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Response of Carrot Seeds to Heat Treatments

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Abstract. **Tolerance of carrot seeds (*Daucus carota* L.) to heat treatments that could eradicate seedborne pathogens was investigated. Germination and emergence of seedlings from seeds treated in hot water at 35, 40, 45, 50, or 55C from 4 to 20 min were not affected, but seeds treated at 60C for 8 min or more were affected adversely. At 45 and 50C, treatment durations as long as 48 min did not affect emergence, but >20 min at 55C reduced emergence. Similar results were obtained when seeds were treated at the same temperatures in water containing 1.1% sodium hypochlorite (NaOCl). Emergence of seeds treated in hot water or 1.1% NaOCl and planted within 5 days generally was similar to that of treated seeds stored for 90 days at 20C in 60% RH before planting. Any existing differences were small and not clearly related to temperature–duration treatment combinations. Percent emergence from seeds of 19 out of 25 hybrid cultivars treated at 50C for 15 min was reduced by an average of 2.9%, but differences for untreated seeds ranged from −13.3% to +4.8%. Emergence from hot water-treated seeds was reduced after 6 weeks of storage at 70% and 80% RH, but not at 20% to 60% RH. Prolonged treatment and the higher temperatures were particularly effective in reducing populations of seedborne *Alternaria dauci*.**

Leaf blights caused by the fungus *Alternaria dauci* (Kühn) Groves & Skolko and the bacterium *Xanthomonas campestris* pv. *carotae* are important foliar diseases of carrot *Daucus carota* L. (2, 10, 11, 14). Both pathogens can be seedborne (1, 6, 7, 12–14), and their exclusion or eradication from carrot seeds are fundamental disease management tactics (3, 11, 14). Seed tests for both pathogens are now available (7, 13), but, despite efforts by seed producers, routine production of pathogen-free seed has not been fully attained (7, 13). Thus, treatments to eradicate these pathogens from stock and commercial seed will continue to be important in disease management programs (1, 3, 10, 12, 14).

In 1944, Ark and Gardner (1) concluded that 10 min at 52C completely eradicated *X. campestris*. pv. *carotae* without affecting seed performance. Since this report, hot water treatment (HWT) near 50C for 10 or 12 min has been recommended to eradicate *X. campestris* pv. *carotae* from seed (1, 2, 4, 10, 11, 14). Protocols have often stressed or implied careful adherence to treatment durations and temperature tolerances of ±0.5C. However, reports of seed damage from HWT (real or perceived) have been common and growers and seed producers are reluctant to use it. Moreover, close temperature control tolerance specifications have increased the cost and complexity of seed treating equipment (4). Rigid control of treatment durations and temperatures as a primary solution to seed damage problems may distract attention from other causes such as improper drying and may have discouraged development of more effective eradication treatments for seedborne pathogens of carrots.

We examined germination and seedling emergence from seeds treated over a wide range of temperatures and durations including the addition of NaOCl to the treatment bath. Extended treatment durations at 50 and 55C reduced survival of *A. dauci*. Seed performance of various carrot cultivars following HWT and performance of HWT seed after storage at high and low relative humidities were also studied.

Materials and Methods

'Caropak' F1 hybrid (Asgrow Seed Co.) was used unless otherwise specified. Lots of 1200 seeds (estimated by weight) were sealed in loose-fitting cheesecloth bags and treated in a temperature-controlled, mechanically stirred water bath. Temperature was increased in increments of 5C from 35 to 60 ± 0.5C; duration was increased from 4 to 20 min in 2-min increments. There were four replications for each of the 54 temperature–duration combinations. HWT for extended periods (20 through

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48 min in 4-min increments at 45, 50, and 55C) was applied with the same methods. Seeds of 25 hybrid carrot cultivars of diverse genetic origin were treated at 50C for 15 min. After HWT, seeds were rinsed in cold tap water, spread on trays, dried for 8 hr in a warm (24 to 30C) greenhouse, and then placed in an air-conditioned room in 40% to 60% RH at 20C for 4 to 5 days before testing. Seeds were also HWT in water containing 1.1% NaOCl (20% commercial bleach).

One hundred seeds from each replicate of the temperature–duration combinations were distributed on filter paper in 10-cm plastic petri dishes and then covered with two additional filter papers; 3 ml of water was added to each dish. After 10 days in the dark at 20C, germinated seeds were counted. A seed was scored as germinated if the emerged radicle was at least 3 times the length of the longest seed axis.

Four lots of 100 seeds from each temperature–duration combination were distributed on the surface of a peat–vermiculite growth medium (400 cm^3; Redi-Earth Peat-Lite Mix, W. T. Grace and Co.) that was lightly packed in 12.5 × 12.5 × 6-cm plastic bedding plant containers; eight units were nested in a master tray without drain holes (Com Pac D801 and F1020, respectively, T.O. Plastics, Minneapolis). Seeds were covered with 1 cm of growth medium. Trays were placed in a greenhouse (ambient air at 24 to 30C) and watered with tap water as needed. Emerged seedlings were counted at 14 and 21 days after seeding.

Portions of HWT seeds were stored at 22C in 25 × 40 × 10-cm sealed plastic boxes containing 2 cm of a glycerine-water mixture (62 g in 38 ml) to maintain 60% RH (5). After 90 days of storage, four lots of 100 seeds from each temperature–duration combination were tested for emergence in the greenhouse as described.

Separate lots of seed were HWT for 15 min at 50C then divided into 100 seed sublots and stored in air-tight plastic 800-ml containers at 20, 40, 60, 70, or 80% RH (maintained with glycerine–water mixtures) at 22C; each RH environment was replicated four times. After 0, 2, 4, 6, and 8 weeks, four sublots of seeds were removed from each RH environment and tested for emergence.

'Hicolor 9' carrot seeds naturally infested with *A. dauci* were HWT at 45, 50, or 55C for 0, 10, 20, 30, 40, 50, or 60 min then tested for survival of the pathogen; seeds were produced and tested as described by Strandberg (13).

Results and Discussion

Below 55C, HWT for up to 20 min did not adversely affect germination. A response surface (data not presented) of the means for percent germination from this 6 × 9 factorial arrangement of treatments was nearly flat except for 60C treatments. This pattern indicated that germination following HWT for 4 to 20 min at 35 to 55C was similar. However, at 60C, for durations exceeding 8 min, and at 55C, for durations exceeding 12 min, the depressed response surface showed that germination was reduced by these treatments. If treatments that obviously damaged seeds were not considered (i.e., data for 60C ommitted), the mean percent germination for all other HWT seeds was 78.2 ± 7.1%; for untreated seeds the mean was 78.3% ± 4.2%.

Effects of HWT on seedling emergence were also evaluated. At 55C and below, a flat response surface for mean emergence values indicated no apparent effects. At 60C, for HWT exceeding 8 min, a depressed response surface showed that seedling emergence was reduced by these treatments (Fig. 1A). With the 60C data omitted, mean emergence for all other HWT was 81.4% ± 4.4%. The mean for untreated seeds in this test was 81.8% ± 5.6%. Results from emergence tests were similar, but less variable, than germination tests (SD = ±4.1% and 7.1%, respectively). Thus, emergence was used to evaluate responses to HWT in all subsequent experiments.

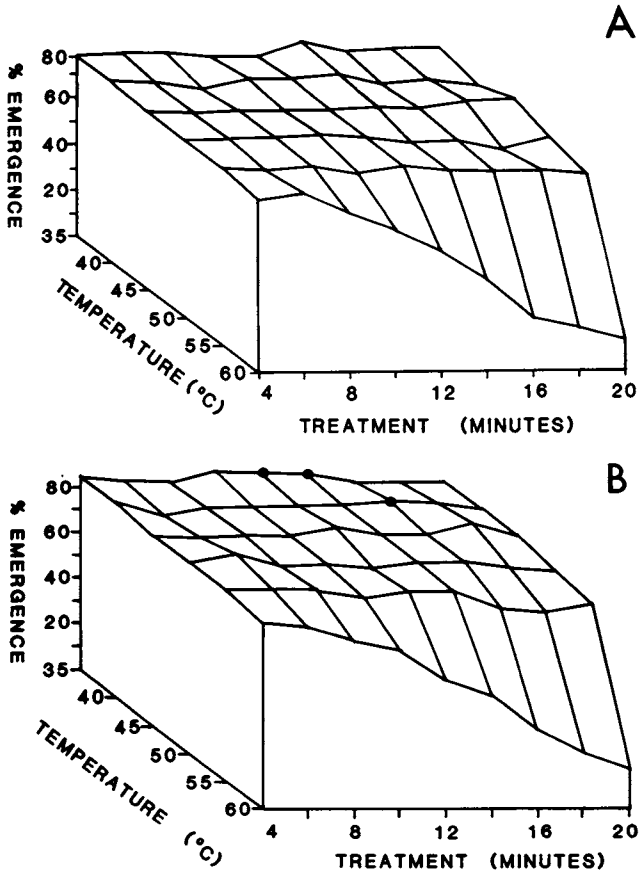

Fig. 1. (**A**) Response surface of mean values of seedling emergence from carrot seed planted within 5 days after hot water treatment at 30 to 60C for 4 to 20 min. (**B**) Response surface for mean seedling emergence from seeds that received the same treatments but were stored 90 days in 60% RH at 20C before planting. Dark elipses at some points on this response surface identify means significantly different (P = 0.05) from means for seeds from corresponding treatments planted without storage.

Emergence from seeds planted within 5 days of HWT and from HWT seeds stored for 90 days at 60% RH was compared; Student's t test (P = 0.05) was applied to paired emergence values. Response surfaces for mean emergence values before and after storage were similar (compare Fig. 1 A and B). Only three means were significantly different (Fig. 1B). The mean emergence for stored HWT seed (data for 60C omitted) was 82.7% ± 11.0%; the mean for untreated, stored seed was 81.5% ± 5.2%.

Seeds treated in 1.1% NaOCl responded similarly to those treated in water alone. A response surface of mean emergence values did not indicate treatment effects at or below 55C, but effects were again obvious at 60C for durations exceeding 8 min (Fig. 2A). Mean percent emergence at 55C and below (60C data omitted) was 76.4% ± 8.3%; for untreated seeds the mean was 77.0% ± 6.2%.

Emergence from NaOCl-treated seeds after 90 days of storage in 60% RH at 20C was similar to that for NaOCl-treated seeds that were planted within 5 days of treatment (compare Fig. 2 A

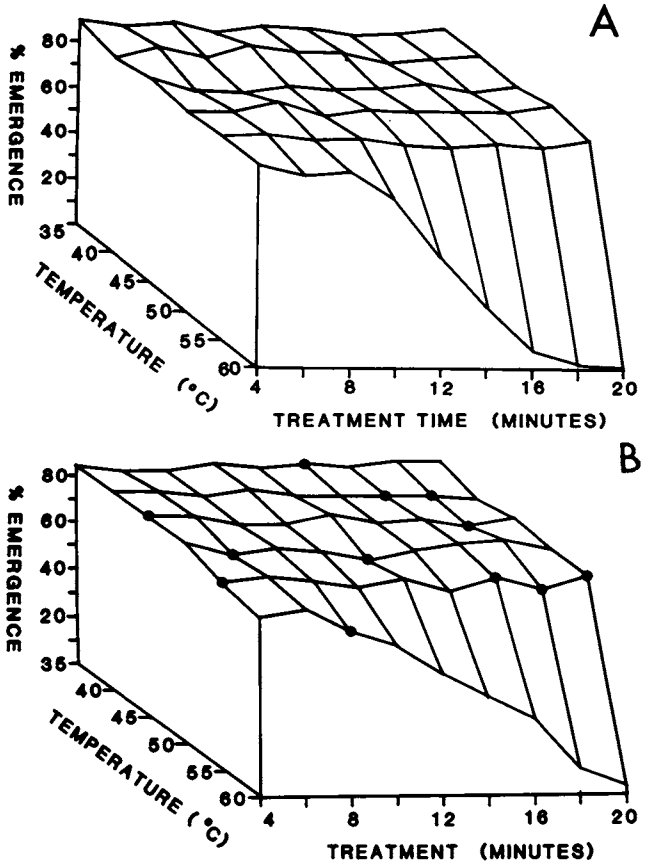

Fig. 2. (**A**) Response surface for mean values of seedling emergence from carrot seed planted within 5 days after treatment with hot water plus 1.1% NaOCl at 30 to 60C for 4 to 20 min. (**B**) Response surface for mean seedling emergence from seeds that received the same treatments but were stored 90 days in 60% RH at 20C before planting. Dark elipses at some points on this response surface identify means significantly different (P = 0.05) from means for seeds from corresponding treatments planted without storage.

and B). With data for 60C omitted, the mean emergence for stored HWT–1.1% NaOCl seeds was 82.6% ± 4.3%; the mean for untreated stored seed 83.2% ± 5.2%. However, mean emergence for 12 NaOCl temperature–duration combinations was significantly different following storage (Fig. 2B).

Similar differences in emergence after storage of HWT and NaOCl-HWT seeds were observed in two experiments. Although these differences were small, and their distribution among treatment combinations did not clearly indicate a cause, most differences for HWT-NaOCl seeds occurred in the longer durations at 50 and 55C. This suggests HWT in 1.1% NaOCl may more adversely affect emergence from seeds than does HWT when seeds are not planted soon after treatment.

Extended HWT durations (20 to 48 min) at 45 and 50C did not affect seedling emergence. Regression lines for emergence with treatment duration (20 to 48 min) for these temperatures were not significant (r^2 = 0.0001 and 0.067, respectively). There were no significant differences among any treatment durations at 45C (P = 0.05, mean = 87.0% ± 3.1%, F = 1.62) or at 50C (mean = 82.6% ± 4.2%, F = 0.34), nor were these values different from untreated seeds in this test (mean = 85.7% ± 3.3%). However, emergence was severely reduced by HWT for durations longer than 20 min at 55C; the regression line for percent emergence with treatment duration (20 to 48 min) at 55C was $Y = -2.7X + 137.3$, $r^2 = 0.94$.

Our results indicate HWT at 50C with or without 1.1% NaOCl for up to 48 min, or at 55C for <20 min, did not greatly affect emergence in 'Caropak' carrot. Temperature (50 to 52C) and duration (10 to 12 min) commonly recommended to eradicate *X. campestris* pv. *carotae* (1, 2, 4, 10–12, 16) are well within the range of temperature and treatment durations that, in our study, did not affect emergence.

When seeds of 25 cultivars were treated at 50C for 15 min, emergence was reduced in 76% of the cultivars (range −0.1 to −13.2%), but only a third of the reductions were significant. Emergence was significantly increased in one cultivar (Table 1). There were no significant (P = 0.05) correlations between changes in emergence after HWT and the percent germination furnished by the supplier of the seed sample (r^2 = 0.01) or with mean seed weight (r^2 = 0.11). Differences in response to HWT among different seed lots of the same cultivar may also be expected, but the lack of seeds prevented us from testing this assumption.

Emergence from HWT or untreated seeds stored at 20% to 80% RH for 0 to 6 weeks was similar. However, after 8 weeks of storage, emergence from HWT seeds stored at 70% or 80% RH was reduced (Fig. 3). Significant regression lines were obtained for decreasing emergence with time for HWT seeds stored at both 70% and 80% RH; $Y = -0.762X + 72.65$, $r^2 = 0.16$

Table 1. Seedling emergence for 25 hybrid carrot cultivars after hot-water-treating seeds at 50C for 15 min.

Experimental hybrid no. or cultivar	Percent emergence[z]		Change[y] (%)
	Not treated	Treated	
12	100	96.0	−4.0 *
35	99.8	97.5	−2.3 NS
9	99.5	93.0	−6.5 *
17	99.5	100	+0.5 NS
16	99.3	96.8	−2.5 *
10	98.5	98.8	−0.1 NS
25	97.3	93.0	−4.3 *
14	96.3	93.0	−3.3 *
37	93.0	87.5	−5.5 *
39	90.5	88.8	−1.7 NS
55	88.5	75.3	−13.2 *
74	87.5	92.3	+4.8 *
66	87.3	79.0	−4.3 *
48	87.3	80.5	−6.9 NS
58	87.3	85.0	−2.3 NS
28	86.5	87.5	+1.0 NS
57	79.8	76.5	−3.3 NS
13	76.0	78.0	+2.0 NS
1	73.5	77.0	+3.5 NS
Fanci Pak	72.8	73.3	+0.5 NS
29	69.8	66.5	−3.3 NS
32	69.0	57.0	−12.0 *
Dagger	58.5	54.0	−4.5 *
64	54.0	51.8	−2.2 NS
4	38.8	38.3	−0.5 NS
		Mean	−2.95
		SD	4.28

[z]Emergence in greenhouse test. Value is average for four replicates of 100 seeds.

[y]NS,*Not significant or significant at P = 0.05 by Student's t test, respectively.

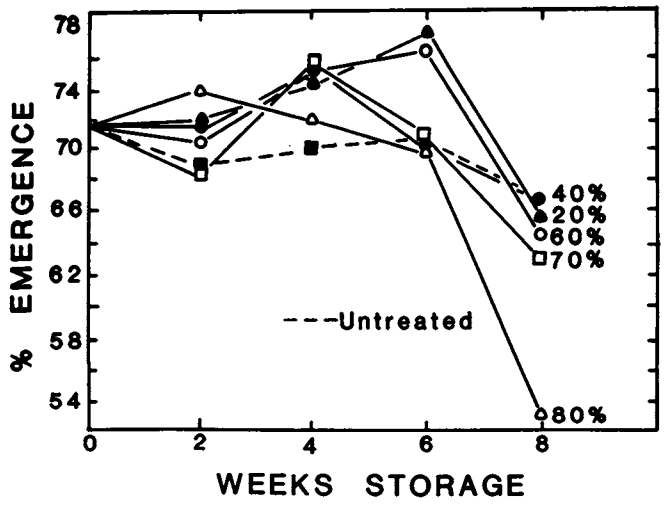

Fig. 3. Emergence of carrot seedlings from untreated and hot water-treated seeds (15 min, 50C) after storage in various relative humidities at 20C.

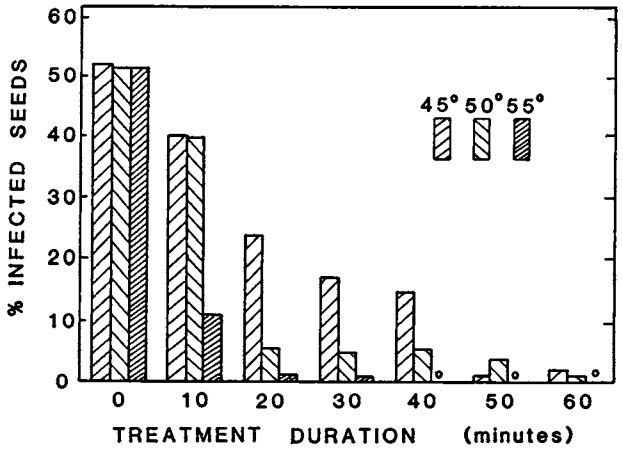

Fig. 4. Survival of *Alternaria dauci* on naturally infested seeds after hot water treatment at 45, 50, or 55C for up to 60 min.

and $Y = -2.025X + 76.25$, $r^2 = 0.45$, respectively. Below 70% RH, storage for short periods (1 to 6 weeks) minimally affected emergence (Fig. 3). HWT of several other vegetable seeds has been reported to not greatly affect storage life up to 1 yr (8).

Although Ark and Gardner (1) reported that HWT at 52C for 12 min completely eradicated the bacterial blight pathogen from carrot seeds, improved detection methods have shown that eradication may not always be attained by this treatment (7). Yet, reduction of primary inoculum in infested seeds is beneficial to reduce the impact of plant diseases (3) and, although HWT will probably continue as a valuable disease management tool, its effectiveness must be improved. Recently, studies to evaluate HWT with NaOCl as an improved treatment to reduce populations of *X. c.* pv. *carotae* in infested seeds have been initiated by other investigators.

Although Strandberg (13) was unable to eradicate *A. dauci* from seeds by HWT at 50C for 12 min, with or without $Ca(OCl)_2$, results of our study demonstrated that pathogen survival could be more effectively reduced by HWT for longer durations, higher temperatures (55C), or combinations of both (Fig. 4). With the seed lot used, emergence was severely reduced by HWT at 55C for longer than 20 min. However, it required at least 40 min at 55C to eradicate the pathogen (Fig. 4). Clearly, HWT alone is insufficient to eradicate *A. dauci* without seed damage. However, it may be possible to combine HWT with fungicides or other agents to eradicate this pathogen.

Our results show that HWT up to stated limits did not greatly affect seedling emergence and that carrot seeds may tolerate temperatures and treatment durations longer than those commonly recommended. Miller and McWhorter (9) treated carrot seeds with steam for 30 min at 62 to 67C and, although *X. c.* pv. *carotae* was not eradicated, emergence was only slightly affected. Clearly, damage from HWT is not likely to occur because of modest fluctuations in treatment duration or temperature. Moreover, extended treatment durations at higher temperatures or other, more rigorous, treatments may be more effective at eradicating pathogens from carrot seed. Based on the results of this study, the general reluctance by growers and seedsmen to employ HWT for many (but not all) applications appears unfounded. Disease control benefits can, in many instances, outweigh the small and deleterious effects of HWT on seed performance. However, differential responses by cultivars (and perhaps seed lots) suggest that treatment of small test samples before treating large seed lots is appropriate.

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