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The Impact of Ensiling at Different Moisture Contents on Germinability and Viability of Selected Weed Species' Seeds

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Abstract: Weeds are an increasingly significant issue inhibiting agricultural production worldwide. Forage conservation could form part of an integrated weed management program if ensiling killed weed seeds. In Experiment 1, seeds of five grass (*Hordeum* spp., *Bromus diandrus*, *Bromus hordeaceum*, *Lolium rigidum* and *Vulpia* spp.) and two broad-leaved temperate weed species (*Echium* spp. and *Raphanus raphanistrum*), that were either untreated, ensiled in pasture (*Trifolium subterranean*/*Lolium rigidum* mixture) forage for a minimum of three months, underwent 48 h *in sacco* digestion in steers or ensiled prior to digestion were tested for germinability and viability. In Experiment 2, seeds of eight tropical weed species (*Cenchrus ciliaris*, *Rumex* spp., *Bidens pilosa*, *Sorghum halepense*, *Urochloa panicaoides*, *Paspalum dilatatum*, *Brachiara eruciformis* and *Choris truncata*) were ensiled in *Sorghum bicolor* forage. In Experiment 3, *L. rigidum* and *R. raphanistrum* seeds were ensiled in either *Medicago sativa* forage wilted to 336.9, 506.5 or 610.7 g/kg dry matter; or in chaff to which water or water plus acid was added at rates to achieve 350, 450 or 550 g/kg dry matter content with lactic plus acetic acid added in the ratio of 3:2 at 80, 45 or 10 g/kg DM, respectively. In Experiment 4, *L. rigidum* and *R. raphanistrum* seeds were ensiled in cotton wool to which water or water plus acid was added at the same rates as in Experiment 3. Germinability of all seeds following ensiling was substantially reduced or nil. The extent of the reduction varied with species and experiment. *In sacco* digestion reduced germinability in Experiment 1, but to a lesser extent than ensiling; while ensiling plus digestion reduced germination rates to 0%.

Keywords: weed seed; silage; ensiling; germination; digestion; seed viability

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1. Introduction

Weeds are a major constraint to agricultural production in both developed and developing countries [1]. Successful weed management relies on preventing weed survival, seed production and replenishment of the seedbank. To achieve this, the management of agricultural crop and pasture weeds in developed countries has relied extensively on the use of herbicides since their rapid development after World War II. The use of herbicides is also increasing in developing countries that traditionally relied on hand weeding or tillage [1]. However, herbicide resistance has reduced the efficacy of a number of key herbicides, with at least 263 weed species confirmed as having developed resistance, with some species resistant to different herbicides with multiple modes of action [2]. More recently the adoption of conservation agriculture with the aim of achieving sustainability has reduced the use of tillage as a management practice for soil preparation and weed control prior to sowing of crops and pastures. This has increased reliance on herbicide usage for weed control which has increased selection pressure and consequently the development of herbicide resistance [3,4]. As world food demand increases, agricultural systems will need

to increase efficiency, and crop and livestock productivity; and successful weed control will be an integral component of these systems. Future weed management will also require alternative strategies that do not include tillage while reducing reliance on herbicides.

Cutting forage is one strategy that has been shown to reduce the number of seeds produced per plant, by reducing the number of tillers or the number of seeds produced per tiller [5]. Cutting most commonly occurs when pastures or crops are harvested and conserved as either hay or silage to create a fodder reserve which is then fed to livestock. Hay is produced when the forage is allowed to air dry before baling to the extent that the lowered dry matter (DM) content prevents the growth of spoilage organisms and the risk of spontaneous combustion when wet/moist hay is stored [6]. In contrast, silage is produced when the cut forage is harvested, stored anaerobically, and undergoes an acid fermentation during which plant sugars are converted to lactic and other acids including acetic acid. Silages also vary in a range of characteristics: quantity and type of acids and other compounds produced, final pH and DM content.

Research has shown that the pattern of fermentation and resultant fermentation products are principally determined by three forage attributes: DM content, sugar content and buffering capacity. Dry matter content directly affects the dominant bacteria species and level of bacterial activity. Desirable lactic acid bacteria, which produce lactic acid as the major fermentation product, are favoured by DM contents > 30%. However, as DM content increases the level of bacterial activity declines, fermentation is inhibited and the pH at which fermentation ceases will be higher. Buffering capacity is the inherent capacity of the forage to resist pH change and determines the amount acid required to reach the required pH for preservation. Sugar (water soluble carbohydrates) content determines the amount of acid that can be produced [7].

Forage conservation is common practice in many countries and can form part of an Integrated Weed Management (IWM) strategy because weeds and weed seeds in the hay or silage are removed from the paddock. To achieve this, forage conservation must occur prior to seed shatter and regrowth must be controlled to minimise possible regrowth seed set so as to restrict weed seed entering the seedbank [8,9]. However, feeding hay or silage that contains viable weed seeds to livestock is a potential source of weed spread. Seeds that are not ingested will fall onto the ground and can potentially germinate. Ingestion by livestock such as sheep and cattle has been shown to reduce seed viability; however, some viable seeds will likely be excreted in faeces [10–12]. The proportion of viable seeds remaining after digestion by sheep and cattle has been shown to vary with plant species, can range from 0 to 80%, is inversely proportional to the level of hard seededness, and lower for cattle than sheep [11–13].

Silage offers an additional advantage compared to hay, in that most weeds seeds are rendered non-viable by the ensiling process [14–16], whereas those in hay are not. The proportion of viable seeds recovered from silage has been shown to vary with species, for example seeds from temperate grass weeds were more likely to be rendered non-viable than hard seeded species [16,17]. Various commentators have speculated that acids produced during the ensiling process are responsible, but there are no reported data to confirm this. Furthermore, the mechanism by which ensiling renders weed seeds non-viable is unknown, and therefore it is not possible to know which of these characteristics are important or what threshold levels of acid are required.

Previous studies investigating the effect of ensiling on weed seed viability were predominantly conducted using seeds of winter growing weed species and were ensiled in chopped forage (to produce fine-chop silage). In developed countries there is a trend towards producing silage in large square or round bales. Baled silages have higher DM contents than traditional chopped silages (approximately 500 g/kg compared to 350 g/kg) and have a longer particle length because the forage is either not chopped or only minimally chopped. Consequently, baled silages have a less extensive (restricted) fermentation and produce less acid than traditional chopped silages [7,18].

Our paper reports on experiments conducted at the Wagga Wagga Agricultural Institute, New South Wales (NSW), Australia to test the hypotheses that (1) ensiling in high DM, unchopped pasture or in chopped sorghum forage reduces weed seed viability; (2) increasing forage DM content lessens the effect of ensiling on viability of temperate weed species seeds, and (3) moisture content per se rather than silage acids are principally responsible for rendering weed seeds non-viable. The effect of ensiling in high DM, unchopped pasture on seed viability was also compared with *in sacco* rumen digestion or both to simulate the feeding of these seeds to livestock. Results from this work will provide farmers with evidence that ensiling weed seeds can be an effective weed management strategy. The use of rumen cannulated steers in this experiment was approved by the NSW Department of Primary Industries' Animal Ethics Committee (ORA 12/15/005).

2. Materials and Methods

In Experiment 1, mature seeds from selected eight grass and broadleaf weed species (Table 1) were hand harvested from crop and pasture paddocks at the Wagga Wagga Agricultural Institute in the year prior to ensiling and stored in paper bags at room temperature. Seeds (n = 50) of each species were placed in 12 individual Dacron bags of the type used for *in sacco* degradability studies (total of 96 bags) and allocated to one of four treatments: ensiled, digestion (*in sacco* degradability), ensiled plus digestion and control: hereafter referred to as E, D, ED and C, respectively. The E and ED treatments were ensiled in polythene bag mini-silos as described by Piltz et al. [16] on 11 November 2016 in mini-silos of wilted, unchopped pasture forage composed of *Trifolium subterraneum* L. (650 g/kg), *L. rigidum* L. (333 g/kg) and 20 g/kg of other species, and with a DM content of 587.9 g/kg. Two Dacron bags of each weed species were placed into each mini-silo (n = 3), with each mini-silo representing a replicate. The bags containing weed seeds were layered in the forage to ensure each was in contact with silage. The mini-silos were sealed and stored in 200 L drums surrounded by damp sand at room temperature in a weatherproof shed until they were opened on 5 April 2017. The remaining six bags of each weed species were retained and stored in paper bags at room temperature.

Table 1. Effect of ensiling, *in sacco* digestion or both on germinability of selected temperate weed species' seeds.

Weed Species	Common Name	Germinability (%)				Control Seed Viability (%)
		Control	Ensiled	Digestion	Both	
Avena fatua L.	Wild oats	88.7 (±4.67)	20.7 (±11.10)	0	0	91.3 (±2.40)
Bromus diandrus Roth.	Great brome	94.0 (±3.46)	0	0	0	96.0 (±2.00)
Bromus hordeaceum L.	Soft brome	100 (±0)	5.3 (±3.53)	9.3 (±9.30)	0	100 (±0)
Echium spp.	Paterson's curse	24.0 (±10.58)	0	11.1 (±4.88)	0	45.3 (±7.42)
Hordeum spp.	Barley grass	69.3 (±6.57)	0	11.6 (±6.38)	0	70.7 (±6.36)
Lolium rigidum Gaud	Annual ryegrass	82.7 (±3.71)	12.7 (±3.53)	63.6 (±7.87)	0	85.3 (±3.33)
Raphanus raphanistrum L.	Wild radish	34.0 (±5.29)	0.7 (±0.67)	3.6 (±2.57)	0	82.7 (±3.71)
Vulpia spp.	Silvergrass	63.3 (±31.80)	24.0 (±8.08)	19.9 (±10.00)	0	64.0 (±32.08)

Values in parentheses are standard errors of the mean. Ensiled: more than 3 months in unchopped subterranean clover dominant pasture with a dry matter content of 587.9 g/kg. Digestion: 48 h *in sacco* in the rumen of mature Red Poll steers. There were n = 3 replicate bags/treatment and n = 50 seeds per bag. Viability includes the proportion of germinated plus viable ungerminated seeds.

Upon opening the silages, one bag of each weed species from each mini-silo was paired with a bag of the same weed species that had not been ensiled. Both bags were placed in the rumen of a mature Red Poll steer for 48 h, which is the equivalent length of time as the rumen phase when determining in vitro digestibility [19]. All bags from each mini-silo were placed in the rumen of the same steer, and bags from different mini-silos

were placed in different steers. The steers were fed a diet consisting of lucerne hay, oaten chaff, barley grain and oat grain at 300, 300, 200 and 200 g/kg of the diet, respectively, on an as-fed basis each morning for 10 days prior to and the duration of the *in sacco* component of the experiment, with the amount fed calculated to provide approximately 1.2 times maintenance energy requirements. Diets were fed for 10 days prior to the digestion phase to ensure the rumen had adjusted to a standard diet, and for the 48 h digestion phase.

At the completion of the ensiling or digestion phase depending on the treatment, all seeds were placed on Whatman No. 2 filter paper moistened with 4 mL of distilled water in a 9 cm Petri dish. Petri dishes were sealed with Parafilm®M and incubated for 21 days at 25 °C/15 °C day/night temperatures with a 12 h photoperiod; after which the number of germinated seeds was recorded. Germination tests for the D and ED treatments occurred 48 h after the C and E treatments; when the digestion phase was complete. Ungerminated seeds that retained their physical integrity (firmness) were tested for viability using the tetrazolium test [20].

In Experiment 2, mature seeds from a range of summer growing species (Table 2) were hand harvested from crop and pasture paddocks near the Tamworth Agricultural Institute, NSW, Australia; and stored in paper bags at room temperature. Seeds (n = 50) of each species were placed into six individual Dacron bags prior to ensiling in bag mini-silos (n = 3). There was one bag of each weed species per bag. The bags were ensiled on 15 March 2017 in sorghum (*Sorghum bicolor* (L.) Moench) forage with a dry matter content of 310.4 g/kg using the same technique as in Experiment 1. One Dacron bag of each weed species was placed in each mini-silo bag. The bags were opened on 10 July 2017 and the ensiled and control seeds were tested for germination and viability as in Experiment 1.

Table 2. Effect of ensiling in sorghum (*Sorghum bicolor* (L.) Moench) forage with a dry matter content of 310.4 g/kg on seed germinability of selected summer growing weed species.

Species	Common Name	Germination (%)		Control Seed Viability (%)
		Control	Ensiled	
Cenchrus ciliaris L.	Buffel grass	17.8 (±8.85)	0	29.0 (±5.34)
Rumex spp.	Dock	63.5 (±18.2)	0	64.9 (±17.78)
Bidens pilosa L.	Farmers friend	75.0 (±8.80)	0	74.9 (±8.80)
Sorghum halepense L.	Johnsons grass	29.5 (±2.10)	0	41.9 (±3.35)
Urochloa panicoides P. Beauv.	Liver seed grass	22.0 (±1.95)	0	28.4 (±3.19)
Paspalum dilatatum Poir.	Paspalum	19.8 (±2.43)	0	53.8 (±1.31)
Brachiaria eruciformis (Sm.) Griseb.	Sweet summer grass	70.1 (±9.73)	0	70.8 (±10.28
Chloris truncata R. Br.	Windmill grass	48.4 (±1.96)	0	48.4 (±1.96)

Values in parentheses are standard errors of the mean. There were n = 3 replicate bags/treatment and n = 50 seeds per bag.

In Experiment 3, artificial silage was produced from *M. sativa* chaff to determine the effects of moisture and acid content, on the presumption that no fermentation would occur. Seeds (n = 50) of *R. raphanistrum* or *L. rigidum* from the same batch used in Experiment 1 were sealed in Ankom F57 Fiber Filter Bags (ANKOM Technology, Macedon, NY, USA) and ensiled in *M. sativa* chaff to which varying quantities of distilled water were added to achieve DM contents of 350, 450 or 550 g/kg, hereafter referred to as C350, C450 and C550, respectively. A second cohort of treatments had distilled water plus organic acids added at the rate of 80, 45 or 10 g/kg DM for the 350, 450 and 550 g/kg DM chaff silages, respectively, and hereafter referred to as C350A, C450A and C550A, respectively. The organic acids were lactic acid (UNIVAR AR grade; 99.7%; Univar Solutions Inc., Seattle, IL, USA) and acetic acid (Chemsupply AR grade; 88%; ChemSupply Australia, SA, Australia) in the ratio of 3:2 and the rate of distilled water adjusted to account for the volume of acid to achieve the same DM content. These acid levels were typical for silages of similar DM content, and reflective of the reduction in fermentation that occurs as DM content

increases. As a comparison *R. raphanistrum* and *L. rigidum* seeds were also ensiled in *M. sativa* forage which had been wilted to achieve a target DM content of 350, 450 or 550 g/kg, hereafter referred to as F350, F450 and F550, respectively. The bags containing seeds were placed between layers of forage or chaff bag mini-silos (n = 3) as described in Experiment 1 on 11 or 12 December 2017. Duplicate bags of seed for each species were ensiled in each mini-silo replicate, hence there were six estimates generated per treatment. The mini-silos were subsequently stored in damp sand for four months prior to opening on 11 April 2018. Upon opening the ensiled and control seeds were tested for germination and viability as in Experiment 1.

Data from Experiment 3 showed the *M. sativa* chaff silage had unexpectedly fermented, prompting a fourth experiment to assess the effects of moisture and acid content on seed viability. Prior to commencing the experiment, a pilot study using sterile cotton wool and distilled water was conducted to ensure that natural fermentation would not occur. Distilled water was added to sterile cotton wool to achieve 350, 400 or 500 g/kg DM content, vacuum sealed and stored for 15 days in damp sand. Upon opening, the cotton wool bags were tested for pH and all were found to be in the range of pH 6.7 to 7.0 so it was concluded that no fermentation had occurred. Consequently, a fourth experiment was conducted.

Seeds (n = 50) of *R. raphanistrum* or *L. rigidum*, also from the same batch as Experiment 1, were sealed in individual Ankom F57 Fiber Filter Bags. Micro-silages were prepared using sterile cotton wool to which varying quantities of distilled water or distilled water plus organic acids were added to achieve a DM content of 350, 450 or 550 g/kg, hereafter referred to as CW350, CW450 and CW550, respectively (Experiment 4). As with Experiment 3, lactic and acetic acid at the ratio of 3:2 were added at 80, 45 or 10 g/kg DM for the 350, 450 and 550 g/kg DM artificial silages, respectively, hereafter referred to as CW350A, CW450A and CW550A; and the rate of distilled water adjusted to account for the volume of acid. Bags containing seeds of *L. rigidum* or *R. raphanistrum* seeds (n = 50 per bag), of the same batch used in Experiments 1 and 3, were placed between layers of the cotton wool within plastic bags (n = 3) prior to vacuum sealing. In this experiment triplicate bags of seed for each species were ensiled in each plastic bag, hence there were nine estimates generated per treatment. The sealed bags were subsequently stored in damp sand similar to the previous experiments for two weeks prior to opening. Germinability and viability was determined as for previous experiments.

For all experiments, means and standard errors were calculated for each treatment within species for all three experiments [16,21]. To test for significant differences between two treatment means a treatment- specific pairwise least significant difference (LSD) was calculated from an approximate treatment-specific pairwise standard error of difference (SED ($p < 0.05$)); the approximate pairwise SED was calculated using the formula SED = $\sqrt{2} \times$ SE, where SE is the mean SE of the two means being tested.

3. Results

3.1. Experiment 1

Germinability of all species' seeds was reduced ($p < 0.05$) following the E, D and ED treatments (Table 1). *Bromus diandrus*, *Hordeum* spp. and *Echium* spp. showed zero seeds germination following E, while the germinability of *R. raphanistrum* (0.7%) was not significantly different to zero. *Avena fatua* and *B. diandrus* had zero germination following D, and all species' seeds had zero germination following ED. The germinability of seeds treated with E was lower ($p < 0.05$) for *Hordeum* spp., *L. rigidum* and *Echium* spp. but higher ($p < 0.05$) for *A. fatua* compared to D. The ED treatment further reduced germinability ($p < 0.05$) for *B. hordeaceum*, *L. rigidum* and *Vulpia* spp.

Viability testing of ungerminated seeds that still retained their physical integrity following the E, D and ED treatments was inconclusive, with the proportion of apparently viable non-germinated seeds appearing to increase for some species and is therefore not reported. Viability of the control seeds is reported.

3.2. Experiment 2

Viability in some unensiled summer growing species seeds was low and data for species with control seed viability rates < 20% were not presented due to concerns that that seed was not representative of a typical population. None of the seeds of the remaining species germinated after ensiling (Table 2), and viability following ensiling was inconclusive, as in Experiment 1, and therefore is not reported.

3.3. Experiment 3

Unexpectedly, all *M. sativa* chaff silages showed signs of fermentation and silage composition was assessed (Table 3). The level of acetic and total volatile fatty acids was higher ($p < 0.05$) for the chaff plus distilled water and acid compared to the chaff plus distilled water treatments with equivalent DM content. Conversely the level of lactic acid was higher ($p < 0.05$) for the chaff plus distilled water treatments. The levels of lactic, acetic and total volatile fatty acids were higher ($p < 0.05$) for the chaff treatments compared to the forage treatments of similar DM content. The pH and level of ammonia (% of total nitrogen) was higher ($p < 0.05$) for forage compared to chaff treatments of similar DM content.

Table 3. Composition and pH of silages produced from *Medicago sativa* L. forage wilted to achieve different dry matter contents, or chaff with either water or water plus acid added to achieve a similar dry matter content.

Treatment	Silage Type	Dry Matter Content (g/kg)	Acid [1] Added (g/kg DM)	pH	Composition (g/kg DM)						Ammonia (% of Total *N*)
					DM [2] Content	Lactic	Acetic	Propionic	Butyric	Total VFA	
F350	Forage	350	0	5.3 ^{d}	336.9 ^{a}	36.7 ^{d}	15.7 ^{cd}	0.2 ^{bc}	0.2 ^{a}	16.3 ^{cd}	13.1 ^{e}
F450	Forage	450	0	5.6 ^{e}	506.5 ^{c}	8.3 ^{b}	5.0 ^{a}	0.1 ^{ab}	0.0 ^{a}	5.3 ^{a}	8.1 ^{d}
F550	Forage	550	0	5.8 ^{f}	610.7 ^{d}	0.6 ^{a}	1.9 ^{a}	0.0 ^{a}	0.1 ^{a}	2.2 ^{a}	7.7 ^{cd}
C350	Chaff	350	0	4.4 ^{b}	322.2 ^{a}	65.0 ^{h}	19.4 ^{e}	0.2 ^{bc}	1.6 ^{b}	21.5 ^{e}	7.1 ^{bcd}
C450	Chaff	450	0	4.5 ^{b}	417.6 ^{b}	59.1 ^{g}	12.5 ^{bc}	0.2 ^{bc}	0.1 ^{a}	13.0 ^{bc}	6.1 ^{bcd}
C550	Chaff	550	0	4.8 ^{c}	523.6 ^{c}	41.8 ^{e}	10.0 ^{b}	0.3 ^{cd}	0.0 ^{a}	10.7 ^{b}	5.4 ^{abc}
C350A	Chaff	350	80	4.1 ^{a}	346.1 ^{a}	39.5 ^{de}	34.5 ^{f}	0.5 ^{e}	0.1 ^{a}	35.7 ^{f}	3.1 ^{a}
C450A	Chaff	450	45	4.5 ^{b}	447.1 ^{b}	22.6 ^{c}	16.4 ^{de}	0.4 ^{de}	1.9 ^{b}	19.3 ^{de}	3.7 ^{a}
C550A	Chaff	550	10	4.7 ^{c}	524.9 ^{c}	45.8 ^{f}	13.6 ^{cd}	0.2 ^{bc}	0.1 ^{a}	14.5 ^{bc}	4.8 ^{ab}
p value				<0.001	<0.001	<0.001	<0.001	<0.001	0.066	<0.001	<0.001
l.s.d ($p < 0.05$)				0.14	32.42	3.72	3.56	0.10	1.38	4.07	2.38

[1] Acid added treatments: lactic acid plus acetic acid at the ratio of 3:2 [2] Oven dry matter content. There were $n = 3$ replicates/treatment. Values in the same column with different superscripts are significantly different ($p < 0.05$).

No *L. rigidum* or *R. raphanistrum* seeds germinated following ensiling although some were viable (Table 4). Viable *L. rigidum* seeds remained after ensiling for all treatments, but rates were ≤1.5%, and C350 and C450 did not differ from zero. Viability of *R. raphanistrum* seeds ranged 0–2% for all treatments except F450 (9.1%) and F550 (29.0%); with C550, C350A, C450A and C550A rates not different to zero.

Table 4. Viability (%) of *Lolium rigidum* L. and *Raphanus raphanistrum* L. seeds following ensiling in *Medicago sativa* L. forage wilted to achieve different dry matter contents or chaff with water and acid added to achieve a similar dry matter content.

Treatment	Silage Type	Dry Matter Content (g/kg)	Acid Added (g/kg DM)	*Lolium rigidum* (%)	*Raphanus raphanistrum* (%)
F350	Forage	350	0	2.7 (±1.43)	1.3 (±0.84)
F450	Forage	450	0	2.3 (±1.20)	9.1 (±3.43)
F550	Forage	550	0	6.3 (±1.50)	29.0 (±5.88)
C350	Chaff	350	0	1.3 (±0.99)	1.1 (±0.74)
C450	Chaff	450	0	0.3 (±0.33)	1.3 (±0.67)
C550	Chaff	550	0	2.7 (±1.43)	2.0 (±1.63)
C350A	Chaff	350	80	2.3 (±1.20)	0
C450A	Chaff	450	45	1.3 (±0.67)	0.3 (±0.33)
C550A	Chaff	550	10	1.3 (±0.67)	0.3 (±0.33)

Values in parentheses are standard errors of the mean. The germinability and viability of untreated *L. rigidum* and *R. raphanistrum* seeds was 82.7% and 85.3%; and 34.0% and 82.7%, respectively. There were $n = 6$ replicate bags/treatment and $n = 50$ seeds per bag.

3.4. Experiment 4

None of the *R. raphanistrum* and only a small number of L. rigidum seeds germinated after ensiling. We observed that seeds that did not germinate were soft and non-viable. *Lolium rigidum* seeds that germinated were distributed across all silages except the 350 g/kg DM plus acid treatment, which exhibited no seed germination (Table 5). Germination rates did not differ between CW350, CW450 and CW550, but were lower for CW350A compared to CW450A and CW550A. When compared at the same DM content, germination rate for CW350A was lower ($p < 0.05$) than CW350. Germination of CW450 did not differ from zero.

Table 5. The proportion of germinating *Raphanus raphanistrum* seeds present after ensiling in artificial cotton wool silage with water and acid added to achieve a range in dry matter contents.

Treatment	Dry Matter Content (g/kg)	Acid Content (g/kg DM)	Number of Silage Bags Containing Viable Seed	Germination (%)
CW350	350	0	2	0.9 (±0.68)
CW450	450	0	1	0.5 (±0.49)
CW550	550	0	2	0.9 (±0.66)
CW350A	350	80	0	0
CW450A	450	45	1	0.2 (±0.24)
CW550A	550	10	1	0.3 (±0.29)

Values in parentheses are standard errors of the mean. The germinability and viability of untreated *R. raphanistrum* seeds was 34.0% and 82.7%, respectively. There were $n = 9$ replicate bags/treatment and $n = 50$ seeds per bag.

4. Discussion

Ensiling in pasture or sorghum forage reduced weed seed viability in all weed species evaluated, supporting previous experimental findings obtained by Piltz et al. [9,16]. Digestion *in sacco* also reduced germinability for most species, but to a lesser extent than ensiling, while ensiling plus digestion eliminated germination. Increasing forage DM content did not reduce the impact of ensiling on seed viability of winter growing weed species, based on the artificial silage results obtained in Experiments 3 and 4. However, the proportion of viable *R. raphanistrum* seeds remaining after ensiling increased as DM content of *M. sativa* forage increased in Experiment 3. We conclude that available moisture content per se rather than silage acids is likely responsible for rendering weed seeds non-viable, as postulated in our initial experimental hypotheses.

Piltz et al. previously speculated that reduced seed viability due to ensiling was caused by imbibition of available moisture which led to seed germination and subsequent seed death due to anoxic conditions [16]. The corollary in this case being that seeds which are dormant do not imbibe moisture and therefore do not germinate, consequently escaping damage during the ensiling process. In Experiment 1, the reduction in germination following either ensiling or digestion was less than previously reported for *Hordeum* spp., *L. rigidum* and *Vulpia* spp. by Piltz et al., but similar for the other five winter growing species [16]. Possible reasons for this discrepancy with *Hordeum* spp., *L. rigidum* and *Vulpia* spp. could include length of ensiling, differences in innate seed dormancy caused by genetic or environmental effects or other unknown factor(s). These unknown factors could include differences between silages in moisture availability for imbibition, i.e., water activity or fermentation products.

Seed viability has been shown to decline with increasing duration of ensilage [17,22,23]; with three months generally sufficient to render the seed of most species non-viable [14,17,22,23]. Since the duration of this experiment was three months, a period identical to that of the previous experiment conducted by Piltz et al., we conclude that ensiling duration was not the explanation for differences in germinability between these experiments. The authors also excluded innate dormancy as a possible explanation, because the difference between germination and viability of the untreated seeds was < 3% for all three species, suggesting that only a small portion of

seeds were dormant. Physical dormancy (hard seed coat) has also been suggested as a means by which seeds can escape damage during ensiling; species with a hard seed coat generally require a longer ensiling period to achieve a similar reduction in seed viability [22]. Hard coated species are also typically less susceptible to damage following ingestion [11]. However, *Hordeum* spp., *L. rigidum* and *Vulpia* spp. are not considered to be hardseeded.

The higher DM content in our experiment compared to Piltz et al. [16] may have reduced moisture imbibition by those species, but these results infer differences in moisture imbibition requirements between weed species. Further research is required to determine if there is an interaction between silage DM content and species on seed viability post ensiling. However, the reduced effect on germination and viability also observed after digestion implies that another, currently unknown, factor may have contributed. It is conceivable that seeds vary in their readiness to imbibe moisture under short term exposure such as during digestion. Therefore, we suggest that further research should also consider seed source and age effect on viability after both ensiling and digestion.

Previous reported studies into the effect of ensiling on weed seed viability have mainly evaluated seed of winter growing species. Although testing for viability post ensiling was inconclusive in this study, ensiling clearly reduced germination rates for all summer growing species tested in Experiment 2. We conclude that seeds of both winter and summer growing species are equally susceptible to the effect of ensiling.

Experiments 3 and 4 were designed to independently assess the effects of silage DM content and the presence of acid on seed viability. Our initial attempt to produce artificial silages from lucerne chaff clearly failed due to the obvious fermentation that occurred. The results indicated that both substrate (plant sugars) and viable bacteria were still present after the drying and chaffing process. Nevertheless, we report our results to inform others attempting to produce an artificial silage from dried forage.

Interestingly, the level of fermentation exhibited from ensilage was greater for the chaff than the silage. This may be due to the fact that the chaff had higher plant sugar content and/or lower buffering capacity which resulted in more acid being produced [7]. The variable lactic acid contents observed in the three chaff plus acid silages (C350A, C450A and C550A) cannot be explained. Similarly, the higher lactic acid content for C350 compared to C350A and C450 compared to C450A. Perhaps the addition of higher acid rates restricted fermentation for C350A with lower lactic acid levels the consequence. In contrast the acetic acid content of the C350A, C450A and C550A silages are more as expected. Regardless of the cause, our results show that dried forage can still have sufficient sugar and dormant bacteria to undergo a fermentation. Future research using wilted forage that is sterilised to remove bacteria would determine if this is a viable option.

Dry matter content had no major effect on either germinability or viability of *R. raphanistrum* or *L. rigidum* seeds ensiled in Experiment 3 and Experiment 4. Acid reduced seed viability in Experiment 3 and for the 350 g/kg DM treatment in Experiment 4 when compared at the same DM content, but the differences were numerically small and of no practical significance. In contrast, seed viability of both species increased with increasing DM content of the lucerne forage silages in Experiment 3. This suggests dormant but viable seeds remain after ensiling. The proportion of potentially dormant seeds in the untreated control was 2.6% of *L. rigidum* and 48.7% of *R. raphanistrum*. The slightly higher number of potentially dormant *L. rigidum* seeds for the F550 treatment (6.3%) requires further investigation, but since the difference was relatively small it may be error. We recommend that future research addresses the effects of ensiling on seed dormancy over a broader range of DM contents in silage.

Viability of *L. rigidum* and *R. raphanus* seeds in Experiments 3 and 4 was lower after ensiling in chaff or cottonwool compared to *M. sativa* forage at 450 g/kg or 550 g/kg DM content. This suggests an inherent difference between the two silage types, unrelated to acid content. We speculate that by adding water to dried forage (hay) rather than drying (wilting) fresh forage to achieve the desired DM content increased relative water activity, at least until most of the free water was absorbed. However, that period may have been

sufficient for seeds to imbibe. Therefore, even though DM content of the F550 treatment (587.9 g/kg) content was only slightly higher than the dried artificial silages in Experiments 2 and 3, water activity would have considerably lower. Consequently, moisture imbibition by seeds was reduced and thus germination.

5. Conclusions

Our results confirm that ensiling eliminates or severely reduces germinability of weed seeds, with the most probable cause being imbibition of moisture. Similar effects were observed for seeds ensiled with or without acid in artificial silages, suggesting that moisture per se is the causal factor. However, the potential effect of acids in promoting moisture uptake by seeds cannot be discounted. Weed seed viability declines across the standard range in DM content at which forages are ensiled. However, at the drier end of the spectrum, the effect of ensiling on seed germination was reduced. It is likely the magnitude of the response was related to water activity (level of free moisture) or seed imbibition potential. Further research is required to determine the contributing factors to seed germination decline during ensiling. Development of techniques that clearly delineate the effects of moisture content, chop length, fermentation profile and water activity is recommended. Possibly using silages which have already fermented to completion and can be sterilised to prevent further bacterial activity prior to the seeds being 'ensiled'. Complementary research that encompasses a range of forage types, moisture contents, chop lengths and seeds of different species and from different sources is also recommended. This research is required to provide well-defined guidelines to the agricultural community on the reduction in seed viability achievable for various species ensiled using chopped or baled systems. Our research suggests that producing silage from pastures and forage crops containing weeds and their seeds has a role in Integrated Weed Management.

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