

Emergence and seed persistence of *Echinochloa colona*, *Urochloa panicoides* and *Hibiscus trionum* in the sub-tropical environment of north-eastern Australia

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Summary

Better understanding of seed-bank dynamics of *Echinochloa colona*, *Urochloa panicoides* and *Hibiscus trionum*, major crop weeds in sub-tropical Australia, was needed to improve weed control. Emergence patterns and seed persistence were investigated, with viable seeds sown at different depths in large in-ground pots. Seedlings of all species emerged between October and March when mean soil temperatures were 21–23°C. However, *E. colona* emerged as a series of flushes predominantly in the first year, with most seedlings emerging from 0–2 cm. *Urochloa panicoides* emerged mostly as a single large flush in the first two years, with most seedlings emerging from 5 cm. *Hibiscus trionum* emerged as a series of flushes over three seasons, initially with majority from 5 cm and then 0–2 cm in the later seasons. Longevity of the grass seed was short, with <5% remaining after burial at 0–2 cm for 24 months. In contrast, 38% of *H. trionum* seeds remained viable after the same period. Persistence of all species increased significantly with burial depth. These data highlight that management strategies need to be tailored for each species, particularly relating to the need for monitoring, application times for control tactics, impact of tillage, and time needed to reduce the seed-bank to low numbers.

Keywords: Emergence patterns, seed persistence, awnless barnyard grass, liverseed grass, bladder ketmia.

Introduction

Echinochloa colona (L.) Link (awnless barnyard grass), *Urochloa panicoides* P.Beauv. (liverseed grass), and *Hibiscus trionum* L. (bladder ketmia) are major summer weeds of the grain and cotton cropping systems in the sub-tropical cropping region of north-eastern Australia (Rew *et al.* 2005, Walker *et al.* 2005, Osten *et al.* 2007). They are a costly constraint to agricultural production in this region, and not effectively or consistently well managed (Walker *et al.* 2005).

The recent development of resistance in *E. colona* and *U. panicoides* to atrazine (Adkins *et al.* 1997, Heap 2009) and glyphosate (Storrie *et al.* 2008, Heap 2009, Preston 2009), major herbicides used in this region, has increased their adverse impacts and management costs. There are no reported herbicide resistant populations of *H. trionum* in Australia or overseas (Heap 2009). However, the similarity between *H. trionum* and cotton's phenology and physiology makes this weed very difficult to control in this situation (Chachalis *et al.* 2008). *E. colona* and *H. trionum*, and to a lesser extent *U. panicoides*, are also problem weeds in the Mediterranean region of Europe, Africa, tropical Asia, North and South America, Pacific and Indian sub-continent, particularly in rice and cotton crops (Westra *et al.* 1996, Raju and Reddy 1999, Uremis *et al.* 2003, Valverde 2007, Chachalis *et al.* 2008).

The sub-tropical cropping area of Australia has a summer-dominant but variable rainfall pattern that enables both summer and winter crops to be grown in rotation with moisture-storing fallows (Webb *et al.* 1997). Although the environment and cropping system have favoured evolution of a diverse weed flora, as evident with 80–139 species identified in various field surveys (Felton *et al.* 1994, Wicks *et al.* 2000, Rew *et al.* 2005), *E. colona*, *U. panicoides* and *H. trionum* dominate the weed flora during the warmer months (Walker *et al.* 2005, Osten *et al.* 2007).

These weeds germinate in flushes in the Australian spring and summer (Wilson and Duff 1981, McGillion and Storrie 2006), as they require warm moist conditions for germination, and thus infest the summer crops and fallows. However, only limited studies on ecology and seed-bank dynamics of these weeds have been recorded. Uremis and Uygur (1999) found the minimum, optimum, and maximum germination temperatures were 15, 30 and 40°C for *E. colona* and 10, 30 and 40°C for *H. trionum*. Similarly, Chachalis *et al.* (2008) stated the optimum germination

temperature for *H. trionum* was 30/20°C with 12 hour photoperiod, with no germination at 10 or 45°C. Emergence of *E. colona* and *H. trionum* was highest from soil surface layers but failed to emerge below 5 or 8 cm respectively (Guru and Govindra 2005, Chachalis *et al.* 2008). Persistence of grass seed was short, with no *U. panicoides* seed persisting after four years in Australia (Walker *et al.* 2006) and 1% of *E. colona* seed after seven years in Turkey (Uremis and Uygur 2005). In contrast, seed of *H. trionum* was long-lived with 23% remaining after seven years (Uremis and Uygur 2005). Seed persistence increased with burial depth from 2 to 10 cm for *H. trionum* (Westra *et al.* 1996), and from the soil surface to 20 cm for *E. colona* (Chaves *et al.* 1997), but did not differ for 15 and 30 cm for *E. colona* and *H. trionum* (Uremis and Uygur 2005). The ecology of *E. crus-galli*, a minor weed of cropping in north-eastern Australia (Keenan *et al.* 2006) has been widely researched (for example Brod 1968, Li *et al.* 1999). Whilst the morphology and molecular characteristics of *E. colona* and *E. crus-galli* have been compared (Danquah *et al.* 2002, Keenan *et al.* 2006, Ruiz-Santaella *et al.* 2006), no ecological comparisons have been published.

To assist with the development of improved weed management strategies, including those using computer models (Renton *et al.* 2008, Thornby *et al.* 2008), more information is needed on *E. colona*, *U. panicoides* and *H. trionum* seedling establishment and seed persistence, as these are key drivers of the seed-bank dynamics. This paper reports on the emergence patterns and seed-bank dynamics of these three weeds when seeds were sown at different depths in a sub-tropical environment.

Materials and methods

Seed collection

Seeds of *E. colona*, *U. panicoides* and *H. trionum* were collected between late February and early March 2003 from populations in multiple fallow paddocks in areas with no known resistant weeds on the Darling Downs, west of Toowoomba, Queensland, Australia. Inflorescences with mature seeds were harvested manually, and dried at ambient temperature in a glasshouse for several weeks. After sorting from other plant material, mature seeds were stored in the dark at 10°C until the experiments commenced. Initial seed viability was determined using tetrazolium tests (International Seed Testing Association 1985).

Burial experiments

In experiment 1, viable seeds of *E. colona* (430 seeds per pot), *U. panicoides* (350) and *H. trionum* (350) were sown together at 0–2 cm, 5 cm and 10 cm depths in weed-free vertisol soil, obtained from the experiment

site, in black plastic pots (25 cm deep and 25 cm diameter). Initial seed-bank density was similar to that found in weedy paddocks in this region, and the soil was representative of the agricultural soils of north-eastern Australia (Webb *et al.* 1997). Seeds were initially mixed with a small quantity of soil, placed at the nominated depth in each pot, and covered with a circle of plastic mesh (1 cm grid pattern) to assist with relocating the seeds following exhumation (Taylor *et al.* 2005). The pots were filled with soil to 5 cm below the rim, buried on 8th June 2003 at the Queensland Primary Industries and Fisheries research station 9 km south-west of Toowoomba (27°33'S, 151°51'E), with rims protruding 5 cm, so that the soil surface in the pot was level with that in the surrounding field. The soil in the pots was not disturbed until exhumation. The pots were arranged in randomized complete blocks using a factorial design with three burial depths, seven exhumation times, and three replicates. The surrounding area was kept weed-free to prevent any contamination with new seeds.

As the initial emergence patterns in the first experiment differed for the two grasses, a second experiment was initiated with viable seed of *E. colona* (430) and *U. panicoides* (350) sown together at 0–2 cm and 5 cm depths in weed-free vertisol soil in black plastic pots. These were buried in the field on 30th March 2004, using the same procedure as for the first experiment. The experimental design was factorial with two burial depths and four exhumation times with three replicates in randomized complete blocks.

The soil used in both experiments was not sterilized to maintain natural fauna and flora, and was collected from areas not naturally infested with these three weeds.

Measurements

After each rainfall event, emerged seedlings were counted in those pots nominated for the last exhumation, and then all pots were sprayed with a lethal dose of glyphosate (0.72 kg a.e. ha⁻¹).

Nine pots (3 burial depths × 3 replicates) were exhumed at 2, 4, 6, 12, 18, 24 and 36 months following burial in the first experiment, and six pots (2 burial depths × 3 replicates) were exhumed at 6, 12, 18 and 24 months following burial in the second experiment. The soil layer containing the weed seeds was extracted from the pot and washed through a series of sieves to recover the seed. Within two days of being exhumed, the extracted seeds were counted and a sub-sample (30 seeds) was placed into Petri dishes in a growth cabinet at 20°C in 12 h light. Germination was recorded after 14 days. The non-germinated seed were dissected and subjected to tetrazolium viability testing. Red coloured embryos were considered viable dormant

seed. Percentage viable seed was calculated as the sum of germinated seed and dormant seed as a portion of that sown initially. Technical errors prevented data collection for 36 month exhumation of *U. panicoides* (all depths) and *E. colona* (10 cm depth).

Daily rainfall and air temperatures were recorded using an automated weather logger at the experimental site.

Statistics

Cumulative emergence, as percent of seeds initially sown, was compared using analysis of variance following one, two (both experiments) and three (experiment 1) years of burial for the different depths and species. Seed viability data were analysed using parallel curve analysis with exponential decay curves, initially combined for the 13 data sets, comprising 3 species × 3 depths in the first experiment plus 2 species × 2 depths in the second experiment, and then separately for the 5 species × depth data sets. The general form of the exponential decay curve was $y = A + B R^t$, where y is viable seed as percentage of seed sown at duration of burial time t (months), A is the asymptote, B is the range of the curve between the value at $t = 0$ and the asymptote, and R is a nonlinear parameter defining the rate of exponential decay, $k = \log(R)$. Each curve was tested to see whether it differed significantly from a straight line by comparing the change in regression sums of squares against a common residual mean square across depths with an F-test. All data variance was initially compared for homogeneity, which showed that cumulative emergence in experiment 1 required transformation (arcsine) and back-transformed means are presented. All analyses used Genstat 11th edition.

Results

Environmental conditions at the experimental site

Between June 2003 and August 2006, the average monthly rainfall was 71 mm for the months between October and March, whereas it was 19 mm for the other months (Figure 1). Summer rainfall was greater in 2003 than the other years. The mean air minimum and maximum temperatures between October and March for the three years were 16.3 and 27.4°C, and 9.6 and 21.2°C for the other months.

Seedling emergence

Emergence of the three species was restricted to the wetter and warmer months of October to March, apart from one flush of *U. panicoides* in July 2005 following 80 mm in the previous two weeks (Figure 2). As analysis of the combined data across both experiments showed significant 4-way interaction ($P < 0.001$), each experiment was analysed separately, revealing significant year × species × depth interaction in both experiments ($P < 0.001$).

In both experiments *E. colona* emergence was greatest from the surface 2 cm and decreased significantly with burial depth with minimal emergence from 10 cm depth (Table 1). Cumulative emergence after two years was 27.1 and 13.6% of seeds sown in the surface in experiments 1 and 2 respectively. Emergence was predominantly during the first warm season following burial, with flushes emerging at regular intervals between October and March, with minimal emergence in the third year of seed burial in experiment 1 (Figure 2).

Emergence patterns and response to burial depth differed markedly for *U. panicoides* compared with *E. colona* (Figure 2).

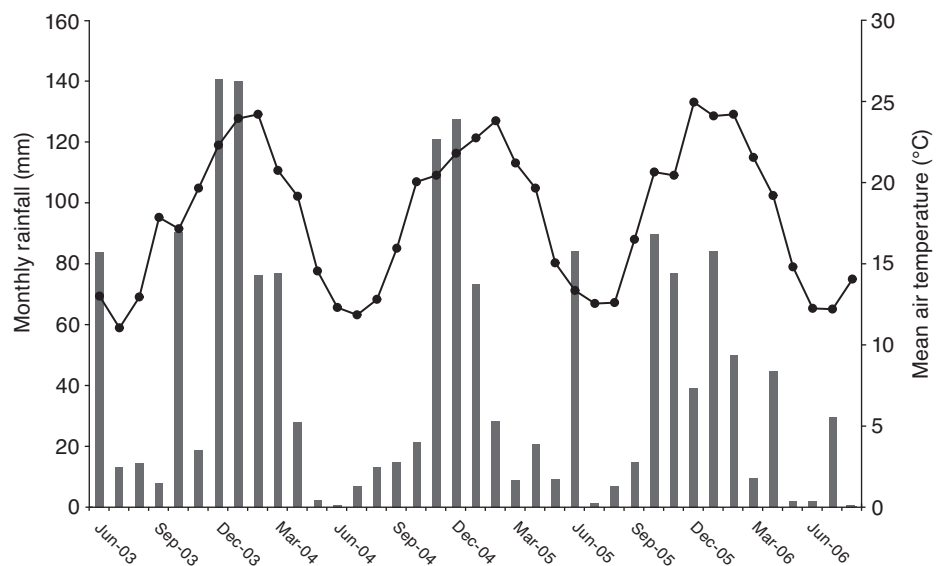


Figure 1. Monthly rainfall (mm) and mean air temperature (°C) for June 2003 to August 2006.

In both experiments, *U. panicoides* emergence was significantly greater from 5 cm than the surface 2 cm, and a substantial portion emerged from 10 cm in the first experiment (Table 1). Cumulative emergence after two years was 73.5 and 73.2% from 5 cm depth in experiments 1 and 2 respectively. Emergence was restricted generally to one major flush in October 2003 in the first experiment, and December 2004 and October 2005 in both experiments. More *U. panicoides* emerged in the third year of burial compared with *E. colona*.

Emergence patterns of *H. trionum*

changed with time and were different to the emergence patterns of the grasses (Figure 2). During the first 12 months of burial, emergence was significantly greater from the 5 cm depth than the other depths (Table 1). However in the following year, cumulative emergence was significantly greater from the surface 2 cm (11.9%) than from the other depths. Small flushes emerged over six months during the first year of burial, which was followed by an annual substantial flush from seed sown at the 0–2 cm depth in the second and third year. Emergence in the third year of burial

was minimal from 5 and 10 cm depths but was 8.2% from the surface 2 cm.

Seed persistence

Each species × depth set of data was analysed separately and presented, as there was highly significant interaction ($P < 0.001$) between 13 groups of species × depth data. Burial depth had a significant effect on seed persistence; *E. colona* ($P = 0.004$), *U. panicoides* ($P = 0.019$) and *H. trionum* ($P = 0.039$) in experiment 1, and *E. colona* ($P = 0.013$) and *U. panicoides* ($P = 0.009$) in experiment 2. In each instance, persistence increased with burial depth, although the impact differed among the species and to a lesser extent between experiments. The exponential model showed a good fit to all data sets, with adjusted R^2 values ranging from 0.88 to 0.94 (Figure 3), although the difference between non-linear and linear regression was not significant for *U. panicoides* ($P = 0.36$) and *H. trionum* ($P = 0.09$) at 10 cm depth in experiment 1 and *E. colona* ($P = 0.98$) and *U. panicoides* ($P = 0.68$) at 5 cm depth in experiment 2.

For both *E. colona* and *U. panicoides*, decay was rapid with seed buried in the surface 2 cm and 5 cm depth in experiment 1 (Figures 3a, 3b). An average of 1 and 2%, and 9 and 10% viable seeds was measured remaining after 24 months of burial at 0–2 and 5 cm depths for *U. panicoides* and *E. colona* respectively, whereas 21 and 24% remained at 10 cm depth. Similarly in the second experiment, persistence increased with burial depth, with 0.1 and 5% and 7 and 28% viable seeds measured as remaining after 24 months of burial at 0–2 and 5 cm depths for *U. panicoides* and *E. colona* respectively. Loss of *U. panicoides* seed at the 0–2 cm depth was particularly rapid initially with a measured 91% loss in the first six months. Persistence of *H. trionum* seed was much greater than for the two grasses. After 24 months of burial, 38, 51, and 64% viable seeds persisted at 0–2, 5 and 10 cm depths respectively. Even after 36 months, 31, 34, and 52% viable seeds remained at these depths.

Discussion

Seedlings of *E. colona*, *U. panicoides* and *H. trionum* commenced emerging after September and ceased by March in most instances. This period coincides with the normal growing season for summer crops, such as sorghum and cotton, in the north-eastern cropping region of Australia (Webb *et al.* 1997). In many instances, the major flushes of these weeds were in October – December, when the young crops are most susceptible to competition from weeds. These flushes emerged when the mean air temperatures were between 20 and 25°C, which aligns with that found for *E. colona* and *H. trionum* by Uremis and Uygur (1999) and for *H. trionum* by

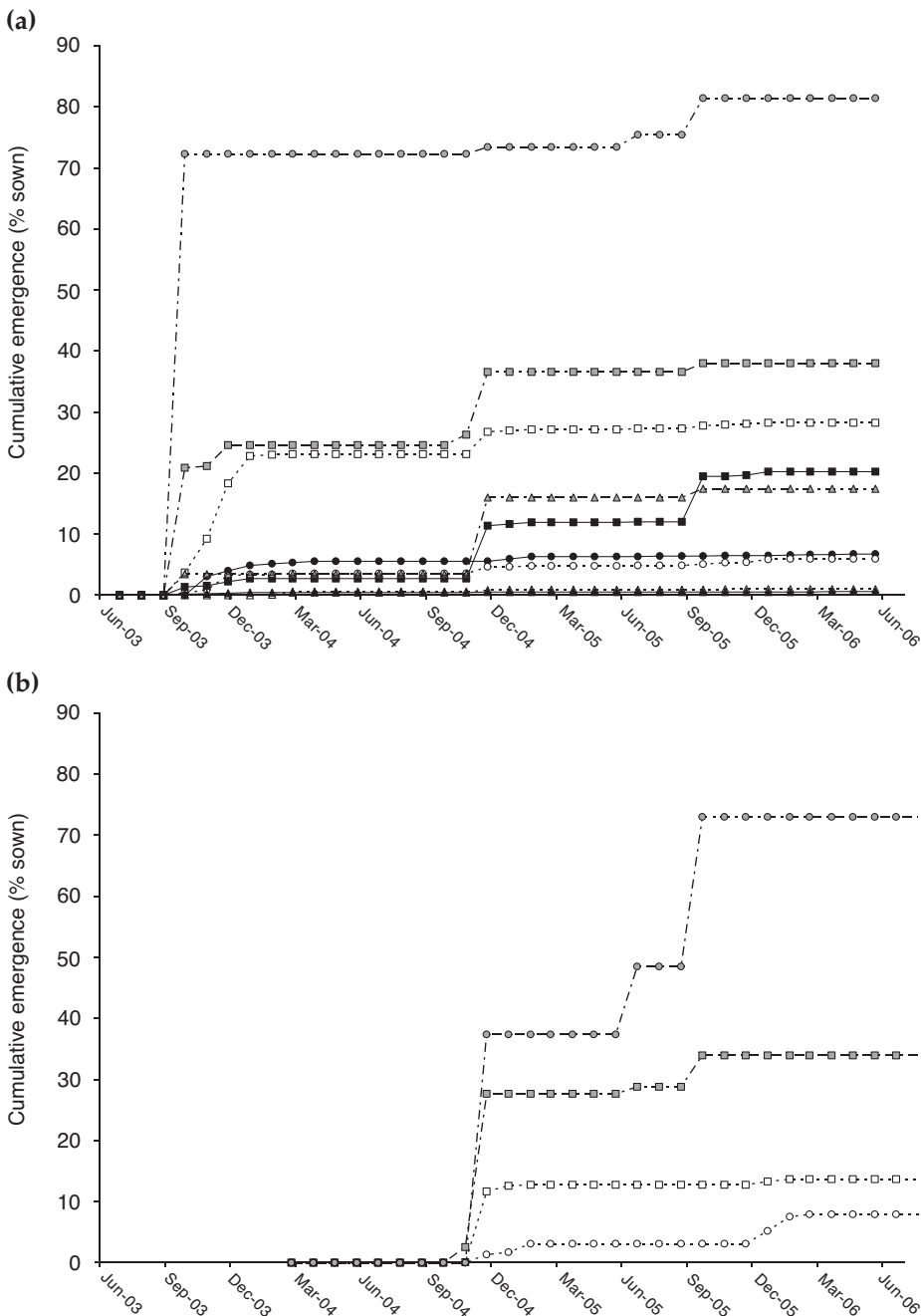


Figure 2. Cumulative emergence (% of seed initially sown) of: (a) *Echinochloa colona* (open), *Urochloa panicoides* (grey), and *Hibiscus trionum* (black) following seed burial at three depths in June 2003 (Experiment 1) and (b) *Echinochloa colona* and *Urochloa panicoides* following seed burial at two depths in March 2004 (Experiment 2). Depths were 0–2 (square), 5 (circle) and 10 cm (triangle).

Chachalis *et al.* (2008). As well as these temperature requirements, the timing of the first and sequential flushes was dependent on rainfall. This was evident with the first flushes emerging in October 2003 and 2005, which had monthly rainfall of 90 mm in both years, whereas the first flush in 2004 was delayed to December as only 21 mm were received in October.

The number of seedlings emerging was significantly influenced by the depth of seed burial, although the impact of depth differed between the three species. Emergence of *E. colona* and *H. trionum* decreased with increasing burial depth following a year's burial, which is consistent with that found by Guru and Govindra (2005) and Chachalis *et al.* (2008) in other environments, and for other weeds such as *Phalaris paradoxa* L. (Taylor *et al.* 2005) and *Sonchus oleraceus* L. (Widderick *et al.* 2004) in this environment. Less than 1% of *E. colona* and *H. trionum* emerged from 10 cm depth, and thus these weeds would be favoured under zero tilled systems that leave the weed seeds in the soil surface. In contrast, emergence of *U. panicoides* was substantially greater from 5 cm than the surface soil, and a significant portion emerged from 10 cm, indicating that germination of *U. panicoides* seeds may be inhibited by exposure to light. Taylor *et al.* (2004, 2005) also found that emergence of *P. paradoxa* was significantly lower on the soil surface than buried at 2.5 cm, as seed germination was suppressed by high intensity white light. Therefore, *U. panicoides* would be not favoured by zero tilled systems, and may explain, to some extent, why *E. colona* was more prevalent than *U. panicoides* in the north-eastern cropping region (Wicks *et al.* 2000, Rew *et al.* 2005, Osten *et al.* 2007).

In general, seed viability in the soil seed-bank followed an exponential decline with time, as found for other weed species (Widderick *et al.* 2004, Taylor *et al.* 2005, Walker *et al.* 2006), particularly for seed in shallow depths. However, seed persistence differed substantially between the grasses and *H. trionum* in this sub-tropical environment. *E. colona* and *U. panicoides* had only a short persistence, particularly in the surface soil, which is consistent with other grasses such as *Avena* spp. and *P. paradoxa* in a similar environment (Walker *et al.* 2006) and other studies with *E. colona* and *U. panicoides* (Uremis and Uygur 2005, Walker *et al.* 2006). Only 1–2% of the sown seed of these summer grasses persisted after two years of burial in the surface soil. In contrast, seed persistence of *H. trionum* was much longer than the annual grasses with 38 and 31% of the sown seed remaining viable after two and three years of burial in the soil surface. This persistence is similar to that found in other studies on *H. trionum* (Uremis and Uygur 2005), and for other weeds with hard seed coats, such as *Rapistrum rugosum* (L.) All., *Fallopia*

convolvulus (L.) Link and *Polygonum aviculare* L. (Walker *et al.* 2006).

Seed persistence increased with depth of burial for all species, although the im-

act of burial depth varied between species initially and experiments. During the first year of burial, the impact of depth was greatest for *U. panicoides* and the least for

Table 1. Accumulative emergence (% of seed sown) of *Echinochloa colona*, *Urochloa panicoides* and *Hibiscus trionum* at 12 and 24 months following burial in June 2003 (Experiment 1) and March 2004 (Experiment 2) and at 36 months for Experiment 1. The same letter indicates no significant difference among treatment means within each experiment.

Duration of seed burial (months)	Depth of burial (cm)	Experiment 1			Experiment 2	
		<i>E. colona</i>	<i>U. panicoides</i>	<i>H. trionum</i>	<i>E. colona</i>	<i>U. panicoides</i>
12	0–2	23.1 ab	24.5 ahn	2.7 d	12.7 a	27.8 c
	5	3.6 de	72.3 q	5.5 egij	3.1 b	37.5 d
	10	0.3 c	3.3 di	0.4 c		
24	0–2	27.1 af	36.3 r	11.9 lm	13.6 a	34.1 d
	5	4.7 deg	73.5 q	6.3 egj	7.9 a	73.2 e
	10	0.8 c	15.6 mo	0.6 c		
36	0–2	28.2 fh	37.8 r	20.1 bnop		
	5	5.9 ijk	81.6 s	6.7 gk		
	10	0.9 c	17.0 lp	0.6 c		

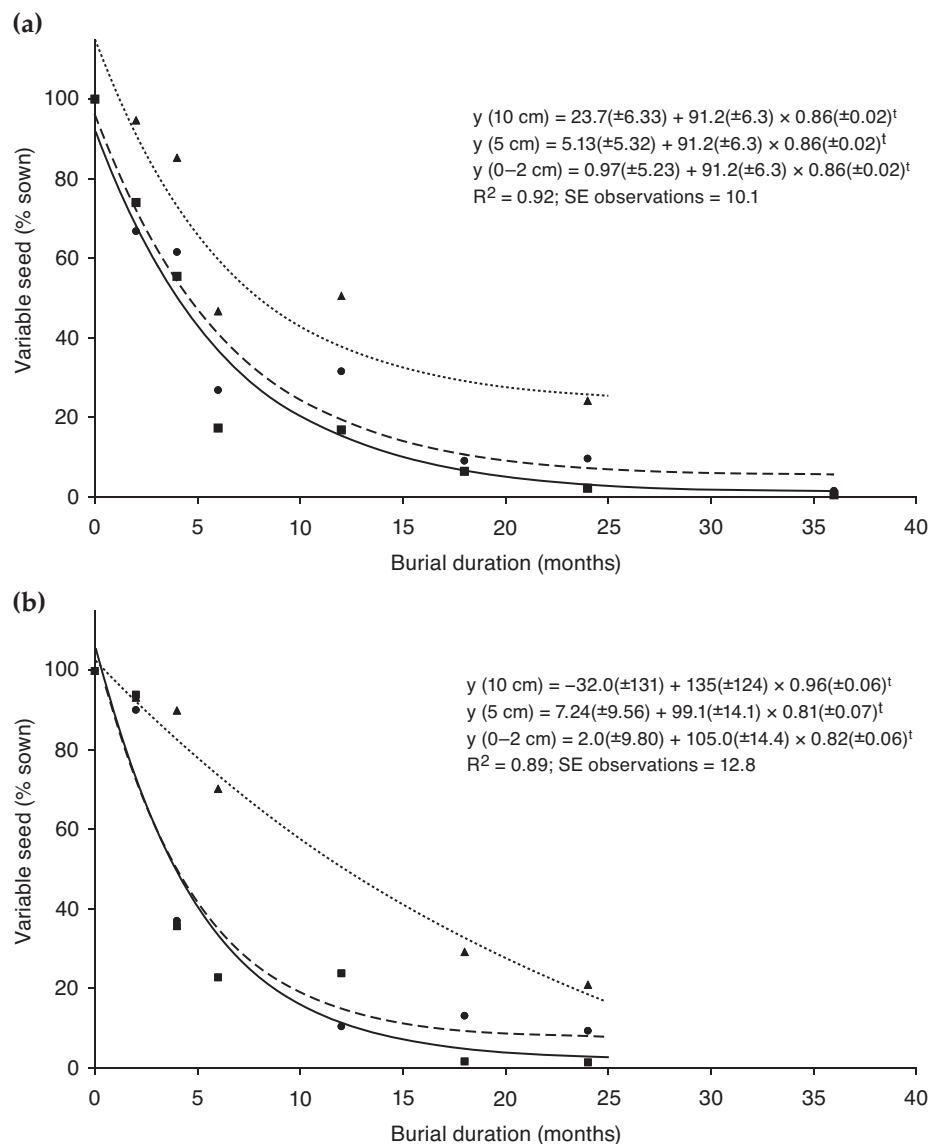


Figure 3. (See next page for caption).

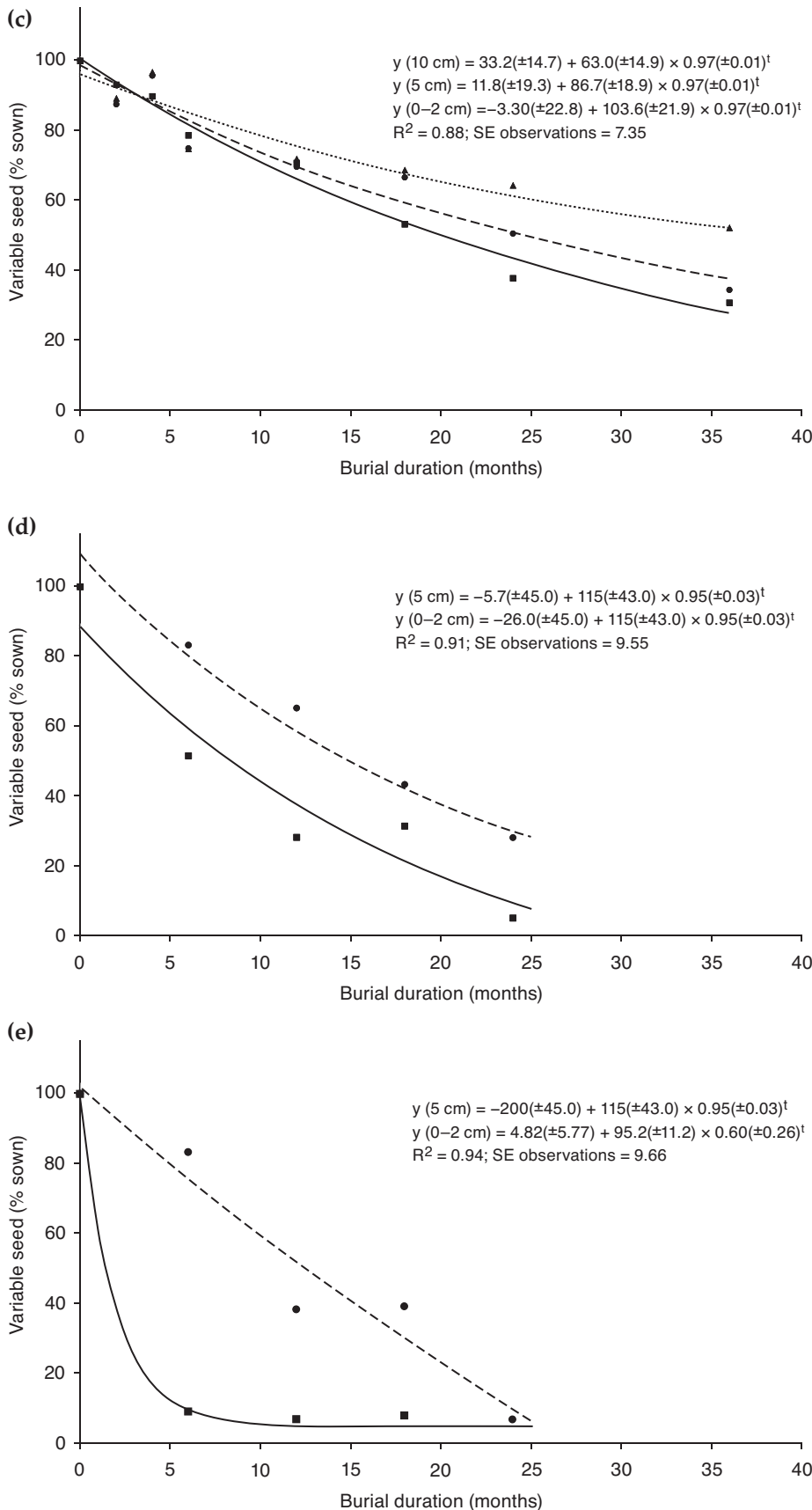


Figure 3. Changes in viable seed (% of seed initially sown) of: (a) *Echinochloa colona*, (b) *Urochloa panicoides*, and (c) *Hibiscus trionum* seed following burial at three depths in June 2003 (Experiment 1); and (d) *Echinochloa colona* and (e) *Urochloa panicoides* seed following burial two depths in March 2004 (Experiment 2). Depths were 0–2 (square), 5 (circle) and 10 cm (triangle). The fitted models are solid line for 0–2 cm, dashed line for 5 cm and dotted line for 10 cm data.

H. trionum. This difference in initial loss from the shallower depths was probably due to greater emergence of the grasses, particularly for *U. panicoides*, from these depths during the first year of burial. Also, the difference in persistence of *E. colona* was greater in the second experiment compared with the first. A likely reason is the difference in the environmental conditions during the experiments, with more rain received in summer 2003 (543 mm) promoting greater emergence compared to summer 2004 (380 mm). The impact of burial depth on persistence was similar with that found in other studies with *E. colona* (Westra *et al.* 1996) and *H. trionum* (Chaves *et al.* 1997), although persistence of these two weeds did not increase further when buried at greater depths to 30 cm (Uremis and Uygur 2005).

The differences among these weed species in emergence patterns and seed persistence have implications with regard to their management strategies. It is preferable to leave weed seeds in the surface soil, as this will lead to a more rapid decline in the seed-bank. In general, *E. colona* seedlings will need to be controlled regularly throughout the warmer months, whereas control of *U. panicoides* seedlings can focus on the first main flush. These management strategies that control all emerged seedlings over two years will lead to $\geq 95\%$ reduction in the seed-bank in the surface soil. *U. panicoides* and *E. colona* have developed resistance to atrazine and glyphosate in the north-eastern grain region of Australia (Adkins *et al.* 1997, Storrie *et al.* 2008, Heap 2009, Preston 2009). For paddocks with resistant annual grasses, management strategies that prevent weed seed burial and seed rain, and do not rely heavily on herbicides to which resistance has developed, will lead to a rapid decline in herbicide resistant populations over two to three years.

Whilst the same approach could be applied to weeds with hard seedcoats, such as *H. trionum*, the management strategy will need to be in place for considerably longer to substantially reduce the seed-bank. For these weeds, it is much more critical to prevent any seed rain, given that their seeds are much more persistent, and flushes can emerge for at least three years and potentially longer. It is also important to implement preventive strategies for herbicide resistance prior to these weeds developing resistance, due to the long persistence of these weed seeds in the soil seed-bank.

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