Thermotherapy of Soybean Seeds to Control Seedborne Fungi

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ABSTRACT

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Refined soybean oil was evaluated as a medium for heating soybean (*Glycine max*) seeds to control seedborne fungi, particularly *Phomopsis* spp. Seeds of various cultivars were heated in hot oil for periods and temperatures ranging from 5 min at 70 C to 20 sec at 160 C. Treatments that decreased the recovery of *Phomopsis* spp., with a concomitant increase in the number of germinable, pathogen-free seeds, ranged from 5 min at 70 C to 10 sec at 140 C. Seeds placed in soybean oil at 27 C did not imbibe oil, swell, or slough their seed coats.

Heat treatment of seeds is a common technique used to control certain plant diseases. Thermotherapy inactivates or kills a pathogen while leaving the host tissue viable. Baker reviewed the use of thermotherapy for control of plant diseases (2,3).

Many media have been used to heat-treat seeds: water, steam, air, carbon tetrachloride (CCl_4), petroleum oils, vegetable oils, and microwave irradiation (2,7,9). Hot water is the most commonly used medium. However, seeds of large-seeded legumes, such as soybeans (*Glycine max* (L.) Merr.) and green beans (*Phaseolus vulgaris* L.), rarely are heated in hot water because they quickly imbibe water, swell, and slough off their seed coats. To avoid the problems of hot water treatment, Watson et al (9) tested various nonaqueous fluids to find a compatible medium for heating seeds of large-seeded legumes. They found that seeds of green beans and of lima beans (*Phaseolus lunatus* L.) survived longer when treated in motor oil at 90 C than in water at 90 C. Seeds heated for 60 min in boiling CCl_4 (76.8 C) also survived. However, they did not show that the technique controlled seedborne pathogens. In this study, immersion of soybean seeds in heated soybean oil was tested as a means of eliminating the seedborne pathogen complex caused by *Phomopsis* spp., the causal agents of pod and stem blight and Phomopsis seed decay of soybeans (6). These fungi are found commonly in the seed coat of soybeans and other large-seeded legumes and occasionally in the embryo (4,5). Seedborne mycelia serve as a source of primary inoculum in the development of pod and stem blight (6).

MATERIALS AND METHODS

Seed source. Soybean seeds of various cultivars and levels of *Phomopsis* spp. infection were obtained from E. G. Jordan (U.S. Department of Agriculture, APHIS, University of Illinois at Urbana-Champaign). Several cultivars were used because infection was frequent enough in any one cultivar for application of statistical analyses.

Reaction of soybean seeds placed in soybean oil. Ten Wells soybean seeds each were placed in 10 ml beakers containing either refined soybean oil (Durkee Food Service, SCM Corp., Cleveland, OH 44115), tap water, or nothing. Observations were made on imbibition and swelling of the seeds and on the condition of the seed coat after 30 min at 27 C.

Measurement of moisture content. Seeds of low moisture content (5.4–7.5%) were used because seeds of higher moisture levels are more susceptible to heat damage (2) (Fig. 1). Seed moisture content was measured by one of two ways. One method employed a Burrows Digital Moisture Computer 700 (Burrows Equipment Co., Evanston, IL 60204), while the second was by a modification of the U.S. Department of Agriculture, Agricultural Marketing Service method (1). The second method involved weighing four lots of approximately 15 g of soybeans to within 0.1 g. The weighed seeds were heated for 72–96 hr at 105 C in a hot oven and then reweighed. Any loss in weight was attributed to water loss. Percent moisture content (MC) was calculated by the formula:

$$\% \text{ MC} = 100\ (W_1 - W_2)/\ W_1$$

in which W_1 = the weight before heating, and W_2 = the weight after heating. The MC value used is an average of four replications.

Oil thermotherapy of soybean seeds. Seeds of known moisture content were placed in cheesecloth bags with 50 or 100 seeds per bag. Bags were selected randomly for treatment. Seedbags to be heated were soaked for 5 min in refined soybean oil at 27 C. All bags for any one treatment were heated in a 4-L beaker containing at least 3 L of soybean oil heated to the proper temperature on a hot plate. The oil was agitated with a magnetic stir bar to insure even heating. Temperature of the oil was measured by either a mercury or an ethanol thermometer. After each treatment, heated seeds were cooled by immersion for at least 5 min in 2 L of oil at 30 C. Cooled seeds were drained, and then rinsed in 95% ethanol at least twice to remove excess oil. The ethanol and oil formed an emulsion that separated into two phases after a few hours; both the ethanol and oil could be recovered and reused. Bags containing ethanol

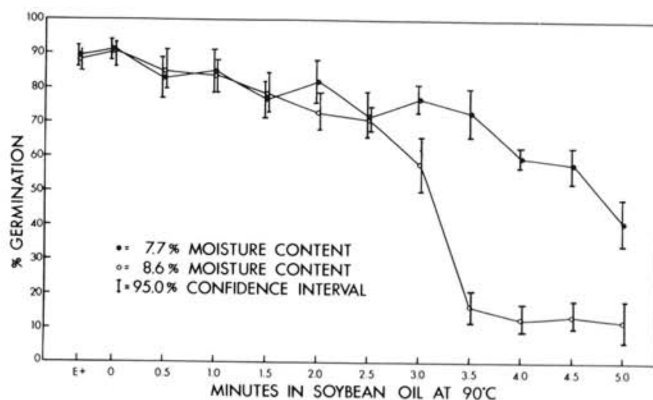

Fig. 1. Germination of Wells soybean seeds on potato-dextrose agar (6 days at 25 C) of two moisture contents after being heated in soybean oil at 90 C. Means based on either replications of 50 seeds each.

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control seeds were not placed in oil but were immersed in 95% ethanol for 4 min. Bags with untreated control seeds were not rinsed in ethanol. All seeds were surface disinfested by immersion in 0.05% NaOCl for 4 min and then rinsed at least twice in double distilled water. Seeds were plated on potato-dextrose agar (Difco Laboratories, Detroit, MI 48232) (PDA) and incubated in the dark for 3–8 days at 25 C. The percentage of germination and the presence of *Phomopsis* spp. were recorded. A seed was considered "germinated" if its radicle was at least as long as its cotyledons, and "clean" if no fungi were present; if *Phomopsis* spp. were present, it was placed within the "Phomopsis" category.

Thermal effect on germination. To determine whether the heat treatment would stimulate germination independent of any control of seedborne *Phomopsis* spp., Wells seeds with a low incidence of *Phomopsis* spp. infection were heated from 0 to 5 min in 30-sec increments in oil at 90 C.

RESULTS AND DISCUSSION

Reaction of soybean seeds placed in soybean oil. Both untreated seeds and seeds soaked 30 min in soybean oil at 27 C showed no increase in size, imbibition of oil, nor slippage of the seed coats. Seeds placed in tap water swelled and the seed coats slipped and were sloughed off.

Oil thermotherapy of soybean seeds. All treatments reduced the recovery of *Phomopsis* spp. and there was a larger percentage of germinated-clean seeds when Wells seeds were heated for 0 to 5 min in oil at 90 C (Table 1). Germination was higher than both controls in all but the 3.5- and 4.5-min treatments. The recovery of *Phomopsis* spp. was lower in the ethanol control than in the unheated control. Treatments as short as 30 sec in oil at 90 C decreased recovery of *Phomopsis* spp. below the ethanol control. The recovery of less than 0.3% *Phomopsis* spp. after 4 min or more in oil at 90 C indicated that longer treatments would be unnecessary unless eradication of *Phomopsis* spp. was essential. Thus, the

TABLE 1. Effect of oil thermotherapy on germination and recovery of *Phomopsis* spp. from seeds of several soybean cultivars[x]

Cultivar and % moisture	Temperature (C)	Time (min)	Germinated[y] clean (%)	Germinated[y] (%)	*Phomopsis spp.*[y] recovery (%)
Wells (6.6%)	Unheated control[z]	-	24.2 e	67.5 e	69.5 a
	Ethanol control[z]	-	38.2 d	72.7 de	55.2 b
	90	0.5	67.2 c	83.0 ab	19.0 c
	90	1.0	79.2 ab	86.0 a	1.7 cd
	90	1.5	82.0 a	84.0 a	1.0 d
	90	2–2.5	81.2–79.2 ab	83.0–82.7 ab	0.5 d
	90	3.0	78.3 ab	84.5 a	0.5 d
	90	3.5	72.3 bc	76.0 cd	0.8 d
	90	4.0	80.2 ab	81.8 abc	0.0 d
	90	4.5	74.5 b	77.0 bcd	0.0 d
	90	5.0	81.0 ab	82.0 abc	0.2 d
FLSD ($P = 0.05$)			7.2	6.6	5.8
L-22 (7.5%)	Unheated control		51.7 g	73.8 bcd	26.2 a
	Ethanol control		66.0 def	78.5 abcd	18.0 bcd
	70	1.0	50.7 g	77.0 abcd	22.5 ab
	70	2.0	56.8 fg	74.2 bcd	20.5 b
	70	3.0	54.0 g	73.5 cd	19.8 bc
	70	4.0	61.7 efg	76.8 abcd	15.2 cd
	70	5.0	69.0 bcde	81.8 abc	6.8 e
(7.5%)	80	1.0	67.0 cdef	81.8 abc	14.0 d
	80	2.0	75.0 abcd	79.8 abcd	3.2 ef
	80	3.0	80.0 ab	83.8 ab	1.2 f
	80	4.0	78.2 abc	81.5 abc	1.5 f
	80	5.0	80.5 a	83.2 abc	0.0 f
	90	1.0	79.7 ab	85.2 a	4.0 ef
	90	2.0	70.2 abcde	71.0 d	0.0 f
	90	3.0	56.5 fg	57.0 e	0.0 f
	90	4.0	11.7 h	11.8 f	0.0 f
	90	5.0	15.7 h	16.0 f	0.0 f
FLSD ($P = 0.05$)			11.4	10.2	4.5
Kent (6.1%)	Unheated control		40.0 ghi	89.2 ab	8.8 a
	Ethanol control		54.0 de	90.0 a	4.8 c
	70	1.0	38.8 hi	84.2 cdefg	5.2 c
	70	2.0	35.0 i	79.7 g	7.5 bc
	70	3.0	40.2 ghi	83.2 efg	5.2 c
	70	4.0	40.8 ghi	84.0 cdefg	5.2 c
	70	5.0	40.8 ghi	82.0 fg	5.7 bc
	80	1.0	43.2 fgh	86.8 abcdef	2.5 d
	80	2.0	45.7 fg	87.2 abcde	1.2 de
	80	3.0	48.0 ef	83.5 defg	0.5 de
	80	4.0	56.0 cd	88.2 abcd	0.8 de
	80	5.0	63.4 b	88.2 abcd	0.3 e
	90	1.0	62.5 bc	89.7 a	0.0 e
	90	2.0	79.7 a	88.0 abcde	0.0 e
	90	3.0	83.0 a	84.5 bcdefg	0.0 e
	90	4.0	82.0 a	88.5 abc	0.0 e
	90	5.0	80.2 a	86.0 abcdef	0.0 e
FLSD ($P = 0.05$)			6.8	4.9	2.2

(continued on next page)

shorter immersion time at 90 C is an advantage over more conventional cooler treatments.

L-22 seeds at 7.5% moisture treated for 3, 4, and 5 min at 80 C, and for 1 min at 90 C, had higher rates of germinated-clean seeds than did the ethanol control (Table 1). The ethanol control had a greater rate of germinated-clean seeds than the unheated control. Seeds treated 1 min in oil at 90 C had a higher germination than the unheated control, but not significantly higher than the ethanol control. Recovery of *Phomopsis* spp. was lower than the ethanol control in the following hot oil treatments: 5 min at 70 C; 2, 3, 4, and 5 min at 80 C; and 1, 2, 3, 4, and 5 min at 90 C.

Kent seeds at 6.1% heated for 3, 4, or 5 min in oil at 90 C had the highest rate of germinated-clean seeds (Table 1). However, no treated seeds had a higher germination than the controls. All seeds heated in oil at 70 C and those for 4 min in oil at 80 C germinated lower than the unheated control. Recovery of *Phomopsis* spp. in the 80 and 90 C treatments was lower than in the ethanol control. Seeds heated in 70 C oil showed no change in recovery of *Phomopsis* spp.

The Kent and the L-22 seeds differed in three ways: the cultivars were different; Kent seeds had a lower moisture content; and L-22 seeds had a higher rate of *Phomopsis* spp. infection than the Kent seeds. The majority of the L-22 seeds treated for 4–5 min in oil at 90 C did not germinate and the majority of the Kent seeds heated in the same way did germinate. The difference may have been due in part to cultivar variation, but it was probably due to a lower moisture content of the latter, since moisture is critical in seed survival of heat treatment (2).

The treatment of Williams seed lots at 5.4% moisture that resulted in more germinated-clean seeds than the ethanol control was for 30 sec in oil at 110 C (Table 1). Three treatments had germination rates similar to the controls: 30 and 60 sec at 100 C, and 30 sec at 110 C. All other treatments had germination rates lower than the controls.

The preceding experiment was repeated using Union seeds at 6.0% moisture. The treatments for 60 sec at 100 C and 30 sec at 110

TABLE 1 (continued). Effect of oil thermotherapy on germination and recovery of *Phomopsis* spp. from seeds of several soybean cultivars[x]

Cultivar and % moisture	Temperature (C)	Time (min)	Germinated[y] clean (%)	Germinated[y] (%)	*Phomopsis* spp.[y] recovery (%)
Williams	Unheated control		57.6 bc	81.6 ab	9.6 a
(5.4%)	Ethanol control		65.6 bc	82.4 ab	7.3 a
	100	0.5	61.4 bc	80.0 ab	8.3 a
	100	1.0	68.6 b	71.6 b	0.0 b
	110	0.5	82.0 a	84.3 a	0.0 b
	110	1.0	56.0 c	56.6 c	0.3 b
	120	0.5	57.4 bc	58.0 c	0.0 b
	120	1.0	0.0 d	0.0 d	0.0 b
	130	0.5–1.0	0.0 d	0.0 d	0.0 b
FLSD ($P = 0.05$)			12.4	11.5	2.6
Union	Unheated control		63.3 c	89.0 ab	2.0 a
(6.0%)	Ethanol control		76.3 b	89.3 ab	1.3 ab
	100	0.5	77.6 b	93.6 a	0.7 bc
	100	1.0	86.6 a	93.6 a	0.3 bc
	110	0.5	86.6 a	88.3 b	0.3 bc
	110	1.0	40.6 d	41.0 d	0.0 c
	120	0.5	55.6 c	56.0 c	0.0 c
	120	1.0	0.0 e	0.0 e	0.0 c
	130	0.5–1.0	0.0 e	0.0 e	0.0 c
FLSD ($P = 0.05$)			7.6	5.3	1.1
(7.5%)	Unheated control		77.4 b	92.4 a	1.0 ab
	Ethanol control		74.6 b	89.4 a	2.0 a
	130	0.16	81.6 ab	90.0 a	1.7 a
	130	0.33	56.0 c	56.0 b	0.0 b
	130	0.50	0.0 d	0.0 c	0.0 b
	140	0.16	85.0 a	86.2 a	1.0 ab
	140	0.33	0.0 d	0.0 c	0.0 b
	140	0.50	0.0 d	0.0 c	0.0 b
	150	0.16	3.6 d	3.6 c	0.0 b
	150	0.33	0.0 d	0.0 c	0.0 b
	160	0.16–0.33	2.4–0 d	2.4–0 c	0.0 b
FLSD ($P = 0.05$)			7.4	6.6	1.1
Wells	Unheated control		17.7 d	52.6 a	78.6 a
(5.7%)	Ethanol control		24.6 c	53.0 a	68.3 b
	130	0.16	49.0 a	55.3 a	29.3 c
	130	0.33	18.3 d	18.3 c	1.6 ef
	130	0.50	0.0 e	0.0 d	0.0 f
	140	0.16	41.6 b	42.3 b	8.3 d
	140	0.33	0.0 e	0.0 d	0.0 f
	140	0.50	0.0 e	0.0 d	0.0 f
	150	0.16	3.0 e	3.0 d	5.0 de
	150	0.33	0.0 e	0.0 d	0.0 f
FLSD ($P = 0.05$)			6.2	7.3	4.2

[x]Seeds were surface disinfested by immersion for 4 min in 0.05% NaOCl, rinsed twice in double distilled water, plated on potato-dextrose agar, incubated in the dark at 25 C and observed at 6 to 8 days after plating.

[y]A seed was considered germinated if its radicle was at least as long as its cotyledons, and clean if no micoorganisms were visible on the seed. *Phomopsis* spp.-infected seeds were placed under the *Phomopsis* spp. recovery column. Mean of at least six replications of 50 seeds per replication. Means followed by a common letter do not differ significantly, $P = 0.05$, according to Fisher's least significant difference.

[z]Neither control was placed in oil. Ethanol control was immersed 4 min in 95% ethanol before surface disinfestation.

C had more germinated-clean seeds than either control (Table 1). Germination was not increased by any treatment and germination was lower in all but the 100 C treatments.

Soybean seeds survived immersion for 30 sec in oil at 120 C, and treatments as short as 30 sec in oil at 110 C showed a higher rate of germinated-clean seeds compared to the controls. Such brief, high-temperature treatments also decreased the recovery of *Phomopsis* spp. Length of treatment became critical at 110 C; with Williams seeds, an increase from 30 to 60 sec caused a decrease in germination from 84 to 57%. Even more pronounced was the drop in germination caused by an increase from 30 to 60 sec in oil at 120 C with a decrease in germination from 58 to 0% (Table 1).

The highest temperatures used in our studies were 130, 140, 150, and 160 C. Union seeds at 7.5% moisture were placed for 10, 20, or 30 sec in oil at 130 and 140 C and for 10 and 20 sec at 150 and 160 C. An increase in germinated-clean seeds occurred in the 10 sec in oil at 140 C treatment, but not the 10 sec in oil at 130 C treatment, compared to the controls (Table 1). Germination in the two treatments was not different from the controls. Seeds heated for 10 sec in oil at 150 and 160 C showed 3.6 and 2.4% germination, respectively.

The high-temperature experiments were repeated without the 160 C treatments and using Wells seeds at 5.7% moisture. Two treatments, 10 sec in oil either at 130 or 140 C, had a higher percentage of germinated-clean seeds than the ethanol control, while all other treatments had a lower rate (Table 1). Germination was reduced by all treatments except 10 sec in oil at 130 C. Although treatments reduced the recovery of *Phomopsis* spp., they did not eradicate the fungus.

The treatment for 10 sec in oil at 140 C approached the limit of thermotherapy of soybean seeds in soybean oil for the given moisture content. As temperatures were raised, the time spread between when the *Phomopsis* spp. was killed and the seed was killed decreased.

There are two options for effective high-temperature therapy of soybean seeds to control *Phomopsis* spp.: use seeds of a low moisture content so they can survive the longer treatments at 130 and 140 C, or if seeds of higher moisture content are used, then use lower temperatures and a longer treatment time, such as 30 sec at 110 C, so that seeds can survive and *Phomopsis* spp. be reduced.

Thermal effect on germination. No evidence of a direct thermal effect on germination was found. Any increase in germination of seeds heated for 30 sec or more in oil at 90 C was due to the control of seedborne *Phomopsis* spp. that inhibits or kills infected seeds during germination. When seeds of cultivar Wells with 7.7% moisture content and a low (2%) incidence of *Phomopsis* spp. were heated for 0.5 to 5.0 min in oil at 90 C, germination was reduced below the control (Fig. 1). Seeds at 7.7% moisture content had less loss of viability when heated for 3 min or longer, than seeds at 8.6%. Thus, seeds must have a low moisture content before being exposed to soybean oil thermotherpy.

The immersion of soybean seeds for 5 min in oil at 90 C controlled *Phomopsis* spp. without affecting germination, and the treatment for 10 sec in oil at 140 C decreased the recovery of *Phomopsis* spp. with a concomitant higher rate of germinated-clean seeds. This is the first report on the use of treatment temperatures greater than 100 C on soybean seeds. The highest temperature previously reported to which soybean seeds have been treated to control seedborne fungi was 94 C (8).

The use of such high soybean oil temperatures (130–140 C) for brief periods to treat soybean seeds raised the question of the effect of high temperatures on the internal parts of the treated seeds. Investigation of that problem was beyond the scope of this study. However, it may not be necessary for an entire seed to reach a temperature of the heating medium for the treatment to be effective. If the pathogen was limited to or concentrated in the seed coats, as are *Phomopsis* spp. in soybean seeds (5), it may be desirable to heat the seed coat without heating the rest of the seed. This may explain the results of the treatment of 10 sec in oil at 140 C; where the fungus-infected seed coat reached a high temperature, but since the treatment was brief it was likely that the remainder of the seed remained cooler than the seed coat.

There is the question of whether the embryo of hot-oil-treated seeds reached treatment temperature. Seeds heated for 30 min in oil at 90 C germinated 30%, indicating that the embryo survived such high temperatures (10). The possibility that the internal tissues of soybean seeds reached such a high temperature and survived may have implications in the control of other pathogens, especially seedborne viruses. Few plant viruses are transmitted in true seeds, and even fewer are controlled by thermotherapy (7). However, now that soybean oil has been shown to be a compatible medium for heating soybean seeds, it may be desirable to investigate whether seedborne viruses, such as soybean mosaic virus, can be inactivated either by a short, high temperature treatment, or by a more conventional long, cooler (35–60 C) treatment.

The oil technique can be used to disinfect small lots of seeds for germ plasm exchange or by plant pathologists wanting to have seeds with reduced levels of seedborne fungi, but it may not be feasible for a commercial operation. The technique is simple and inexpensive. It has been used to control seedborne fungi in soybean seeds to be used in experiments in which a fungicide might interfere with the results (T. L. Whatley, *personal communication*, Dept. of Plant Pathology, University of Illinois at Urbana-Champaign).

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