

Effects of ensiling on seed germinability and viability in *Rumex crispus* L

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Abstract

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In this study, experimental data show that at least 52% of seeds of *Rumex crispus* L. survive ensiling for 100 days. The aim of the study was to elucidate how retention time in silage along with plant population, dry matter content and green forage length in silage affect seed germinability and viability. To study this, ensiling was carried out in glass containers at the laboratory and in plasticized round bales under field conditions. Seeds were recollected and tested in germinability and viability tests.

The results from the laboratory experiment show that about half of the seeds survive ensiling. However, no significant differences between the treatments (retention time in silage, population of origin, high/low dry matter content, chopped/intact green forage) were observed. An interesting observation was that all seeds in the laboratory experiment, recollected after one month or later, were in secondary dormancy. Plausible causes to this are discussed. The results from the field experiment show that 100% of the seeds remain viable after 141 days in round bale silage.

The agricultural significance of this subject depends on whether the seeds survive the whole chain silage – rumen digestion – storage in farmyard manure or not. This has not been sufficiently investigated. Nevertheless, my conclusion is that ensiling alone does not render seeds of *Rumex crispus* harmless on arable land.

Agrovoc: silage, Rumex, seed germinability, seed viability, weed control

Keywords: curled dock, ensilage, grobarhet, krusskräppa, livsduglighet, ogräs, *Rumex crispus*

Sammanfattning

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Den här studien visar att minst 52% av frön från krusskräppa, *Rumex crispus* L., överlever ensilering i 100 dagar. Målet med studien var att utreda om ensileringstidens längd kombinerat med frönas ursprungspopulation, torrsubstanshalt och strålängd i ensilaget påverkar grobarhet och livsduglighet hos ensilerade frön. För att studera detta utfördes ensilering med frön i glasbehållare i laboratorium och med ett mindre antal frön i inplastade rundbalar i fält. Fröna plockades upp och testades med avseende på grobarhet och livsduglighet.

Resultaten från laboratorieexperimentet visar att ungefär hälften av fröna överlever ensileringen. Det kunde däremot inte påvisas några statistiskt säkerställda skillnader mellan försökets olika behandlingar (ensileringstid, ursprungspopulation, hög/låg torrsubstanshalt, korthackad/långsträig grönmassa). En intressant iakttagelse var att alla frön som plockades upp ur glasbehållarna efter en månad eller senare var i sekundär gröningsvila. Tänkbara orsaker till detta diskuteras. Resultaten från rundbalsexperimentet visar att 100% av fröna är livsdugliga efter 141 dagar.

Spridningen av frön från *Rumex crispus* via ensilage är beroende av hur fröna klarar hela kedjan ensilage – idisslarnas fodermältning – lagring i stallgödsel. Fler studier måste göras för att få tillräcklig kunskap om detta. Slutsatsen av den här studien är dock att enbart ensilering inte oskadliggör frön från *Rumex crispus*.

Agrovoc: silage, Rumex, seed germinability, seed viability, weed control

Nyckelord: curled dock, ensilage, grobarhet, krusskräppa, livsduglighet, ogräs, *Rumex crispus*

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2. Introduction

2.1 Agricultural significance

Curled dock (*Rumex crispus* L.) is one of the five most widely distributed plants in the world (Cavers & Harper, 1964) and is known as a noxious weed in several countries (Frankow-Lindberg, 1998). The distribution in the Fenno-Scandinavian countries is shown in Figure 1.

The weed problems in organic farming due to *Rumex crispus* have accelerated lately and are no longer problems unique to permanent grazing leys. Studies have shown that seeds of *Rumex crispus* are able to pass through the rumen digestive tract, endure storage in manure and still retain their germinability (Atkeson *et al.*, 1934). Accordingly there is a possibility that weed seeds are dispersed with farmyard manure. However, Pleasant & Schlater (1994) claims that farmyard manure is less important than the seed bank as a weed source for New York farms.

The number of seeds from *Rumex crispus* was in this study estimated to 2100-10 700 seeds / 1000 kg manure. Investigations of other weed species (kochia *Kochia scoparia*, redroot pigweed *Amaranthus retroflexus*, common lambsquarters *Chenopodium album*, black bindweed *Polygonum convolvulus* (*Fallopia convolvulus*), round-leaved mallow *Malva pusilla* and field pennycress *Thlaspi arvense*) have shown that the seeds can survive both ensiling and rumen digestion (Blackshaw & Rode, 1991). Mayer *et al.* (2000) studied weed seed germinability and found that *Trifolium arvense* kept its germinative ability through 12 months storage in silage. Fitch & Zahnley (1941) showed that field bindweed (*Convolvulus arvensis*), morning glory (*Ipomoea purpurea*) and velvetleaf (*Abutilon theophrasti*) germinated after 1636 days in silage.

At the beginning of the century, when most of the work on weed seed viability after storage in farmyard manure was done, it was far more common to compost solid manure. There are not many studies that evaluate how weed seed viability is affected by the manure storage systems of today. However, Šarapatka *et al.* (1993) showed that seeds from broad-leaved dock *Rumex obtusifolius* in the sub-surface layer (40 cm depth) of anaerobically fermented liquid manure (biogas production) had a germination rate of 19% after 30 days. On 180 cm depth all seeds lost their germination power within 30 days. In addition, Mayer *et al.* (2000), showed that seeds from corn spurrey *Spergula arvensis* and long-headed poppy *Papaver dubium* germinated after three months storage in semi-liquid manure. Elema *et al.* (1990) considered liquid cattle manure to be a source of certain weed species. However, percentage germinated seeds is not a measure of seed viability. A much higher amount of the seeds can be viable than the ones that germinate.

The answer to *Rumex crispus* success as a weed is among many its ability to establish quickly from seed, to flower in the first year and to stay dormant in the soil for a very long time (Cavers & Harper, 1964). When the plant is damaged by soil cultivation, the formed root segments can result in new plants (Weidow, B., 1993; Cavers & Harper, 1964). Moreover, even after having been cut the green perianth segments can provide the seed with assimilate and thereby make maturation possible (Maun, 1974). Picking the weed plants by hand is still an often-practised way of control though very time-consuming.

2.2 Silage

Silage has during the last 20 years become one of the most important feeding stuffs for ruminant animals. The fundamental idea is to preserve as much of the nutritive value in the green forage as possible and obtain a high digestibility. Thus, degradation and growth of undesired microorganisms must be prevented, preferably by eliminating oxygen in the green forage and supporting lactic acid bacteria growth (McDonald *et al.*, 1991). Anaerobic conditions are accomplished in hermetically sealed containers e. g. tower silos, bunker silos and plasticized bales. The small amount of air present after sealing the silo is rapidly removed by respiratory enzymes

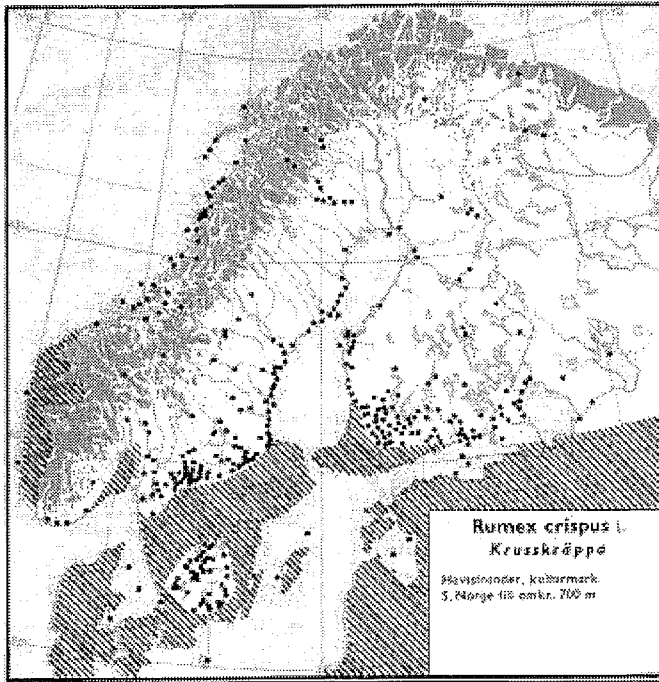


Figure 1. The distribution of *Rumex crispus* in the Fenno-Scandinavian countries (Nordiska Riksmuseet, 2002, <http://linnaeus.nrm.se/flora>).

in the plant material. Chopped material is more easily packed which reduces the amount of air trapped.

To promote lactic acid bacteria growth it is favourable if substrate is readily available. This is achieved when chopped green forage is used for ensiling. Growth of lactic acid bacteria decreases pH of the silage, which result in impaired growth conditions for many other microorganisms. Dry matter content of the silage is another important factor to avoid growth of undesired microorganisms such as Clostridia (McDonald *et al.*, 1991).

2.3 Seed longevity and dormancy

Several studies have shown that the seeds of *Rumex crispus* can survive many years of unfavourable conditions (Darlington & Steinbauer, 1961; Lewis, 1973). According to Darlington & Steinbauer (1961) *Rumex crispus* is among the three longest surviving species in Dr Beal's 80 years viability test. *Rumex crispus* showed a germinability of 2% that indicates that 80 years is near the maximum of the species longevity.

As many of our common weeds, seeds of *Rumex crispus* exhibit non-deep physiological dormancy. Characteristic for seeds of some species with non-deep physiological dormancy is that dormancy is broken by a relatively short period (5-90 days) of cold stratification. In other species, including *Rumex crispus*, high temperatures ($\geq 15^{\circ}\text{C}$) break dormancy. Dormancy breakage is incomplete or does not occur at all if the seeds are cold stratified preceding the higher temperature. The period required for dormancy loss at high temperature is relatively longer than the time needed for seeds of species with cold stratification requirements, from several weeks to many months. In contrast to many other species with this kind of dormancy, *Rumex crispus* does not show any signs of having a dormancy cycle. Hence, the seeds do not reenter secondary dormancy when it is once broken (Baskin & Baskin, 1998).

The ageing processes continue slowly in a dormant seed. As the age of the seed increases there is a risk for accumulated genetic damages. Proteins within the seed may also break down irreparably (Milberg, 1990). Went & Munz (1949) and Went (1969) claim that low oxygen levels cause the seeds respiratory processes to proceed more slowly, thus enable seeds to germinate at high ages.

2.4 Biology

Curled dock, *Rumex crispus* L., belongs to the family *Polygonaceae* and is a 40 - 100 cm tall perennial herb with erect flowering stems (Mossberg *et al.*, 1997). It has a fleshy tap root that can reach a length of 160 cm. The crisped basal leaves that become narrower to the base, are up to 40 cm long and 11 cm wide. The flowers have no nectar and are usually wind pollinated. *Rumex crispus* can grow on almost all soils apart from of the most acid (Cavers & Harper, 1964).

The fruit is a nut (here referred to as a seed) with three distinct edges, enclosed in three perianth segments. At the base of each perianth segment there is a nodule, which makes *Rumex crispus* easy to distinguish from northern dock, *Rumex longifolius*. One plant can produce up to 40 000 seeds. The average weight of 1000 seeds is somewhere between 1 and 3 g, the higher weight for maritime plants (Cavers & Harper, 1964). Seeds from different populations may differ in their germination responses, for example have different requirements for dormancy breakage (Baskin & Baskin, 1998).

2.5 Aim of the study

I have in my study investigated how the seeds of *Rumex crispus* are affected by different kinds of silage. I sought the answer to the following questions:

- What are the effects on seed germinability and viability of different retention time in silage?
- In what way does dry matter content of the silage affect seed germinability and viability?
- Are there any differences in seed germinability and viability between seeds from different populations?
- How does green forage length in the silage affect seed germinability and viability?

3. Methods

3.1 Seed

Seeds were chosen from two different populations, one from northern Öland (57° N 17° E), collected in summer 2000, the other from Uppsala (60° N 18° E), collected in winter 2000/2001. Seeds from the population in Uppsala showed a slightly lower germinability in previous studies (Pye, unpublished) than seeds from Öland. By using these two populations in my experiment it was possible to analyse any occurring differences. Until the examination, the seeds were stored in a freezer at -20°C.

To ensure that the seeds could be placed in the silage and still be easy to find, they were put into permeable nylon bags, about 5 × 5 cm. Every bag contained 15-18 seeds.

3.2 Ensiling - laboratory experiment

The experiment ley was situated in Säby, about 4 km south of Uppsala city. The included species was timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*) and red clover (*Trifolium pratense*). The silage process may proceed differently, depending on different sugar content etc., if the botanical composition of the silage is altered. The relationship between different species in this experiment was assumed to be constant.

The ensiling experiment was carried out in glass containers of 4 dm³. The lid was equipped with water seal to enable gases to leak out although no oxygen could get inside. When needed, silicon was used to make the container airtight. The method used to imitate the real process during ensiling in a tower silo, bunker silo or round bale, is the standard method in feedstuff experiments (Henderson, 1990; McDonald *et al.*, 1991).

Two types of green forage were used for silage; low dry matter content (29%) and high dry matter content (41%). To examine if green forage length have effects on seed germinability and viability, each of these treatments were divided into chopped and intact material. Each container was filled and tightly packed with 750 g of green forage.

To examine how the seed germinability and viability were affected by retention time, seeds were recollected at six dates with four replicates. This resulted in totally 96 containers. Two bags with seeds from each of the two populations were placed, equally distributed, in each container resulting in 192 bags. The time interval between recollection of seeds from the silage containers was two weeks, except from the last date when seeds were recollected 100 days after start of the experiment.

The ensiling was carried out without any additives and the temperature was held constantly at 25°C in the room where the containers were placed.

3.3 Ensiling - field experiment

To ensure that the results from the ensiling experiment using glass containers are comparable with practical ensiling methods, a number of seeds were put into plasticized round bales. The same type of permeable nylon bags as the ones used in the glass container silage were used in this experiment. Seeds were collected from the same populations and at the same time as in the container experiment. The experiment was started in early July and seeds were recollected after 61, 100 and 141 days.

3.4 Germination test

Seeds retrieved from the silage containers were put in 9 cm Petri dishes on two filter papers (filter paper: Munktell 1003) moistured with 4.5 ml tap water. After three weeks in a germination incubator the seeds were taken out and the number of germinated seeds was counted at the laboratory. The temperature in the germination incubator was set at 16 °C for 12 hours (lights on) and 6 °C for the next 12 hours (lights off) with a light flow of $70 \pm 10 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Four sets of seeds that had not been placed in silage were tested in the same way to serve as control.

3.5 Viability test

To examine if seeds that did not germinate were dead or dormant they were put in a new Petri dish with 4.5 ml gibberellic acid and potassium nitrate (GA 0.5 g · l⁻¹, KNO₃ 2 g · l⁻¹). Gibberellic acid is a plant growth hormone that give signals to the aleurone layer in the seed to produce α -amylase and other digestive enzymes. The enzymes hydrolyse molecules in the endosperm to sugar and other nutrients which are needed in the germination process (Campbell, 1996).

Seeds that had not germinated after three weeks were considered alive but dormant if they could resist a light pressure from a pair of tweezers. This way of testing viability of dormant seeds is described by Pleasant & Schlater (1994).

3.6 Tetrazolium test

The tetrazolium test was carried out to ensure that the seeds I had considered alive in the viability test really were alive.

2,3,5-triphenyltetrazolium chloride (tetrazolium) is a colourless, water soluble salt. In contact with the enzyme dehydrogenase, which is active in viable cells, tetrazolium is reduced to formazan, a red-coloured, non-soluble compound (Ellis *et al.*, 1985a). Thus, it is possible to distinguish between viable and dead seeds.

To enable the tetrazolium to diffuse into the seed embryo, the seeds were moistened overnight on wet filter paper in Petri dishes. Five grams 2,3,5-triphenyltetrazolium chloride were dissolved in 500 ml phosphate buffer solution (pH 7). The seeds were soaked in the tetrazolium solution for 24 hours in 30°C. The concentration of the solution and the staining time was taken from earlier tests on black bindweed, *Fallopia convolvulus* (Andersson, 2001). After staining, the seeds were rinsed in deionised water. To be able to see the embryo, every seed was dissected and examined in a stereo magnifier. A sample of unstained, dissected seeds were placed in the tetrazolium solution for another 24 hours to exclude that the solution had been unable to diffuse through the seed coat.

3.7 Statistics

Analysis of variance was carried out using logistic regression analysis in the GENMOD procedure available in SAS software. The results were analysed to elucidate correlations between recollection date, population, dry matter content, green forage length and seed germinability and viability. A significance level of 5% was used in the ANOVA.

4. Results

Seeds that had not been placed in silage germinated at a remarkable high rate (Table 1). In contrast, only a few seeds from the first recollection date germinated in the glass container experiment (Table 2).

Table 1. Seed germination and viability in *Rumex crispus* from two populations. Results from germination and viability tests of the control seeds

Population	Germination, water (%)	Germination, gibberellic acid + potassium nitrate (%)
A	100	-
B	99	0

Statistical analyses showed no significant differences in seed germinability and viability between the treatments (recollection date, population, dry matter content, green forage length) of the experiment.

Table 2. Germination and viability of *Rumex crispus* seeds recollected from silage (glass container experiment) with two straw lengths at six testing dates. Values are means over testing dates

Recollection date	Green forage length	Germination, water (%)	Germination, gibberellic acid + potassium nitrate (%)	Viable seeds, "press test" (%)	Viable seeds, tetrazolium test (%)
1	short	11.5	8.5	91.5	47
	long	0.8	6.1	81.1	16
2	short	0	0	97.6	50
	long	0	0	97.3	47
3	short	0	0	95.6	50
	long	0	0	92.1	36
4	short	0	0	93.0	65
	long	0	0	94.7	47
5	short	0	0	98.5	52
	long	0	0	95.6	75
6	short	0	0	91.2	64
	long	0	0	97.4	38

Even when gibberellic acid/potassium nitrate - solution was added to the seeds no germination was observed at other recollection dates than the first (Table 2, Table 3). The values presented in the column "Viable seeds, "press test" (%)" in Table 2 and Table 3 derive from the test described in 3.5.

Table 3. Germination and viability of *Rumex crispus* seeds from two plant populations recollected from silage (glass container experiment) with two straw lengths at low and high dry matter content

Population	Green forage length	Dry matter content	Germination, water (%)	Germination, gibberellic acid + potassium nitrate (%)	Viable seeds, "press test" (%)	Viable seeds, tetrazolium test (%)
A	short	low	0.7	1.0	91.1	59
		high	6.9	3.3	96.5	67
	long	low	0	0	92.7	37
		high	0.5	2.6	95.8	59
B	short	low	0	0	93.9	48
		high	0	1.1	97.2	42
	long	low	0	0	95.6	28
		high	0	1.3	96.3	40

The tetrazolium test showed that 52% (average over green forage length) of the seeds are viable even after 100 days (recollection date 6) in silage (Table 2, Table 3). Relations between plant population, green forage length, dry matter content and percentage viable seeds are presented in Table 4.

The round bale ensiling experiment gave varying results (Table 4). However, it is possible to draw the conclusion that the majority of the seeds survive ensiling in round bales.

Table 4. Germination and viability of *Rumex crispus* seeds from one plant population recollected from round bale silage

Retention time in silage (days)	Population	Germination, water (%)	Germination, gibberellic acid + potassium nitrate (%)	Viable seeds, tetrazolium test (%)
61	B	100	-	-
	B	0	47	78 (9)*
100	B	100	-	-
	B	0	44	100 (9)*
141	B	100	-	-
	B	88	6	100 (1)*
	B	86	7	100 (1)*

*) number of remaining seeds tested in the tetrazolium test

5. Discussion

One interesting question that the results gave rise to, is why secondary dormancy was induced in the ensiled seeds. The seeds that were tested without preceding ensiling showed a germination rate close to 100% (Table 5). At the first testing date, 19% of the seeds (Table 1) retrieved from silage germinated, to be followed by 0% at the following dates. Seeds put into silage are exposed to very low oxygen levels, which, according to Baskin & Baskin, (1998) may cause seeds to enter secondary dormancy. To break this dormancy, the seeds were exposed to the conditions in the germination incubator; alternating temperatures and light. In this case, this treatment in combination with the added gibberellic acid and potassium nitrate was insufficient to break the induced secondary dormancy. To increase the knowledge in this matter, it is necessary to further investigate how dormancy of ensiled seeds of *Rumex crispus* is broken. This will help to estimate the potential of weed seeds from silage and if it is to consider a problem on arable land. According to Went & Munz (1949) it is possible that seed longevity in silage is extended due to low oxygen levels.

Different retention time in silage proved to be of little or no importance from the aspect of weed control. The few germinating seeds from recollection date 1 would cause no harm in practise since almost no silage is used before at least a couple of months in the silo or plasticized round bale. As regards dry matter content there seem to be a tendency for higher viability in seeds from silage with high dry matter content. In the same way, seeds recollected from silage with short green forage length tend to be viable to a higher extent than seeds from silage with intact green forage. High dry matter content silage may influence the seeds less than silage with low dry matter content since the lower occurrence of silage effluent would affect the seeds to a lower extent. However, these results are in a way contradicting since it would be expected that silage with short green forage length would, in the same way as with low dry matter content, influence the seeds more thoroughly.

Studies have shown that *Rumex crispus* has an optimal germination temperature of 30/15°C (alternating) which is considerably higher than 16/6°C used in my experiment (Baskin & Baskin, 1998; Ellis *et al.*, 1985b). However, 16/6°C is a more realistic temperature for Swedish climate. Thus, it is possible that the results from the germination and viability tests would have been different at higher temperatures.

The discrepancy between the viability results from the "press test" and the tetrazolium test can not be fully explained due to lack of knowledge about how seeds of *Rumex crispus* respond to these tests. Yet, the results from the tetrazolium test can be regarded as minimum values of viability after ensiling.

Weed seeds from silage can reach the field in generally two ways; falling from the feeding-rack when supplementary feeding in the pasture or through farmyard manure. The question whether the latter is any problem is dependent on the survival of the seeds through ensiling, animal digestion *and* manure storage. Not much work has been done on weed seed viability including the whole chain: field – silage – animal – manure – field. Investigations where the whole chain is studied are crucial to get knowledge about weed infestation through seeds from silage.

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