Hot Water Treatment for Elimination of Seed-Borne Phoma betae and Other Microbial Contaminants from Sugarbeet Seed[1]

LEONARD J. HERR[2]

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Introduction

Seeds of certain sugarbeet breeding lines, being used in gnotobiotic black root disease investigations, were contaminated with seed-borne *Phoma betae* Frank. A seed surface-sterilization technique using silver nitrate (1) [3] controlled the contaminating microorganisms present on commercial sugarbeet seed lots (*P. betae*-free in our tests), but failed to control the seed-borne *P. betae* of the contaminated breeding lines. An alternative method for eliminating seed-borne *P. betae* was therefore required.

Problems with seed-borne *P. betae* are by no means a new occurrence. In 1915, Edson (2) described a hot water treatment developed by Peters (4) in Germany in 1907 for reducing the incidence of *Phoma*-contaminated sugarbeet seed (one contaminated seedling/300-400 treated seeds). This treatment consisted of heating seeds in water at 60° C for 10 min, drying on filter paper for 24 hr, followed by a second heating in water at 60°C for 10 min. The method was rather thoroughly investigated. Temperatures above 60° C were found to cause serious injury to seeds; whereas temperatures below 60°C were ineffective. Substitution of one heat treatment for the recommended two was unsuccessful. Edson (2) also reported that the hot water treatment reduced germination.

Research goals of this investigation were three-fold. First, because *P. betae* is one of the several pathogens involved in the black root disease complex of sugarbeet, its uncontrolled occurrence as a seed-borne pathogen in black root studies is intolerable. Second, gnotobiotic (known mixtures of organisms) studies initially require elimination of all contaminating microorganisms from the sugarbeet seeds. If the hot water treatment effectively controlled seed-borne *Phoma,* would it also control other microbial

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[2] Associate Professor of Plant Pathology, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691 and the Ohio State University, Columbus, Ohio 43210.

[3] Numbers in parentheses refer to literature cited.

contaminants? Third, considering the changes in sugarbeet varieties since Peters' and Edson's studies (2, 4), would the heat tolerance of modern varieties be sufficiently similar for effective heat treatment without unacceptable reductions in seed germination.

An abstract of a portion of these results has been published (3).

Materials and Methods

Seeds of beet breeding line 685, highly contaminated with *P. betae,* were used in most tests. Small quantities of seed (40/treatment) were tied in cheesecloth bags to facilitate handling during hot water treatments. Seeds were immersed in deionized water heated to 60°C in small beakers (100 or 250 ml capacity) in a controlled temperature water bath. Temperature of the water within the beaker was measured with a thermometer before and during treatment. After the appropriate treatment time (8-15 min) the seeds were dried overnight at 28°C in a forced-air incubator. A second (8-15 min) hot water treatment was applied, after which the seeds were surface-sterilized three min in a 1:4 dilution of 5.25% sodium hypochlorite. Seeds were then plated (4 plates of 10 seeds/plate) on 2% water agar and incubated 5 days at 20°C. Following incubation, seeds were examined for germination and for *P. betae* and other microbial contaminants. Results are expressed as percentages based on the number of seeds/treatment. (Deviations from this general procedure are specifically noted.)

Hand-polished sugarbeet seed was polished by use of a box lined with corrugated rubber matting and a corrugated rubber covered polishing block similar to that of Coe (G. Coe, personal communication). Machine-polished seed had been passed through a rice polisher prior to their acquisition.

Commercial varieties are referred to by their standard designations and breeding lines have been given abbreviated designations.

Results

Hot water treatments were compared with seed surface-sterilization by silver nitrate (1) for effectiveness in elimination of *P. betae* and other microbial contaminants from beet line 685 seeds (Table 1). Silver nitrate treatment failed to eliminate all *P. betae* colonies and was relatively ineffective in reducing other contaminants. In this test heat-treated seed (no additional surface-sterilization) controlled *P. betae* but had relatively high other contamination. Seed germination was markedly affected by the different heat treatment procedures.

Table 1.—Germination, contamination and *Phoma betae* colonies of sugarbeet seed surface-sterilized with silver nitrate or hot water treated at 60°C for various time intervals.

Percentage	Treatments				
	2 Periods 10 Minutes 2 Days	2 Periods 10 Minutes Same Day	1 Period 20 Minutes	Silver Nitrate	No Treatment (Control)
Germination	93	35	70	83	85
Other Contamination	10	13	10	20	50
Phoma Colonies	0	0	0	8	48

Successful control of *P. betae* by heat therapy afforded an opportunity to evaluate the pathogenic potential of this fungus. Heat-treated and non-treated seed were planted in sterilized soil or gnotobiotic perlite-nutrient solution culture in deep culture dishes and seedling survival was ascertained after two weeks. Naturally occurring seed-borne *P. betae* greatly reduced seedling survival. In sterilized soil the percentage survival of heat-treated and non-treated seeds was 93 and 43 percent respectively; whereas, in perlite the percentages were 78 and 45 respectively.

At this point the principal problem remaining seemed to be improved control of "other" contaminants. Heat therapy with and without additional surface-sterilization was compared for control of contaminants (Table 2). Sodium hypochlorite appeared better than silver nitrate for this purpose and was much simpler to use. However, *P. betae* occurred in heat-treated seed, which necessitated modification of the hot water treatment method.

Table 2.—Effect of heat treatment (60°C, 10 min on 2 days) followed by: a) no further treatment, b) silver nitrate surface-sterilization, or c) sodium hypochlorite surface-sterilization, on sugarbeet seed germination, contamination and *Phoma betae* colonies.

Percentage	Treatments		
	Heat Only	Silver Nitrate	Sodium Hypochlorite
Germination	88	88	85*
Other Contamination	5	5	0
Phoma colonies	3	0	0

Two factors of the technique were examined: 1) increased water temperature and 2) increased treatment time. Raising the water temperature to 65°C eliminated *P. betae,* but depressed seed germination and gave high contamination percentages (Table 3). The high incidence of contamination was due exclusively to spore-forming bacteria in the single-period heat treatments. Best results for control of *P. betae* and other contaminants were obtained by increasing the treatment time interval at 60°C to 15 min. (Table 4).

Table 3.—Germination and contamination of beet seed treated with hot water (65°C) for various time intervals.

Percentage	Treatments			
	10 Min on 2 days	15 Min on 2 days	20 Min on 1 day	30 Min on 1 day
Germination	40	25	35	0
Contamination	40	23	98[a]	100[a]

[a] Spore-forming bacteria

Table 4.—Effect of hot water treatment of seed at 60°C on two consecutive days for 10 and 15 minute intervals on germination, contamination and *Phoma betae* colonies.

Variety	Percentage	Treatments		
		Controls	10 Min	15 Min
322	Germination	100	119	119
	Other Contamination	48	13	3
	Phoma colonies	28	8	0
261	Germination	100	106	97
	Other Contamination	70	5	0
	Phoma colonies	45	5	0

The combination of heat treatment and surface-sterilization by sodium hypochlorite appeared to be the most effective procedure for elimination of microbial contaminants from sugarbeet seed. Therefore, the heat tolerance of seed of commercial varieties was investigated. Germination of seeds of the monogerm varieties GW H-1 and US H-20 was markedly reduced by the 15 min treatment at 60°C on successive days, whereas that of the multigerm US 401 was essentially unaffected (germination of US 401=140%, GW H-1=23%, US H-20=25% and 685=80%). Reduction of the treatment time to 8 min freed seeds of GW H-1 and US H-20 of contaminants and permitted reasonably high germination (Table 5). Because commercial seed lots were *P. betae*-free in our tests, only "other" contaminants had to be controlled and thus the 8 min treatment period was acceptable.

Table 5.—Effect of length of hot water treatment periods (all 60°C on 2 days) on germination and contamination of two commercial sugarbeet varieties.

Variety	Percentage	Treatments			
		Control	8 Min	10 Min	12 Min
GW H-1	Germination	83	73	55	42
	Contamination	20	0	0	0
US H-20	Germination	100	70	63	55
	Contamination	43	0	0	0

The possibility that varietal heat tolerances were related to seed weight was explored by ranking varieties according to average weight/100 seeds and designating varieties as either heat tolerant or non-tolerant, based on results of previous tests (Table 6). No correlation was evident.

Table 6.—Sugar beet varieties and breeding lines ranked according to average weight (per 100 seeds) in relation to heat tolerance.

Variety	Av. Weight/ 100 Seed (mg)	Percent germination[1] 8 Min	10 Min	15 Min	Heat[2] Tolerance
685	49	....	93	93	T
GW H-1	77	73	55	23	N
US 401	112	..	.	140	T
US H-20	114	70	63	25	N
261	185	....	106	97	T
322	187	..	119	119	T

[1] Percentage based on number of seeds plated.
[2] T=tolerant N=non-tolerant

Testing of varietal heat tolerance, elimination of *P. betae* and elimination of other microbial contaminants was extended using six additional beet breeding lines. Presumably, seed polishing should reduce seed contaminants, so this variable also was evaluated. All seed used in this test were surface-sterilized with sodium hypochlorite prior to plating on water agar. Only two of the six beet lines tested were contaminated with *P. betae* (Table 7). Hand polishing neither controlled *P. betae* nor definitely reduced contaminants. Use of only one machine polished seed sample did not permit adequate evaluation of its effectiveness. Heat treatment plus surface-sterilization controlled both *P. betae* and other contaminants. Heat tolerance in the different breeding lines ranged from no to high germination.

Table 7.—Effects of seed polishing and heat treatment (60°C, 15 min on 2 days) on seed germination, contamination, and *Phoma betae* colonies of six sugarbeet breeding lines.

Treatments	Percentage[1]	Varieties 633	687	340	AJP	BP-2	103
Control	Germination	167	78	123	161	101	134
	Contamination	5	14	0	0	2	8
	Phoma colonies	0	3	0	0	0	5
Hand polished	Germination	136	68	83	152	86	15
	Contamination	3	9	1	0	3	7
	Phoma colonies	0	6	0	0	0	4
Heat treated	Germination	78	82	47	43	23	0
	Contamination	0	0	0	0	0	0
	Phoma colonies	0	0	0	0	0	0
Machine polished	Germination	163	....	....	....	....	....
	Contamination	2	....	....	....	....	....
	Phoma colonies	0	....	....	....	....	....

[1] Percentages calculated on number of seeds planted (100).

Discussion

The heat treatment method, as described by Edson (2), failed to eliminate *P. betae*. Successful application of heat therapy is based on differences in the thermal death points of *P. betae* and sugarbeet seeds. Thermal death (within limits) can be considered the resulant of the interaction of temperature and time. Thus, increase of either treatment temperature or time of exposure should increase thermal death. Increased treatment time best preserved the differential between beet seed and *P. betae* death points, but varietal variations in heat tolerance occurred. Of nine breeding lines tested, five had high heat tolerance, three had medium, one had low and one was not tolerant to heat, as based on seed germination tests following treatment. Similarily, of three commercial beet varieties tested one had high and two had low heat tolerance. Knowledge of varietal heat tolerance thus is essential for successful application of the technique and, in some cases, may limit its use. The lack of correlation between seed weight and heat tolerance indicated that a higher degree of complexity is involved than simply the seed mass heated.

Seed polishing undoubtedly helps reduce the microbial contamination load on seeds by removing portions of contaminated seed ball, but additional treatment is necessary to eliminate remaining contaminants.

One difficulty encountered during these heat therapy studies with experimental sugarbeet breeding lines was the limited seed supplies available for testing. However, the generally high *P. betae* and other contaminant percentages of untreated controls added to confidence in the results despite the relatively small numbers of seeds tested.

Summary

Seeds of some sugarbeet breeding lines were contaminated with the black root pathogen, *Phoma betae*. The reported effectiveness of heat therapy for elimination of this pathogen was reexamined and expanded upon. Seeds contaminated with *P. betae* were treated in hot water 15 min at 60°C, dried overnight at 28°C, treated a second 15 min at 60°C, and surface-sterilized in a 1:4 dilution of 5.25% sodium hypochlorite. This procedure controlled both *P. betae* and other microbial contaminants. However, not all beet varieties tested were equally tolerant to heat. Heat tolerance was not correlated with seed weight. Shorter treatment times (8 min) were used to eliminate microbial contaminants from two relatively heat intolerant commercial monogerm varieties (non-contaminated with *P. betae*).

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