

Use of Hot-Water Treatment as a Means of Controlling *Alternaria* spp. on Safflower Seeds

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ABSTRACT

Zazzerini, A., Cappelli, C., and Panattoni, L. 1985. Use of hot-water treatment as a means of controlling *Alternaria* spp. on safflower seeds. *Plant Disease* 69:350-351.

A comparison between hot-water and fungicide treatment for the control of leaf spot disease caused by *Alternaria* spp. in safflower showed that hot-water treatment significantly reduced the percentage of safflower seeds with viable *Alternaria* spp. without altering the germination of the seeds.

Leaf spot disease of safflower (*Carthamus tinctorius* L.) is caused by two pathogenic fungi, *Alternaria alternata* (Fr.) Keissl. and *A. carthami* Chowdhury. Preliminary studies carried out near Perugia in Central Italy indicated that *Alternaria* spp. cause severe infections in the field and that they are transmitted by seeds (1,5).

Inoculum of these organisms was found both outside and inside the teguments (1), and in some cases, it reached the embryo (2). Infested seeds are mainly responsible for the spread of these pathogens to newly cultivated areas.

This paper reports the effect of hot-water treatments on survival of *Alternaria* spp. in naturally infected or contaminated seeds as an alternative to chemical treatment, which until now has given poor results (2,4).

MATERIALS AND METHODS

Safflower seeds (cultivar UC1) were harvested from infected plants grown near St. Apollinare (Perugia). These seeds were used to determine 1) whether the conidia of *Alternaria* were on the pericarp or not, 2) the percentage of seeds

infected and contaminated with *Alternaria* spp., and 3) the location of the pathogens in seeds.

The numbers of conidia of *Alternaria* spp. on the seed surfaces were determined by weighing 50 g of seeds and washing them with sterile distilled water. The suspension was centrifuged at 7,000 g, and the pellet was recovered in 6 ml of sterile distilled water. The number of conidia of *Alternaria* in the suspension was determined with a hemacytometer. This procedure was replicated four times.

Localization of fungi was determined on 100 whole seeds as well as various anatomical parts removed aseptically from another 100 seeds. Seeds were surface-sterilized in 4% NaOCl solution for 4 min. Whole seeds and their parts (pericarp, episperm, and embryo) were incubated separately for 10 days at 20 ± 2 C in petri dishes containing potato-dextrose agar (PDA) at pH 6. Development of pathogens on the seeds or in their parts on the plates was examined under a stereomicroscope and a compound microscope.

For the hot-water treatment, seeds were placed in nylon bags and immersed in a Dubnoff water bath (model 3160 D, Valentini O., Milano) at different temperature and time combinations (Table 1). Uniformity of water temperature was maintained by shaking (45 strokes per minute).

Three fungicides, TMTD (50% a.i.), iprodione (Rovral 50% a.i.), and captafol (Sipafol L 43.5% a.i.), were tested as seed treatments and compared with untreated (control) seeds and NaOCl surface-sterilized seeds.

To determine the survival of *Alternaria* spp. on safflower compared with that on untreated seeds, 400 seeds per treatment were plated on PDA at 10 seeds per petri dish.

A randomized block design (four replicates of 100 seeds per treatment) was adopted for all experiments and the percentage of germination determined

Table 1. Effect of hot-water and fungicide treatments on survival of *Alternaria* spp. in naturally infected safflower seeds and on germination of seeds^y

Treatments	Percentage of safflower seeds with viable <i>Alternaria</i> spp.	Percentage of germination of safflower seeds
Hot-water		
50 C		
60 min	0.3 ^z	72.5 j
45 min	1.0 kl	80.2 be
30 min	1.3 kl	80.2 be
15 min	7.7 ij	80.5 be
45 C		
60 min	4.3 jk	84.2 ac
45 min	9.7 hi	84.2 ac
30 min	10.0 hi	83.7 ac
15 min	22.7 h	81.5 ad
40 C		
60 min	37.3 f	86.7 a
45 min	32.3 g	84.7 ab
30 min	36.7 fg	82.2 ad
15 min	47.7 e	83.7 ac
Fungicide		
Captafol	62.7 c	75.0 ef
(261 g a.i./g seed)		
Iprodione	60.7 c	76.5 df
(150 g a.i./g seed)		
TMTD	66.7 b	78.5 ce
(150 g a.i./g seed)		
Sodium hypochlorite	50.0 d	87.0 a
(8% Cl active)		
Untreated	72.0 a	84.5 ab

^y *Alternaria* spp. include *A. alternata* and *A. carthami*.

^z Average of four replicates for each treatment. Each replicate had 100 seeds. Numbers followed by the same letter do not differ significantly ($P=0.01$) according to Duncan's multiple range test.

This work was supported by the M.A.F. (Ministero Agricoltura e Foreste).

Accepted for publication 11 October 1984.

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according to the International Seed Testing Association method (3). All data were transformed in the respective angular values, then elaborated by Duncan's multiple range test.

RESULTS

Location of *Alternaria* in seeds. The pathogens were found both outside and inside the pericarp. About 100 conidia of *Alternaria* spp. per seed were recovered from the seed surfaces. Colonies of the pathogens were observed on 79% of whole seeds and in 69% of separate pericarps after surface-sterilization.

Effect of hot-water treatment on survival of *Alternaria* spp. in naturally infected seeds and germination of seeds.

The effect of hot-water treatment on the survival of the pathogens and on the percentage of germination of the treated and untreated seeds are shown in Table 1 and Figure 1. The dose of heat administered (expressed as temperature per time) and the percentage of treated seeds that still had viable *Alternaria* spp. were negatively correlated. Significantly fewer treated seeds than untreated seeds still had viable *Alternaria*. The percentage of germination of the treated seeds for each treatment, except 50 C for 60 min, was not significantly different from that of the untreated seeds.

Effect of fungicide treatment on survival of *Alternaria* spp. in naturally infected seeds and germination of seeds.

The percentage of fungicide-treated seeds that still had viable *Alternaria* spp. was significantly less than that of untreated seeds. However, a great number of fungicide-treated seeds (60.7–66.7%) still had viable *Alternaria* spp. (Table 1). The percentage of germination in the fungicide-treated seeds was significantly less than that of untreated seeds.

DISCUSSION

Results of these studies confirm that mycelium of *Alternaria* spp. is in and on safflower seeds and that a large number of seeds treated with fungicides have viable *Alternaria* spp.

The percentage of safflower seeds that still had viable *Alternaria* spp. was significantly reduced by hot-water treatments. *Alternaria* spp. in or on safflower seeds can be eradicated by hot-water treatment at 50 C for 30 min or longer. Hot-water treatments did not reduce germination significantly.

Although the long temperatures required to kill the pathogens should make hot-water treatment convenient to use, this method warrants further

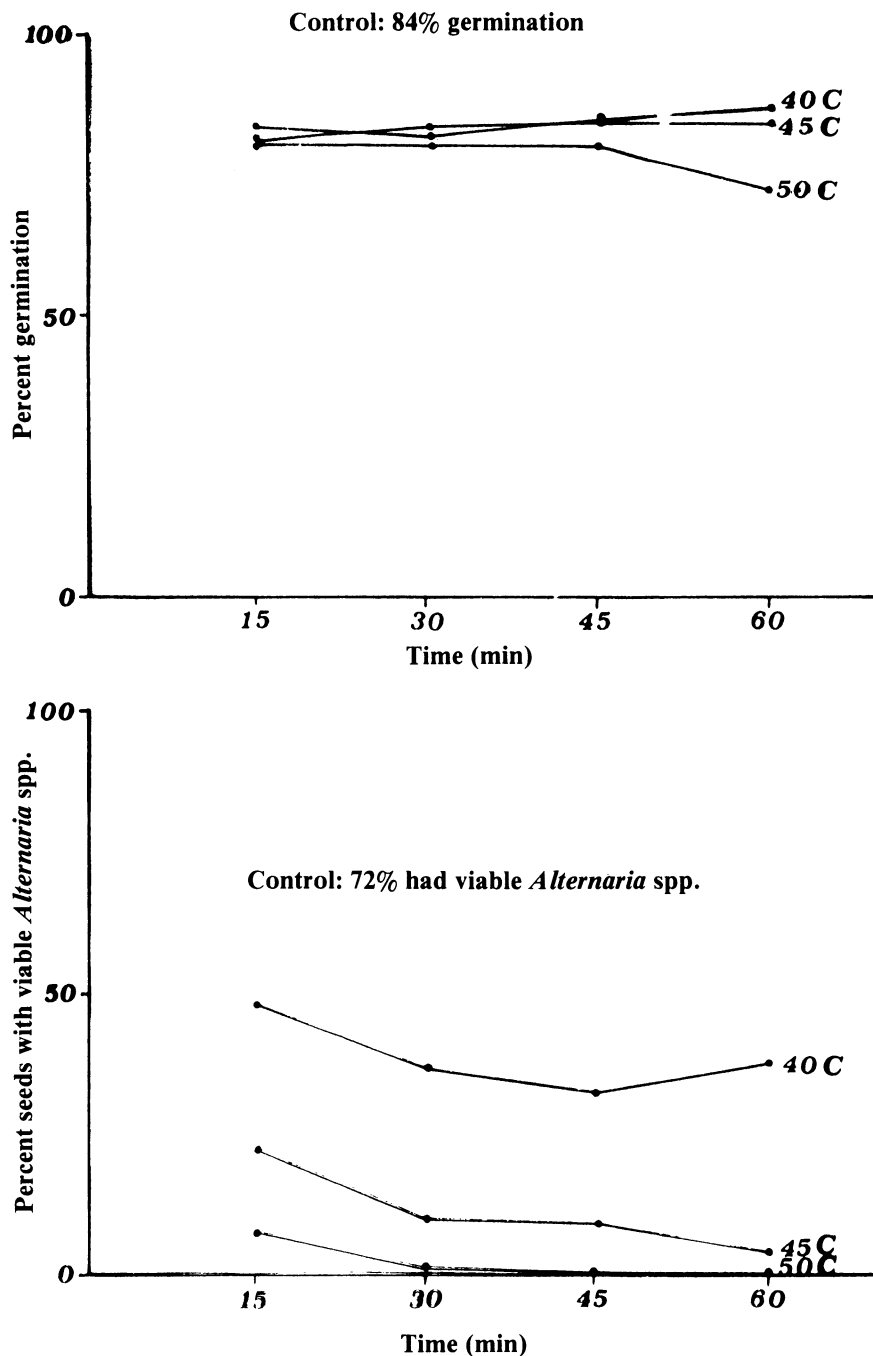


Fig. 1. Effect of hot-water treatment (top) on germination of safflower seeds and (bottom) on survival of *Alternaria* spp. in naturally infected seeds.

research to design the machinery to carry out large-scale treatments and to determine the effect of hot-water treatment on long-term seed storage.

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