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Short Communication

Seed persistence of the invasive aquatic plant, *Gymnocoronis spilanthoides* (Asteraceae)

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Abstract. Seed persistence of *Gymnocoronis spilanthoides* (D.Don) DC.; Asteraceae (Senegal tea), a serious weed of freshwater habitats, was examined in relation to burial status and different soil moisture regimes over a 3-year period. Seeds were found to be highly persistent, especially when buried. At the end of the experiment, 42.0%, 27.3% and 61.4% of buried seeds were viable following maintenance at field capacity, water logged and fluctuating (cycles of 1 week at field capacity followed by 3 weeks' drying down) soil moisture conditions, respectively. Comparable viability values for surface-situated seeds were ~3% over all soil moisture regimes. Predicted times to 1% viability are 16.2 years for buried seed and 3.8 years for surface-situated seed. Persistence was attributed primarily to the absence of light, a near-obligate requirement for germination in this species, although secondary dormancy was induced in some seeds. Previous work has demonstrated low fecundity in field populations of *G. spilanthoides*, which suggests that soil seed banks may not be particularly large. However, high levels of seed persistence, combined with ostensibly effective dispersal mechanisms, indicate that this weed may prove a difficult target for regional or state-wide eradication.

Introduction

Gymnocoronis spilanthoides (D.Don) DC.; Asteraceae (Senegal tea) is a semi-aquatic, perennial herb that is native to the tropical and sub-tropical regions of the Americas, between Mexico and Argentina (Parsons and Cuthbertson 2001). It has been widely and purposefully introduced beyond its native range as an aquarium plant, an aquatic garden feature, a water purifier and a host for butterflies. *G. spilanthoides* occurs in wetlands and other freshwater habits as an emergent species that can form rounded bushes up to 1–1.5 m in height or develop masses of stems along the edges of watercourses, often extending over the water surface to form dense mats (Vivian-Smith *et al*. 2005). It is considered to be an established or emerging serious weed in most of its introduced range, which includes Australia and New Zealand (Vivian-Smith *et al*. 2005), China (Gao and Liu 2007), Japan (Kadono 2004; Nobuyuki and Yasuro 2005) and Hungary (Török *et al*. 2003). Among its impacts are the blockage of waterways (leading to flooding) and the suppression and exclusion of submerged native plant species and their associated fauna.

G. spilanthoides has been ranked highly as an invasive plant in south-eastern Queensland (Batianoff and Butler 2002) and is declared a Class 1 weed in Queensland (to be targeted for eradication). It is also a declared weed in all Australian states and territories, except the Northern Territory and Victoria (Gunaskera *et al*. 2002). *G. spilanthoides* is also listed on the Northern Australia Quarantine Strategy Alert List and the National Weed Alert List for Environmental Weeds (Cooperative Research Centre for Weed Management 2003; Vivian-Smith *et al*. 2005). Since this plant poses a considerable threat to tropical, sub-tropical and warm temperate Australia, studies of its biology are needed to maximise the efficiency of control programs. While there has not been a dedicated effort to delimit the extent of the *G. spilanthoides* incursion in Queensland, the total area of infestation appears to be ~200 ha (B. Gray, pers. comm.).

In earlier work, Vivian-Smith *et al*. (2005) observed that while fecundity (per cent of florets setting achenes per capitulum) of *G. spilanthoides* is not high, viable achenes (hereafter referred to as 'seeds' [0.8–1.2 mm long, 0.5 mm diameter; air dry weight ~20 mg]) are produced and seeds germinate over a broad range of temperatures, so long as they are exposed to light. Hence, while germination may occur soon after seeds shedding, the near-obligate light requirement for germination implies a potential for developing a persistent soil seed bank. Seedling recruitment following non-selective control with glyphosate (T. Anderson, pers. comm.) is further evidence of the existence of a seed bank for this species. Indeed, Vivian-Smith *et al*. (2005) concluded that the presence of a seed bank should be considered when developing and refining eradication strategies for *G. spilanthoides*.

Knowledge of seed persistence is vitally important at all stages of weed eradication programs, from assessing eradication feasibility at the outset (Panetta and Timmins 2004) to determining when eradication has been achieved (Regan *et al*. 2006; Panetta 2007; but see Rout *et al*. 2009). Eradication feasibility cannot be divorced from the amount of resources available to achieve this objective (Rainbolt and

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Coblenz 1997; Panetta and Timmins 2004; Parkes and Panetta 2009) and seed persistence is a major driver of the duration and cost of eradication programs (Cacho *et al*. 2006; Panetta 2009). Unfortunately, little or nothing is known about seed persistence when decisions are being made on how best to manage incipient weed incursions (Panetta 2004). The aim of the work reported in this paper was to assess the persistence of *G. spilanthoides* seeds in relation to burial status and different soil moisture regimes.

Methods and materials

Seed collection and initial germination assessment

Mature inflorescences (capitula) were collected from an infestation situated along an urban creek at Strathpine, Pine Rivers Shire (28°18′S, 152°59′E) on 24 February 2006. While this infestation had been subjected to control efforts for several years, there was no evidence of treatment with herbicide at the time of collection.

Seeds were extracted from the bulk collected material and stored dry at prevailing laboratory temperatures for 3 months until an assessment of germinability and viability was undertaken, as follows. Twenty seeds were placed on moistened filter paper that had been wrapped over an inverted watchglass, which was then placed within a Petri dish, with a surplus of water in its base. This procedure ensured that there was little or no need to add additional water during the assessment. On 30 May 2006, five replicates of the above setup were placed in a growth cabinet and were subjected to 20°C and 30°C under intermittent light (12 h photo- and thermoperiod). These conditions had been found previously to be optimal for germination (Vivian-Smith *et al*. 2005).

Replicates were generally checked daily and newly germinated seeds removed at these times. When no further germination had been observed for at least 1 week, all ungerminated seeds were examined under a dissecting microscope. Those with white, firm embryos were considered to be viable, but dormant. The assessment therefore yielded estimates of initial viability, seed dormancy and germination rate (calculated as number of days to 50% of total germination).

Seed persistence experiment

This trial was initiated on 9 June 2006. The small seeds (see Introduction) of this species posed problems for quantitative seed recovery. They were therefore mixed with a small quantity of a commercial garden loam and placed in 30 mm × 40 mm woven stainless steel wire mesh packets (with 263 μm apertures). Fifty seeds were enclosed in each packet. Packets were either placed on the surface of the same soil within 12-cm diameter black plastic pots (‘surface-situated’ seeds) or were buried at 2 cm (‘buried’ seeds).

Because *G. spilanthoides* is a Class 1 species in Queensland, this experiment had to be undertaken within a covered, secure enclosure. This created a challenge in terms of simulating conditions that could be considered as reasonably similar to what would occur in the field. Different soil moisture regimes were established by holding pots in a large circular black PVC tank (96 cm tall × 200 cm diameter) that was partially filled with water. Three moisture regimes were established: ‘field capacity’, where the bottom 2 cm of pots containing the seed packets were immersed, such that the soil surface was moist to the touch at all times; ‘water logging’, where the level of the water surrounding the pots was maintained at 2–3 cm below the soil surface (i.e. at or just below the level of the buried seed packets); and ‘fluctuating’, where pots were maintained on a 4-week cycle of 1 week at field capacity, followed by 3 weeks of drying down after removal from the tank. The water level in the tank was maintained continuously at a depth that imposed water-logged conditions in pots subjected to that treatment; field capacity was established by placing pots on inverted plastic containers such that only the bottoms of the pots were immersed.

The experiment had a completely randomised factorial design, with the factors soil moisture regime (three levels), seed burial (two levels) and time of harvest (three levels), replicated four times.

Observations were made of emergence from non-packaged seeds by sowing 50 seeds on the surface of four pots for each of the soil moisture regimes. No equivalent observations were attempted for buried seeds. Seedlings were counted and removed at weekly intervals. No attempt was made to retrieve non-germinated seeds from these sowings and pots were fumigated to destroy any remaining viable seeds 2 months after emergence had ceased.

Seed retrieval and testing

Harvests were undertaken after 1 (6 June 2007), 2 (2 June 2008) and 3 years (10 June 2009). At these times the seed packets were removed from the pots. In the laboratory, packet contents were placed into a small amount of water in the bottom of a Petri dish so that seeds could be recovered under a dissecting microscope. Seeds that were firm were prepared for a germination test under conditions identical to those in the initial assessment. Once germination had ceased, any ungerminated seeds were assessed for viability status as explained earlier. Total seed viability, seed dormancy and germination rate (time to 50% germination) were estimated from the primary data collected.

Data analysis

Seed viability data (as proportions) were analysed by three-way ANOVA for the factors ‘burial’, ‘soil moisture regime’ and ‘time of harvest’, following arcsine transformation in order to normalise the data and stabilise their variance. Since very few seeds persisted when surface-situated, seed dormancy data for buried treatments only were analysed by a two-way ANOVA for the factors ‘soil moisture regime’ and ‘time of harvest’, following arcsine transformation. Untransformed data for the rate of germination were analysed in a manner similar to those for seed viability. However, it was not possible to include all levels of soil moisture in the ANOVA for this variable, since few seeds were recovered from 24 months onwards in the surface-situated, water-logged treatment and all of the recovered buried seeds that had been water-logged were dormant at 36 months. Hence only two levels of soil moisture (field capacity and periodic wetting) were utilised in the analysis for germination rate. Untransformed values are presented in all figures herein.

Since viable seeds remained in all treatments at the end of the experiment, linear regressions were fitted to $\log_{10}$-seed viability data (%) in order to predict potential seed persistence of buried seeds (data aggregated across all soil moisture regimes) and surface sown seeds (data similarly aggregated).

Results

Seed viability at the commencement of the seed persistence experiment was 88.0 ± 3.4% (mean ± s.e.). Germinability (expressed as a percentage of viable seeds) was 83.0 ± 0.8%, corresponding to as little as 5.0 ± 0.8% of seeds being dormant. Germination was rapid (Fig. 1*a*), with 4.9 ± 0.05 days to 50% of the total cumulative value. However, emergence from surface-sown seeds in the pot experiment was much slower (18.8 ± 1.9 days to 50% of total emergence) and total emergence (25.1 ± 1.8%) (Fig. 1*b*) did not approach the germination potential expressed in the initial assessment.

In the seed persistence experiment, burial ($F_{1,55} = 336$; $P < 0.001$), soil moisture regime ($F_{2,55} = 8.34$; $P < 0.001$) and time of harvest ($F_{2,55} = 19.4$; $P < 0.001$) all had highly significant effects upon seed viability. The first order interactions of burial × water ($F_{2,55} = 9.25$; $P < 0.001$) and burial × time ($F_{2,55} = 3.23$; $P = 0.047$) were also significant. Viability levels of surface-situated seed at 36 months were very low (range of means 1.7–3.4%), in contrast to those of buried seed (range of means 27.3–61.4%) (Fig. 2*a*). Predicted times to 1% viability were 16.2 and 3.8 years for buried and surface-sown treatments, respectively (Fig. 3).

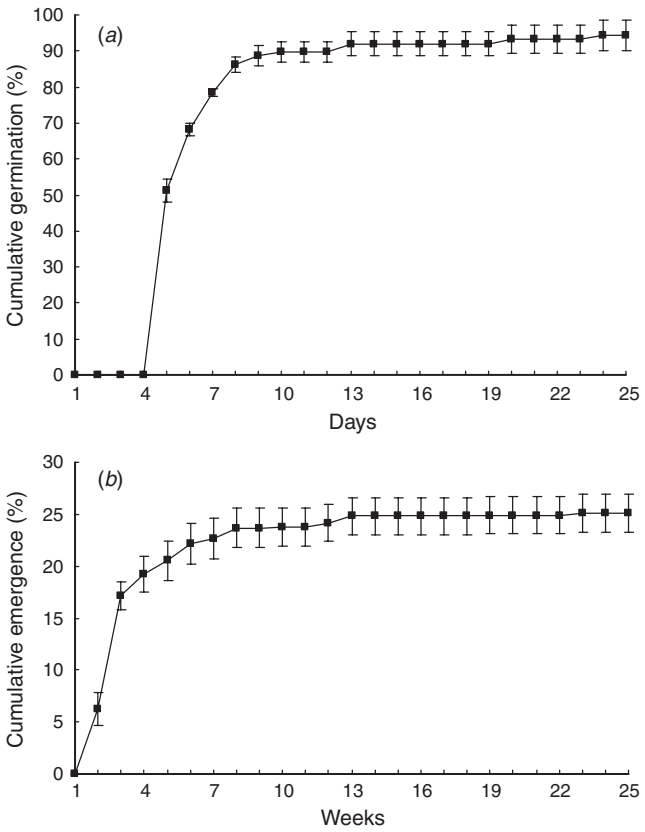

Fig. 1. (*a*) Mean cumulative germination (% of viable seed) (±s.e.) of *Gymnocoronis spilanthoides* seeds at the commencement of the seed persistence experiment. Seeds were sown onto moist filter paper and maintained at 20°C and 30°C under intermittent light (12 h photo- and thermoperiod). (*b*) Mean cumulative emergence (% of viable seed) (±s.e.) from seeds of *G. spilanthoides* sown onto the soil surface in the seed persistence experiment. Data are combined over all soil moisture regimes.

Both soil moisture regime ($F_{2,27} = 34.1$; $P < 0.001$) and time of harvest ($F_{2,27} = 16.7$; $P < 0.001$) had highly significant effects upon the level of dormancy in buried seed, as did their interaction ($F_{4,27} = 4.03$; $P = 0.011$). Notably, 100% of the remaining viable seeds in the buried, water-logged treatment were dormant at 36 months (Fig. 2*b*). This corresponded to 27.3% of the viable seeds originally planted (cf. 5% dormancy at the beginning of the experiment). Also, 16.9% of the planted viable seeds were dormant in the buried, field-capacity treatment at 36 months. A large proportion of the surface-situated seeds that survived for 12 months was dormant (range of means 39.6–63.1%) (Fig. 2*b*), but since overall viability was low (ranging between 10.8 and 14.2%), it is possible that this corresponded to the dormant component of the planted seed lots.

Time of harvest was the only factor whose effect upon germination rate was significant ($F_{2,18} = 6.24$; $P = 0.009$).

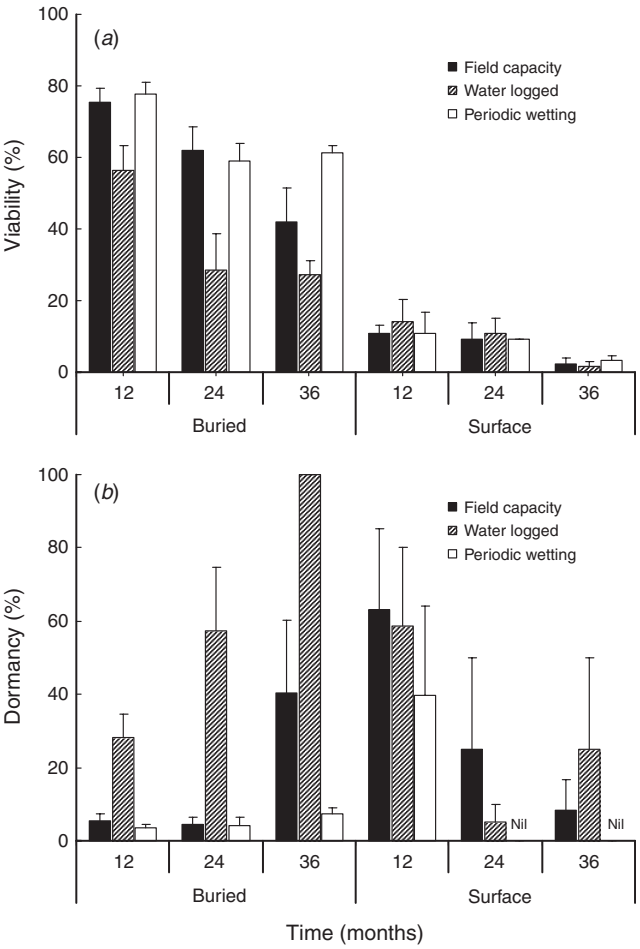

Fig. 2. (*a*) Percentage viability and (*b*) percentage dormancy of seed lots of *Gymnocoronis spilanthoides* that had been buried at 2 cm or were surface-situated in mesh packets for different periods. Following retrieval, seeds were extracted from the packets and maintained on moist filter paper at 20°C and 30°C under intermittent light (12 h photo- and thermoperiod). Values are means and s.e. FC = soil maintained at field capacity; WL = soil maintained under water-logged conditions; PW = soil subjected to periodic wetting to field capacity, followed by drying. Note that in some instances increases in percentage dormancy were associated with decreases in percentage viability and that in all cases few viable seeds remained in surface-situated seed lots.

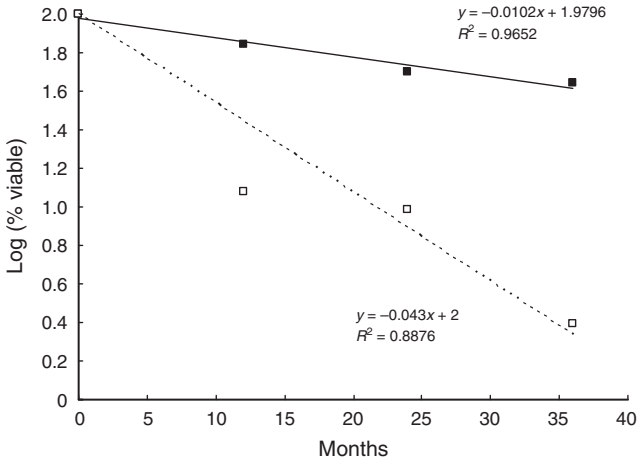

Fig. 3. Seed decay curves for buried (■) and surface-situated (□) treatments, averaged over all soil moisture regimes. Predicted times to 1% viability are 16.2 and 3.8 years for buried and surface-sown treatments, respectively. The regression for surface-situated seed has been forced through the initial seed viability value for the purpose of presentation, but the unforced regression ($R^2 = 0.98$) was used to calculate time to 1% viability.

However, changes in germination rate were not monotonic (Fig. 4) (note that the water-logged soil moisture treatment was not included in the analysis – see aforementioned). Germination was generally rapid following seed retrieval and values did not differ much from that for seeds at the commencement of the seed-persistence experiment.

Discussion

G. spilanthoides seems to belong to the group of species characterised by long-lived (>5 years) seed banks (Thompson *et al*. 1998), especially if its seeds become buried. However, it would appear that exclusion of light is the critical factor, rather

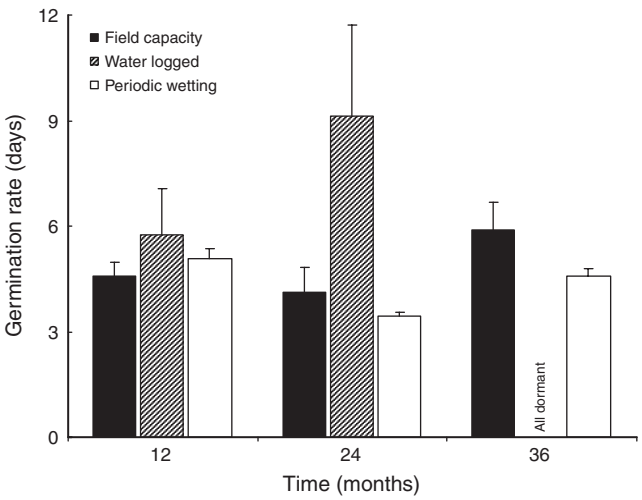

Fig. 4. Germination rate (days to 50% total germination) of seeds of *Gymnocoronis spilanthoides* that were retrieved following burial at 2 cm for different periods. Values are means and s.e. FC = soil maintained at field capacity; WL = soil maintained under water logged conditions; PW = soil subjected to periodic wetting to field capacity, followed by drying.

than burial *per se*. Vivian-Smith *et al*. (2005) reported that only one seed out of 300 germinated in continuous darkness across a range of fluctuating temperatures (from 10°C and 20°C to 20°C and 30°C). It is well known that seeds of many species utilise temperature fluctuation as a cue for germination when shallowly buried (Thompson *et al*. 1977; Thompson and Grime 1983). The lack of germination in continuous darkness in spite of a 10°C diurnal fluctuation (Vivian-Smith *et al*. 2005) suggests that *G. spilanthoides* seeds could persist ungerminated in a thick layer of litter, provided light was excluded. Regardless, burial by soil or litter could be expected to occur relatively frequently in periodically disturbed riparian habitats.

Higher levels of dormancy in retrieved buried seeds, compared with the initial level of seed dormancy, suggest that secondary dormancy was induced by the environment and is an additional mechanism that contributes to *G. spilanthoides* seed persistence. Water-logging may enhance this mechanism for buried seeds, as reflected in the highly significant effect of soil moisture regime (and its interaction with time of harvest) on seed dormancy levels. A degree of dormancy could also be inferred by a reduction in germination rate of buried, water-logged seeds recovered at 24 months (Fig. 4), although a slowing of germination could also reflect a loss of vigour associated with seed ageing under these conditions. Otherwise, germination rates of recovered seeds were generally high and did not differ substantially from those shown by seeds during testing before the initiation of the experiment.

It was not possible to conduct experimental work in the field owing to the legal status of *G. spilanthoides* in Queensland. One positive aspect of the approach taken was that a high degree of control over soil moisture regimes could be maintained, but since a limited range of conditions was employed, the results should best be interpreted as indicating that seeds of *G. spilanthoides* have the potential to be very persistent. Furthermore, estimates of seed persistence might be biased upwards, since some protection from seed-depleting factors (such as predation) would have been afforded by the steel mesh packets. Slower seedling emergence from non-packaged surface-situated seeds (compared with germination rates in the initial assessment) was most likely due to lower temperatures in the enclosure during the winter months, relative to the temperature regime (20°C and 30°C) of the assessment. What remains unexplained is why emergence ceased at the equivalent of 25% of the viable seeds that had been sown (Fig. 1*b*), unless some seeds achieved a degree of burial sufficient to exclude light.

Seeds were markedly less persistent when surface-situated, but even here did not fall into the transient category (persisting for <1 year), as predicted by the controlled ageing test (CAT) of Long *et al*. (2008). These authors noted the discrepancy between the present experimental work, which was ongoing at the time, and the test prediction, but did not advance an explanation for it. However, it is possible that the periodic (or continuous) wetting experienced by seeds in the present study enabled repair enzymes to be active or inhibited deleterious molecular reactions (Vertucci and Farrant 1995; Walters *et al*. 2005; Long 2007). Suffice it to say that while the CAT has shown some promise in broadly categorising species with regard to seed persistence, a wider range of species must be tested before the general reliability of this test can be gauged.

From a management perspective, it is fortunate that *G. spilanthoides* shows rather low levels of seed production, given its degree of seed persistence. Vivian-Smith *et al*. (2005) found that 19% and 6% of florets set seed in two populations of this weed (the former value corresponding to the population that was sampled in the present study). Hence it is possible that seed banks are relatively small, albeit persistent. Assessment of seed banks in field populations is clearly required to complement the present study.

Spread via seed dispersal, while occurring, may be less important than that gained through vegetative fragmentation, since seeds do not bear a pappus and most drop near the parent plant. Spread via fragmentation occurs when any part of a stem that has a node breaks away from a parent plant and is moved via water (Cooperative Research Centre for Weed Management 2003). New plants can establish even from leaf fragments, provided the midrib is present (F. D. Panetta, pers. obs.). The combination of persistent seed banks and effective dispersal mechanisms suggest that regional or state-wide eradication of this species will be an expensive and protracted process.

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