330	69	18	0.73
AU-6	872	724	83	34	0.47
AU-CO DIP	2590	N/A	N/A	100 (Initial)	N/A
AU-7	<10	<10	N/A	N/A	>2.00
AU-8	<10	<10	N/A	N/A	>2.00
AU-9	<10	<10	N/A	N/A	>2.00
AU-10	<10	<10	N/A	N/A	>2.00
AU-11	<10	<10	N/A	N/A	>2.00
AU-12	398	50	N/A	N/A	0.80
AU-CO SPR	2500	N/A	N/A	100 (Initial)	N/A

3. Sprout Irrigation Waters – 48h Sprout Growth

Sample #	Total *Ec* K12 (cfu/ml)
US-1	$< 10^4$
US-2	$< 10^4$
US-3	10^4
US-4	10^4
US-5	10^4
US-6	$<10^4$
US-CO DIP	5×10^3
US-7	$<10^4$
US-8	$<10^4$
US-9	$<10^4$
US-10	$<10^4$
US-11	$<10^4$
US-12	$<10^4$
US-CO SPR	5×10^3

Sample #	Total *Ec* K12 (cfu/ml)
AU-1	10^4
AU-2	10^4
AU-3	2×10^4
AU-4	10^4
AU-5	10^4
AU-6	1.5×10^4
AU-CO DIP	4×10^4
AU-7	$<10^4$
AU-8	$<10^4$
AU-9	$<10^4$
AU-10	$<10^4$
AU-11	$<10^4$
AU-12	$<10^4$
AU-CO SPR	2.5×10^4

Summary:

1. U.S. Seed

A substantial number of *E. coli* K12 in the soak-inoculated (dip) seed survived all treatments. There was no discernible difference between various pasteurization treatments; all appeared equally as effective as treatment with 2% calcium hypochlorite. The percentage of survivors ranged from 22 to 90%. This equated to log reductions from

0.66 to 0.05 logs, respectively. On average, 53% of inoculated *E. coli* survived the treatments, equating to an average reduction of about 0.34 logs. Moreover, a large proportion of total survivors appeared to by sublethally injured, as determined by their inability to grow on the selective medium, ranging from 65 to 87% of the total recoverable population. On average 77% of the recoverable population was injured.

The populations of *E. coli* on the spray-inoculated seeds were more readily reduced by all intervention treatments. Again, there was no discernible difference between various pasteurization treatments; all appeared equally as effective as treatment with 2% calcium hypochlorite. Greater than 2-log reductions were observed in all cases.

2. Australian Seed

As with the U.S. seed, a substantial number of *E. coli* K12 in the soak- inoculated (dip) seed survived all treatments. There was no discernible difference between various pasteurization treatments; all appeared equally as effective as treatment with 2% calcium hypochlorite. The percentage of survivors ranged from 18 to 81%. This equated to log reductions from 0.73 to 0.09 logs, respectively. On average, 35% of inoculated *E. coli* survived the treatments, equating to an average reduction of about 0.49 logs. Moreover, a large proportion of total survivors appeared to by sublethally injured, as determined by their inability to grow on the selective medium, ranging from 69 to 90% of the total recoverable population. On average 81% of the recoverable population was injured.

The populations of *E. coli* on the spray-inoculated seeds were more readily reduced by all intervention treatments. Again, there was no discernible difference between various pasteurization treatments; all appeared equally as effective as treatment with 2% calcium hypochlorite. Greater than 2-log reductions were observed in all cases.

3. Sprout Irrigation Waters – 48h Sprout Growth

In all but two instances, for both U.S. and Australian seed, in soak-inoculated (dip) seed samples, wherever survivors were detected after initial treatments (and in controls) the counts in irrigation water after 48h were equivalent to or greater than 10^4 cfu/ml. For spray-inoculated seed, both U.S. and Australian, where survivors were determined to be less than 10 cfu/g of seed, all populations were less than 10^4 cfu/ml of irrigation water after 48h.

Discussion:

All pasteurization treatments appeared equally as effective as treatment with 2% calcium hypochlorite. However, it was determined that a large proportion of the surviving population in soak-inoculated (dip) seed was sub-lethally injured, and would not be detected as such if, as often is the case, normal selective culture techniques are employed. These organisms have the capability to repair and become fully viable contaminants of the seeds, post-treatment. It is essential that consideration be given to the potential presence of sub-lethally injured survivors in work using heat and/or chemical

intervention treatments applied to seeds. Non-selective enrichment techniques are routinely used prior to selective culture to take into account the possibility for survival of injured bacteria.

Seeds inoculated by spraying were more readily decontaminated by all treatments applied in this study. It is not known how seeds are contaminated in nature. It is possible that contamination could either be on the surface (as represented by spraying) or deep within the seed (as represented by soaking), or at points in between. It is therefore essential that comparative studies compare the worst-case seed contamination scenario (i.e., deep-soaking), and not just the less stringent application of the inoculum (i.e., by surface spraying). As evidenced by the grow out of survivors as tested in the irrigation water after 48h, in seeds where a substantial population survives the intervention treatment (10^2-10^3 cfu/g), the populations in the water (and by inference, on the sprouts) will equal or exceed 10^4/ml after 48h. In the spray-inoculated seeds, where populations were more dramatically reduced (by more than 2-logs), the detectable populations in irrigation waters were consistently less than 10^4/ml after 48h.

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Assuring Quality Worldwide

Sprout Irrigation Water Samples – 48h Sprout Growth

Results reported as cfu/plate

Sample #	Media	1/10¹	1/10²	1/10³	1/10⁴	1/10⁵	1/10⁶
US-1	MAC	0	0	0	0	0	0
US-2	MAC	0	0	0	0	0	0
US-3	MAC	1	1	0	0	0	0
US-4	MAC	1	1	0	0	0	0
US-5	MAC	0	2	0	0	0	0
US-6	MAC	0	0	0	0	0	0
US-CO DIP	MAC	1	0	1	0	0	0
US-7	MAC	0	0	0	0	0	0
US-8	MAC	0	0	0	0	0	0
US-9	MAC	0	0	0	0	0	0
US-10	MAC	0	0	0	0	0	0
US-11	MAC	0	0	0	0	0	0
US-12	MAC	0	0	0	0	0	0
US-CO SPRAY	MAC	0	1	0	0	0	0

Sample #	Media	1/10¹	1/10²	1/10³	1/10⁴	1/10⁵	1/10⁶
AU-1	MAC	2	0	0	0	0	0
AU-2	MAC	1	1	0	0	0	0
AU-3	MAC	4	0	0	0	0	0
AU-4	MAC	1	0	0	0	0	0
AU-5	MAC	1	1	0	0	0	0
AU-6	MAC	2	1	1	0	0	0
AU-CO DIP	MAC	2	6	1	0	0	0
AU-7	MAC	0	0	0	0	0	0
AU-8	MAC	0	0	0	0	0	0
AU-9	MAC	0	0	0	0	0	0
AU-10	MAC	0	0	0	0	0	0
AU-11	MAC	0	0	0	0	0	0
AU-12	MAC	0	0	0	0	0	0
AU-CO SPRAY	MAC	3	2	0	0	0	0

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Assuring Quality Worldwide

AU Seed samples

Results reported as cfu/plate

Sample#	Media	1/10¹	1/10²	1/10³	1/10⁴	1/10⁵	1/10¹	1/10²	1/10³	1/10⁴
AU-1	BHIA	84	84	76	92	66	5	11	0	0
	MAC	33	4	3	3	3	0	0	0	0
AU-2	BHIA	88	82	80	70	54	5	3	0	3
	MAC	10	5	9	8	6	0	1	0	0
AU-3	BHIA	240	172	200	280	160	4	6	2	1
	MAC	63	52	24	21	24	0	1	0	0
AU-4	BHIA	81	34	80	80	34	6	3	0	0
	MAC	32	17	13	7	10	0	1	0	0
AU-5	BHIA	53	62	46	44	34	6	1	0	0
	MAC	30	13	10	11	10	0	0	0	0
AU-6	BHIA	120	64	88	82	82	1	2	2	1
	MAC	22	18	13	5	16	0	0	0	0
AU-CO DIP	BHIA	198	320 EST.	---	---	---	19	31	2	8
	MAC	90	100	---	---	---	1	2	0	0
AU-7	BHIA	1	0	1	0	0	0	0	0	0
	MAC	0	0	0	0	0	0	0	0	0
AU-8	BHIA	1	2	0	0	6	0	0	0	0
	MAC	0	0	0	0	0	0	0	0	0
AU-9	BHIA	1	0	2	1	0	0	0	0	0
	MAC	0	0	0	0	0	0	0	0	0
AU-10	BHIA	0	0	0	0	0	0	0	0	0
	MAC	0	0	0	0	0	0	0	0	0
AU-11	BHIA	0	0	0	0	0	0	0	0	0
	MAC	0	0	0	0	0	0	0	0	0
AU-12	BHIA	22	42	51	44	40	1	0	0	0
	MAC	9	4	6	3	3	0	0	0	0
AU-CO SPRAY	BHIA	240	260	---	---	---	70	50	19	4
	MAC	67	34	---	---	---	7	1	0	0

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SILLIKER
Assuring Quality Worldwide

US Seed samples

Results reported as cfu/plate

Sample#	Media	1/10¹	1/10²	1/10³	1/10⁴	1/10⁴	1/10⁵	1/10⁵	1/10⁵	1/10⁵
US-1	BHIA	92	160	200	150	166	13	8	0	0
	MAC	100	60	50	30	29	2	1	0	0
US-2	BHIA	82	32	60	32	48	4	5	3	4
	MAC	24	21	8	4	9	0	0	0	0
US-3	BHIA	56	70	33	54	66	4	4	1	0
	MAC	21	4	3	3	4	0	1	0	0
US-4	BHIA	140	120	120	80	110	23	5	0	0
	MAC	39	17	14	37	38	0	2	0	0
US-5	BHIA	50	40	36	28	33	2	0	0	0
	MAC	11	6	3	5	2	0	0	0	0
US-6	BHIA	100	120	128	144	130	19	37	4	1
	MAC	110	84	106	176	160	2	3	0	2
US-CO DIP	BHIA	200	140	---	---	---	28	25	3	2
	MAC	65	100	---	---	---	0	0	0	0
US-7	BHIA	2	2	0	0	1	0	0	0	0
	MAC	0	0	0	0	0	0	0	0	0
US-8	BHIA	0	1	1	0	1	0	0	0	0
	MAC	0	0	0	0	0	0	0	0	0
US-9	BHIA	8	3	3	5	2	0	0	0	0
	MAC	0	0	0	0	0	0	0	0	0
US-10	BHIA	2	0	1	1	1	0	0	0	0
	MAC	0	0	0	1	0	0	0	0	0
US-11	BHIA	2	4	1	4	1	0	0	0	0
	MAC	0	0	0	0	0	0	0	0	0
US-12	BHIA	100	32	66	54	80	5	5	0	0
	MAC	19	9	17	11	9	0	0	0	0
US-CO SPRAY	BHIA	120	128	---	---	---	73	50	6	6
	MAC	17	12	---	---	---	4	4	0	1