

Thermal inactivation of weed seeds and tubers during drying of pig manure

C.M.J. BLOEMHARD¹, M.W.M.F. ARTS², P.C. SCHEEPENS¹ & A.G. ELEMA³

¹DLO-Centre for Agrobiological Research (CABO-DLO), P.O. Box 14, NL 6700 AA Wageningen, Netherlands

²DLO-Institute of Agricultural Engineering (IMAG-DLO), P.O. Box 43, NL 6700 AA Wageningen, Netherlands

³Research Station for Arable Farming and Field Production of Vegetables (PAGV), P.O. Box 430, NL 8200 AK Lelystad, Netherlands

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Abstract

Propagules of seven plant species were subjected to heat treatments comparable to industrial manure processing. Propagules of all species, which had been preincubated 1 day in pig slurry, responded to heat treatment in an oven in the range from 50 to 104 °C. *Brassica napus* and *Solanum nigrum* were the most heat-sensitive species. The viability of their seeds was greatly reduced after 15 min at 50 °C. Seeds of *Amaranthus retroflexus*, *B. napus*, *Chenopodium album*, and *S. nigrum* were inactivated after 3 min at 75 °C, seeds of *Echinochloa crus-galli* and tubers of *Cyperus esculentus* after 3 min at 90 °C, whereas seeds of *Abutilon theophrasti* only slightly responded to 3 min at 104 °C. Heating seeds of *A. theophrasti* at 104 °C with steam resulted in much less viability than heating at the same temperature in the oven. More than 1 day preincubation in slurry positively affected thermal inactivation of *E. crus-galli* seeds and *C. esculentus* tubers at 75 °C, but not of *A. theophrasti* seeds at 75, 90, and 104 °C.

Keywords : weed propagules, viability, thermal inactivation, heat-sensitivity, imbibition, manure processing

Introduction

Slurry is a surplus product in some regions in the Netherlands with intensive livestock farming. The excess of nutrients in the slurry contributes to the pollution of soil, water, and atmosphere. The surplus problem may partially be solved by utilizing the slurry as an organic fertilizer for crops on arable land in other regions. The risk that slurry contains viable weed propagules (seeds and vegetative reproduction organs) is an important factor that restricts its use in regions with arable farming. Weeds that are dominant in regions with livestock farming, such as *Echinochloa crus-galli*, and herbicide-resistant biotypes of *Chenopodium album* and *Solanum nigrum*, will probably increase weed control costs if they became

established in regions with arable farming. The same is true for weed species that are not yet widely distributed in regions with livestock farming, but still absent in regions with arable farming. These species include *Abutilon theophrasti*, *Amaranthus retroflexus*, and *Cyperus esculentus*. Fodder with weed propagules may be fed to farm animals (Elema et al., 1990). The propagules can survive subsequent passage through the digestive tract of farm animals (Atkeson et al., 1934; Burton & Andrews, 1948), and retain their viability during exposure to slurry (Rieder, 1966; Takabayashi et al., 1979; Elema et al., 1990).

To facilitate transportation of manure over large distances, technologies have been developed that remove water from slurry. A process that several of these technologies have in common is the drying stage, in which the water content of an intermediate product is decreased to about 15 % by heat treatment. This heat treatment may inactivate viable weed propagules that are present in the intermediate product. In addition to the effects of heat treatment, friction forces involved in pelleting the intermediate product have a detrimental effect on the viability of the weed propagules (C.M.J. Bloemhard, unpublished results).

Dry seeds may be quite resistant to heat. For instance, 94 % of seeds of *Avena fatua* were still viable after heating 1 hour at 100 °C in an oven, whereas all seeds were dead only after heating 1 hour at 140 °C (Metz, 1970). Maun (1977) found similar results for seeds of *E. crus-galli* and *Poa pratensis*. Hopkins (1936) imbibed weed seeds at 25 °C and 50 % relative humidity (RH) before heating them 15 minutes in closed tubes. Under these conditions, the lethal temperature for seeds of *A. fatua* was 105 °C, for seeds of *C. album* 95 °C, and for seeds of *A. retroflexus* and *Thlaspi arvensis* 85 °C. Horowitz & Taylorson (1984) heated seeds of *A. theophrasti* 1 hour, and found a decrease in the germination rate at 70 °C and higher temperatures. The effect of heat was larger if the seeds were heated at high RH than in a dry atmosphere. Immersion in hot water was more effective than dry or humid heat. Under all these conditions, the seeds had lost their viability after treatment at 100 °C. Thermal inactivation was preceded by breaking of dormancy at temperatures of 70 °C and higher. From the available data it can be concluded that the effect of heat treatment on seeds is greatly affected by the moisture content of the seeds, and/or moisture in the environment.

Aim of our investigations was to measure the effect of heat treatments on weed propagules under circumstances that are comparable to those in experimental plants for manure processing. We assumed that weed propagules will have imbibed at least 1 day in the slurry before processing. The intermediate product may be heated at 130-150 °C for at least 1 hour. Other heating processes are feasible with temperatures between 75 °C and just above 100 °C, and a duration of heat treatment of 15 minutes at the utmost.

Our experiment were done under laboratory conditions with pig manure. In addition to the six weed species mentioned in the introduction, *Brassica napus* (a crop species) was included to represent cruciferous weeds with oil-containing seeds.

Materials and methods

Seeds and tubers; manure

Seeds of *Abutilon theophrasti* Med. were imported from the USA in 1985. Seeds of *Amaranthus retroflexus* L., *Chenopodium album* L., *Echinochloa crus-galli* (L.) Beauv., and *Solanum nigrum* L., and tubers of *Cyperus esculentus* L. were collected in the vicinity of Wageningen in 1988 and 1989. After collection, the seeds and tubers were dried at room temperature and stored under dry conditions at 4 °C. Seeds of *Brassica napus* L. were commercially available.

The pig slurry used for preincubation of seeds and tubers contained 3.2 % inorganic and 6.4 % organic dry matter. The intermediate product from one of the experimental manure processing plants used for the heat treatments of seeds and tubers contained 24 % inorganic and 37 % organic dry matter.

Measuring the vitality of seeds and germination of tubers

The viability of seeds was estimated by measuring their vitality with the tetrazolium test (MacKay, 1972). Seeds that felt softly were regarded as non-vital. The other seeds were placed between moist blotting paper for 18 hours. After piercing the seed-coat with a needle, the seeds were put into a solution of 1 % 2,3,5-trifenyltetrazoliumchloride (TTC), and stored 48 hours at 30 °C in the dark. To determine the vitality of the seeds, the colouring of the seed embryos was examined. A seed was considered to be vital if more than half of the embryo was red, or if the whole embryo was pale red. They were considered to be non-vital if more than half of the embryo was uncoloured, or if the embryo was coloured only pinkish.

Tubers of *C. esculentus* have no embryo. Their viability was measured in a germination test. Tubers were placed on blotting paper moistened with water in Petri dishes and incubated during 4 weeks at alternating temperatures of 15 °C for 10 hours in the dark and 25 °C for 14 hours in the light.

During the experiments, the germinal force of untreated tubers of *C. esculentus* was about 80 %, and the vitality of untreated seeds ranged from 95 to 100 % for all species.

Realization of heat treatments in an oven and in a pressure vessel

After incubation in pig slurry, the seeds and tubers were exposed to various temperatures in a convection oven (GFL, type 7106) or in a pressure vessel (toaster). The maximal deviation from the desired temperature in the oven was 1.5 °C; deviation from the mean temperature was 0.5 °C. Relative humidity in the oven varied from 4 % at 75 °C to less than 1 % at 95 %.

In the oven, glass pots of 0.5 l with 75 g of the intermediate product were placed in a buffer of sand. A polyester mesh filter (ø 83 mm, height 50 mm) was put on top of the manure. The lids of the pots were closed, and the pots conditioned 24

hours at the heating temperature. The pots were then opened, the seeds or tubers were placed in the polyester mesh filter and covered with a preheated stainless steel plate (425 g). The purpose of the steel plate was to ensure rapid heat transmission to the seeds and tubers (cf. Scherer & Kutzbach, 1980). In most of the experiments, the lids of the pots remained open during the subsequent heat treatments of the seeds and tubers. One pot, containing seeds or tubers of one species, was treated at a time. All treatments at one temperature of one replicate were done on a single day.

In the toaster, seeds and tubers were heated with saturated steam. Seeds and tubers in polyester mesh bags were placed in a perforated cylinder of metal. The cylinder was placed in the toaster (ϕ 260 mm, height 780 mm). The pressure was set at 116 kPa (abs.), corresponding with a temperature of 104 °C. After the treatment, the steam was blown off rapidly. No manure was present in the toaster. Seeds and tubers for one replicate were treated together.

Experimental design

Four different experiments were done, Experiments 1, 2 and 3 with no replicates, and Experiment 4 with three replicates. Each treatment was carried out with 50 seeds or tubers of the species involved in the respective experiment.

In Experiment 1, the effect of heat treatments on the viability of seeds or tubers was tested for the seven mentioned species, with temperature and heating time as variables. All the seeds and tubers were preincubated 1 day in pig slurry.

In Experiment 2, the effect of humidity of ambient air on the viability of seeds and tubers of the seven species was tested. Pots with seeds or tubers were treated 3 or 6 minutes at 75 °C while the pots were open ('low' humidity) or closed ('high' humidity). The humidity in the closed pots was not measured. Again, all the seeds and tubers were preincubated 1 day in pig slurry. Differences in results were analysed separately for *A. theophrasti*, *C. esculentus* and *E. crus-galli*. The logits of the viability percentages were taken and tested with Chi-square.

In Experiment 3, the effect of preincubation duration on viability of seeds of *A. theophrasti*, *A. retroflexus*, and *E. crus-galli*, and tubers of *C. esculentus* was tested. Seeds and tubers were preincubated 1 or 10 days in pig slurry before the heat treatment. Thereafter, they were 3 minutes heated at 75 °C. Differences in viability percentages for *A. theophrasti*, *C. esculentus*, and *E. crus-galli* were tested with Chi-square.

In Experiment 4, three factors were investigated with seeds of *A. theophrasti*, *A. retroflexus*, *E. crus-galli*, and tubers of *C. esculentus*: duration of preincubation in pig slurry, height of temperature, and duration of heat treatment. In addition, for the heat treatments at 104 °C, two methods (oven and toaster) were compared. After testing for homogeneity and normal distribution with the Cochran- and Barlett-test, differences for *A. theophrasti* were tested with ANOVA. Differences for *C. esculentus* and *E. crus-galli* for the treatments at 75 °C were tested with Chi-square, using logits of the percentages.

Results

None of the seeds and tubers of the seven species survived a heat treatment during 1 hour at 130 °C. The other results of Experiment 1 are presented in Table 1. In general, the higher the temperature, and the longer the duration of the heat treatment, the less propagules remained viable, but large differences were found between the species. The species are ranked according to their heat resistance, with *B. napus* and *S. nigrum* being the most sensitive species, and *A. theophrasti* the most resistant one. Only the ranking of *A. retroflexus* was not quite clear from the experimental results. Seeds of this species tended towards a decrease in viability after the treatments of 50 °C, none were viable after 3 and 9 minutes at 75 °C, but 45 % was viable after 15 minutes at 75 °C. It should be mentioned that in the latter case the pot with manure and seeds had unintentionally been closed before the heat treatment. In the subsequent experiments, there was not even a tendency that seeds of *A. retroflexus* could survive any heat treatment at 75 °C (cf. Tables 2-4).

A factor that is influenced by closing the pots is the humidity of the ambient air. In Experiment 2, the influence of this factor was investigated for propagules of the seven species (Table 2). Seeds of *B. napus*, *S. nigrum* and *C. album* again did not survive any of the treatments at 75 °C. In this experiment, all the seeds of *A. retroflexus* were non-viable after the treatments at 'high' RH (closed pots) as well as 'low' RH (open pots). The RH of the ambient air only had a significant effect on the viability of the seeds of *E. crus-galli* (average of 3 and 6 minutes heat duration): less seeds were viable after treatment at 'high' RH than at 'low' RH. The duration of the heat treatment at 75 °C also had an effect on the seeds of *E. crus-galli*.

In Experiment 3, the influence of the preincubation time of seeds and tubers in slurry prior to 3 minutes heat treatment at 75 °C, was investigated. The experi-

Table 1. Viability (%) of seeds and tubers of seven plant species after 3, 9, and 15 minutes heating in an oven at 50, 75, and 100°C. Seeds and tubers were 1 day preincubated in pig slurry.

Species	Temperature (°C)			75			100		
	Heat duration (min)			3	9	15	3	9	15
<i>Brassica napus</i>	92	78	0	0	0	0	0	0	0
<i>Solanum nigrum</i>	98	98	8	0	0	0	0	0	0
<i>Amaranthus retroflexus</i>	100	82	63	0	0	45	0	0	0
<i>Chenopodium album</i>	91	92	90	0	0	0	0	0	0
<i>Echinochloa crus-galli</i>	98	94	98	69	60	2	0	0	0
<i>Cyperus esculentus</i>	88	75	85	43	83	45	0	0	0
<i>Abutilon theophrasti</i>	90	90	88	82	77	73	65	70	54

Table 2. Effect of humidity of ambient air on viability (%) of seeds and tubers after 3 and 6 minutes heating at 75°C. Seeds and tubers were preincubated 1 day in pig slurry. Differences for *Abutilon theophrasti*, *Cyperus esculentus*, and *Echinochloa crus-galli* were tested with Chi-square, using logits of the percentages.

Species	Humidity	'low'		'high'	
		Heat duration (min)		3	6
<i>Brassica napus</i>		0	0	0	0
<i>Solanum nigrum</i>		0	0	0	0
<i>Amaranthus retroflexus</i>		0	0	0	0
<i>Chenopodium album</i>		0	0	0	0
<i>Echinochloa crus-galli</i>		43	15	35	7
<i>Cyperus esculentus</i>		35	10	25	15
<i>Abutilon theophrasti</i>		35	42	34	34
		humidity		heat duration	
<i>E. crus-galli</i>		$P \leq 0.05$		$P \leq 0.01$	
<i>C. esculentus</i>		$P > 0.05$		$P > 0.05$	
<i>A. theophrasti</i>		$P > 0.05$		$P > 0.05$	

ment was done with propagules of those four species that could survive the heat treatment at 75 °C in one of the foregoing experiments.

Seeds of *A. retroflexus* again did not survive 3 minutes heating at 75 °C (Table 3). The preincubation time affected the viability of seeds of *E. crus-galli* and tubers of *C. esculentus*. The preincubation time did not influence the effect of subsequent heating on viability of seeds of *A. theophrasti*.

Experiment 4 was done with the same four species that were used in Experiment 3. Aim of this experiment was to establish or confirm the conditions that would

Table 3. Effect of preincubation time on viability (%) of seeds and tubers after 3 minutes heating at 75°C. Differences for *Abutilon theophrasti*, *Cyperus esculentus*, and *Echinochloa crus-galli* were tested with Chi-square.

Species	Preincubation time (days)	1	10
<i>Amaranthus retroflexus</i>		0	0
<i>Echinochloa crus-galli</i>		43	15
<i>Cyperus esculentus</i>		37	0
<i>Abutilon theophrasti</i>		36	29
<i>E. crus-galli</i>		$P \leq 0.01$	
<i>C. esculentus</i>		$P \leq 0.01$	
<i>A. theophrasti</i>		$P > 0.05$	

inactivate the seeds or tubers of these species.

Seeds of *A. retroflexus* again did not survive any of the heat treatments (Table 4). Seeds of *E. crus-galli* could survive heat treatment at 75 °C. The survival rate at this temperature decreased with increasing heat duration and increasing preincubation time. At temperatures above 75 °C, seeds of this species did not survive except for 1 % in one case (at 104 °C).

Table 4. Viability (%) of seeds and tubers after 3 and 6 minutes heating in an oven at 75, 90 and 104 °C, and in a toaster at 104 °C. Seeds and tubers were 1, 3 and 7 days preincubated in pig slurry. Differences between treatments for *Abutilon theophrasti* were tested with ANOVA. Differences between treatments at 75 °C for *Cyperus esculentus* and *Echinochloa crus-galli* were tested with Chi-square, using logits of the percentages.

Species	Heat duration (min)	3			6		
		1	3	7	1	3	7
<i>Amaranthus retroflexus</i>							
oven	75 °C	0	0	0	0	0	0
oven	90 °C	0	0	0	0	0	0
oven	104 °C	0	0	0	0	0	0
toaster	104 °C	0	0	0	0	0	0
<i>Echinochloa crus-galli</i>							
oven	75 °C	51	11	0	21	8	1
oven	90 °C	0	0	0	0	0	0
oven	104 °C	0	0	0	0	1	0
toaster	104 °C	0	0	0	0	0	0
<i>Cyperus esculentus</i>							
oven	75 °C	27	17	3	18	1	0
oven	90 °C	1	0	0	0	0	0
oven	104 °C	0	0	0	0	0	0
toaster	104 °C	0	0	0	0	0	0
<i>Abutilon theophrasti</i>							
oven	75 °C	50	63	60	65	58	58
oven	90 °C	62	61	57	53	51	55
oven	104 °C	45	46	45	39	44	37
toaster	104 °C	17	21	19	3	1	1
		<i>E. crus-galli</i>	<i>C. esculentus</i>	<i>A. theophrasti</i>			
Heating in oven, 75 against 90 °C				$P > 0.05$			
Heating in oven, 90 against 104 °C				$P \leq 0.01$			
Oven against toaster, 104 °C				$P \leq 0.01$			
Heat duration (oven):		$P \leq 0.01$	$P \leq 0.01$	$P > 0.05$			
Heat duration (toaster):				$P \leq 0.01$			
Preincubation:		$P \leq 0.01$	$P \leq 0.01$	$P > 0.05$			
Preincubation*heat duration:		$P > 0.05$	$P \leq 0.05$	$P > 0.05$			

Tubers of *C. esculentus* could also survive heat treatment at 75 °C. Preincubation time and duration of heat treatment also affected the results for this species at 75 °C. At temperatures above 75 °C, tubers of this species also did not survive except for 1 % in one case (at 90 °C).

With seeds of *A. theophrasti*, the effects of heat treatments at 90 °C did not differ from treatments at 75 °C. The effect of heat treatments at 104 °C differed from treatments at 90 °C. Treatments at 104 °C in the toaster resulted in less vitality than treatments at the same temperature in the oven. Preincubation time had no effect on the results for *A. theophrasti*. The duration of the heat treatment only influenced the treatments at 104 °C in the toaster.

Discussion

Propagules of seven plant species, which had been at least 1 day preincubated in pig slurry, were submitted to heat treatments of short duration in the range from 50 to 104 °C. The experiments were designed in such a way, that conclusions on heat-sensitivity of each species could be drawn from at least two different experiments.

For four of the weed species, the heat sensitivity of their seeds had been tested previously, but the conditions before or during the testing were different from ours. Not unexpectedly, our results were different too. Maun (1977) found that a fraction of the seeds of *E. crus-galli* survived 1 h at 130 °C in an oven. Under the same heating conditions, our seeds of *E. crus-galli* were inactivated already after 3-6 min at 90 °C. A basic difference in conditions was that Maun heated dry seeds, whereas we incubated the seeds 1 day in pig slurry prior to the heat treatments. Seeds of *E. crus-galli* and tubers of *C. esculentus* became even more sensitive to the heat treatments at 75 °C if they were preincubated longer than 1 day in the slurry.

Hopkins (1934) preincubated seeds 1 day at 50 % RH. Seeds of *A. retroflexus* and *C. album* were less heat-sensitive than under our conditions (1 day preincubation in pig slurry). A comparison of the results of Hopkins (1934) on *A. fatua* with those of Metz (1970) with dry seeds of the same species also strongly suggests that the heat sensitivity of seeds is increased by preincubation in a humid atmosphere.

Horowitz & Taylorson (1984) established that seeds of *A. theophrasti* were killed after heating at temperatures between 70 and 100 °C. Their relatively long heat duration of 1 hour probably explains the difference with our results on the same species. They also demonstrated, that seeds that easily imbibe and germinate were much more heat-sensitive than hard-scaled, dormant seeds. In our experiments, a fraction of the *A. theophrasti* seeds (45-50 %) was not affected after 15 min at 100 °C (Exp. 1), and after 3 and 6 min at 104 °C (Exp. 4) in the oven. However, another fraction of the seeds was already inactivated at 75 °C. Lack of dormancy of the latter seeds may explain their greater heat sensitivity. Longer than 1 day preincubation in slurry did not influence the effect of heating. Viability of *A. theophrasti* seeds was greatly reduced after heating at 104 °C in a

pressure vessel (toaster). The most relevant difference from the heat treatments in the oven probably was the presence of steam, which enables better transmission of heat to the core of the seeds than the dry air in the oven. Nearly all seeds were inactivated after 6 minutes in the toaster.

The results have shown that weed propagules can be inactivated by heat treatments of short duration. For most species, heating the seeds or tubers a few minutes at 90 °C will be sufficient. However, to guarantee that all weed propagules in manure, even those of the most resistant species, are inactivated by heat treatment, temperatures above 100 °C will be needed.

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