# Soybean Seed Thermotherapy with Heated Vegetable Oils

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**ABSTRACT**

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Refined maize (*Zea mays*), palm (*Eleais guinensis*), soybean (*Glycine max*), and sunflower (*Helianthus annuus*) oils were evaluated as a medium for heating soybean seeds to control the seedborne fungi *Alternaria* spp., *Cercospora kikuchii*, and *Phomopsis* spp. Seeds of five cultivars with a moisture content 7.5% or lower ranging in age from 6 to 24 mo were treated in one of the heated oils from 2 to 15 min at 80 or 90 C. All treatments significantly ($P = 0.05$) reduced recovery of the three fungi below that of the controls. *C. kikuchii* was eradicated after 5 min at 90 C, but *Alternaria* spp. were not. There was a concomitant increase in germinable seeds with decreased recovery of *Phomopsis* spp. Heat treatment was more effective in seeds less than 1 yr old than in those more than 1 yr old. No heat treatment affected seed germination in the laboratory. Emergence was significantly reduced by heat treatment in the greenhouse for seeds more than 1 yr old but not for seeds 1 mo old. Emergence was reduced by heat treatment below that in the control in the field, but yield was unaffected.

Thermotherapy of seeds is a common method used to control certain seedborne plant pathogens. It inactivates or kills a pathogen while leaving the host tissue viable. Baker (1,2) reviewed the use of thermotherapy for control of plant diseases.

Many media have been used for heat treatment, including water, steam, air, carbon tetrachloride ($CCl_4$), petroleum oils, and microwave radiation (2,11,14,17). However, seeds of large-seeded legumes such as green beans (*Phaseolus vulgaris* L.) and soybeans (*Glycine max* (L.) Merr.) rarely are heated in hot water because they quickly imbibe water, swell, and slough off their seed coats. To avoid this problem, Watson et al (17) tested various nonaqueous fluids to find a compatible medium. They found that seeds of green beans and lima beans (*P. lunatus* L.) survived longer when treated with motor oil at 90 C than with water at 90 C. Seeds heated in boiling $CCl_4$ (76.8 C) for 60 min also survived. However, they did not test for control of seedborne pathogens. Zinnen and Sinclair (18) showed that soybean seeds treated in heated soybean oil decreased the recovery of *Phomopsis* spp. with a concomitant increase in the number of germinable, pathogen-free seeds.

The advantage of using heated vegetable oils rather than heated water to treat large-seeded legumes and possibly seeds of other crops is that the seeds are not damaged by imbibition of water. The advantages of using heated vegetable oils rather than fungicides are that the seeds can be sold for feed and untreated seeds are not contaminated with fungicide-treated seeds.

In this study, immersion of soybean seeds in heated maize (*Zea mays* L.), palm (*Eleais guinensis* L.), soybean, and sunflower (*Helianthus annuus* L.) oils was tested as a means of eliminating or reducing the levels of seedborne *Alternaria* spp; *Cercospora kikuchii* (T. Matsu. & Tomayasu) Gardner, cause of purple seed stain; and *Phomopsis* spp., involved in Phomopsis seed decay (7,9,15). These fungi are found commonly in the seed coats of soybeans and other large-seeded legumes and occasionally in the embryos (4,16). The hyphae of these fungi in soybean seeds serve as primary inoculum in the development of Alternaria leaf spot and seedling blight, Cercospora leaf spot, and pod and stem blight, respectively (6,8,10,12). A portion of these studies was published as an abstract (13).

## MATERIALS AND METHODS

Soybean seeds of cultivars Amsoy 71, Corsoy 71, Cumberland, Swift, and Williams showing symptoms of natural infection by *Alternaria* spp., *C. kikuchii*, or *Phomopsis* spp. were used (10,16). Seeds of low moisture content were used because seeds of higher moisture levels are more susceptible to heat damage (1,18). Seed moisture content was measured with a Digital Moisture Computer 700 (Seedburrows Equipment Co., Evanston, IL) for each lot before treatment and was always less than 7.5% in all experiments. Seed lots with an initially high seed moisture level were dried to 7.5% or lower, often for 24–48 hr at 32–48 C, depending on initial moisture. The seed lots ranged in age from 6 to 24 mo. Amsoy 71 seed lots were 1 yr old or less, Corsoy 71 and Cumberland lots were more than 1 yr old, Swift lots were 2 yr old, and two seed lots of Williams were either 1 mo or 1 yr old.

**Source of vegetable oils.** Commercially prepared maize (Best Foods, CPC International, Inc., Englewood Cliffs, NJ), soybean (Anderson Clayton Foods, Dallas, TX), and sunflower (Hunt-Wesson Foods, Inc., Fullerton, CA) oils were purchased from local food markets. Commercial palm oil was imported from Zaire.

**Oil thermotherapy of soybean seeds.** Seeds of known moisture content were placed in double cheesecloth bags (120 seeds per bag) and assigned randomly to a treatment. Each oil was heated to 80 or 90 C, and the bags of seeds were placed in the heated oil for periods of 2, 5, 10, or 15 min (18). Seeds (240–1,000) were treated with oil (500–1,400 ml) either by placing the beaker with the oil and seeds on a temperature-controlled hot plate or by placing the beaker with oil in a hot water bath (2,000-ml beaker with 400–500 ml of water) on the hot plate at the required temperature. The hot water bath provided a more stable temperature control of the oil than direct heating. After treatment, seeds were allowed to cool to room temperature (25 ± 2 C), washed in 95% ethanol to remove excess oil, surface-sterilized in 0.5% NaOCl for 4 min, rinsed twice for 3 min each in deionized distilled water, air-dried, and plated on potato-dextrose agar (Difco) acidified (pH 4.5) with lactic acid (APDA). There were four replicates of 100 seeds each, with five seeds per plate. Untreated seeds handled in the same manner served as controls.

After 5–7 days, the numbers of germinated seeds and germinated-clean seeds and recovery of seedborne microorganisms were recorded. A seed was considered germinated if its radicle was at least 2.5 times longer than the

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cotyledons, and clean seeds had no bacterial or fungal growth. Because some treatments caused a slight delay in germination or growth of surviving hyphae, culture plates were incubated for a few additional days. The effectiveness of treatment was based on percent germination and recovery of seedborne mycoflora between heat-treated and untreated seeds. Fungus identification was based on colony characteristics and spore morphology, and with *Phomopsis* spp., pycnidia formation (9,10,16).

**Greenhouse studies.** To determine the effect of heat treatment on seedling emergence, unheated and heated seeds of each cultivar were not washed with ethanol after cooling. They were planted in greenhouse flats (51 × 36 × 7 cm) containing either a steam-sterilized greenhouse mix (field soil-peat-sand-vermiculite [3:4:5:2] or soil-peat-sand mix [3:1:1]). The tests were in a randomized complete block design with four to 10 replicates of 40–100 seeds each.

**Field studies.** The effects of oil thermotherapy on field emergence and colonization of stems and pods by seedborne fungi of plants grown from these seeds were studied in 1983 and 1984. The field plots were located on the Agronomy/Plant Pathology South Farm, UIUC, in a field that had continuous soybeans for more than 30 yr. In 1983, Amsoy 71 seeds with at least 20% *Phomopsis* spp. were either unheated (control) or heated in each oil for 2.5 or 5 min at 90 ± 2 C as described previously and planted by hand in each of four 5-m rows on 75-cm centers. There were four replicates of 640 seeds each. In 1984, Williams seeds with 14% *Phomopsis* spp. were either unheated (control) or heated in soybean oil for 6 and 10 min at 80 C or for 5 min at 90 C and planted in four 3-m rows on 75-cm centers. There were four replicates of 440 seeds each. Both plots were planted in a randomized block design.

To determine the occurrence of fungi in soybean stems, 10 plants from each replicate were harvested 60 days after planting (R6 growth stage) (5) from plots in 1983 and five plants per replicate were harvested in 1984. Stem pieces were cut from the lower two-thirds of the stems, placed in cheesecloth bags, and washed in running tap water for 4 hr, then drained and surface-sterilized in 0.5% NaOCl as described previously. The stem pieces were plated on APDA culture plates with five pieces per plant per replicate. Numbers of colonies of *Alternaria, Fusarium,* and *Phomopsis* spp. were recorded after 4 days in the dark at 25 C.

To determine the occurrence of fungi in pods, 10 pods were harvested at random from the middle of soybean plants at growth stages R6–R8. The pods were placed in cheesecloth bags and washed in running tap water for 7 hr, drained, and dipped in a solution of commercial paraquat (Paraquat 27.1%) as described by Cerkauskas and Sinclair (3). Four 10-pod replicates were placed on cellulose pads (Kimpak) on germination trays and incubated for 4–7 days at 25 C in a seed germinator (Stults Scientific Engineering Co., Springfield, IL). The number of pods with pycnidia characteristic of *Phomopsis* spp. was recorded.

**Table 1.** Effects of thermotherapy with heated (90 ± 2 C) vegetable oils at two exposure times on total seed germination, clean-germinated seeds, and recovery of *Phomopsis* spp. of two soybean cultivars

| Oil | Time (min) | Germinated seeds (%)[w] Total | | Clean | | Recovery (%) of *Phomopsis* spp.[w] | |
|---|---|---|---|---|---|---|---|
| | | Amsoy 71[x] | Swift[y] | Amsoy 71 | Swift | Amsoy 71 | Swift |
| Maize | 2.5 | 96.0 a[z] | 59.5 a | 76.0 a | 51.0 ab | 5.5 cde | 1.5 b |
| | 5.0 | 89.0 abc | 30.0 b | 68.5 abc | 28.5 c | 0.0 f | 0.0 b |
| Palm | 2.5 | 90.5 abc | 59.5 a | 41.5 d | 58.5 a | 8.5 bc | 1.5 b |
| | 5.0 | 73.5 d | 38.5 ab | 57.0 c | 37.0 bc | 0.0 f | 0.5 b |
| Soybean | 2.5 | 84.0 c | 48.5 ab | 56.5 c | 45.0 abc | 3.5 def | 3.5 b |
| | 5.0 | 90.5 abc | 59.0 a | 73.0 ab | 54.5 ab | 0.5 f | 3.5 b |
| Sunflower | 2.5 | 96.5 a | 48.0 ab | 61.0 abc | 45.0 abc | 3.0 def | 3.5 b |
| | 5.0 | 87.0 bc | 45.0 ab | 58.5 c | 44.0 abc | 1.0 ef | 0.0 b |
| Untreated | 0.0 | 85.5 bc | 35.0 b | 64.0 abc | 32.5 c | 26.5 a | 21.5 a |

[w] Means of two replicates of 100 seeds each on acidified (pH 4.7) potato-dextrose agar. Germinated-clean seeds produced seedlings with no evidence of bacterial or fungal growth.
[x] One-year-old seeds; seed moisture content = 7.2%.
[y] Two-year-old seeds; seed moisture content = 6.3%.
[z] Means followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's least significant difference.

**Table 2.** Effects of thermotherapy with heated (90 ± 2 C) vegetable oils at two exposure times on total seed germination, clean-germinated seeds, and recovery of *Alternaria* and *Phomopsis* spp. from 1-mo-old Williams soybean seeds at two moisture levels

| Oil | Time (min) | Germinated seeds (%)[x] Total | | Clean | | Recovery (%) of *Alternaria* spp. | | Recovery (%) of *Phomopsis* spp. | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1[y] | 2[y] | 1 | 2 | 1 | 2 | 1 | 2 |
| Maize | 5 | 93.7 ab[z] | 90.7 b | 87.7 ab | 79.0 c | 1.3 bc | 8.3 b | 0.0 b | 2.7 b |
| | 10 | 89.0 bc | 94.7 a | 85.3 ab | 84.3 abc | 0.0 c | 3.3 c | 0.0 b | 0.0 c |
| Palm | 5 | 95.0 a | 91.7 ab | 86.0 ab | 87.3 a | 5.3 b | 0.3 c | 0.7 b | 0.0 c |
| | 10 | 95.7 ab | 86.7 c | 87.3 ab | 83.0 abc | 2.0 bc | 0.3 c | 0.0 b | 0.0 c |
| Soybean | 5 | 91.3 abc | 92.7 a | 91.7 a | 80.0 bc | 1.0 c | 2.3 c | 0.0 b | 2.3 b |
| | 10 | 88.0 c | 91.3 ab | 83.0 b | 85.7 ab | 1.0 c | 2.3 c | 0.0 b | 0.3 c |
| Sunflower | 5 | 95.0 a | 92.3 ab | 86.3 ab | 81.3 abc | 0.7 c | 2.0 c | 0.0 b | 0.0 c |
| | 10 | 96.0 a | 93.3 ab | 87.7 ab | 86.7 a | 0.3 c | 0.7 c | 0.0 b | 0.0 c |
| Untreated | 0 | 77.3 d | 51.0 d | 40.3 c | 15.0 d | 23.2 a | 27.3 a | 30.7 a | 52.3 a |

[x] Means of three replicates of 100 seeds each on acidified (pH 4.7) potato-dextrose agar. Germinated-clean seeds produced seedlings with no evidence of bacterial or fungal growth.
[y] Seed moisture content = 6.9 and 6.7% for seed lots 1 and 2, respectively.
[z] Means followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's least significant difference.

**Data analysis.** All data were analyzed statistically with the Statistical Analysis System (SAS). Fisher's protected least significant difference (FLSD) was used to separate treatment means. All significant differences are at $P = 0.05$ unless otherwise indicated.

## RESULTS AND DISCUSSION

**Oil thermotherapy of soybean seeds.** The percentages of germinated Amsoy 71 and Swift seeds on APDA generally were greater than those for the controls in all treatments; some treatments were significantly greater (Table 1). The percentages of germinated-clean seeds generally were not significantly different from those in the controls. The percentages of germinated and germinated-clean seeds of Amsoy 71 always were greater than those of Swift in all treatments except for 2.5 min in palm oil. This exception could not be explained. The difference between germinated and germinated-clean seeds between cultivars was due in part to the differences in their ages; Amsoy 71 was 1 yr old and Swift was 2 yr old. All treatments significantly reduced the recovery of *Phomopsis* spp. from seeds of both cultivars, which had similar levels of *Phomopsis* spp. despite their age differences. *Phomopsis* spp. were eradicated in seed lots of both cultivars treated for 5 min in heated (90 ± 2 C) maize oil, in Amsoy 71 seed lots treated with heated palm oil, and in Swift seed lots treated with heated sunflower oil. The lack of consistent eradication probably was due in part to the extent of colonization of embryo tissues by *Phomopsis* spp. (16).

The percentages of germinated and germinated-clean seeds of 1-mo-old Williams on APDA were significantly greater than those of the controls for all treatments in two experiments (Table 2). *Phomopsis* spp. were eradicated from seeds in all treatments of experiment 1 and in all but three treatments in experiment 2. Because the seeds were younger, *Phomopsis* spp. may not have colonized embryo tissues to the extent found in Amsoy 71 and Swift. The level of *Phomopsis* spp. infection of Williams seeds used in experiment 2 was 52.3%. This high level of infection and the possibility of embryo infection may explain the lack of eradication by every treatment.

Recovery of *Alternaria* spp. from Williams seeds on APDA was significantly reduced below that of the control in all treatments in two experiments, and the pathogens were eradicated in a single treatment (10 min in maize oil in experiment 1) (Table 2). In a third experiment with a seed lot of Williams with 6.6% moisture treated in heated (92 ± 2 C) soybean oil, recovery of *Alternaria* spp. was 30.3, 21.7, and 10.3% for the control and for seeds heat-treated for 5 and 10 min, respectively, and the recovery of *Phomopsis* spp. was 33.3, 3.7, and 0.3%, respectively. Totals of germinated seeds for the three treatments were 57.3, 90.7, and 96.3%, respectively, and totals of germinated-clean seeds were 27.3, 69, and 81.3%, respectively. *Alternaria* spp. were shown to colonize soybean seed coats and embryo tissues (10). The failure to eradicate them from heat-treated soybean seeds may be due to embryo colonization and/or heat tolerance of conidia.

Three experiments were done to study the control of seedborne *C. kikuchii* by oil thermotherapy. In the first, Amsoy 71 seeds showing symptoms of purple seed stain were untreated (control) or treated in soybean oil for 5 min at 90 C. The control germinated at 92%, with 100% recovery of *C. kikuchii* on APDA, and treated seeds germinated at 56%, with 0% recovery of the fungus. In the second experiment, with Corsoy 71, and the third, with Williams purple-stained seeds, seeds were either untreated (control) or treated in soybean oil either for 10 min at 80 C or 5 min at 90 C. Percentages of germination for Corsoy 71 were 86% for untreated seeds, 71% for seeds treated for 10 min, and 69% for seeds treated for 5 min; the latter was significantly lower than the control. Percentages of recovery of *C. kikuchii* were 13% for untreated seeds, 3% for seeds treated for 10 min, and 0% for seeds treated for 5 min; the latter was significantly lower than the control. Percentages of germination for Williams were 81% for untreated seeds, 83% for seeds treated for 10 min, and 66% for seeds treated for 5 min; the latter was significantly lower than the control. Percentages of recovery of *C. kikuchii* were 2.5% for untreated seeds and 0% for seeds treated for 10 and 5 min; the last two were significantly lower than the control. Hyphae of *C. kikuchii* are confined to the soybean seed coat before germination and therefore are easily eradicated (16).

Soybean seeds more than 1 yr old are more susceptible to heat treatment by oil thermotheraphy than seeds less than 1 yr old. Emergence of seedlings in the greenhouse from heat-treated seeds was significantly reduced below that of untreated seeds for seeds 1 yr and older regardless of cultivar (Table 3). Emergence of seedlings from heat-treated 1-mo-old Williams seeds was significantly higher than or equal to that of untreated seeds. There was a tendency for reduced seedling emergence in the field from 1-yr-old Amsoy 71 seeds treated with heated oils, with a significantly lower emergence from seeds treated for 5 min with maize, soybean, and sunflower oils at 90 ± 2 C than for unheated seeds in 1983 (Table 4). This reduction in emergence suggests that heat treatment with vegetable oils is either phytotoxic or it increases the susceptibility of seedlings to soilborne microorganisms. Thermotherapy had no measurable effects on seed yields. Similar results were obtained in 1984 (*unpublished*). This sensitivity of old seeds may be due in part to loss of vigor.

The bioassay of stem pieces and pods from plants grown in the field from heat-treated seeds showed no significant differences in the levels of *Alternaria* spp., *C. kikuchii*, or *Phomopsis* spp. between any treatment or the control for 1983 and 1984. Thus, heat treating seeds had no effect on the occurrence of these fungi on plants grown from them.

Zinnen and Sinclair (18) showed that soybeen seeds of moisture content of

**Table 3.** Mean percentages of soybean seedling emergence in soil in the greenhouse of seeds untreated or treated with vegetable oils at 90 ± 2 C 15 days after treatment

| Time (min) | Amsoy 71 (6.7)[w] | Swift (6.5)[w] | Williams (6.3)[x] | | Williams (6.7)[x] | |
|---|---|---|---|---|---|---|
| | 1 yr | 2 yr | 1 mo | 1 yr | 1 mo | 1 yr |
| 0.0 | 67 a[y] | 45 a | 81 b | 72 a | 79 a | 85 a |
| 2.5 | 57 b | 30 b | –[z] | – | – | – |
| 5.0 | 49 c | 23 b | 93 a | – | 76 a | – |
| 10.0 | – | – | 95 a | 50 b | 82 a | 48 b |

[w] Means of 10 replicates of 40 seeds each, treated in soybean oil. Numbers in parentheses indicate percent moisture content.
[x] Means of four replicates of 25 seeds each, treated in palm oil. Numbers in parentheses indicate percent moisture content.
[y] Means followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's least significant difference.
[z] No data.

**Table 4.** Mean field emergence 4 wk after planting and seed yield of Amsoy 71 soybean seeds (1 yr old with seed moisture content = 7.2%) untreated or treated in heated vegetable oils at 90 ± 2 C (1983)

| Oil | Time (min) | Emergence[y] (%) | Yield (kg/ha) |
|---|---|---|---|
| Maize | 2.5 | 64.9 ab[z] | 2,965.3 a |
| | 5.0 | 66.0 ab | 3,212.3 a |
| Palm | 0.5 | 79.6 a | 2,988.0 a |
| | 1.5 | 80.1 a | 2,998.0 a |
| Soybean | 2.5 | 72.5 a | 3,136.8 a |
| | 5.0 | 50.9 b | 2,994.0 a |
| Sunflower | 2.5 | 72.1 a | 2,775.5 a |
| | 5.0 | 50.5 b | 2,742.0 a |
| Untreated | 0.0 | 81.4 a | 2,925.8 a |

[y] Mean of four replicates of 320 seeds each.
[z] Means followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's least significant difference.

7.7% or lower were less susceptible to heat damage than those of higher moisture levels. Our data suggest, in addition, that seeds less than 1 yr old are less susceptible to heat damage than those more than 1 yr old. This has been shown for other seeds but not for soybeans (1).

We also found that *C. kikuchii*, which primarily colonizes the seed coat (16), can be eradicated, whereas *Alternaria* and *Phomopsis* spp., which colonize the embryo as well as the seed coat (10,16), are more difficult to eradicate. This is the first report of the reduction of *Alternaria* spp. and the eradication of *C. kikuchii* from heat-treated soybean seeds. *Phomopsis* spp. are more apt to be eradicated from soybean seeds less than 1 yr old than from those more than 1 yr old, presumably because the fungus colonizes the embryo over time. Also, *Phomopsis* is not as likely to grow at low moisture content. Thus, for the most successful reduction or eradication of seedborne *Alternaria* spp., *C. kikuchii*, and *Phomopsis* spp. from soybean seeds with oil thermotherapy, when seed moisture content is 7.7% or lower, seeds should be heat-treated as soon after harvest as possible for 10 min between 90 and 92 C.

This is the first report on the use of vegetable oils other than soybean oil for thermotherapy of soybean seeds to control seedborne pathogens. The oils used did not show any individual effects different from those reported for soybean oil (18). No visible tissue damage occurred with any oil. There was no visible evidence of absorption of any oil by the soybean seed coat found in culture plates. Thermotherapy did not predispose resulting soybean plants to disease. A major criterion necessary for the selection of a vegetable oil for soybean seed thermotherapy is availability and cost. The vegetable oils can be recycled after use.

Vegetable oil thermotherapy may not be practical for large-scale commercial use because of the problem of heating seeds uniformly in large numbers. However, it can be used to treat small lots of seeds in germ plasm collections or samples that are to be sent into countries where the pathogens may not be established. It may be of practical use for small farmers, primarily in developing countries, who save seeds from year to year, for the control of seedborne pathogens without the use of a fungicide when the latter is not readily available. In contrast to fungicide-treated seeds, heat-treated soybean seeds not used for planting can be fed to animals or used commercially.

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