THE EFFECT OF SEED MOISTURE CONTENT AND THE DURATION AND TEMPERATURE OF HOT WATER TREATMENT ON CARROT SEED VIABILITY AND THE CONTROL OF *ALTERNARIA RADICINA*

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Abstract

Hot water treatment of seeds to control seedborne pathogens is an important tool for organic seed production. Reducing seed moisture content may have the potential to increase carrot (*Daucus carota* L. var. *sativus* D.C.) seed tolerance to treatment. Two hot water seed treatment experiments were conducted. The first studied the effect of seed moisture content (SMC), treatment temperature and treatment duration on germination. Maximum safe treatment temperature and durations were established at 50°C and 30-40 min. Germination decreased slightly from 68% at 5% SMC to 63% at 20% SMC (LSD 1.2) for all durations. The second experiment studied the effect of initial SMC and treatment durations on infestation of seed by *Alternaria radicina* and seed germination. Treatment at 50°C for 30 min for all SMC compared to the control resulted in a decrease in *A. radicina* infestation from 69.2 to 1.7%. Reducing SMC from 20 to 5% for all durations resulted in a small decrease in infestation from 25% to 18% (LSD 1.5). Reducing SMC to 5% prior to hot water treatment may be a commercially viable means of minimising reductions in seed viability and decreasing fungal infestation levels.

Introduction/Problem

Non-organic seed has been routinely treated with synthetic biocides to control seed borne pathogens and pests. These treatments are prohibited in certified organic production and alternative treatments, such as hot water or treating seed with biological control agents (Schmitt *et al.* 2004), are being investigated and used. To be effective, heat treatment of seeds requires the thermal tolerance of the seed to be greater than that of the pathogen. To be a practical option, the 'tolerance window' i.e., the difference between the tolerance of seed and pathogen needs to be sufficiently large so that heat treatment causes a large reduction in viable pathogens with minimum impact on seed viability. Techniques that can increase the size of the tolerance window could improve heat treatments. Research into seed vigour tests has shown that SMC affects seed's heat tolerance (TeKrony 1995). Altering SMC might have the potential to increase the tolerance window and therefore, make hot water treatment more effective.

The use of standard germination tests (ISTA 2004), which measure percentage germination under controlled conditions, to assess the effects of heat treatment is somewhat limited. This issue has been recognised in the area of seed vigour where seed lots that have the same percentage germination in laboratory tests can show significantly different percentage emergence in the field (Hampton 1999). This means that there are aspects of seed quality that a laboratory based analysis of germination cannot measure. One approach used in seed vigour tests is to stress the seed by subjecting it to elevated temperatures and humidity for a period of time, often several days. For example, the accelerated ageing (AA) test for soybean (*Glycine max* (L.) Merr.) exposes the seed to 41°C ± 0.3°C at 100% RH for 72 h ± 15 min (ISTA 2004). There are noteworthy similarities between the AA test and hot water treatment in that both expose seeds to elevated temperatures and moisture. It is therefore possible that heat treatment may have negative impacts on seeds that are not detected by measuring germination. A possible means to detect more subtle effects of hot water treatment could be to conduct a seed vigour test on the heat-treated seed. However, the current means of vigour testing carrot seed is the same as

for soybean, described above, which means that the seed would be subjected to two sequential heat treatments. This repeated exposure to deleterious conditions means the result could be questionable. An alternative approach is to analyse the rate of germination. This avoids subjecting the seed to further harmful conditions while providing a potentially more sensitive measure of the effects of heat treatment.

Methodology

Experiment 1: Effect of hot water treatment on carrot seed viability in the absence of pathogens.

Carrot seeds, of an unknown F1 hybrid cultivar known to be free of *A. radicina* were used in a three factorial hot water experiment, with factors being initial SMC (5, 10,15 and 20%), duration of treatment (0 (control) 10, 20, 30, 40, 50 and 60 min) and temperature (45, 50 and 55°C). SMC was determined using an internationally standardised method (ISTA 2004). SMC was adjusted by either placing seed in a 30°C oven until the target weight was reached (for 5%) or adding the required amount of sterile distilled water and holding for 24 h at 5°C (for 10-20% SMC). Seeds were contained in stainless steel tea infusers and placed in a hot water bath containing 11 l of water, which was agitated by a shaker plate. After treatment, all seeds were immediately plunged into 15 l of tap water at 15°C for five minutes to rapidly cool them. A germination test (ISTA 2004) was then immediately conducted on the seeds except the seeds were placed on rolled towels instead of the surface of a blotter. Seeds were incubated in alternating 8 h/30°C light and 16 h/20°C dark cycles. The number of normal seedlings (ISTA 2004) was counted each day for 14 d.

Experiment 2: Effect of hot water treatment on infestation levels of *A. radicina* on carrot seed and carrot seed viability.

Carrot seed infested with *A. radicina* was obtained from plants that had been inoculated by spraying laboratory-produced conidia onto them two months prior to harvest. A two factorial experimental design with SMC of 5, 10, 15 and 20% and duration of 0 (control) 10, 20, 30 min was conducted using the same methods used for experiment one. In addition to measuring germination, percentage *A. radicina* infestation was determined; ten seeds were placed on blotter paper that had been moistened with sterile distilled water in a 9 cm petri dish, incubated for three days in darkness at 20°C, then killed by placing in a -20°C freezer for 24 hours and then incubated for 7 d at 20°C with alternating periods of 12 h near ultra violet light and darkness. Ten petri dishes (replicates) were completed for each treatment. Infestation was determined by visual identification of conidia on the seeds (ISTA 2004).

Germination and infestation data were arc sine transformed before analysis with ANOVA to ensure equal variance. Germination curve data were not transformed. All data and LSD values presented here are untransformed while the p values are from the transformed data. Results with a p value greater than 0.05 are considered not significant, a p value between 0.05 and 0.001 is considered significant, while $p<0.001$ is considered highly significant. Germination curves were calculated and fitted to a logistic model (Equation 1), where, y is the number of germinated seeds and x is days. The values γ, β and μ for each curve were analysed with ANOVA. γ is a measure of final germination (number of seeds out of a total of 50), β is the germination rate coefficient and μ is the point of inflexion of the curve which is a measure of the 'average' time to germination and is measured in days.

Equation 1. Logistic curve formula.

$$y_i = \frac{\gamma}{1 + \exp(-\beta(x_i - \mu))} + \varepsilon_i$$

Results and brief discussion

Experiment one. Germination was significantly reduced by increasing treatment duration and temperature, and there was a significant interaction between these two factors (Table 1), in that at 45°C germination did not differ among treatment durations, but at 50°C was reduced after 50 minutes, and at 55°C after 20 minutes. Germination fell from 68% at 5% SMC to 63% at 20% SMC. The average time to germination (μ) tended to increase as treatment duration increased, and there was a

significant interaction between treatment duration and SMC (Table 2). Increasing treatment temperature also increased μ (data not presented)

Table 1. Effect of duration × temperature on germination (%) (LSD 4.3)

		Duration (mins)						
		0	**10**	**20**	**30**	**40**	**50**	**60**
Temp°C	**45**	82	84	81	77	81	80	82
	50	81	81	81	80	80	72	65
	55	78	77	62	38	19	5	1

Table 2. Effect of SMC × duration on μ (days) (LSD 0.81)

		Duration (mins)						
		0	**10**	**20**	**30**	**40**	**50**	**60**
SMC	**5**	3.9	3.6	4.1	4.8	4.6	6.1	6.2
	10	4.0	3.9	4.1	4.9	5.0	4.8	6.9
	15	4.2	3.8	4.5	4.5	4.7	6.0	6.2
	20	4.5	3.6	4.5	4.8	5.7	5.0	7.0

Experiment two. SMC (Table 3) had a significant effect and duration (Table 4) had a highly significant effect on infestation levels. The effect on percentage germination was not significant for either SMC or duration; however, the interaction was highly significant (data not presented). The grand mean for germination for the whole experiment was 16%.

Table 3. Effect of initial SMC on infestation of seeds by *A. radicina* (LSD 1.5)

	SMC			
	5	**10**	**15**	**20**
Infested seeds %	18	23	20	25

Table 4. Effect of treatment duration on infestation of seeds by *A. radicina* (LSD 1.5)

	Duration (mins)			
	0	**10**	**20**	**30**
Infested seeds %	69	9	5	2

The first experiment established the thermal treatment limits of carrot seed. Fifty-five degrees Celsius was clearly too hot causing a significant decrease in germination after just 20 min. In comparison, germination started to decrease only after 40 min at 50°C, so 50°C is taken to be the maximum safe temperature and 40 minutes the maximum duration.

The germination rate analysis conducted in this experiment is in general agreement with the percentage germination data. However, the average time to germination (μ), while following similar trends to percentage germination for treatment temperature and duration, demonstrated an earlier onset of negative effects (after 30 min for all SMC). This may indicate that damage to seeds is occurring at more quickly than can be detected by percentage germination. The experience of seed vigour tests suggests that laboratory and field based percentage germination of heat treated seeds may not be in agreement and that treatments found to be 'safe' under controlled condition tests may not be 'safe' when the seed is planted in the field.

While treatment temperature and duration were highly significant for germination rate (β) there was no clear trend. The high level of significance gained where no clear trend exists is due to the large statistical power of the experimental design. This is also partly true of SMC, where although the change in germination data is highly statistically significant, biologically the change is not sizeable, especially when compared to the large effects of temperature and duration on germination. However, at the 5% SMC level there was a slight increase indicating that lowering SMC before treatment could be beneficial in improving percentage germination.

The second experiment showed treatment duration had a very large and biologically highly significant, effect on the infestation levels of *A. radicina* reducing them to very low, agronomically acceptable, levels. The effect of SMC on infestation was less clear because there is no apparent trend in the data. Although the difference between the lowest and highest SMC was biologically significant this needs qualifying as the SMC of carrot seed in ambient conditions is around 10%, so such a large reduction

would not be gained in commercial operations. The high infestation levels and low overall germination of the infested seeds limits the ability to draw any clear conclusions from the germination analysis.

There is a methodological issue that should be considered, in that the process of altering SMC may have a direct effect on the pathogen, as it does on the carrot seed. To be precise the experiment measures the effect of altering SMC on the pathogen as well, and to be completely thorough the effect of altering MC should be tested on the pathogen in isolation. However, the experiment is aimed at understanding and simulating a commercial practice so it is suggested this issue does not overly impinge the value of the study.

Conclusions

The safe treatment duration and temperature established for carrot seed is much greater that that required to cause a large reduction in viable *A. radicina*, showing that it is a practical and effective means of reducing the infestation levels of carrot seed lots. The effect of altering SMC prior to treatment was less pronounced, but there was an improvement in germination of treated seed and a decrease in the infestation level. It may be commercially and practically valuable to decrease SMC to 5% prior to treatment to reduce the loss of seed viability while also reducing viable fungal infestation levels. Even if this is not the case, care should be taken to ensure that SMC is not raised above ambient levels prior to treatment, as this will reduce the effectiveness of the treatment. Further work using seed that has infestation levels more likely to found in commercial practice would be valuable to confirm the observed effects of altering SMC and also to test the method on other seed borne carrot pathogens such as *A. dauci* and *C. carotae*.

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