Efficacy of Dry Heat Treatment in Reducing *Salmonella* Contamination on Sprout Seeds

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Abstract

This study evaluated the efficacy of dry heat in reducing *Salmonella* on sprout seeds, as affected by treatment time, temperature, relative humidity, treatment scale and seed type. The impact of treatment on seed germination, sprout yield and *Salmonella* re-growth during sprouting was examined. Ten g of seeds/beans inoculated with *Salmonella* or 1 kg of mung beans spiked with 10% of inoculated beans were subjected to dry-heat treatment in a humidity-controlled chamber. Treated seeds/beans were analyzed for *Salmonella* by plate count and culture enrichment. One hundred alfalfa seeds or 50 beans were germinated in a petri dish and numbers of germinated seeds/beans were recorded for 5 days. Sprout yields were determined after 7 days. Two hundred g of treated beans were sprouted in glass jars for 4 days and levels of *Salmonella* were analyzed daily. A greater log kill was observed when treatment was conducted at higher temperatures, under higher relative humidities (RH), or for longer time. Optimal treatment conditions that reduced *Salmonella* to below detection (< -0.3 log cfu/g) while maintaining germination and sprout yield at > 90% of that of untreated controls were identified. Treatment scale did not affect efficacy as a similar log reduction was achieved whether 10 g or 1 kg of mung beans were treated under optimal conditions. *Salmonella* re-growth was observed during sprouting of treated beans, although could be delayed. Dry-heat treatment can be an effective means in reducing *Salmonella* on sprout seeds, but pathogen could re-grow during sprouting. The delay in pathogen re-growth needs to be considered when conducting microbial testing of sprout production batches.

Introduction

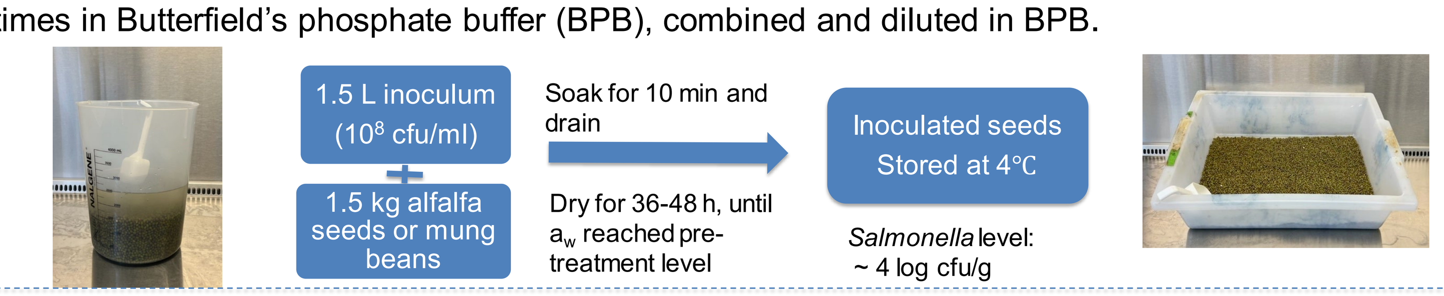
The Produce Safety Rule requires that seeds used to grow sprouts be treated to reduce pathogens using scientifically valid methods (*1*). Treatments may be applied at sprout operations or by seed suppliers. Although chemical treatments are the most studied, their use by sprout growers are limited due to the lack of EPA approved seed treatment chemicals. Physical methods, such as dry heat, have increasingly been evaluated (*2,3,4*). Dry-heat treatments have the added advantages in that they are scalable and can avoid the need for a post-treatment drying step. The efficacy of dry heat for decontamination of seeds differed among published studies. While some studies have reported that dry-heat treatment could reduce populations of *E. coli* O157:H7 or *Salmonella* on seeds by > 5 logs, others showed a much lower efficacy (*3,4*). Many studies did not examine the impact on seed germination or sprout yield. Research is needed to better understand factors that may affect treatment efficacy and to identify conditions that can effectively decontaminate seeds while preserving their germination capability. Research is also needed to examine pathogen re-growth during sprouting of treated seeds to determine the overall impact of treatment by dry heat in reducing microbial contamination in sprouts.

Objectives

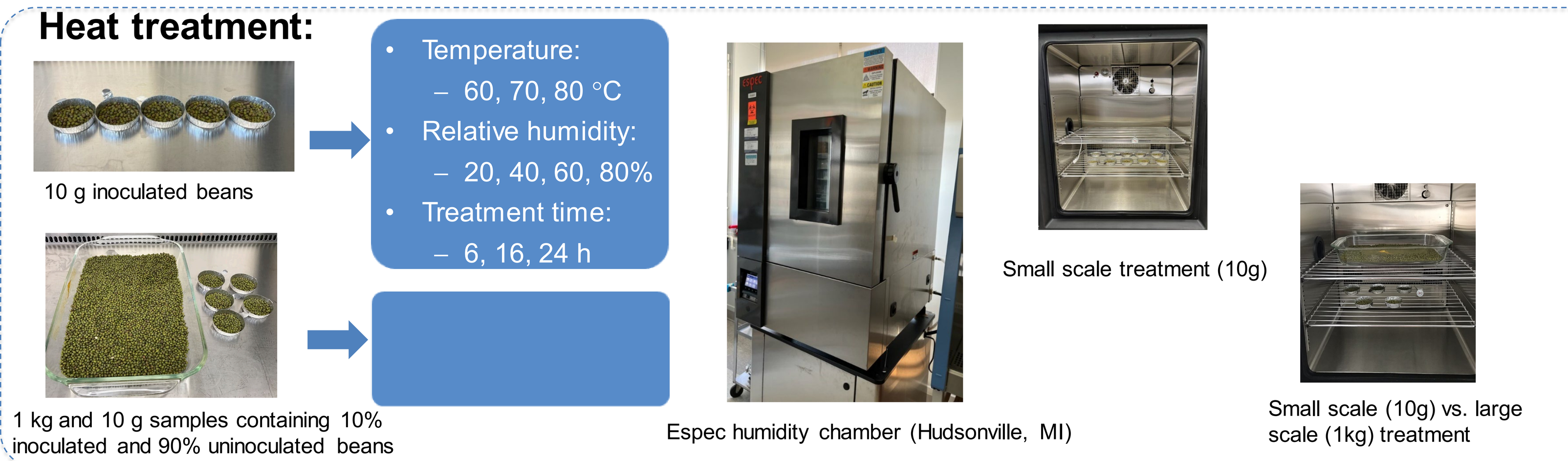
- Determine the efficacy of dry heat treatment in reducing *Salmonella* contamination on alfalfa seeds and mung beans as affected by
  - Temperature
  - Treatment time
  - Relative humidity
  - Treatment scale
- Determine the impact of dry-heat treatment on germination and sprout yield
- Determine whether post-treatment soak could alleviate the negative impact of heat treatment on germination and sprout yield
- Examine pathogen re-growth during sprouting of heat-treated mung beans

Materials and Methods

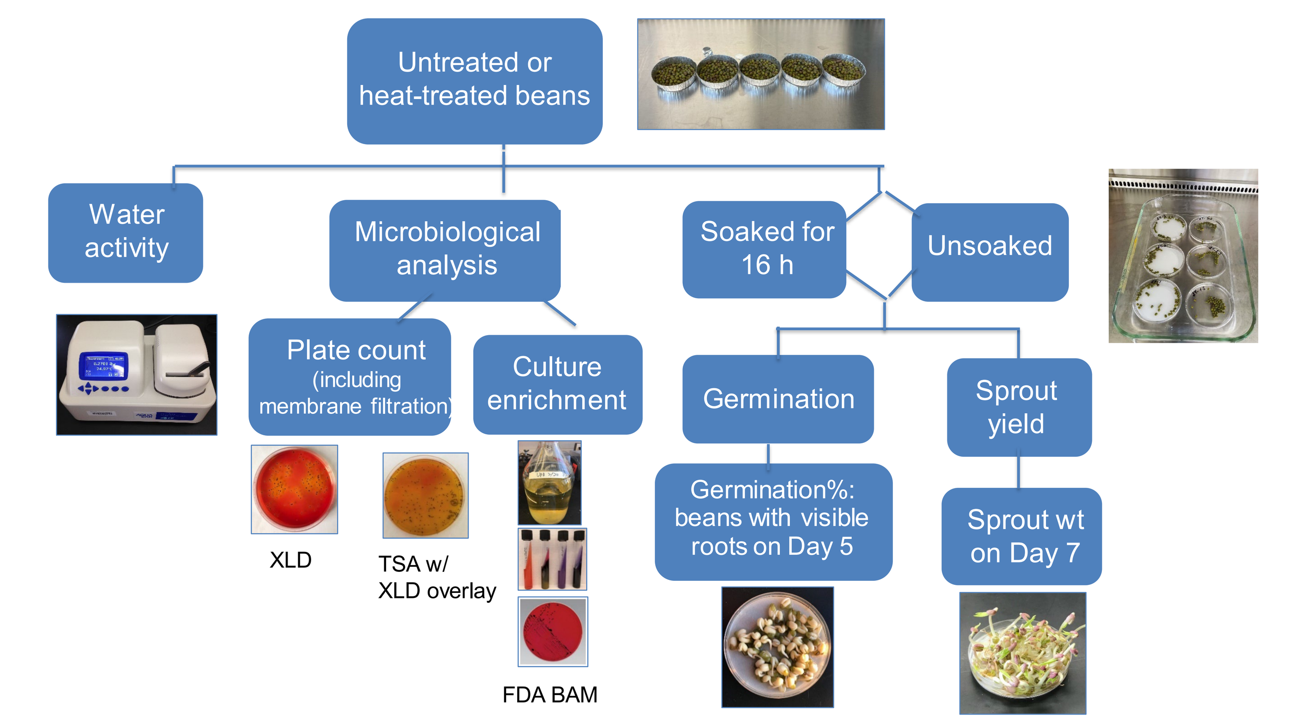
Seed inoculation: Five serotypes of *Salmonella* (Tennessee, Muenchen, Cubana, Saintpaul, and Mbandanka) isolated from sprout outbreaks were individually grown in BHI at 35°C for 18-24 h, washed 3 times in Butterfield's phosphate buffer (BPB), combined and diluted in BPB.



Heat treatment:

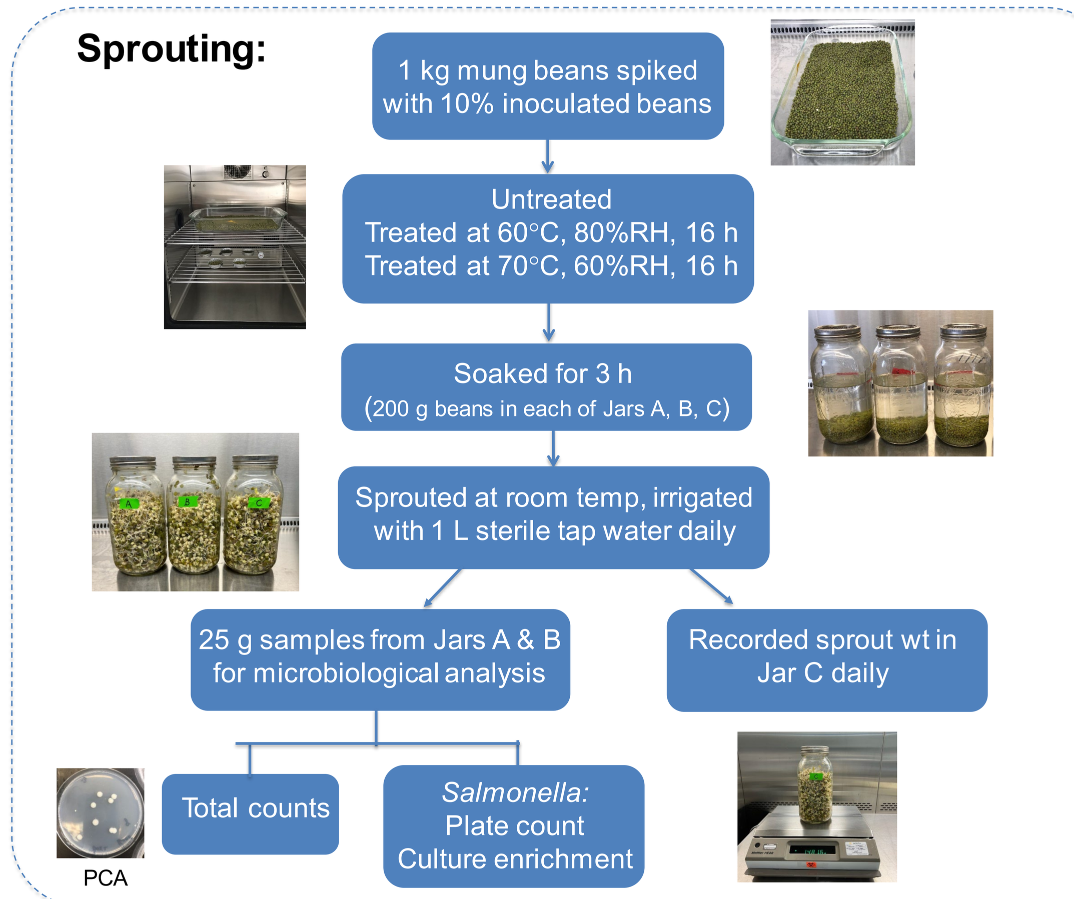


Post-treatment tests:



Salmonella reduction = *Salmonella* level in untreated beans – *Salmonella* level in treated beans
Relative germination = germination% of treated seeds/germination% of untreated beans
Relative sprout yield = sprout yield of treated beans/sprout yield of untreated beans

Sprouting:



Results

Table 1. Impact of dry-heat treatment on *Salmonella* reduction, seed germination and sprout yield for alfalfa seeds, as affected by treatment temperature, relative humidity, and treatment time

60°C					70°C					80°C				
60C	20%RH	40%RH	60%RH	80%RH	70C	20%RH	40%RH	60%RH	80%RH	80C	20%RH	40%RH	60%RH	80%RH
	Salmonella reduction (log cfu/g)					*Salmonella* reduction (log cfu/g)					*Salmonella* reduction (log cfu/g)			
6 h	1.02	1.28	1.74	>3.69	6 h	1.63	3.00	2.85	>3.92	6 h	2.52	>3.67	>3.72	>3.49
16 h	1.60	1.81	2.08	>3.69	16 h	1.79	3.64	>3.94	>3.92	16 h	3.30	>3.67	>3.72	>3.49
24 h	1.84	2.16	2.99	>3.69	24 h	1.85	2.90	>3.94	>3.92	24 h	3.61	>3.67	>3.72	>3.49
	Relative germination					Relative germination					Relative germination			
6 h	0.99	1.01	0.99	0.97	6 h	1.01	1.02	0.89	0.15	6 h	1.04	0.23	0.05	0.08
16 h	0.98	0.99	1.00	0.36	16 h	0.99	0.98	0.27	0.12	16 h	1.02	0.10	0.04	0.00
24 h	1.03	0.99	1.00	0.21	24 h	0.98	0.96	0.17	0.06	24 h	1.01	0.04	0.02	0.01
	Relative sprout yield					Relative sprout yield					Relative sprout yield			
6 h	0.93	0.89	0.89	0.87	6 h	0.99	0.96	0.30	0.11	6 h	0.80	0.21	0.05	0.06
16 h	0.98	0.97	0.91	0.33	16 h	0.94	0.90	0.12	0.09	16 h	0.63	0.08	0.04	0.00
24 h	0.91	1.01	0.86	0.18	24 h	0.98	0.86	0.08	0.06	24 h	0.84	0.04	0.02	0.00

Detection limit = -0.3 log cfu/g

Table 2. Impact of dry-heat treatment on *Salmonella* reduction, seed germination and sprout yield for mung beans, as affected by treatment temperature, relative humidity, and treatment time

60°C					70°C					80°C				
60C	20%RH	40%RH	60%RH	80%RH	70C	20%RH	40%RH	60%RH	80%RH	80C	20%RH	40%RH	60%RH	80%RH
	Salmonella reduction (log cfu/g)					*Salmonella* reduction (log cfu/g)					*Salmonella* reduction (log cfu/g)			
6 h	0.29	1.39	0.58	3.51	6 h	1.50	2.66	>3.87	>4.21	6 h	2.7	3.4	>3.71	-
16 h	1.55	1.79	2.45	>4.01	16 h	1.23	3.67	>3.87	>4.21	16 h	2.75	3.5	>3.71	-
24 h	1.14	3.46	2.90	>4.01	24 h	1.28	>4.51	>3.87	>4.21	24 h	>3.65	>3.76	>3.71	-
	Relative germination					Relative germination					Relative germination			
6 h	1.00	1.00	1.00	1.00	6 h	0.99	1.00	0.99	0.97	6 h	0.99	0.97	0.24	-
16 h	0.99	1.00	1.00	0.97	16 h	1.00	1.00	0.99	0.91	16 h	0.99	0.82	0.00	-
24 h	1.00	0.97	1.00	0.89	24 h	1.00	1.00	0.98	0.90	24 h	1.00	0.85	0.00	-
	Relative sprout yield					Relative sprout yield					Relative sprout yield			
6 h	1.01	1.14	1.00	0.98	6 h	0.84	1.03	0.94	0.97	6 h	0.80	0.59	0.38	-
16 h	0.94	1.00	0.96	0.91	16 h	0.84	1.06	0.91	0.88	16 h	0.74	0.46	0.38	-
24 h	1.05	1.00	0.93	0.85	24 h	0.76	0.92	0.80	0.76	24 h	0.68	0.41	0.37	-

Detection limit = -0.3 log cfu/g
- Not done

Table 3. Impact of post-treatment soak on germination and sprout yield of treated mung beans*

	20%RH	40%RH	60%RH	80%RH
	%G of soaked beans / %G of unsoaked beans			
60°C	-	-	1.00	1.02
70°C	-	1.00	1.01	0.95
80°C	1.00	0.97	-	-
	Yield of soaked beans / Yield of unsoaked beans			
60°C	-	-	0.97	1.08
70°C	-	0.98	0.97	1.26
80°C	0.92	0.98	-	-

All beans were treated for 16 h.
* Each value represents the average of results obtained from at least two separate runs.
%G = % germination
- Not done

Table 4. Impact of dry-heat treatment on *Salmonella* reduction in mung beans, as affected by treatment scale (10 g vs. 1 kg)*

Treatment	Scale	*Salmonella* reduction (log cfu/g)	Culture enrichment∃,#
untreated		2.2	
60°C/80%RH/16h	10 g	> 2.5	2/6ᴬ
	1 kg	> 2.5	1/9ᴬ
untreated		2.2	
70°C/ 60%RH/16h	10 g	> 2.5	2/4ᴮ
	1 kg	> 2.5	1/6ᴮ

* Values represent results from two or three separate experiments conducted at 60°C/80%RH/16h or 70°C/60%RH/16h, respectively.
Detection limit = -0.3 log cfu/g
∃ Number of positive samples/number of samples tested
\# Values in column followed by the same letter are not significantly different ($p > 0.05$).

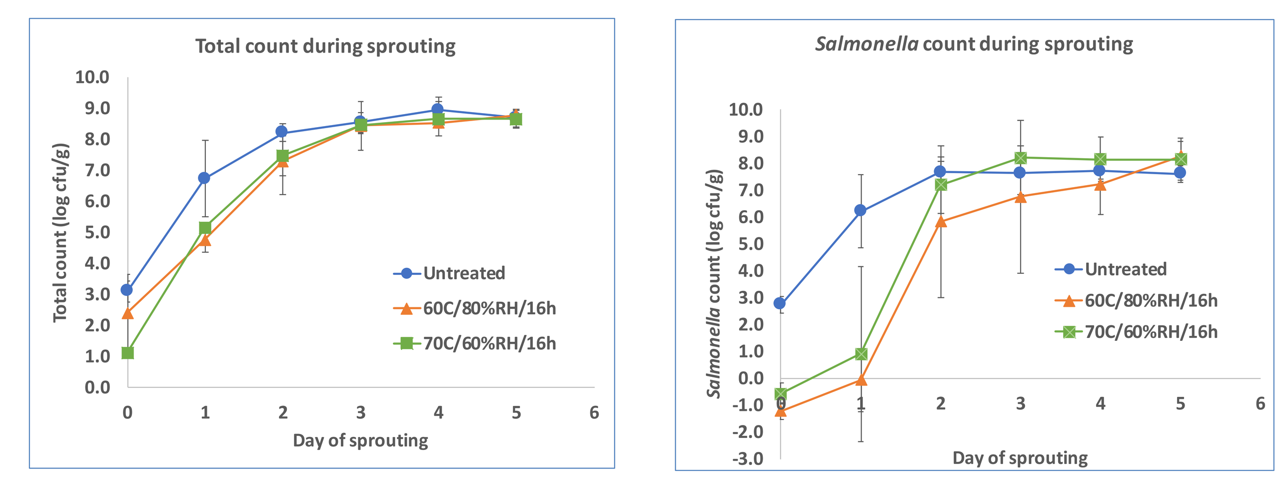


Figure 1. Proliferation of *Salmonella* and background microflora during sprouting of untreated or beans treated under optimal conditions (60°C/80%RH/16h (n=3); 70°C/60%RH/16h (n=2).

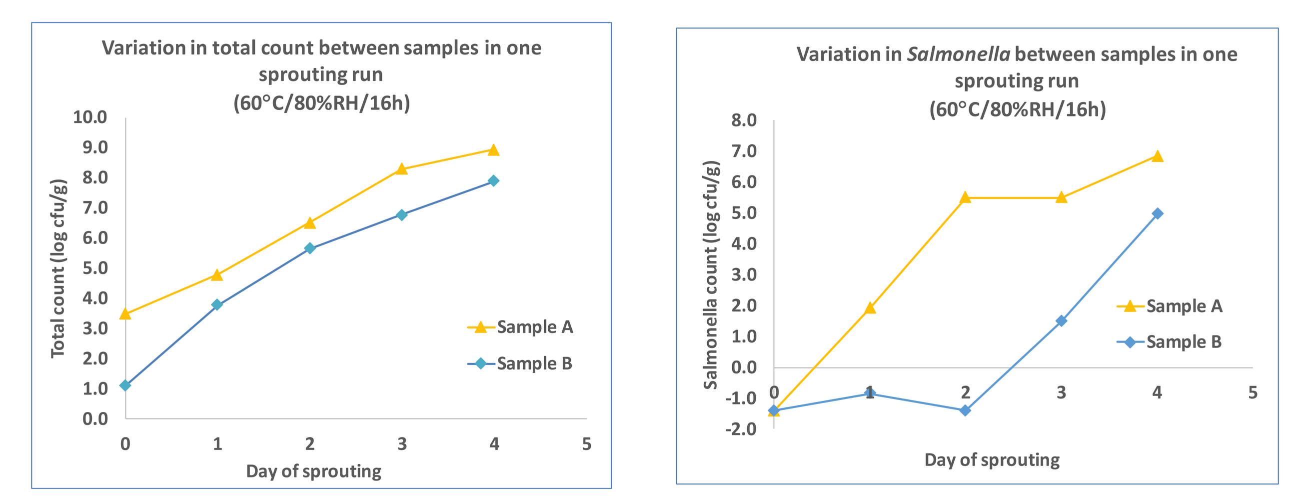


Figure 2. Microbial proliferation during sprouting can differ between samples treated under the same condition. In one replicate sample, *Salmonella* was undetectable after 2 days of sprouting but increased to 5.0 logs after 4 days.

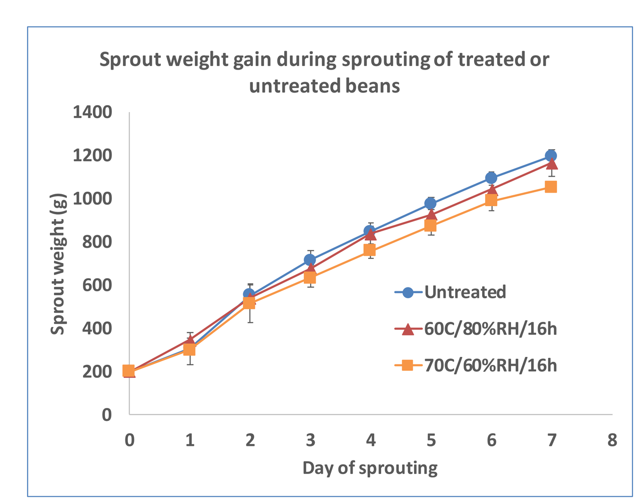


Figure 3. Impact of dry-heat treatment on sprout yield.

Discussion

- A greater log kill was observed when treatment was conducted at a higher temperature, under a higher RH, or for a longer time (Tables 1 and 2).
- Heat treatment can negatively affect germination and sprout yield. Treatment at 80°C, under all conditions, lowered sprout yield to ≤ 80% (Tables 1 and 2).
- Optimal treatment conditions need to be determined for individual seed type. For mung beans, the best conditions, where *Salmonella* was reduced to below detection and relative germination and sprout yield were at > 90% of that of untreated control, were 60°C/80%RH/16 h, 70°C/60%RH/16 h, 70°C/80%RH/6 h or 70°C/80%RH/16 h (Table 2).
- For alfalfa seeds, treatment at 60°C/80%RH/6 h was able to reduce *Salmonella* to below detection while maintaining germination and sprout yield at 97% and 87% , respectively, of that of untreated control (Table 1).
- Post-treatment soak did not result in improved germination or sprout yield of treated mung beans (Table 3).
- Efficacy was not affected by treatment scale. A similar *Salmonella* reduction was achieved whether 10 g or 1 kg of mung beans were treated (Table 4).
- Populations of background microflora and *Salmonella* in mung beans were lowered after dry-heat treatment but increased during sprouting to levels similar to those in sprouts grown from untreated beans (Figure 1).
- Delay in *Salmonella* re-growth during spouting of treated beans was observed. The extent of delay may differ between samples (Figure 2). This delay may result in *Salmonella* not being detected by typical sprout testing programs (*5*).
- Sprouts grown from mung beans treated under optimal conditions had a similar weight gain as that of untread beans (Figure 3).
- Overall, dry-heat treatment can be an effective means in reducing *Salmonella* on sprout seeds but pathogen re-growth during sprouting needs to be considered in sprout testing programs.

References

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