

## **PART C: WEED SEED SURVIVAL AS AFFECTED BY MANURE HANDLING**

### **Introduction**

The potential for weed seed introduction to cropland through the application of manure is a question faced by many farmers. The application of manure may increase the number of seeds already present in the weed seedbank or introduce seeds from a weed species not yet present in a field. Time of harvest and type of feed storage may affect weed seed dormancy and viability (Mt. Pleasant and Schlather 1994). Processes, such as ensiling or rumen digestion can also affect weed seed viability in manure by reducing the number of viable weed seed present (Blackshaw and Rode 1991). Anaerobic manure digestion may also reduce weed seed germination and viability. In a field study, this project determined the effect of anaerobic digestion on germination of weed seeds common to the Anoka sand plains region.

### **Materials and Methods**

Because of changes in weed seed germination and viability that can occur during seed storage, newly matured weed seeds were collected from plants the fall of the initiation of the experiment. Weed seed were characterized for potential germination and viability to establish a baseline prior to inclusion in the study. Six weed species were chosen in part to reflect species typically found in manure in the region and to include representatives of weed seed 'groups'. Seed groups included were grass and broadleaf species, large and small seeded species, true seeds and achenes (smartweeds), and species with known impermeable, protective seed coats (velvetleaf). Weed species included in this experiment are as follows:

1. velvetleaf (*Abutilon theophrasti*)
2. common lambsquarters (*Chenopodium album*)
3. redroot pigweed. (*Amaranthus retroflexus*)
4. wild proso millet (*Panicum miliaceum*)
5. giant foxtail (*Setaria faberi*)
6. ladysthumb smartweed (*Polygonum persicaria*)

Weed seed were collected from the Rosemount Experiment Station during the fall of 2002 and 2003 for the first and second trials of the experiment, respectively. Seeds were cleaned and stored at room temperature until used in experiments. Seed germination was tested by placing seed in a petri dish between moistened filter paper at 24 C for 14 days in the light. Seeds with emerged radicals were counted as germinated (Hartzler et al. 1999). Viability of 400 seed from each species was determined by placing seed in a petri dish between moistened filter paper for a minimum of 48 hours in a germinator at 24 C, then treated with a 1% (w/v) solution of tetrazolium. Seeds were considered viable if the embryo stained red.

Tracking this seed lot through a farm-scale digester is like the “needle in a haystack” analogy. The seed needed to be contained and retrievable and so were placed in nylon mesh bags. For each treatment, all six species of weed seed were combined into one mesh bag. One hundred seed per species was added to each bag. Each treatment was replicated six times for a total of six bags. Since most, (but not all) of the weed seed in the system will pass through a dairy cow, we subjected seed to an *in vitro* rumen fermentation procedure (Marten and Halgerson, 1980) proven to simulate conditions of a cow’s digestive system. In this procedure, weed seeds were soaked in rumen fluid for 48 hours in an Ankom Daisy fermentor oven. Next, the seeds were immersed in a pepsin and hydrochloric acid solution for 24 hours to simulate passage through the stomach. Seed was then subjected to one of three manure or fertilizer treatments: 1.) anaerobic digested manure, 2.) conventional manure storage, and 3.) an inorganic fertilizer control.

For the two manure storage treatments, mesh bags were placed in either the anaerobic digester or in raw, undigested manure prior to its entering the digester. The mesh bags were placed into a larger mesh bag along with a temperature monitor and weighted with fishing sinkers to prevent the seed from remaining on the surface of the digestate. Seed bags were in manure treatments for 20 days (length of time for one batch of manure to pass through the digester though weed seeds may pass through at different rates depending on size, solution/suspension density, etc.). Seeds were placed in the end of the anaerobic digester where the manure exits prior to entering the storage lagoon, as this position was the only available access point from which to introduce the seeds and ensure they could be retrieved again. Though not ideal, we feel this approach most closely reflects anaerobic digester conditions, considering limitations of internal access to the digester and the need to track a seed lot know origin and viability in lieu of using a simulated digester.

To determine germination of seed once it was removed from the digester, seed were assayed in field boxes. Germination and emergence of the weed seed was monitored for two growing seasons. The experiment was placed in an area previously in sod as it was assumed that the *in situ* weed seed bank under sod would be low (Hartzler et al. 1999). The sod was removed and the soil worked to a depth of approximately 3 inches. The wooden frame field assay boxes (15 by 18 inches) were placed 2 inches into the soil. In late November of 2001 and 2002, weed seeds were removed from the digester or conventional manure storage, placed in the appropriate

wooden frame and incorporated with a small rake to a depth of approximately .5 inches into the soil. Digested or non-digested manure was added to the wooden frames at a rate of 6000 gal/A, a rate used by Dennis Haubenschild in fields where corn will be planted the following year. Each experimental treatment was duplicated without the addition of weed seed to determine the level of the *in situ* seedbank for a total of 12 assay boxes for each run of the experiment.

In mid-April of 2002 and 2003, ammonium nitrate (34-0-0) at a rate of 180 lb N/A as ammonium nitrate) was added to each plot requiring inorganic fertilizer, approximating the level of N available in the 6000 lb/A manure applications. Levels of P and K were found to be at acceptable levels based on a soil test conducted the fall of 2001. The number of germinating weed seeds was recorded on approximately a monthly basis throughout the growing season and weeds were hand pulled after counting. In April of 2003 and 2004, soil within each frame was mixed to a depth of two inches and germinating seedlings were again counted for the second season for each trial. To account for the number of possible weeds in the wooden assay frames that germinated from seeds already present in the soil at the initiation of the experiment, the number of “background” weeds was determined from assay boxes without added weed seed and were subtracted from the number of weeds that germinated in plots with added weed seed. Each treatment was replicated six times and the experiment was repeated in time. Results from the experiment were analyzed as a randomized complete block design and means were separated with a Least Significant Difference test at the 0.05 level of significance. Results did not differ between experimental runs and data was combined from both trials. Due to funding limitations, a non-rumen seed treatment check was not included, and viability of weed not expressed through germination in the field assay boxes was not determined.

Table 1.  
Timing of experiment initiation and data collection.

	Trial 1	Trial 2
Date of experiment initiation	November 2001	November 2002
First season of field assay data collection	2002	2003
Second season of field assay data collection	2003	2004

## Results and Discussion

Viability of weed seed used in Trials 1 and 2 ranged from 99% for velvetleaf to 82% for wild proso millet (Table 2). Results of the weed seed germination tests ranged from 11% for common lambsquarters to 0% for ladythumb smartweed, wild proso millet and velvetleaf. The low weed seed germination values compared to the high weed seed viability results indicate that the majority of weed seeds were dormant when placed into the anaerobic digester or conventional manure storage.

Table 2

Trials 1 and 2. Percent germination and viability of weed seed used in anaerobic digestion experiment, prior to rumen digestion, collected from Rosemount, MN, Fall 2001 or Fall 2002.

Species	Germination*		Viability*	
	(%)		(%)	
	2001	2002	2001	2002
redroot pigweed	2	5	92	100
common lambsquarters	11	12	88	100
wild proso millet	0	0	82	98
Ladysthumb smartweed	0	0	95	88
velvetleaf	0	14	99	99
giant foxtail	0.8	0.8	88	87

\*Mean of 4 lots of 100 seeds each.

In Trials 1 and 2, no giant foxtail, wild proso millet or ladythumb smartweed seeds germinated in plots from any treatment during spring or summer of 2002 through 2004. This is most likely due to destruction of the weed seed in rumen fluid and simulated stomach acid. Similar results were documented by Blackshaw and Rode (1991) who reported less seed survival of the grassy weeds, green foxtail, downy brome and barnyardgrass seeds compared to broadleaf weed seeds after rumen digestion.

During the first summer of data collection in each trial, there were no differences in number of broadleaf seeds germinating between conventional lagoon and anaerobic digestion manure treatments, with one exception. Velvetleaf seeds had higher rates of germination after

the anaerobic manure treatment, with an average of 14 seeds germinating compared with nine seeds for the conventional manure treatment (Table 3). The increase in velvetleaf germination the first year of the study may have been due to scarification of the hard seed coat during anaerobic digestion. The scarification process may have enabled these velvetleaf seed to break seed dormancy and germinate sooner. During the second year of the study, the situation was reversed with the conventional and organic fertilizer treatments resulting in higher numbers of velvetleaf seed germinating compared with the anaerobic digestion treatment. In total, the same number of velvetleaf seed germinated over the course of two years of assay, but the number varied between the first and second season depending on manure treatment. After two years, it must be noted that the majority of velvetleaf seed did not germinate and were either destroyed by the initial rumen digestion treatment or remained dormant in the soil.

Table 3

First and second season data on numbers of velvetleaf seeds germinating in the spring and summer after 20 days of fall storage in different manure storage systems compared to seeding with inorganic fertilizer application. Trials 1 and 2 were combined. Haubenschild Farms, MN.

<b>Manure/fertilizer system</b>	<b>Velvetleaf</b>	
	<b>First season</b>	<b>Second season</b>
<b>Anaerobic Digestion</b>	14	2
<b>Lagoon Storage</b>	6	6
<b>Inorganic Fertilizer</b>	9	6
<b>LSD (0.05)</b>	3	3

When examining the timing of velvetleaf germination, most seed germinated between mid-May to mid-June (Julian dates of 130 to 170) (Figures 1 and 2). During the first season of both trials, more velvetleaf seed germinated from the anaerobic digester treatment during this time period (Figures 1a and 2a). In Trial 1, there were no differences in velvetleaf emergence among treatments during mid-May to mid-June for the second season (Figure 1b). However, in the second season of data collection in Trial 2, there were significantly more velvetleaf seed germinating at 126 Julian days (May 5) for the conventional manure storage treatment than for the anaerobic digestion treatment (Figure 2b).

There were no differences in the cumulative number of velvetleaf, common lambsquarters or pigweed spp. seeds germinating over two seasons among manure/fertilizer

treatments in each trial. No ladythumb smartweed seed germinated in either trial (Table 4). Jayanayagam and Collins (1984) reported that dormant weed seed were less likely to be destroyed by anaerobic digestion than non-dormant seed. The weed seed used in our study was harvested a few months prior to the initiation of the experiment. Germination and viability tests indicated that 80% or more of the seed were likely to be dormant and less susceptible to damage caused by anaerobic digestion. Our results demonstrate that it is critical to characterize the age and dormancy of weed seed used to study the effect of anaerobic digestion on weed seed viability.

Table 4

Cumulative number of weed seed germinating in a field germination assay for two seasons following 20 days in different manure storage systems at Haubenschild Farms. Trials 1 and 2 were combined.

Manure/fertilizer system	Weed species					
	Vele <sup>1</sup>	Colq	Rrpw	Lasw	Gift	Wipm
Anaerobic digestion	16	12	1	0	0	0
Conventional storage	12	18	5	0	0	0
Inorganic fertilizer	14	11	4	0	0	0
LSD (0.05)	NS	NS	NS	NS	NS	NS

<sup>1</sup> Vele = velvetleaf                      Lasw = smartweed spp.  
 Colq = common lambsquarters      Gift = giant foxtail  
 Rrpw = redroot pigweed.              Wipm = wild proso millet

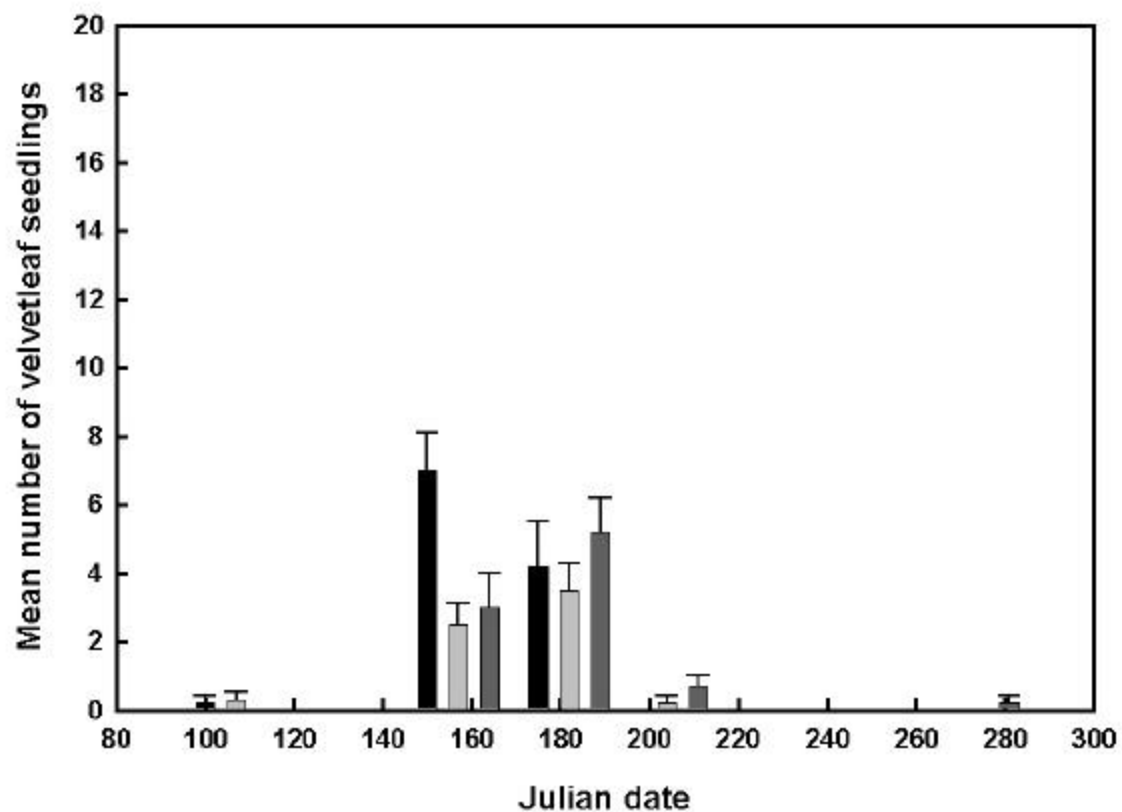
The temperature of the anaerobic digester during the 20 days that the weed seed were suspended in the anaerobic digester ranged from 95 to 105 F. The Haubenschild's digester is heated in the floor near the manure entry point such that temperatures most likely are higher near the floor near the entry area, with some convection and kinetic mixing occurring. However, overall, much of the manure and weed seed therein will not be exposed to temperatures significantly higher than the 95 to 105 F measured in the vicinity of the seed bags. This temperature is below the estimated 140 F required to kill the majority of weed seed in compost or manure piles (Larney and Blackshaw 2003). Some weed seed requires even higher temperatures to be killed. Wiese et al. (1998) reported that seven days of 180 F temperatures were necessary to kill field bindweed seed in a compost pile. Sarapatka et al. (1993) demonstrated that temperatures in a monitored anaerobic digester that ranged from 133 to 122 F

were more effective in reducing weed seed viability than temperatures in the 86 F temperature range. From the results of our study, we concluded that temperatures in the anaerobic manure digester did not attain the level required to kill viable dormant weed seeds.

### **Conclusion**

In conclusion, the majority of the freshly harvested weed seed were dormant at the time the seeds were exposed to the manure storage treatments. Other studies have documented that dormant weed seed are less likely to be destroyed by anaerobic digestion. Seeds of wild proso millet, giant foxtail and ladysthumb smartweed did not germinate during the course of the experiment and were most likely killed as a result of the rumen and simulated stomach treatment used prior to the manure storage treatments. Although higher numbers of velvetleaf seed germinated during the first year of both trials, there were no cumulative differences in weed seed germination among the anaerobic or conventional manure storage or the inorganic fertilizer treatment for any weed species tested. Anaerobic digester temperatures ranging from 95 to 105 F may have been too low to kill the weed seed during the period when the weed seeds were suspended in the digester. It may also be concluded that the majority of common lambsquarters, pigweed spp. and velvetleaf seed had not germinated after two seasons and most likely remained dormant in the soil. Although anaerobic manure digestion has many advantages, such as odor control and production of electricity via methane production, our results did not document a reduction on viability of weed seed after anaerobic manure digestion.

**Figure 1a. Seed Germination as Affected by Manure Storage  
Trial 1, First Season of Data Collection**



- Anaerobic digestion
- Conventional manure storage
- Inorganic fertilizer control

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