Is heat treatment a viable option for destroying weed seeds in biosecurity risk goods?

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Abstract The prevention of biosecurity threats is an international priority and the development of improved phytosanitary treatment technologies is part of New Zealand's biosecurity science strategy. Twelve temperature/time treatments were investigated as potential methods to devitalise weed seeds. Six weed species, *Digitaria violascens*, *Eleusine indica*, *Lepidium virginicum*, *Plantago lanceolata*, *Portulaca oleracea* and *Sonchus oleraceus* were exposed to treatments of 50°C, 60°C or 70°C each for 1.5 h, 4 h, or 15 h. The efficacy of these treatments was compared with those of two industry standards: (i) 85°C for 15 h at 40% RH; and (ii) methyl bromide (80 g/m³) for 24 h. The standard treatments were significantly more effective at devitalising weed seeds than the experimental treatments. This study showed that temperatures below 85°C are generally insufficient for devitalising seeds of the weed species examined.

Keywords heat treatment, weed seeds, biosecurity, seed devitalisation.

INTRODUCTION

International trade facilitates the spread of unwanted species directly through their unintentional introduction as contaminants of cargo or stowaways within different modes of transport (Pimentel et al. 2005; Hulme 2014). As such, the prevention and mitigation of biosecurity threats has become an international priority. The aim of New Zealand's Biosecurity Science Strategy Objective 1.7 is to develop improved treatment technologies which are more cost effective, safer and environmentally acceptable for at-risk goods (MAF Biosecurity 2007). The risks associated with phytosanitary invertebrate pests are relatively easily estimated because: (a) the target crop species is known; (b) the potential pool of pests of a specific crop is relatively small and well recognised; and (c) the risks are quantifiable in monetary terms

(Hulme 2012; Leung et al. 2012). Quantifying the risks posed by weeds is more complicated than those from phytosanitary pests, however. Weeds have historically been considered a low-priority biosecurity risk due to their slow rate of invasion leading to long periods of time before having economic impacts. Extensive, but complicated, approved biosecurity treatment standards already exist (MPI 2012) but capability to meet these standards varies among countries and localities. Therefore, exporters, importers and biosecurity authorities would be more likely to employ biosecurity treatments having a wide spectrum of pest control; not specifically targeting weeds, but encompassing them. With open markets and greater demand for goods from developing countries, it is important that phytosanitary treatments are easy to understand

and apply, require minimal resourcing and do not affect the goods hosting the potential pest. One such broad-spectrum treatment could be a relatively simple heat treatment. Heat treatment is currently an alternative approved biosecurity treatment of soil in response to arthropod biosecurity breaches on shipping and air containers as well as soil fungal spores, insects and seeds on agricultural machinery (MPI 2012). It has also been shown to successfully control weed seeds in agriculture (van Loenen et al. 2003; Vidotto et al. 2013). Therefore, using heat would be a logical alternative to the current practice of using methyl bromide (MeBr) for treatment of seeds in biosecurity risk goods. Heat treatment standards for biosecurity are generally based on maintaining the goods at a specific temperature for a specified time. These treatments, as with other quarantine treatments, are designed to kill plant pests without destroying or appreciably devaluing the infested commodity. Some common heat treatments include: hot water immersion; steam; vapour heat and forced hot air (USDA 2013; Dahlquist et al. 2007). The aim of this study was to review existing literature for suitable heat treatments and to test the effect of these on six species of weed seed representing both grass and broadleaf weeds that have been previously intercepted at the border (James et al. 2014).

MATERIALS AND METHODS

Preparation of weed seeds

Fresh seed was collected from plants of *Digitaria violascens*, *Eleusine indica*, *Lepidium virginicum*, *Plantago lanceolata*, *Portulaca oleracea* and *Sonchus oleraceus* grown in a glasshouse at Ruakura, Hamilton, New Zealand. Seed were collected over the summer of 2014–15 and stored at 5°C until required. Measures of seed health were calculated in early January 2016, following the rules prescribed by the International Seed Testing Association (ISTA 2015). However, as most seeds studied were not specifically listed in the rules, some generalisations had to be made.

The initial germination rate of the seeds was determined by placing 50 seeds of each species

onto a paper towel, wetted with tap water, and placed into sealable plastic bags. These bags were kept on the bench in a Controlled Environment Room with 16 h dark at 20°C ($\pm 0.5^\circ\text{C}$) and 8 h light at 30°C ($\pm 0.5^\circ\text{C}$). Germination was monitored for 6 weeks. Germination was considered to have occurred after radical emergence. This was replicated four times for each species.

The thousand seed weight of each of the test species was determined by sampling eight replicates of 1000 seeds counted by hand and weighed. The thousand seed weight was then calculated as the average weight of the eight replicates for each species.

Seed moisture content was assessed by sampling four replicates of 1 g of seed, taken from each of the test species, and weighed into metal dishes. These were then placed into the oven at 103°C for 17 h. Once dried, the metal dishes were covered and placed into a desiccator to cool at ambient temperature, before being re-weighed. The moisture content was calculated as the percentage difference between the seed fresh weight and dry weight.

Preparation of “host goods” to be treated

Seed bags were prepared during January 2016 by counting 50 seeds each of *D. violascens*, *E. indica*, *L. virginicum*, *Plantago lanceolata*, *Portulaca oleracea* and 25 seeds of *S. oleraceus* into individual fine mesh bags (40 x 40 mm, 0.3 mm mesh). The bags were folded at the top and stapled shut to prevent seed escape. Fifty-six bags were prepared for each species (14 treatments x four replicates, Table 1).

Weed seeds are likely to be introduced into New Zealand via contamination of host goods. An artificial “host good” (Daltons washed No. 1 Sand) was used in this study. The initial moisture content of the sand was determined gravimetrically by drying a known weight at 80°C for 24 h. To prepare the “host goods”, autoclave bags (300 x 560 mm) were filled with sand (4 kg). Each “host goods” bag was artificially contaminated by placing one bag of each of the six test seed species in the middle of the sand within the bag.

Treatment details

The range of temperatures and time scales for treatments were determined from the current heat treatments approved by the Ministry for Primary Industries quarantine heat treatment standards for various commodities such as batteries and timber (56°C, 30 minutes), bulk containerised products (60°C, 10 minutes) and winches, wire ropes and thick timber (70°C, 4–26 h) (MPI 2012) (Table 1).

Table 1 Detailed list of the treatment duration and temperature

Treat- ment #	Treat- ment temper- ature (°C)	Humidity (%)	Time (h)
1	22 (Untreated)	65	1.5
2	22 (Untreated)	65	4
3	22 (Untreated)	65	15
4	50	23	1.5
5	50	23	4
6	50	23	15
7	60	18	1.5
8	60	18	4
9	60	18	15
10	70	20	1.5
11	70	20	4
12	70	20	15
13 ¹	85	40	15
14 ¹	MeBr ² (80 g/m ³) 10–15	n/a	24

¹ Industry treatment standards for comparison (MPI 2012)

² MeBr = methyl bromide

Treatments 1–12 were applied in the Plant Protection Glasshouse Facility, AgResearch Ruakura Research Centre, Hamilton, during January 2016. Three Contherm Digital Series 5 ovens set at either 50°C (Treatments 4–6), 60°C (Treatments 7–9) or 70°C (Treatments 10–12) were used for heat treatments. The untreated bags (Treatments 1–3) were placed on the floor in the glasshouse laboratory which was air conditioned

to 22°C. The open autoclave bags were placed in the centre of the oven once the set temperature had been reached. The bags were treated in four replicate blocks on different days. The humidity within the autoclave bags was monitored during treatment using an Egmont mini humidity meter (code HIMHM).

Treatments 13 and 14 were applied at the Quarantine Treatment Centre (QTC) in Mangere, Auckland during January 2016. These were treated within the biosecurity quarantine system as the treatment standards for comparison of the effectiveness of the heat treatments. The heat treatment chamber was maintained at 40% RH for Treatment 13 but the relative humidity during Treatment 14 was not measured.

Determination of viability

Seed viability was assessed through a germination test following International Seed Testing Association (ISTA) methodology (ISTA 2015), within 24 h following the heat treatments. As most of the species are not specifically mentioned in the ISTA protocols the standard conditions for the germination of small, temperate seeds was used. The seeds from each mesh bag were placed onto a paper towel, wetted with tap water, and placed into sealable plastic bags. These bags were kept on the bench in a Controlled Environment Room with 16 h dark at 20°C (±0.5°C) and 8 h light at 30°C (±0.5°C). Germination was monitored for 6 weeks. Germination was considered to have occurred after radical emergence.

Statistical analyses

Prior to statistical analysis, the germination count data for each species, treatment and replicate were square root transformed to stabilise the variance. These transformed data were analysed independently for each species using ANOVA blocked by replicate. The treatments were analysed as a four temperature (untreated, 50, 60, 70) by three exposure times (1.5, 4, 15) factorial plus two additional standard treatments: MeBr at 24 h; and 85°C at 15 h). To test for the main effects of temperature and/or time, the data were first analysed using the factorial + two

added treatment structure in the ANOVA. If the interaction between temperature and time was not statistically significant at the 5% level, the main effects of temperature and time were examined. If the interaction was significant, an ANOVA with a single fixed effect of treatment was fitted (thereby comparing directly the means between the 14 treatments). A protected Fisher's least significant differences (LSD) analysis (at the 5% level) was used to compare the mean of all treatments.

RESULTS

The weight of one thousand seeds was determined for each of the six test species (Table 2). The lightest seed, *Portulaca oleracea*, generally withstood the heat treatments more successfully than the other species, but a plot of the data did not indicate that there was a relationship between seed weight and seed survival. The heaviest seed, *Plantago lanceolata*, was intermediate in its ability to survive heat treatment. Further, species with seeds of similar weight: *E. indica*, *L. virginicum* and *S. oleraceus*, performed quite differently from each other, with *S. oleraceus* being the most susceptible to heat treatments and *L. virginicum* being the most resistant to methyl bromide (Table 3).

The interaction between temperature and time for the heat treatments was highly significant ($P<0.001$) showing a strong relationship between the effect of temperature by time on seed germination across all species and when compared with the standard treatments. Germination decreased as either temperature

or time increased or both variables together increased (Table 3).

Results of the LSD analysis showed that Treatments 4–12 differed in their success across species when compared with the standard treatments (Treatments 13 and 14) (Table 3).

In most cases, both the standard treatments were significantly more effective at devitalising seed than the other non-standard treatments. However, the 85°C/40% RH standard treatment was significantly more effective than MeBr for *L. virginicum* and *Portulaca oleracea*. Non-standard treatments at 70°C for either 4 h or 15 h and 20% RH resulted in the lowest levels of subsequent seed germination. With the exception of *Portulaca oleracea*, these treatments significantly reduced the germination of all species when compared with the other non-standard treatments and, for *E. indica*, these treatments were as good as the standard treatments. For *S. oleraceus*, all treatments, except 50°C for 1.5 h and 4 h, were as good as the standard treatments.

DISCUSSION

The industry standard heat treatment of 85°C/40% RH at 15 h was the most successful of all the treatments tested, resulting in no germination of any seed of the six weed species evaluated in this trial. However, reducing the temperature to $\leq 70^{\circ}\text{C}$ did not result in significant seed death, except for *S. oleraceus* at 70°C. Therefore, no changes to the industry standard heat treatment can be recommended as

Table 2 Pre-treatment germination, thousand-seed-weight (g) and moisture content of the seed.

Species	Germination (%)	Thousand-seed-weight	
		(g)	Moisture content (%)
<i>Digitaria violascens</i>	98	0.391	9.92
<i>Eleusine indica</i>	38	0.524	9.31
<i>Lepidium virginicum</i>	100	0.626	12.25
<i>Plantago lanceolata</i>	76	2.350	11.65
<i>Portulaca oleracea</i>	100	0.198	8.36
<i>Sonchus oleraceus</i>	93	0.520	6.58

Table 3 Summary of the square-root transformed germination means and the least significant differences (LSD) for each species. Actual mean germination rate is shown as a percentage in parentheses.

Treatment #	Treatment temperature (°C)	Time (h)	Humidity (%)	<i>Digitaria violascens</i>	<i>Eleusine indica</i>	<i>Lepidium virginicum</i>	<i>Plantago lanceolata</i>	<i>Portulaca oleracea</i>	<i>Sonchus oleraceus</i>
1	22 (Untreated)	1.5	65	6.96 (97%)	4.01 (32%)	7.07 (100%)	5.71 (65%)	7.04 (99%)	4.63 (43%)
2	22 (Untreated)	4	65	6.81 (93%)	3.84 (29%)	7.05 (99%)	6.01 (72%)	7.07 (100%)	4.57 (42%)
3	22 (Untreated)	15	65	6.98 (97%)	3.85 (30%)	7.07 (100%)	5.92 (70%)	7.07 (100%)	4.79 (46%)
4	50	1.5	23	6.85 (94%)	3.49 (24%)	7.04 (99%)	6.23 (78%)	6.96 (97%)	1.70 (6%)
5	50	4	23	6.95 (96%)	3.03 (18%)	6.95 (96%)	6.39 (82%)	6.76 (91%)	1.96 (8%)
6	50	15	23	4.89 (48%)	2.24 (10%)	4.48 (40%)	3.92 (31%)	5.41 (59%)	1.46 (4%)
7	60	1.5	18	6.36 (81%)	3.75 (28%)	7.07 (100%)	6.54 (85%)	6.98 (97%)	0.85 (1%)
8	60	4	18	6.11 (75%)	2.71 (15%)	6.44 (83%)	4.76 (45%)	6.95 (96%)	0.50 (1%)
9	60	15	18	6.14 (75%)	2.10 (9%)	0.35 (0%)	4.43 (39%)	6.57 (86%)	0.00 (0%)
10	70	1.5	20	6.49 (84 %)	1.76 (6%)	6.74 (91%)	4.66 (43%)	6.38 (81%)	0.00 (0%)
11	70	4	20	3.63 (26%)	0.60 (1%)	0.00 (0%)	0.25 (0%)	6.32 (80%)	0.00 (0%)
12	70	15	20	0.25 (0%)	0.00 (0%)	0.00 (0%)	0.00 (0%)	0.85 (1%)	0.00 (0%)
13	85°C	15	40	0.00 (0%)	0.00 (0%)	0.00 (0%)	0.00 (0%)	0.00 (0%)	0.00 (0%)
14	MeBr ¹	24	n/a	0.00(0%)	0.00 (0%)	2.44 (12%)	0.00 (0%)	1.39 (4%)	0.00 (0%)
P-value				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD (5%)				0.618	0.933	0.932	0.697	0.300	0.788

¹MeBr = methyl bromide

a broad application for weed-seed devitalisation. *S. oleraceus* were neither the lightest or heaviest seeds tested so other aspects of seed morphology, such as seed coat thickness and permeability, are likely to be more important factors of persistence than seed weight. However, because these aspects of seed morphology were not measured it may only be surmised that the hard black seed coat of *Portulaca oleracea* may have contributed to the seed's ability to survive the heat treatments (Kelly et al. 1992).

Investigations are being carried out on the effectiveness of ozone as a fumigation treatment for invertebrates (Mendez et al. 2003; Isikbera & Athanassioub 2014), and extending these studies to include weed seeds is worthy of further investigation.

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