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Seed germination and viability of herbicide resistant and susceptible *Chenopodium album* populations after ensiling, digestion by cattle and manure storage

J APER*, B DE CAUWER†, S DE ROO†, M LOURENÇO‡, V FIEVEZ‡, R BULCKE† & D REHEUL§

**Plant Sciences Unit, Applied Genetics and Breeding, Institute for Agricultural and Fisheries Research, Melle, Belgium, †Weed Science Unit, Department of Plant Production, Ghent University, Gent, Belgium, ‡Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Production, Ghent University, Gent, Belgium, and §Plant Breeding and Sustainable Crop Production Unit, Department of Plant Production, Ghent University, Gent, Belgium*

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Summary

Chenopodium album became a problem weed in sugar beet production, due to resistance to metamitron, a key herbicide in this crop. Dispersal of the seeds from resistant biotypes may occur due to spread by wind, animals, agricultural machinery or manure. This study examined the effect of ensiling, digestion by cattle and storage in slurry and farmyard manure on the germination and viability of the seeds of one susceptible and three resistant *C. album* populations. After 4 weeks in a maize silo, seed viability of *C. album* populations was reduced drastically to 0–5%. Incubation for 24 h in the rumen followed by a post-ruminal digestion *in vitro* of intact seeds only resulted in a small reduction in viability in one *C. album* population. Storage in a slurry cellar for 16 weeks reduced the viability of intact seeds of the *C. album* populations to 25–60%. Only 0–1% of the seeds remained viable after storage in a farmyard manure heap for 4 weeks. An accelerated ageing experiment showed seed persistence to be population specific and less related to seed weight. Keeping a fresh maize silo closed for at least 4 weeks and heaping farmyard manure are excellent preventive measures to limit the spread of resistant *C. album* seeds between fields.

Keywords: herbicide resistance, fat-hen, common lambsquarters, seed persistence, accelerated ageing, slurry, seed spread.

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Introduction

Since the beginning of the 21st century, unsatisfactory control of *Chenopodium album* L. (common lambsquarters, fat-hen) has been observed in Belgian sugar beet fields. These control failures were mainly attributed to evolved resistance to metamitron, a key herbicide in sugar beet (*Beta vulgaris* L. subsp. *vulgaris* var. *altissima* Döll) that inhibits photosystem II (PSII) electron transport (Mechant *et al.*, 2005). Metamitron resistance in *C. album* is caused by a mutation on the *psb*A gene of the chloroplast genome. This mutation alters the target site of metamitron in the chloroplast and prevents binding of the herbicide (target-site resistance)

Correspondence: J Aper, Plant Sciences Unit, Applied Genetics and Breeding, Institute for Agricultural and Fisheries Research, Caritasstraat 21, B-9090 Melle, Belgium. Tel: (+32) 477 355527; Fax: (+32) 272 2901; E-mail: jonas.aper@ilvo.vlaanderen.be

(Powles & Yu, 2010). Actually, three point mutations (Ser_{264} to Gly, Ala_{251} to Val and Leu_{218} to Val) on the *psb*A gene of the chloroplast genome are known to cause metamitron resistance in *C. album* (Mechant *et al.*, 2008; Petersen & Varrelmann, 2010). The Ser_{264} to Gly mutation is also known to cause cross-resistance to herbicides used in other rotational crops, such as metribuzin in potato (*Solanum tuberosum* L.), and terbuthylazine and atrazine in maize (*Zea mays* L.) (Hirschberg & McIntosh, 1983; Mechant & Bulcke, 2006). A survey of the Belgian sugar beet area conducted in 2008 by Mechant *et al.* (2010) showed the metamitron-resistant Ser_{264} to Gly-mutated biotype to be present in 95% of the surveyed sugar beet fields with an unsatisfactory control of *C. album* and in 74% of the surveyed fields with satisfactory control.

Although resistance may have evolved *in situ*, it is very likely that seed dispersal among fields may partly account for the high prevalence of the resistant Ser_{264} to Gly biotype across the sugar beet area. Seed dispersal of *C. album* in and across fields can occur via agricultural machinery, wind, animals or manure application (Holm *et al.*, 1977; Bassett & Crompton, 1978). Several authors mentioned the risk of spreading viable weed seeds with manure (Thill & Mallory-Smith, 1997; Mohler, 2001; Goldwasser *et al.*, 2011). Manure transport between Flemish farms is common and can lead to seed migration over hundreds of kilometres.

After unsuccessful weed control, seeds of surviving weeds may end in the harvested produce and feed. This is particularly risky for fields with resistant *C. album* plants that escaped weed control in fields of forage maize, newly sown grassland, fodder beet and tuber and root crops that are occasionally used as a feed (e.g. potato, sugar beet). The feed gets contaminated with *C. album* seeds because the weed plants are harvested along with the crop (forages) or because weed seeds end up in soil attached to roots and tubers. During ensiling, weed seeds are subjected to unfavourable conditions such as a low pH (2–4), a (temporarily) high temperature, a high pressure and the absence of oxygen (Seglar, 2003), which drastically reduce the germination and viability of *C. album* seeds (Blackshaw & Rode, 1991; Westerman *et al.*, 2012).

After ensiling, the seeds are ingested by animals. Blackshaw and Rode (1991) reported a reduction in germination and viability of *C. album* from 76% and 87% to 40% and 52%, respectively, after rumen digestion for 24 h. However, Van Renterghem *et al.* (1991) found no reduction in germination of *C. album* seeds that were subjected to an *in vitro* simulation of the digestive tract of a cow.

Most seeds enter manure via the digestive tract of animals. Viability of seeds in manure may vary with manure type, storage duration and manure temperature. In addition, the position in the farmyard manure heap may influence the survival of the seeds. Elema and Scheepens (1992) and Edwards and Younger (2006) investigated the germination and viability of weed seeds in cattle slurry and farmyard manure respectively. Both studies showed large reductions in germination ability with increasing storage time, burial depth and storage temperature. In a study conducted by Mt Pleasant and Schlather (1994), *C. album* seeds were found in 12 of 20 surveyed farms in a range from 5 to 413 viable seeds per one metric ton of manure.

The aim of this study was to assess the risk of spreading *C. album* through manure by examining the effect of ensiling, digestion by cattle and storage in slurry and farmyard manure on the seed germination and viability of one susceptible and three resistant *C. album* populations. In addition, an accelerated ageing experiment was performed to assess the influence of seed weight on seed persistence. The accelerated ageing test subjects seeds to moisture and temperature stress and is found to be a good predictor of soil seed persistence (Long *et al.*, 2008) and seed vigour (Hampton & TeKrony, 1995). No attempt is made to associate the observed seed persistence with the resistance trait. According to Neve *et al.* (2009), many studies compared resistant and susceptible weed populations to determine the existence of a fitness cost with resistant biotypes, but this is problematic, as the observed characteristics may also be related to a different genetic background, aside from a resistance mutation.

Materials and methods

Plant material

Four *C. album* populations were used in the experiments: Herbiseed (S), a susceptible reference population, purchased from Herbiseed (Twyford, United Kingdom); Melle (Ra), a Belgian atrazine-resistant population (and cross-resistant to metamitron), harvested in 2004 in an experimental field under continuous maize cropping for 22 years; Kortessem (Rm), a Belgian metamitron-resistant population, harvested in a sugar beet field and selected after a metamitron treatment to ensure a homogeneous-resistant population and Hammarlunda, a Swedish metamitron-resistant population (Rms), harvested in a sugar beet field. The Ra and Rm populations have the Ser_{264} to Gly mutation, while the Rms population, the Ala_{251} to Val mutation.

Seeds used in this study were collected in July 2010 from isolated glasshouse-grown plants. After seed harvest, seeds of each population were air-cleaned and fractionated into three weight categories (heavy,

middle and light) using a densimetric table (Dubois, Sint-Niklaas, Belgium) (Table 1) and afterwards stored at 4°C in the dark until the start of the experiments. Four replicates of 1000 seeds were used to determine the thousand kernel weight per fraction. The middle weight category was used in the ensiling experiment, the digestion experiment and manure storage experiment (see further), whereas the heavy and lightweight categories were used for the accelerated ageing experiment.

For the ensiling, digestion and manure storage experiment, seed samples were enclosed in fine-mesh (45-μm pore size) nylon bags (3 by 10 cm in size). Each nylon bag was subdivided into separate compartments each containing 120 seeds of one particular *C. album* population. Each of the four experiments (ensiling, digestion, storage in slurry and farmyard manure, accelerated ageing) started with intact seeds.

Ensiling experiment

Twelve nylon bags, each divided into three compartments with seeds of the S, Rm and Ra populations, were buried in a fresh-made maize silage pile at approximately 50 cm depth. The nylon bags were divided into three groups of four bags (for three exhumation dates) and attached separately to three large high-density polyethylene (HDPE) net bags (45 × 65 cm in size, 12 mm pore size). After seed burial, the pile was rolled again using a tractor to remove the air and covered using a polyethylene (PE) plastic sheet. Nylon bags were exhumed 4, 7 and 16 weeks after burial date (6 October 2010) while unpacking the pile. The pH of unpacked ensiled maize was measured by dissolving 50-g maize in 50 ml of demineralised water. The average pH 4 and 7 weeks after seed burial was 3.73 (SE: 0.005) and 3.76 (SE: 0.030) respectively.

Digestion experiment

Sixteen nylon bags, each divided into four compartments with seeds of the S, Rm, Ra and Rms populations, were placed into the rumen of a fistulated Holstein–Friesian cow, fed a diet mainly composed of maize and grass silage. The bags were removed from the rumen after 0, 4, 16 and 24 h of incubation and washed immediately (2 times 10 min, with tap water). After ruminal digestion, stomach (abomasum), small intestine and large intestine digestion were simulated *in vitro* based on the method utilised by Tilley and Terry (1963). The digestion in the abomasum was simulated by incubation in flasks with 150 mL of HCl (0.075 M) with pepsin (2 g L^{-1}) at 39°C for 2 h. After this digestion step, pH of the incubation fluid was brought to 7.5 with NaOH (1 M), and 150 mL of pancreatin solution (0.2 M $NaH_2PO_4.H_2O$ with 1 g L^{-1} pancreatin) was added. This solution was shaken for 2 h at 39°C to simulate small intestine digestion. Large intestine digestion was simulated by submersing the recovered nylon bags for 24 h at 39°C in a 1:4 mixture of fresh faecal material (40 g; obtained from the fistulated cow) and phosphate buffer (160 ml; 28.8 g L^{-1} Na_2HPO_4, 6.1 g L^{-1} $NaH_2PO_4.H_2O$ and 1.4 g L^{-1} NH_4Cl, flushed with CO_2). Finally, nylon bags were recovered and washed two times with tap water for 10 min. The seeds that did not undergo ruminal digestion (i.e. 0 h rumen digestion) were also exposed to these *in vitro* processes, to evaluate the effect of the *in vitro* digestion processes on seed germination and viability. During evaluation of germination and viability, an additional control was used that consisted of seeds that were kept in the dark at 4°C after harvest.

Table 1 Thousand kernel weight (±SE) of the three seed weight categories in four *Chenopodium album* populations: Ra, atrazine-resistant population; Rm and Rms, metamitron-resistant populations; S, susceptible population

	Thousand kernel weight (g)		
Population	Light	Middle	Heavy
Ra	0.80 ± 0.005	0.88 ± 0.001	0.91 ± 0.005
Rm	0.76 ± 0.010	0.77 ± 0.004	0.81 ± 0.001
Rms	1.42 ± 0.010	1.44 ± 0.007	1.50 ± 0.001
S	0.67 ± 0.020	0.75 ± 0.005	0.79 ± 0.001

Slurry and farmyard manure experiment

The same net bags as in the ensiling experiment were used to group the nylon bags, each again divided into three compartments with seeds of the S, Rm and Ra populations. One net bag was placed at a depth of 40 cm in a farmyard manure heap, and another net bag was submerged at 100 cm of depth in cattle slurry (=liquid manure) for 4, 7 and 16 weeks. To keep the net bag submerged in slurry, two bricks were added.

Accelerated ageing experiment

The heavy and lightweight categories of seeds of the S, Rm, Ra and Rms populations were placed in an incubator (Model 1535, Shel Lab, Sheldon Manufacturing, Cornelius, USA) at 100% relative humidity and 45°C following the guidelines of the International Seed Testing Association (Hampton & TeKrony, 1995). Five incubation times were tested: 0, 2, 4, 8 and 16 days. The seeds were washed after incubation and soaked for 48 h in a 0.05 M KNO_3-solution in the dark at 4°C to enhance germination. After the soaking step,

germination and viability were determined. The accelerated ageing experiment allowed the calculation of the incubation times causing 50% and 90% reduction in viability (hereafter called persistence indices P_{50} and P_{90}), which are considered good predictors of seed vigour and persistence (Hampton & TeKrony, 1995; Long *et al.*,2008).

Evaluation of germination and viability

At the end of each experiment (ensiling, digestion, manure storage and accelerated ageing), a germination test was performed. In each germination test and for each population, a set of untreated (control) seeds (dark-stored at 4°C) was included as a reference. Four replications per treatment and per population were placed in blocks on a germination table and exposed to 21 day of alternating day/night temperature (25/10°C) under a 14-h-light/10-h-dark regime. This alternating temperature regime was chosen to enhance germination (Vincent & Roberts, 1977; Bouwmeester & Karssen, 1993). Each replicate consisted of two filter papers (Rotilabo Type 112A, Carl Roth GmbH + Co. KG, Karlsruhe, Germany), each bearing 100 seeds which were moistened by paper strips (Schleicher & Schuell, Dassel, Germany). Germinated seeds were counted and removed every 3 days for 3 weeks. Seeds were considered germinated when the emergent radicle had reached a length of 2 mm.

Seeds that did not germinate were subjected to a crush test followed by a tetrazolium viability test (Hampton & TeKrony, 1995; Sawma & Mohler, 2002). In the crush test, all empty seeds or seeds that were crushed by gentle pressure with a pair of tweezers were classified as unviable. The seeds that remained firm were subjected to the tetrazolium test. Seeds were cut longitudinally, and one half of each seed section was placed on a filter paper moistened with 2.5 mL of 1% tetrazolium (2,3,5-triphenyltetrazoliumchloride, UCB, Leuven, Belgium). After 255 min of dark incubation at 20°C, the seeds were evaluated under a binocular microscope. Seeds were considered viable when embryos were uniformly stained red/dark pink. The number of viable seeds was calculated as the sum of the number of germinated seeds in the germination test and the number of viable seeds indicated by the tetrazolium test.

Statistical analysis

All data were expressed as percentages. All treatment combinations were treated as a randomised complete block design with four replicates using ANOVA in R (R Development Core Team, 2009). Treatments comprised all combinations of four *C. album* populations with three burial times (ensiling experiment), four digestion times (digestion experiment) and three storage times (manure storage experiment) (four replications). ANOVA analysis was performed with the use of contrasts within populations, based on the method used by Blackshaw and Rode (1991). Because homogeneity of variances was not met in the data of farmyard manure incubation for the Rm and S populations, no analysis was performed. The accelerated ageing experiment was treated as a completely randomised design with four replications and three factors: incubation time, seed weight category and population. In this study, the incubation times causing 50% and 90% reduction in viability (persistence indices P_{50} and P_{90}) were calculated using the four-parameter logistic regression model (Seefeldt *et al.*, 1995; Knezevic *et al.*, 2007):

$$y = c + \frac{d - c}{[1 + \exp[b(\log(x) - \log(P_{50}))]]} \qquad (1)$$

where y is the viability of the seeds, x the incubation time, c is the lower limit, d is the upper limit (i.e. the initial viability) and b is the slope of the curve around the P_{50}. Regression analysis of the accelerated ageing experiment was performed using the *drc* package in R (Ritz & Streibig, 2005; R Development Core Team, 2009).

Results

Ensiling experiment

A dramatic reduction in seed germination and viability was observed after 4 weeks of ensiling (Table 2). Only 5%, 0% and 2% viable seeds were left for populations Ra, Rm and S respectively. After 7 weeks of ensiling, only one non-germinated viable seed was found in one replication of S. After 16 weeks of ensiling, only one germinated viable seed was found in one replication of Ra.

A significant reduction in seed germination and viability was observed for the control seeds of the Ra population between 4 weeks and the average of 7 weeks and 16 weeks (Table 2). This decreased viability followed the trend of a significant decrease in germination and seems biologically unrealistic. This may be explained by an underestimation of viable seeds from the control set at 7 and 16 weeks. It is possible that viable, dormant seeds giving a population-specific and time-dependant suboptimal colouration with the tetrazolium test were wrongly catalogued as non-viable, while they were still able to germinate.

Table 2 Germination (G) and viability (V) percentages (±SE) of three *Chenopodium album* populations after ensiling of intact seeds that had been dark-stored at 4°C

			Incubation period (weeks)†			Contrasts‡		
Population§	Treatment		4	7	16	Control vs. silage	4 week vs. 7 week-16 week§,¶	7 week vs. 16 week§
Ra	Control	G (%)	70 ± 6.5	50 ± 5.5	54 ± 5.2	–	**	NS
		V (%)	86 ± 5.3	70 ± 3.9	71 ± 5.1	–	***	NS
	Silage	G (%)	3 ± 1.6	0 ± 0.0	0 ± 0.3	***	NS	NS
		V (%)	5 ± 1.1	0 ± 0.0	0 ± 0.3	***	NS	NS
Rm	Control	G (%)	60 ± 4.3	59 ± 3.2	58 ± 8.0	–	NS	NS
		V (%)	79 ± 2.4	78 ± 2.5	74 ± 5.0	–	NS	NS
	Silage	G (%)	0 ± 0.0	0 ± 0.0	0 ± 0.0	***	NS	NS
		V (%)	0 ± 0.3	0 ± 0.0	0 ± 0.0	***	NS	NS
S	Control	G (%)	59 ± 2.6	62 ± 4.4	62 ± 2.3	–	NS	NS
		V (%)	80 ± 1.6	82 ± 1.9	82 ± 1.6	–	NS	NS
	Silage	G (%)	1 ± 0.0	0 ± 0.0	0 ± 0.0	***	NS	NS
		V (%)	2 ± 0.4	0 ± 0.2	0 ± 0.0	***	NS	NS

†The seeds of the control treatment were dark-stored at 4°C during each incubation period.

‡Contrasts were determined within a population and for germination and viability separately, NS, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

§Abbreviations: Ra, atrazine-resistant population; Rm, metamitron-resistant population; S, susceptible population; w, weeks.

¶The significant decreased viability of the control Ra seeds at the 7 and 16 weeks incubation period underestimates the real number of viable seeds and is related to a population-specific suboptimal colouration of the non-germinated seeds.

Digestion experiment

There was a clear increase in germination percentage of Rm and Ra with increasing rumen incubation time, compared with the control (Fig. 1). The Rms population showed a significant reduction in germination and viability after 16 and 24 h of ruminal incubation. The post-ruminal digestion processes increased the germination percentage for the Rm and S population, but did not have a large effect on seed viability.

We found an increased viability for the Ra and Rm populations with increasing rumen incubation time. This was also attributed to a suboptimal colouration of the control set of seeds with the tetrazolium test.

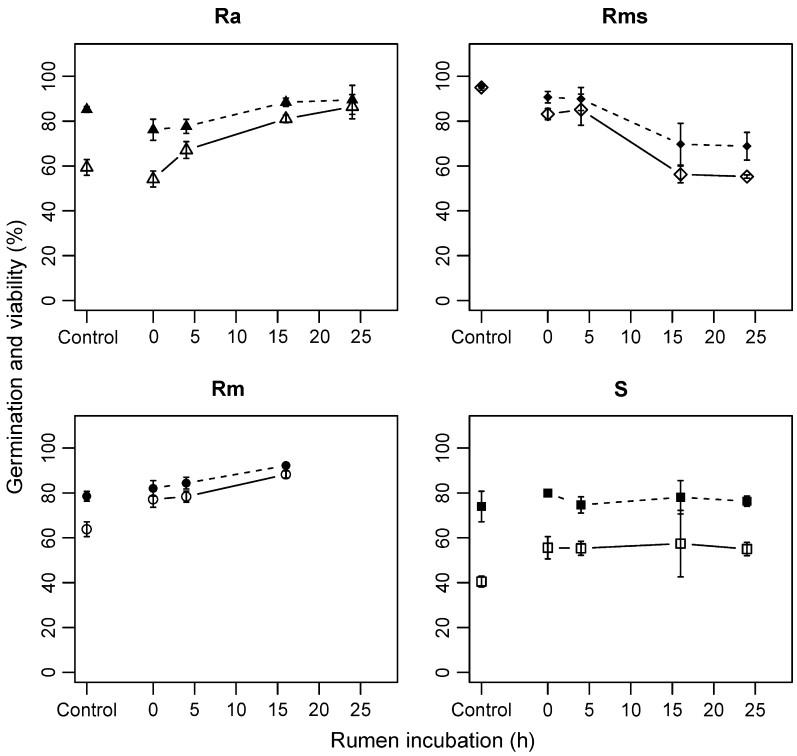

Fig. 1 Germination (white symbols) and viability (black symbols) percentages (±SE) of four *Chenopodium album* populations after rumen and *in vitro* digestion of intact seeds that had been dark-stored at 4°C. Two replications of four of Rms after 16 h of ruminal incubation and all the replications of Rm after 24 h of ruminal incubation were lost. Abbreviations: Ra, atrazine-resistant population; Rm and Rms, metamitron-resistant populations; S, susceptible population.

Unfortunately, two replications of four of Rms after 16 h of ruminal incubation and all the replications of Rm after 24 h of ruminal incubation were lost, which put limitations on the statistical accuracy of this last incubation period.

Slurry and farmyard manure experiment

The viability of the seeds of the three tested *C. album* populations was unaffected after 4 weeks of slurry incubation (Table 3). The viability of the S population started to decrease after 7 weeks of slurry incubation. After 16 weeks, both the germination and viability percentages of the seeds of the three populations decreased significantly compared with 7 weeks of slurry incubation (Table 3). Incubation in farmyard manure reduced germination and viability of the seeds of the three tested populations dramatically after 4 weeks of storage.

Accelerated ageing experiment

No viable seeds for the three *C. album* populations and only a few viable seeds were found for the S population (0.5 ± 1.02%) after 16 days in the incubator at 45°C and 100% RH. The regression parameters and persistence indicators are shown in Table 4. The *d*-parameter is an estimation of the initial percentage of viable seeds in the seed lot prior to any incubation. For the populations Ra, Rm and S, a higher *d*-parameter and thus a higher initial viability was found for the heavyweight category in comparison with the lightweight category (Table 4). Within populations, no differences in the P_{50} indices were found between the two weight categories. For the P_{90} index, the heavyweight category of the Rms population had a lower P_{90} index in comparison with the light fraction (4.80 days versus 6.55 days). The P_{50} index was highest for the Rm and Ra population, followed by S and the lowest was found for Rms.

Discussion

This study confirmed that it is possible for resistant *C. album* seeds to survive ensiling, digestion by cattle and manure storage. Farmers should be cautious when using manure as a fertiliser, because it can introduce metamitron-resistant *C. album* to their fields.

All performed experiments started with intact fresh dark-stored unimbibed seeds. In practice, however, when seeds enter the slurry cellar or the farmyard manure heap, most of them have already been subjected to ensiling and digestion. These ensiled or digested seeds are expected to be more susceptible to

Table 3 Germination (G) and viability (V) percentages (±SE) of four *Chenopodium album* populations after storage in slurry of farmyard manure of intact seeds that had been dark-stored at 4°C

			Incubation period (weeks)†			Contrasts‡		
Population§	Treatment		4	7	16	Control vs. Slurry/Farmyard manure	4 week vs. 7 week-16 week§	7 week vs. 16 week§
Ra	Control	G (%)	78 ± 2.3	65 ± 3.7	66 ± 3.0	–	**	NS
		V (%)	86 ± 2.3	86 ± 2.0	88 ± 1.6	–	NS	NS
	Slurry	G (%)	76 ± 4.3	75 ± 1.9	32 ± 3.2	**	***	***
		V (%)	82 ± 4.9	85 ± 1.6	60 ± 6.8	**	NS	***
	Farmyard manure	G (%)	0 ± 0.0	4 ± 4.3	0 ± 0.0	***	NS	NS
		V (%)	0 ± 0.0	5 ± 5.0	0 ± 0.3	***	NS	NS
Rm	Control	G (%)	68 ± 2.9	59 ± 2.8	62 ± 2.3	–	*	NS
		V (%)	80 ± 3.6	77 ± 2.5	84 ± 1.2	–	NS	*
	Slurry	G (%)	86 ± 0.7	73 ± 3.4	25 ± 1.4	NS	***	***
		V (%)	89 ± 0.9	82 ± 2.6	48 ± 1.8	***	***	***
	Farmyard manure	G (%)	1 ± 0.5	1 ± 0.8	0 ± 0.0	–	–	–
		V (%)	1 ± 0.5	1 ± 0.8	0 ± 0.3	–	–	–
S	Control	G (%)	64 ± 5.4	51 ± 2.2	56 ± 3.1	–	**	NS
		V (%)	79 ± 2.8	80 ± 1.5	85 ± 2.1	–	NS	NS
	Slurry	G (%)	82 ± 1.8	65 ± 0.9	9 ± 0.6	*	***	***
		V (%)	86 ± 1.4	73 ± 1.7	21 ± 4.2	***	***	***
	Farmyard manure	G (%)	0 ± 0.0	0 ± 0.0	0 ± 0.0	–	–	–
		V (%)	0 ± 0.0	0 ± 0.0	0 ± 0.0	–	–	–

†The seeds of the control treatment were dark-stored at 4°C during each incubation period.

‡Contrasts were determined within a population and for germination and viability separately, NS, not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

§Abbreviations: Ra, atrazine-resistant population; Rm, metamitron-resistant population; S, susceptible population; w, weeks.

Table 4 Regression parameters and persistence indicators P_{50} and P_{90} in the accelerated ageing test. Seeds were incubated for 0, 2, 4, 8 and 16 days at a temperature of 45°C and a relative humidity of 100%

Population*	Seed weight category	Regression parameters (± SE)†			
		b	*d*	P_{50}‡	P_{90} (±SE)‡
Ra	Heavy	9.8 ± 12.68	0.91 ± 0.019	9.0 ± 1.41	11 ± 5.0
	Light	6.0 ± 2.70	0.84 ± 0.021	8.6 ± 0.34	12 ± 2.0
Rm	Heavy	7.6 ± 7.00	0.89 ± 0.020	8.6 ± 0.56	11 ± 21.1
	Light	8.4 ± 15.79	0.81 ± 0.020	8.5 ± 0.94	11 ± 8.2
Rms	Heavy	4.1 ± 0.55	0.90 ± 0.033	2.8 ± 0.14	4.8 ± 0.54
	Light	2.8 ± 0.39	0.90 ± 0.032	3.0 ± 0.17	6.6 ± 0.26
S	Heavy	2.9 ± 0.55	0.68 ± 0.030	5.2 ± 0.40	11 ± 0.6
	Light	4.5 ± 1.31	0.50 ± 0.026	5.4 ± 0.45	8.7 ± 2.59

*Ra, atrazine-resistant population; Rm and Rms, metamitron-resistant populations; S, susceptible population.
†*b*; the slope of the line at the inflection point; *d*, the upper limit; *c*, the lower limit, estimated for all the regression curves together at −0.002 ± 0.0163.
‡P_{50}; P_{90}, incubation time causing a reduction in viability of 50% and 90% respectively.

the subsequent processes, like storage in farmyard manure or slurry. Thus, the seed viabilities found in this study are only expected in worst-case scenarios.

Our results show that seed survival of *C. album* biotypes after 4 weeks of ensiling at a depth of 50 cm was extremely low, confirming the results of Elema and Scheepens (1992). However, in the study of Van Renterghem *et al.* (1991), 5% of the *C. album* seeds germinated after a 6-week stay in the maize silo at a depth of 40 cm. Nevertheless, ensiling can significantly reduce the viability of *C. album* seeds. Besides storage time, seed survival also depends on burial depth, which was not tested in our study. It is expected that the ensiling conditions are less harsh (less anaerobic, higher pH) at the surface of a silage pile. Elema and Scheepens (1992) found that viability of *C. album* seeds reduced from 96% to 3% after 4 weeks at a depth of 70 cm in a maize silo, whereas viability was reduced to 45% at a depth of 5 cm. When the seeds remained less than 2 weeks in a maize silo at a depth of 70 cm, viability was reduced to 7%. Thus, it is important that farmers respect the time (10–21 days) required for the fermentation process to reduce and stabilise the pH to 4 or below when ensiling forages/crops (Seglar, 2003), not only for the nutritional value and stability of the silo, but also to reduce the viability of weed seeds.

After ruminal digestion and *in vitro* simulation of post-ruminal digestion, a remarkable increase in seed germination was noticed for the populations Ra, Rm and S. Presumably, the acid environment found in parts of the digestion system influences integrity and permeability of the hard seed coat of *C. album* seeds. This is not surprising, because it is known that treating seeds of *C. album* with an acid (e.g. H_2SO_4; so-called acid scarification) improves germination (Buhler & Hoffman, 1999). The treatment with 0 hours of rumen incubation takes only the *in vitro* digestion into account, which has no biological meaning, but this treatment shows that this part of the digestive tract of a cow also plays a role in an increase in the germination, but showed only minor effects on seed viability. Our results show a limited effect of digestion (only for the Rms population), which could be explained by the hard seed coat (Haidar *et al.*, 2010), acting as a protective layer against microbial and digestive enzymes. Other studies, however, reported higher reductions in viability for *C. album* after a 24-h ruminal digestion (Blackshaw & Rode, 1991). As fermentation rate, pH and retention time are determined by the diet of the animal, it would be interesting to quantify the effect of diet on the reduction in germination and viability of weed seeds. Mastication is also expected to cause a reduction in seed viability, but this was not investigated here, due to the chosen *in sacco* methodology.

The germination and viability of *C. album* seeds of the tested populations were significantly reduced after a 16-week storage in slurry. Our findings are not in line with those of Van Renterghem *et al.* (1991), where no loss in *C. album* seed survival was found after 4 months of incubation in Weck-bowls at 4°C and 18°C in cattle slurry. In contrast, Elema and Scheepens (1992) recorded a reduction in germination and viability of *C. album* seeds after 16 weeks at 4°C in agreement with our results. The longer the seeds stayed in a slurry cellar, and the warmer the slurry temperature, the higher the reduction in seed germination and viability. Farmyard manure storage completely impaired germination and viability of the seeds. A possible reason is the high temperature generated by the fermentation process. As for a maize silo, burial depth may affect the observed reduction in germination and viability. Weed seeds that are at the surface of the silage or manure pile will escape the effects of pH and temperature.

The accelerated ageing experiment showed that only the heavy seed weight category of the Rms population tended to be less resilient to high relative humidity and high temperature, compared with the light seed weight category. The seed weight categories within *C. album* populations Ra, Rm and S did not differ in P_{50} or P_{90} index, despite their difference in initial viability, that is, the '*d*'-parameter in the regression model. Long *et al.* (2008) suggested that the relationship between seed physical characteristics and seed persistence is not robust, which seems to be the case for *C. album* seeds. Furthermore, a high P_{50} index may point towards a better seed persistence, not only in the soil, but also during ensiling, digestion and manure storage.

In general, the resistant populations Rm and Ra have similar and even higher seed vigour and persistence compared with the susceptible population S. In order to minimise further spread of viable seeds of resistant *C. album* biotypes via manure, we recommend: (i) to keep silos closed for at least 4–5 weeks, (ii) to avoid the input of intact, non-ensiled *C. album* seeds in the slurry cellar and (iii) to store farmyard manure in large solid heaps.

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References

Bassett IJ & Crompton CW (1978) Biology of Canadian weeds 32. Chenopodium album L.. *Canadian Journal of Plant Science* **58**, 1061–1072.

Blackshaw RE & Rode LM (1991) Effect of ensiling and rumen digestion by cattle on weed seed viability. *Weed Science* **39**, 104–108.

Bouwmeester HJ & Karssen CM (1993) Seasonal periodicity in germination of seeds of *Chenopodium album* L. *Annals of Botany* **72**, 463–473.

Buhler DD & Hoffman ML (1999) *Andersen's Guide to Practical Methods of Propagating Weeds & Other Plants*. Weed Science Society of America, Lawrence, USA.

Edwards AR & Younger A (2006) The dispersal of traditionally managed hay meadow plants via farmyard manure application. *Seed Science Research* **16**, 137–147.

Elema AG & Scheepens PC (1992) *Verspreiding van onkruiden en plantenziekten met dierlijke mest*. Proefstation voor de Akkerbouw en de Groententeelt in de Vollegrond, Lelystad, The Netherlands.

Goldwasser Y, Miron J, Sibony M, Sorek P, Yosef E & Rubin B (2011). Spread of weed seeds by cattle manure - is there a danger of infesting farmers' fields with weeds? In: *Proceedings of the 3rd International Conference on Novel and Sustainable Weed Management in Arid and Semi-Arid Ecosystems*. 5. Huesca, Spain.

Haidar MA, Gharib C & Sleiman FT (2010) Survival of weed seeds subjected to sheep rumen digestion. *Weed Research* **50**, 467–471.

Hampton JG & TeKrony DM (1995) *Handbook of Vigour Test Methods*. The International Seed Testing Association, Zürich, Switzerland.

Hirschberg J & McIntosh L (1983) Molecular basis of herbicide resistance in *Amaranthus hybridus*. *Science* **222**, 1346–1349.

Holm LG, Pucknett DL, Pancho JV & Herberger J (1977) *The World's Worst Weeds: Distribution and Biology*. University Press of Hawaï, Honolulu, USA.

Knezevic SZ, Streibig JC & Ritz C (2007) Utilizing R software package for dose-response studies: the concept and data analysis. *Weed Technology* **21**, 840–848.

Long RL, Panetta FD, Steadman KJ *et al.* (2008) Seed persistence in the field may be predicted by laboratory-controlled aging. *Weed Science* **56**, 523–528.

Mechant E & Bulcke R (2006) Cross-resistance profile of metamitron-resistant *Chenopodium album* L. biotypes from sugar beet. *Journal of Plant Diseases and Protection*, **20**, 147–153.

Mechant E, Bulcke R & Maeghe L (2005) Resistance to metamitron in *Chenopodium album* from sugar beet. A tale of the (un)expected?. *Communications in Agricultural and Applied Biological Sciences* **70**, 501–506.

Mechant E, De Marez MT, Hermann O, Olsson R & Bulcke R (2008) Target site resistance to metamitron in *Chenopodium album* L. *Journal of Plant Diseases and Protection*, **21**, 37–40.

Mechant E, De Marez MT, Vroman G *et al.* (2010) Distribution of metamitron-resistant *Chenopodium album* L. in Belgian sugar beet. *Communications in Agricultural and Applied Biological Sciences* **75**, 53–59.

Mohler CL (2001) Weed life history: identifying vulnerabilities. In: *Ecological Management of Agricultural Weeds*. (eds M Liebman, CL Mohler & CP Staver), 40–98. Cambridge University Press, Cambridge, UK.

Mt Pleasant JM & Schlather KJ (1994) Incidence of weed seed in cow (*Bos* sp.) manure and its importance as a weed source for cropland. *Weed Technology* **8**, 304–310.

Neve P, Vila-Aiub M & Roux F (2009) Evolutionary-thinking in agricultural weed management. *New Phytologist*, **184**, 783–793.

Petersen J & Varrelmann M (2010) Ein neuer Gänsefuß-Biotyp mit Resistenz gegenüber Metribuzin und Metamitron. In: *Vortragsveranstaltung Herbizidsymposium Rübe von FCS*. (ed M Karthika), 11–12. Göttingen, Germany.

Powles SB & Yu Q (2010) Evolution in action: plants resistant to herbicides. *Annual Review of Plant Biology*, **61**, 317–347.

R Development Core Team (2009) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org

Ritz C & Streibig JC (2005) Bioassay Analysis using R. *Journal of Statistical Software* **1**, 2.

Sawma JT & Mohler CL (2002) Evaluating seed viability by an unimbibed seed crush test in comparison with the tetrazolium test. *Weed Technology* **16**, 781–786.

Seefeldt SS, Jensen JE & Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationships. *Weed Technology* **9**, 218–227.

Seglar B (2003) Fermentation analysis and silage quality testing. In: *Proceedings of the Minnesota Dairy Health Conference, 18*. University of Minnesota, USA.

Thill DC & Mallory-Smith CA (1997) The nature and consequence of weed spread in cropping systems. *Weed Science* **45**, 337–342.

Tilley JMA & Terry RA (1963) A two-stage technique for the *in vitro* digestion of forage crops. *Journal of the British Grassland Society* **18**, 104–111.

Van Renterghem B, Carlier L, Huysman F & Dendooven L (1991) Milieuhygiënische implicaties. In: *Mengmest: implikaties voor de landbouw en het milieu*. (eds B Van Renterghem & W Verstraete), 165–190. IWONL, Brussels, Belgium.

Vincent EM & Roberts EH (1977) Interaction of light, nitrate and alternating temperature in promoting germination of dormant seeds of common weed species. *Seed Science and Technology* **5**, 659–670.

Westerman PR, Hildebrandt F & Gerowitt B (2012) Weed seed survival following ensiling and mesophilic anaerobic digestion in batch reactors. *Weed Research* **52**, 286–295.