JARQ 32, 55-60 (1998)

Effect of Cattle Digestion and of Composting Heat on Weed Seeds

Tomoko NISHIDA*[1], Norihiro SHIMIZU*[2], Motohiko ISHIDA*[3], Tohko ONOUE*[1] and Norikazu HARASHIMA*[4]

*[1, 4] Department of Grazing Animal Production, National Grassland Research Institute (NGRI) (Nishinasuno, Nasu, Tochigi, 329-27 Japan)
*[2, 3] Department of Forage Production and Utilization, NGRI

Abstract

Two experiments were conducted to determine whether alien weed seeds mixed in imported fodder crops could become established in forage crop fields via cattle excreta. Horsenettle (*Solanum carolinense* L.) seeds were fed to cows which received 2 diets with different concentrates, i. e. flaked barley and corn, and the seeds recovered from excreta were tested for germination and viability. The 2 diets had no effect on the seed recovery, or on the germination or viability of recovered seeds. The mean percentages of seed recovery, and germination and viability of recovered seeds were 83, 61 and 76%, respectively. Among the seeds estimated to be contained in excreta placed outdoors, 15% emerged. Likewise, the germination percentage of 15 weed species exposed to compost incubation was determined. Weed seeds were placed in 39 composting tubes (52.1 L) for 7-25 days, and the regression equation was calculated between the maximum compost temperature and the percentage of species which retained their germinability. The effects were not conspicuous until the maximum temperature reached 46°C. Thereafter there was a rapid decline in the percentage of germinable species, and none of the species germinated when the temperature was above 57°C. The results showed that imported fodder crops containing weed seeds could become a source of weed infestation unless animal excreta were treated adequately.

Discipline: Weed control
Additional key words: alien weed, imported fodder crop, weed infestation

Introduction

Recently, many new alien weeds have been detected in forage crop fields and artificial pastures, and they have become a problem in Japan. Shimizu et al.[14] reported that many weed seeds were mixed in imported fodder crops. Each year, 18 million tons of fodder crops were imported during the period between 1984 and 1994[6]. Therefore, alien weed seeds mixed in imported fodder crops could be a source of alien weed infestation in forage crop fields and pastures in Japan, suggesting that countries which import a large amount of fodder crops may encounter the same problem.

For weed seeds mixed in imported fodder crops, however, there are several possible barriers before they are spread over forage crop fields and pastures. Approximately 70% of imported fodder crops are initially fumigated with methyl bromide in Japan. In this treatment, however, only imbibed seeds are killed. The seeds in fodder crops are dry in general and they are not affected. Secondly, a large amount of fodder crops are crushed into pieces which pass through a 2 mm sieve used with a rotary crusher in factories. Mixed weed seeds, especially small ones, possibly pass through this process and maintain their viability. Third, animal digestion is considered to reduce weed seed viability. However, it was reported that some weed seeds remained viable after being digested by cattle[1,5,16]. Therefore, viable weed seeds in cattle excreta are a possible source of weed

Present address:
*[1] Department of Grassland Management, NGRI (Nishinasuno, Nasu, Tochigi, 329-27 Japan)
*[2] Division of Plant Ecology, National Institute of Agro-Environmental Sciences (Tsukuba, Ibaraki, 305 Japan)

infestation in fields.

In the experiments reported here, 1) the percentages of recovery, germination and viability of horsenettle (*Solanum carolinense* L.) seeds passing through the digestive tract of cattle fed 2 different diets were calculated, 2) seedling emergence from feces placed outdoors was examined, and the germination percentage of 15 weed species exposed to composting heat was determined. These data should be useful to determine whether alien weed seeds mixed in imported fodder crops could become established in forage crop fields and pastures via cattle excreta, and develop strategies to reduce the spread of alien weed seeds.

Materials and methods

Exp. 1: Effect of cattle digestion

(1) Horsenettle seed

Experiment 1 was conducted at the National Grassland Research Institute (NGRI), Tochigi, Japan, from October to November 1995.

Horsenettle was selected for the experiment because it was one of the alien weeds which had become common in Japan, recently[13]. Horsenettle seeds were collected at NGRI in the spring of 1995 and stored at room temperature until use in this experiment. To determine the percentage of germination and viable seeds, 200 seeds were placed on moistened filter paper in 2 petri dishes in a growth chamber (each petri dish contained 100 seeds). The seeds were kept under alternate temperature and light conditions, i.e. 35°C and light for 12 h, and 25°C and dark for 12 h. Germination count was performed over a 25-day period. Seeds that did not germinate

Table 1. Composition and nutritive value of the diet (DM%)

	Diet	
	Barley	Corn
Composition		
Alfalfa silage	17.0	17.0
Grass silage	31.0	34.0
Flaked barley	40.2	–
Flaked corn	–	33.2
Soybean meal	11.0	15.0
Mineral mix	0.5	70.5
Vitamin mix	0.3	0.3
Nutritive value		
CP	16.90	16.98
OCW	41.12	41.38
TDN	70.80	71.70

were subsequently tested for viability using the tetrazolium test[12].

(2) Cattle and diets

Two diets with different concentrates were fed to 2 dry Holstein cows at maintenance level, respectively. Diet barley contained 40% flaked barley (on a DM basis) and 48% silage. Diet corn contained 15% flaked corn and 51% silage. The nutritive value of the 2 diets was identical (Table 1). Cows were kept in digestion trial stall[7] separately, and fed the diet in the morning and evening. After the diets were offered for 14 days, 10,000 horsenettle seeds (18.5 g) mixed in the concentrates were fed to the cows once. All the feces were collected during a period of 6 days after feeding of the seeds. The trial was repeated 3 times, every 3 weeks. Mean weights (± s.d.) of the 3 cows fed the diet barley and diet corn were 660 ± 57 kg and 687 ± 16 kg, respectively.

(3) Feces collection, and recovery of seeds and seedling emergence from feces

Total feces were collected twice a day for the 1st day (18 and 24 h after feeding of the seeds), 4 times a day for the 2nd day (30, 36, 42 and 48 h after feeding of the seeds), twice a day for the 3rd, 4th and 5th days (60, 72, 84, 96, 108 and 120 h after feeding of the seeds) and once a day for the 6th day (144 h after feeding the seeds). Thirteen fecal collections were weighed and a subsample (0.25 – 4 FW kg, mean 1.2 FW kg) was taken from each fecal collection to determine the seed recovery, percentage of germination and viability. Subsamples were put in nylon bags (300 mesh), washed and then air-dried at room temperature. Sound horsenettle seeds were collected from dried subsamples and then their number was counted. Seed recovery, expressed as a percentage of seed input, was calculated by multiplying the number of seeds recovered per gram of subsample by the total fecal output per collection, and adding the number of seed recovered from 13 collections and then dividing by the number of seeds fed. The seeds recovered from feces were tested, as described in (2), for the determination of the percentages of germination and viability.

Other subsamples were taken from feces collected during the 2nd day and 3rd day after feeding of the seeds. Subsamples 3, 4, 5 and 6, and subsamples 7 and 8 were grouped into days 2 and 3, respectively. A subsample for each day weighed about 2 kg. Subsamples were put on cheesecloth in plastic plates containing small holes and then placed outdoors. Seedling numbers were counted in May 1996.

Table 2. Tested weed species

Family	Species
Asteraceae	*Ambrosia trifida* L., *Bidens frondosa* L., *Cirsium vulgare* Tenore
Solanaceae	*Solanum americanum* Mill, *S. carolinense* L.
Malvaceae	*Abutilon theophrasti* Medic.
Phytolaccaceae	*Phytolacca americana* L.
Amaranthaceae	*Amaranthus patulus* Bertoloni, *A. spinosus* L., *A. viridis* L.
Polygonaceae	*Persicaria lapathifolia* S. F. Gray, *P. longiseta* Kitag.
Poaceae	*Digitaria ciliaris* Koeler, *Echinochloa crus-galli* Beauv., *Panicum dichotomiflorum* Michx

Percentage of emergence from feces was calculated by dividing the number of seedlings by the number of seeds calculated in the subsample.

(4) Statistical analyses

Data were arcsin-transformed prior to analyses[9].

The effects of the diet on the percentages of seed recovery and germination and viability of total seeds recovered were analyzed in a randomized block design, where the diets were treatments and the trial periods were blocks.

The difference between the percentages of germination and viability of total seeds recovered and of non-ingested seeds was tested in relation to that of non-digested seeds as population mean with population variance unknown.

The effect of the number of days after ingestion on germination and viability was analyzed in a randomized block design, where the number of days after ingestion were treatments and the trial periods were blocks, for each diet.

Exp. 2: Effect of composting heat

(1) Composting tubes and materials

Experiment 2 was conducted at NGRI from December 1993 to November 1994.

Composting tubes were made of chloridized vinyl. The diameter and height of the outside part of a tube were 46 cm and 46 cm, respectively. The capacity was 52.1 L. To obtain various composting heat temperatures, tubes were packed with cattle feces and sawdust by varying the ratios, densities or sizes of materials, and ventilation conditions. The temperature was measured at the center of the tube every hour.

(2) Weed seeds

Fifteen weed species consisting of alien species which had become common[13] or common native species in forage crop fields in Japan were selected for the experiment (Table 2). Weed seeds were collected in Tochigi Prefecture in the autumn of 1993 and stored at room temperature until use in this experiment. Weed seeds were placed in small cheesecloth bags, with one species per bag which contained 50 seeds each. The 15 cheesecloth bags were placed in larger nylon mesh bags. The large bags were placed in the center of a composting tube. Seeds were exposed to composting incubation for 7–25 days and then removed to test for germination. The seeds were placed on a moistened filter paper in petri dishes in a growth chamber. The seeds were kept under alternate temperature and light conditions, i.e. 35°C and light for 12 h, and 20°C and dark for 12 h. Germination count was performed over a 7–25-day period. Eight trials were conducted over the course of 1 year, using a total of 39 tubes. Control seeds were tested for germination in January, April and October 1994, using 100 seeds for each species for each test.

Results

Exp. 1: Effect of cattle digestion

The difference between the diets had no effect on the percentage of seed recovery, percentages of

Table 3. Effect of diets on percentages of seed recovery[a)], germination and viability of total seeds recovered, and seedling emergence[b)] from feces

(%)

Diet[c)]	Recovery	Germination	Viability	Emergence
Barley	84	58	73	18
Corn	81	65	78[d)]	12

a): Total recovery 6 days after ingestion was calculated as percentage of seeds ingested.

b): Subsamples collected during the 2nd and 3rd days were used for the determination.

c): The composition and nutritive value of the 2 diets were almost the same, except for concentrates.

d): Viability of a subsample collected 48 h after ingestion in block 1 could not be measured, and it was not included.

No significant difference between diets at $P = 0.05$.

germination or viability of total seeds recovered, or on the percentage of seedling emergence from feces (Table 3). Seeds fed to the cows were recovered at a rate of 83% (mean of the 2 diets). The germination and viability were 61 and 76% (mean of the 2 diets), respectively. The germination and viability of non-ingested seeds were 63 and 83%, respectively. The germination was not affected by cattle digestion, while the viability of the seeds recovered decreased ($P<0.01$). Among the seeds contained in the subsamples placed outdoors, 15% (mean of the 2 diets) emerged, and it was revealed that horsenettle seeds ingested by cattle could emerge from feces, outdoors.

The percentages of germination and viability of the seeds recovered each day after ingestion are presented in Table 4. Only the results from days 2–5 were included in the analysis of variance, because more than 100 seeds were collected and tested for germination and viability, except on the 5th day in block 3 in the diet barley (only 37 seeds were collected). Difference between the diets was not compared. The duration of the period during which seeds remained in the digestive tract had no effect on the percentages of germination or viability which did not change over the period, regardless of the diets. There were no major differences in the percentages of germination or viability between the number of days after ingestion.

Table 4. Effect of number of days after ingestion on the percentages of germination and viability of seeds recovered (%)

Diet		Days after ingestion					
		1	2	3	4	5	6
Barley	G	58	59	59	58	49	58
	V	70	75	71	73	68	75
Corn	G	58	59	70	64	61	62
	V	71	78	79	79	73	77

G: Germination, V: Viability.
No significant difference in the percentages of germination or viability in each diet at $P=0.05$ between days after ingestion (the 2nd – the 5th day).

Table 5. Germination of control seeds (%)

Species	Test time		
	Jan.	Apr.	Oct.
A. trifida	8	9	8
B. frondosa	42	37	6
C. vulgare	23	4	14
S. americanum	77	55	58
S. carolinense	9	45	46
A. thophrasti	53	69	100
P. americana	51	18	34
A. patulus	65	94	96
A. spinosus	87	94	100
A. viridis	3	77	98
P. lapathifolia	45	70	0
P. longiseta	46	73	0
D. ciliaris	51	72	88
E. crus-galli	15	100	96
P. dichotoomiflorum	3	1	84

Exp. 2: Effect of composting heat

Moisture content of the compost at the beginning of the exposure of seeds was 72 ± 4% (mean of 39 tubes). The maximum temperatures of 39 tubes ranged from 18.5 to 75.3°C.

Control seeds of *P. lapathifolia* or *P. longiseta* did not germinate in October 1994 (Table 5), but the maximum germination percentage of the 2 species which were exposed in October was 16%. Therefore, it was considered that 15 weed species remained viable during the experimental period. Since the germination of control seeds of *S. carolinense, A. theophrasti, A. patulus, A. viridis, D. ciliaris, E. crus-galli* and *P. dichotomiflorum* increased with time, the dormancy of these seeds was considered to be broken gradually during the experimental period.

The relationship between the maximum temperature to which weed seeds were exposed and the percentage of species which retained their germinability is depicted in Fig. 1. A set of models of straight lines with 2 intersecting points[10] was calculated by letting the maximum temperature be an independent variable and the percentage of the species which retained their germinability a dependent variable. The

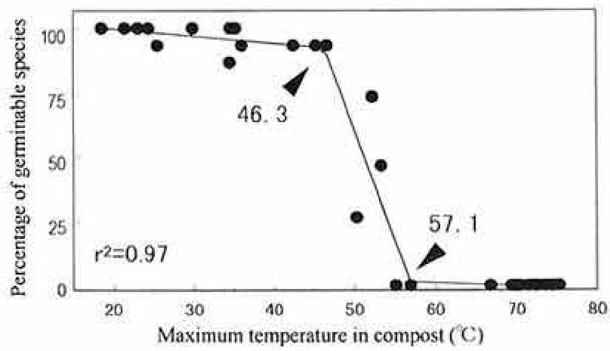

Fig. 1. Percentage of weed species which retained their germinability after being exposed to varying temperatures in compost

Table 6. Percentage of species which retained germinability, maximum temperature and duration of the period during which temperature was higher than 45°C

Maximum temperature in compost (°C)	≦42.4	45.2	46.6	50.3	52.2	53.3	≧55.1
Tube number	11	1	1	1	1	1	23
Germination of species (%)	97(5)	93	93	27	73	47	0
Duration (h)	0	9	27	76	40	109	142(40)

Values in parentheses represent s. d.

effects were not obvious until the maximum temperature reached 46°C. Thereafter there was a rapid decline in germinability, and all the species lost their germinability when the temperature rose above 57°C.

The percentage of the species which retained their germinability, the maximum temperature and duration of the period during which temperature was higher than 45°C are presented in Table 6. The duration was 142 h in the 23 tubes in which the maximum temperatures were higher than 55.1°C and all the species lost their germinability.

Discussion

Exp. 1: Effect of cattle digestion

Total recovery of horsenettle seeds from feces was not affected by the diets and the mean of the 2 diets was 83%. This percentage was much higher than that reported in similar experiments using pasture species[15,19]. The viability of all the seeds recovered was high (76%, mean of the 2 diets), though it was lower than that of non ingested seeds ($P<0.01$). The viability of other weed species recovered from cattle feces[1] and exposed to rumen digestion by cattle[2] was much lower than the values obtained in this experiment, and it was considered that the tolerance of horsenettle seeds to cattle digestion was relatively higher than that of other species.

The width, length and thickness of horsenettle seeds which were collected in Tochigi in 1993 were 1.87 ± 0.16, 2.36 ± 0.17 and 0.68 ± 0.09 mm, respectively (Nishida, unpublished data). Particles which pass through a 2.36 mm sieve are considered to flow out from the rumen of cattle[11]. Since many sound horsenettle seeds were recovered in this experiment, it was considered that they could avoid physical destruction caused by mastication and rumination. Hence the size of horsenettle seed is considered to contribute to the tolerance. Besides, as the viability of the seeds recovered did not change over the period during which they remained in the digestive tract, horsenettle seeds may not be very susceptible to the chemical effect of digestion.

The germination percentage of all the seeds recovered was 61% (mean in the 2 diets), and there was no difference between that of ingested and non-ingested seeds. Soft seeds of legumes are readily digested, and the percentage of hardseedness among the recovered seeds increased with the duration of the retention time[15,18]. The percentage of dormancy of horsenettle seeds recovered did not change with the duration of the retention time.

Among the seeds which were estimated to be contained in the subsamples placed outdoors, 15% (mean of the 2 diets) emerged, a lower value than the percentage of emergence expected from the viability of seeds recovered. Emergence might have continued after seedling counts were performed in May 1996. However, 59 seedlings emerged from a subsample on an average and the possibility for horsenettle to become established in the fields via cattle feces was confirmed. Yamada et al.[19] reported that there was a close correlation between the number of seedlings from feces and the number of sound seeds in the feces. The recovery and viability of horsenettle seeds were very high and seedlings emerged from feces placed outdoors in this experiment. Therefore, cattle feces are a possible source of weed infestation in forage crop fields and pastures if weed seeds are mixed in feed.

It is considered that flaked barley disintegrates faster than flake corn in the rumen and induces a lower ruminal pH. However, there was no difference between the recovery of horsenettle seeds in diet barley and in diet corn in this experiment. Ishida and Kayo measured the ruminal pH (every 2 h from 2 to 8 h after ingestion) using fistulated cows which were fed the same diets as in this experiment (2 cows for each diet). The lowest pH values in diet barley and diet corn were 5.69 and 5.86, respectively, and there was no significant difference (at $P=0.05$) between the 2 diets (unpublished data). This was considered to be one reason why the difference in diets did not affect the recovery of horsenettle seeds in this experiment.

Exp. 2: Effect of composting heat

The intersecting points of the set of models fitted between the maximum temperature and the percentage of species which retained their germinability corresponded to 46 and 57°C. These values indicate that some species lost their germinability when temperatures rose above 46°C and all the species lost their germinability when temperatures rose above 57°C in this experiment. It is considered that the germinability of seeds is affected not only by the maximum temperature but also by the duration of the period during which seeds are exposed to temperatures higher than a certain degree. Duration of the period during which the temperature was higher than 45°C was 142 h in tubes where the maximum temperatures were higher than 55.1°C and all the species lost their germinability. Therefore, raising temperature above 60°C and keeping the duration longer than 142 h enable to kill non-dormant seeds in compost. However, it is considered that the higher the maximum temperature, the shorter the duration required to kill seeds. Hence, further studies about the maximum temperature and the duration of the period required to kill seeds are needed.

Weed seeds were considered to imbibe enough water in this experiment because the water content of the compost was 72% which is considered to be relatively high. Lethal temperature and duration of the period during which seeds are exposed to temperatures vary with the water content of seeds[3,8,17]. Hence, the water content of compost for which seeds imbibe enough water is important to kill weed seeds in the composting process.

Lethal temperature for non-dormant weed seeds in compost was determined in this experiment. However, it is considered that dormant seeds could be more tolerant to heat[4]. Dormancy is not broken in a short time after seed setting. Hence, if there are many young seeds in the compost, higher temperature and longer duration are required to kill them. Further studies on lethal temperature for dormant seeds should be carried out to control weed infestation in fields via compost.

References

1) Atkeson, F. W., Hulbert, H. W. & Warren, T. R. (1934): Effect of bovine digestion and of manure storage on the viability of weed seeds. *J. Am. Soc. Agron.*, **26**, 390-397.
2) Blackshaw, R. E. & Rode, L. M. (1991): Effect of ensiling and rumen digestion by cattle on weed seed viability. *Weed Sci.*, **39**, 104-108.
3) Horowitz, M. & Taylorson, R. B. (1983): Effect of high temperatures on imbibition, germination and thermal death of velvetleaf (*Abutilon theophrasti*) seeds. *Can. J. Bot.*, **61**, 2269-2276.
4) Horowitz, M. & Taylorson, R. B. (1984): Hardseedness and germinability of velvetleaf (*Abutilon theophrasti*) as affected by temperature and moisture. *Weed Sci.*, **32**, 111-115.
5) Itoh, M. (1993): Introduction of weed science. Yokendo, Tokyo, pp.99 [In Japanese].
6) Livestock Industry Bureau, MAFF (1985-1995): Bull. commercial feed [In Japanese].
7) Morimoto, H. (ed.) (1971): Experimental methods of animal nutrition. Yokendo, Tokyo [In Japanese].
8) Noguchi, M. & Egawa, T. (1994): Method for killing of upland weed seeds. (3) Effect of heat. *Weed Res., Jpn.*, **39**(I), 250-251 [In Japanese].
9) Okuno, T. (ed.) (1978): Applied statistics handbook. Yokendo, Tokyo [In Japanese].
10) Ootsuka, Y. (1978): Fitting a set of models of intersecting straight lines. *Bull. Comp. Cent. Res. Agric., Forest. Fish.*, **14**, 1-31 [In Japanese].
11) Poppi, D. E., Hendricksen, R. E., & Minson, D. J. (1985): The relative resistance to escape of leaf and stem particles from the rumen of sheep and cattle. *J. Agric. Sci, Camb.*, **105**, 9-14.
12) Shimizu, N. (1984): Seed vigor testing in various tropical grasses by TTC method. *Kyushu Agric. Res.*, **46**, 187 [In Japanese].
13) Shimizu, N. et al. (1994): Recent situation of emergence of alien weeds invading grasslands and forage crop fields. *Weed Res. Jpn.*, **39**(I), 228-229 [In Japanese].
14) Shimizu, N., Kurokawa, S. & Uozumi, S. (1995): Establishment and diffusion mechanisms of alien weeds invading grasslands and forage crop fields (II). *Weed Res. Jpn.*, **40**(I), 178-179 [In Japanese].
15) Simao Neto, M., Jones, R. M. & Ratcliff, D. (1987): Recovery of pasture seed ingested by ruminants. 1. Seed of six tropical pasture species fed to cattle, sheep and goats. *Aust. J. Exp. Agric.*, **27**, 239-246.
16) Takabayashi, M., Abe, R. & Kubota, T. (1975): Studies on the dissemination of weed seeds by livestock. I. Viability of weed seeds passed through digestive tract of dairy cow. *Weed Res. Jpn.*, **19**, 36-40 [In Japanese with English abstract].
17) Takabayashi, M., Kubota, T. & Abe, R. (1978): Dissemination of weed seeds by livestock. II. Methods to kill weed seeds. *J. Cent. Agric. Exp. Stn.*, **27**, 69-91 [In Japanese with English abstract].
18) Yamada, T. & Kawaguchi, T. (1972): Dissemination of pasture plants by livestock. II. Recovery, viability, and emergence of some pasture plant seeds through digestive tract of dairy cow. *Grassl. Sci.*, **18**, 8-15 [In Japanese with English abstract].
19) Yamada, T., Matsuo, S. & Tamura, K. (1972): Dissemination of pasture plants by livestock. III. Recovery of some pasture plants seeds passed through digestive tract of beef cattle and emergence of seedlings from seeds recovered in feces. *Grassl. Sci.*, **18**, 16-27 [In Japanese with English abstract].

(Received for publication, May 28, 1997)