Effect of duration of heat exposure on upland weed seed viability

Tomoko Nishida*, Shunji Kurokawa**, Shohei Shibata* and Norihisa Kitahara*

Abstract: A study was conducted to determine the length of exposure to heat necessary to kill water-imbibing weed seeds at 55 and 60°C. The examination covered ten upland weed species: *Solanum carolinense* L., *S. americanum* Mill., *Abutilon theophrasti* Medic., *Phytolacca americana* L., *Amaranthus spinosus* L., *A. patulus* Bertoloni, *Persicaria lapathifolia* (L.) S. F. Gray, *Panicum dichotomiflorum* Michx., *Echinochloa crus-galli* (L.) Beauv. var. *crus-galli* and *Digitaria ciliaris* (Retz.) Koeler. All seeds of each species were killed by exposure to heat at 55°C for 72 hr and at 60°C for 24 hr except for those of *Abutilon theophrasti* (dormant seed rate 80%) which were killed by exposure for 120 and 30 hr at the respective temperatures.

These weed species fell into four groups, categorized by a combination of short-duration heat-tolerance (SDHT) and long-duration heat-tolerance (LDHT). SDHT was determined by the viability of weed seeds when treated at 60°C for 3 hr, with low and high subcategories of tolerance. LDHT was determined by the duration required to kill all the seeds at 1% level of significance when treated at 60°C, into three subcategories (low, medium and high tolerance). *Persicaria lapathifolia*, *E. crus-galli*, and *D. ciliaris* were low for both SDHT and LDHT (Group I), and *Amaranthus spinosus* and *Panicum dichotomiflorum* were low-SDHT and medium

-LDHT (Group II). *Solanum* species, *Phytolacca americana*, and *Amaranthus patulus* were high-SDHT and medium-LDHT (Group III), while *Abutilon theophrasti* was low-SDHT and high-LDHT (Group IV). The viability of seeds at short-duration heat treatment for Group I, II and IV was low, but the durations required to kill all seeds were different among the groups. Group III consisted of species for which the viability of seeds at short-duration heat treatment was high, and the fatal duration was medium.

For *Abutilon theophrasti* which required the longest duration of heat exposure to kill all seeds at each temperature, 95% confidence intervals of LD₉₀ values were calculated using the probit method, and were 42 to 58 and 10 to 17 hr at 55 and 60°C, respectively.

Key words: weed seed, heat death, heat exposure duration, heat tolerance, *Abutilon theophrasti*

Introduction

Imported concentrate is considered a major invasion route of the alien weed seeds that afflict forage-crop fields and pastures in Japan¹⁷⁾. Since some weed seeds retain their viability after passing through the bovine digestive tract,^{4,9,18)} weed seeds in animal excreta must be killed in the process of composting before manure is spread over fields. Effective control of weed seeds in the composting process requires adequate temperatures^{13,19)}. We showed that weed seeds were unable to germinate after exposure in compost to temperatures above approximate-

* Department of Grassland Management, National Grassland Research Institute, Nishinasuno, Nasu, Tochigi, 329-2793 Japan

** Department of Forage Production and Utilization, NGRI

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ly 60°C¹³), and that almost all of the tested seeds in plastic petri dishes were dead when they were exposed to the temperature of 55°C for 5 days after imbibing water at 30°C for 24 hr¹²). However, the relationship between the rate of heat death of weed seeds and shorter terms of heat exposure remains unclear. Additional experiments are required to reveal this relationship because the process of composting cannot always provide the conditions necessary to kill seeds, especially during the wintertime¹¹).

The heat tolerance of seeds varies with their water uptake, and water-imbibing seeds have lower heat tolerance than air-dried seeds^{6,15,19}). The water content of manure is usually about 65 to 70% when composting begins¹), and it is assumed that seeds embedded in manure absorb water from manure before being exposed to fermentative heat. In this study, we examined the death rate of weed seeds which absorbed water at 15°C for 24 hr before heat treatment. The water uptake of seeds in manure, before being exposed to heat, varies depending on several factors such as the water content and temperature of manure, the length of time that the seeds are embedded in the manure and the degree of their dormancy, etc.

Although it is reported that the temperature of 45°C adversely affected the germination of *Solanum nigrum*⁵) and *Abutilon theophrasti* seeds⁶), in our previous study some seeds of the tested weed species retained their viability after exposure to a 45°C temperature over the course of an entire week¹²). The maximum temperature in manure tends to rise above 45°C when the manure temperature remains above 45°C for more than one week^{3,11,19}). Weed seeds lose germinating abilities following exposure in compost to temperatures above approximately 60°C¹³). Therefore, we conducted this study to exam-

ine the effect of duration of heat exposure at 55 and 60°C, respectively, on the viability of weed seeds.

Materials and Methods

The experiments were conducted from March to September in 1997.

Weed seeds. We selected ten weed species that commonly occur in forage-crop fields and pastures in Japan: *Solanum carolinense* L., *S. americanum* Mill., *Abutilon theophrasti* Medic., *Phytolacca americana* L., *Amaranthus spinosus* L., *A. patulus* Bertoloni, *Persicaria lapathifolia* (L.) S. F. Gray, *Panicum dichotomiflorum* Michx., *Echinochloa crus-galli* (L.) Beauv. var. *crus-galli* and *Digitaria ciliaris* (Retz.) Koeler. Among these, *Persicaria lapathifolia*, *E. crus-galli* and *D. ciliaris* are native to Japan; the other weeds were introduced from overseas after 1854 when Japan was opened to foreign intercourse. The seeds for the experiment were collected in Tochigi Prefecture during 1994 and 1996, air-dried and stored at room temperature.

Water uptake. Samples consisted of 150 air-dried seeds for *Amaranthus* species, 90 for *S. americanum*, *Panicum dichotomiflorum* and *D. ciliaris*, 60 for *E. crus-galli* and 30 for others. A sample of each species was weighed, placed on filter paper and absorbent cotton in a glass petri dish (d. 6-cm), and supplied with 20 ml of deionized water. The seeds were incubated at 15°C for 24 hr in darkness and re-weighed for water uptake after removal of external moisture by blotting.

Heat treatment. Thirty seeds of each species which absorbed water under the conditions given above were exposed to a temperature of 55°C for a period of 24 hr, and to a temperature of 60°C for 3 hr. After exposure to heat stress, they were incubated for a germination test at alternate temperature and light condi-

tions of 30°C/12 hr light and 20°C/12 hr dark. A germination count was made over a 20-day period. Sound seeds that did not germinate were subsequently tested for viability by tetrazolium (Tz) test²⁾. Seeds that germinated or were stained with Tz were classified as 'viable seeds'. Those that collapsed when they were pinched with a pair of tweezers or were not stained were classified as 'dead seeds'. For the species of which seeds remained viable after this heat exposure, the duration of exposure at each temperature was extended until all seeds were killed. The germination and viability rates of the seeds were recorded at each duration and temperature.

At the beginning of the experiment, untreated seeds were also tested for germination and viability, as were the treated seeds. Dormancy rates were calculated by subtracting the germination rate from the viable seed rate for untreated seeds. As the seed dormancy rate for *Abutilon theophrasti* was quite high at the beginning of the experiment, we re-examined this rate with untreated seeds at the end to determine if there were any changes in the rate of dormancy over the duration of the experiment using the germination test.

Statistical analyses. In all cases, the experimental design was Completely Randomized Design with three replications. Germination and viability rates were arcsin transformed prior to analyses of variance¹⁶⁾. Means of germination and viability within each species were tested between treatments by the l. s. d. method¹⁶⁾, and viability means for the 3 hr treatment at 60°C were tested between species using the same method.

For *A. theophrasti*, which required the longest period of heat exposure to kill all seeds at each temperature, 95% confidence intervals of LD₉₀ values were calculated using the

probit method¹⁶⁾. The probit method assumes a normal distribution for the dose that induces response. The response rate at each dose is converted into 'surveyed probit' using a normal distribution table and a transformation equation. 'Estimated probits' are calculated from surveyed probits, and then modified in order to obtain 'practical probits'. A weighted regression equation between the doses and practical probits is calculated. Then 95% confidence interval of LD₉₀ value is calculated using the equation.

Results and Discussion

Water uptake rate. Following water absorption, the water uptake of the various species of seeds ranged from 6 to 60% (Table 1). Water uptake was lowest for *Phytolacca americana* and highest for *E. crus-galli*.

Dormancy rate. The highest dormancy rate of 80% was recorded for *A. theophrasti* seeds. Dormancy rates for *E. crus-galli* and *D. ciliaris* seeds were 34 and 14%, respectively, and those for seeds of other species were very low (Table 1).

The germination rate for *A. theophrasti* seeds at the beginning and at the end of the experiment was 13 and 11%, respectively. The dormancy rate for *A. theophrasti* appeared to remain unchanged during the experiment.

Although the dormancy rates for *S. carolinense*, *Persicaria lapathifolia* and *Panicum dichotomiflorum* were quite low in this experiment, it has been reported that their seeds do demonstrate dormancy^{8,9,20)}. It appears that the dormancy dissipated during the storage period of one to two years before the beginning of the present study.

Heat treatment.

1) Effect of heat treatment. Seeds of all the species except for *A. theophrasti* were killed by exposure to temperatures of 55 and 60°C

Table 1. Rates of water uptake and dormancy of untreated seeds

species	water uptake ^{a)}	Dormancy rate ^{b)}
	%	
<i>Solanum carolinense</i>	44(2) ^{c)}	2(2)
<i>S. americanum</i>	37(2)	0(0)
<i>Abutilon theophrasti</i>	12(5)	80(6)
<i>Phytolacca americana</i>	6(1)	2(4)
<i>Amaranthus spinosus</i>	33(3)	0(0)
<i>A. patulus</i>	29(1)	1(2)
<i>Persicaria lapathifolia</i>	24(3)	0(0)
<i>Panicum dichotomiflorum</i>	20(2)	6(2)
<i>Echinochloa crus-galli</i>	60(3)	34(13)
<i>Digitaria ciliaris</i>	54(3)	14(4)

Note:

^{a)} Weed seeds were supplied 20 ml of deionized water, and the water uptake was measured after incubation at 15°C for 24 hr.

$$\text{Water uptake(\%)} = \frac{\text{WISW} - \text{ADSW}}{\text{ADSW}} \times 100$$

WISW: water-imbibing seed weight

ADSW: air-dried seed weight

^{b)} The dormancy rate was calculated by subtracting the germination rate from the viable seed rate after absorption of water and incubation for 20 days at 20/30°C

$$\text{Dormancy rate(\%)} = \text{Viability(\%)} - \text{Germination rate(\%)}$$

^{c)} Values in parentheses represent s. d.

for the respective durations of 72 and 24 hr (Table 2). The germination rate for *A. theophrasti* was 0% following 24 hr of heat treatment at both 55 and 60°C but the seeds remained viable. The time required to kill all *A. theophrasti* seeds at temperatures of 55 and 60°C was 120 and 30 hr, respectively.

According to the result of analyses of variance, there was no significant difference between untreated seeds and those undergoing the briefest heat exposure for *A. theophrasti* germination rate at 60°C, or for *S. americanum* viability at either 55 and 60°C (Table 2). Except for these cases, even the

briefest heat treatment, 24 hr at 55°C and 3 hr at 60°C, significantly reduced the germination rate and viability of all species compared to untreated seeds. The length of exposure required to kill all *A. theophrasti* seeds at 1% level of significance was 96 and 30 hr at 55 and 60°C, respectively, the longest among the species studied. The briefest fatal exposures were for *Persicaria lapathifolia*, *E. crus-galli*, and *D. ciliaris*, with minimum durations of 24 hr at 55°C and 3 hr at 60°C.

In a comparison of brief heat-exposure tolerance among the species based on the viability in the 3-hr treatment at 60°C, *S. carolinense*, *S. americanum*, *Phytolacca americana*, and *A. patulus* with high viability showed high tolerance. The lowest viability was observed for *Persicaria lapathifolia*, *E. crus-galli*, and *D. ciliaris*.

2) Grouping by a combination of short-and long-duration heat-tolerance. The tested weed species were grouped by a combination of short-duration heat-tolerance (SDHT) and long-duration heat-tolerance (LDHT). SDHT was determined by viability of the seeds when treated for 3 hr at 60°C. Species with viabilities below and above the 50% mark were subcategorized as low-SDHT and high-SDHT, respectively. LDHT sorted the species into three groups, depending on the duration required to achieve 100% death rate of seeds at 1% level of significance when the seeds were treated at 60°C. The subcategories were described as low (3 hr), medium (6 hr), or high tolerance (30 hr). The ten species fell into four groups (Table 3). *Persicaria lapathifolia*, *E. crus-galli*, and *D. ciliaris* were low for both SDHT and LDHT (Group I), and *A. spinosus* and *Panicum dichotomiflorum* were low-SDHT and medium-LDHT (Group II). *Solanum* species, *Phytolacca americana*, and *A. patulus* were high-SDHT and medium-LDHT (Group III), while *A. theophrasti* was

Table 2. Rates of germination and viability of weed seeds exposed to heat for different periods at 55 and 60°C

	Un- treated	55°C					60°C			
		hours					hours			
		24	48	72	96	120	3	6	24	30
%										
<i>Solanum</i>	G ^{a)} 97a	19bc	3cd	0d			32b	0d	0d	
<i>carolinense</i>	V ^{b)} 99a	72b	7c	0c			67b AB	9c	0c	
<i>S. americanum</i>	G 97a	28c	0d				78b	0d	0d	
	V 97a	79a	0b				84a A	6b	0b	
<i>Abutilon</i>	G 13a	0c	0c	0c	0c	0c	9ab	3b	0c	0c
<i>theophrasti</i>	V 93a	23bc	12cd	9cde	2ef	0f	39b BCD	23bc	7de	0f
<i>Phytolacca</i>	G 92a	0c					49b	0c	0c	
<i>americana</i>	V 94a	0c					57b ABC	3c	0c	
<i>Amaranthus</i>	G 94a	2b	0b				20b	0b	0b	
<i>spinosus</i>	V 94a	2c	0c				24b CDE	1c	0c	
<i>A. patulus</i>	G 96a	38b	0c				70b	0c		
	V 97a	38c	0d				74b AB	0d		
<i>Persicaria</i>	G 83a	0b					0b			
<i>lapathifolia</i>	V 83a	0b					0b F			
<i>Panicum</i>	G 90a	0c					26b	0c		
<i>dichotomiflorum</i>	V 96a	0c					46b BC	0c		
<i>Echinochloa</i>	G 41a	0b	0b	0b			3b	0b		
<i>crus-galli</i>	V 76a	6b	6b	0b			6b EF	0b		
<i>Digitaria ciliaris</i>	G 52a	0b					7b	0b		
	V 67a	0b					10b DEF	0b		

Note:

Values in the same row (small letters) or column (capital letters) followed by the same letter are not different at 1% level of significance.

^{a)}germination rate ^{b)}viability

low-SDHT and high-LDHT (Group IV). The viability of seeds at short-duration heat treatment for Group I, II and IV is low, but the durations required to kill all seeds differed among the groups. Group III consists of species for which the viability of seeds at short-duration heat treatment is high, and the fatal duration is intermediate.

It has been reported that non-dormant seeds of *A. theophrasti* are heat-sensitive¹²⁾, while dormant ones are heat-tolerant⁷⁾. This may explain *A. theophrasti*'s grouping into Group IV.

Dormancy rates for *S. carolinense* and *S. americanum* classified as high-SDHT were

quite low for untreated seeds, but dormant seeds were observed for these species after 24- and 6-hr treatments at 55 and 60°C, respectively (Tables 1 and 2). Dormancy is thought to increase heat tolerance, but *D. ciliaris* and *E. crus-galli*, of which untreated seeds were dormant, rated low for both SDHT and LDHT (Tables 1 and 3).

For non-dormant *A. theophrasti* seeds, it has been reported that water uptake is negatively correlated with heat tolerance⁹⁾. We found in this study that *Phytolacca americana*, with the lowest water uptake among the tested species, showed high-SDHT, while *D. ciliaris* and *E. crus-galli*, with high water

Table 3. Heat tolerance of tested weeds

		Short-duration heat-tolerance ^{a)}	
		Low	High
Long-duration heat-tolerance ^{b)}	Low	Group I <i>Persicaria lapathifolia</i> <i>Echinochloa crus-galli</i> <i>Digitaria ciliaris</i>	
	Medium	Group II <i>Amaranthus spinosus</i> <i>Panicum dichotomiflorum</i>	Group III <i>Solanum carolinense</i> <i>S. americanum</i> <i>Phytolacca americana</i> <i>Amaranthus patulus</i>
	High	Group IV <i>Abutilon theophrasti</i>	

Note:

^{a)} Short-duration heat-tolerance (SDHT) was determined by the viability of seeds when treated at 60°C for 3 hr, with low (below 50%) and high (above 50%).

^{b)} Long-duration heat-tolerance (LDHT) was determined by the duration required to kill the seeds at 100% rate at 1% level of significance when treated at 60°C, with low (3 hr), medium (6 hr) and high (30 hr).

uptake, showed low heat tolerance for both long and short periods (Tables 1 and 3). On the other hand, we observed that *S. carolinense* and *S. americanum* demonstrated high-SDHT, despite relatively high water uptake, while *A. patulus* and *A. spinosus* demonstrated different heat tolerance, despite similar water uptake levels.

The dormancy and water uptake of seeds are correlated with the heat tolerance of a species, but it is apparent that other factors also need to be considered to explain the difference of heat tolerance between species. For example, seed coat structure, seed reserves, and the period over which an embryo is sensitive to the temperature after the seed has started absorbing water may influence heat tolerance. Further studies are required.

3) Fatal exposure duration for *A. theophrasti*. Because the duration of exposure to heat needed to kill all the seeds was longest for *A. theophrasti*, the viability of the seeds can be considered as an index of

fatal exposure for the tested species. In order to quantify the relationship between death rate and duration for which *A. theophrasti* seeds were exposed, a detailed analysis was carried out. We calculated 95% confidence intervals of LD₉₀ values using the probit method and obtained values of 1.62 to 1.76 or 42 to 58 hr at 55°C and 1.01 to 1.24 or 10 to 17 hr at 60°C (Fig. 1).

4) Conclusion. Reports indicate the process of composting can provide temperatures above 55°C for 96 to 144 hr^{3,11,19)} and temperatures above 60°C for 72 to 120 hr. Thus, given normal composting conditions, both temperature and length of heat exposure are sufficient to kill all weed seeds, including dormant *A. theophrasti* ones. The study design does not rule out the possibility that other factors, including ammonia^{10,14)} and microorganisms, may have significant effects on the viability of weed seed in manure.

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Fig.1. Relationship between logarithms of heating duration and death rate of *Abutilon theophrasti* seeds

Circles show 'estimated probits' at each temperature (○ 55°C, ● 60°C). Weighted regression equations between logarithms of heating duration and 'practical probits' that were modified 'estimated probits', were calculated.
 55°C $y = 2.884 + 2.009x$ $\chi^2 = 4.794 < \chi^2_{(3,0.05)}$
 60°C $y = 4.469 + 1.648x$ $\chi^2 = 4.115 < \chi^2_{(2,0.05)}$
 According to χ^2 of the equations, their linearity was undeniable.

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畑雑草種子の生存に及ぼす加熱時間の影響

西田智子*・黒川俊二**・柴田昇平*・北原徳久*

摘 要

現在、草地・飼料畑で大きな問題になっている外来雑草の侵入経路の一つとして、雑草種子の混入した輸入濃厚飼料が家畜に採食され、糞が堆厩肥として圃場に還元される過程で、生存したままの種子が圃場に散布されることがあげられている。家畜の消化作用ではすべての種子を死滅させることは不可能なため、堆肥製造過程で雑草種子を死滅させることが、外来雑草を蔓延させないために必要である。堆厩肥中の雑草種子は、堆肥の最高温度が約60°C以上になれば、発芽力を失うことが明らかにされているが、温度の持続時間と種子の死滅率の詳細は不明である。そこで、本実験は、55及び60°Cにおいて、雑草種子がそれらの温度にさらされる時間と死滅率との関係について調査した。

10種類の畑雑草種子（ワルナスビ、アメリカイヌホオズキ、イチビ、ヨウシュヤマゴボウ、ハリビユ、ホソアオゲイトウ、オオイヌタデ、オオクサキビ、イヌビエ及びメヒシバ）を、15°C暗条件で24時間吸水させた。種子の吸水率は6～60%の範囲であった（Table 1）。これらの種子について、55°C及び60°Cの処理で、死滅率が100%となる時間を調査した。イチビを除く9種類の雑草は、55°Cで72時間、60°Cで24時間処理すれば全ての種子が死滅した。イチビ（休眠率80%）については、55°Cでは120時間、60°Cでは30時間の加熱が必要であった（Table 2）。

調査した10種類の雑草の加熱耐性を、短時間の熱処理に対する耐性（SDHT）と、長時間の熱処理に

対する耐性（LDHT）とを組み合わせで分類した。SDHTは60°C 3時間処理での生存率により、50%を境に低及び高耐性の2群に分けた。LDHTは、60°C処理において1%の有意水準で生存率0%と差がなくなるまでにかかった処理時間により、低、中、高耐性の3群に分類した。その結果、SDHT、LDHTともに低いオオイヌタデ、メヒシバ及びイヌビエ（第1群）、SDHTが低くLDHTが中位のハリビユ及びオオクサキビ（第2群）、SDHTが高くLDHTが中位のワルナスビ、アメリカイヌホオズキ、ヨウシュヤマゴボウ及びホソアオゲイトウ（第3群）、SDHTが低いLDHTの高いイチビ（第4群）の4群に分かれた（Table 3）。第1、2及び4群は、短時間の熱処理で高い死滅率を示すが、100%の種子が死ぬまでにかかる時間はそれぞれ異なるといえる。また、第3群は、短時間の熱処理では生存率が高いが、100%の種子が死ぬまでにかかる時間はそれほど長くない群である。

調査した10種類の雑草種子を死滅させるための時間はイチビ種子の死滅を目安として設定すればよいと考えられたので、イチビについて、プロビット法によりLD₉₀となる時間の95%信頼区間を計算した。その結果、55°Cでは42～58時間、60°Cでは10～17時間となった（Fig. 1）。

本実験の結果と既往の堆厩肥の発酵温度に関する報告とからみて、堆厩肥が順調に発酵した場合は、イチビを含むすべての種子が死滅する温度と時間を確保できるといえる。

キーワード：雑草種子、熱死、加熱時間、熱耐性、イチビ

*草地試験場 草地生産基盤部 飼料基盤管理研究室

**草地試験場 飼料生産利用部 栽培生理研究室