Effects of burial and age on viability of rubber vine (*Cryptostegia grandiflora*) seeds

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Abstract

One of the main difficulties in controlling the woody weed rubber vine (*Cryptostegia grandiflora* Roxb. ex R.Br.) is its capacity to regenerate from seed, particularly in riparian habitats. It is not known how long seeds can remain viable under natural conditions that favour germination, such as during wet seasons, or under conditions that preclude germination, such as during drought, or dry storage. Two experiments were undertaken to determine the viability of rubber vine seeds exposed to a range of contrasting conditions. Experiment 1 compared changes in the viability of seeds that were placed at six positions within the soil profile (0 cm on bare ground, 0 cm ground level with seeds covered with 10 cm thick, slightly pressed, dry vegetation mulch and 5, 10, 20 and 40 cm below ground) and exposed to either natural rainfall or had rainfall excluded. Retrieval of seed lots was undertaken annually for a maximum of four years. Experiment 2 compared the viability and vigour of rubber vine seeds that had been stored for different durations (0, 1, 9, 11 and 20 years) under conditions conducive to prolonged life (dry storage at 7 ± 1°C).

In the field, the most rapid decline in viability occurred under natural rainfall conditions – with no viable rubber vine seeds remaining in the soil seed bank after one year, irrespective of burial depth. In contrast, viability of seed lots under conditions where rainfall was excluded averaged 68, 29 and 0% after 1, 2 and 3 years, respectively. Under dry storage, viability of 1 year old seed was extremely high (99%) and not significantly different to that of freshly collected seed. In comparison, viability of 9 and 11 year old seed averaged 87%, and only 20% of 20 year old seeds remained viable. Almost all viable seed had sufficient vigour to develop into seedlings, irrespective of age.

Introduction

Rubber vine (*Cryptostegia grandiflora* Roxb. ex R.Br.) is an invasive woody weed of the dry tropics of Queensland. It predominantly infests rivers and creeks (Tomley 1995) but can also invade open pastures where the water table is high (Dale 1980). It can set seed at least twice a year and produce more than 8000 seeds per large shrub in a single reproductive episode (Grice 1996). This magnitude of reproduction may lead to the establishment of dense infestations. Deleterious consequences of dense infestations of rubber vine include degradation of native ecosystems, loss of biodiversity, reduced pasture production, increased costs of mustering and stock deaths due to poisoning (McGavin 1969). The cost of rubber vine to primary industry in the State of Queensland has been estimated to be at least 18 million dollars per annum (Mackey 1996).

Because of the seriousness of the problem, rubber vine was declared a noxious weed in Queensland in 1955 (Mackey 1996). It was allocated a weed category status of P2 and P3. A category P3 status demands that the area under infestation be reduced; category P2 status dictates that the plant be destroyed. Prior to this declaration, rubber vine was sold in Queensland as an ornamental plant under the name of 'Indian Rubber Vine'. The only hint of its invasive character was that 'regular trimming is needed to keep it under control' (Jeffrey 1987). The unabated spread of rubber vine in Queensland has led the Northern Territory to raise its weed status to level P1, which prohibits its introduction into the Territory. Rubber vine is now a Weed of National Significance (WONS) and its propagation is illegal in both the Northern Territory and Queensland (Thorp and Lynch 2000). However, dispersal mechanisms for rubber vine (wind, water and human activity) are not inhibited by legislative edict. Furthermore, this status does not restrict the propagation and sale of rubber vine in other states even though it may pose a major threat to the grazing industry and natural ecosystems throughout semi-arid Australia. Stores of rubber vine seeds may be held in these states and stored seeds may remain in Queensland from times when propagation of the weed was legal. Seeds may also survive under conditions that can prolong their life, for example, in dry soil. A better understanding of the longevity of rubber vine seed would provide an indication of the risk latent in both fresh and stored seed and the limits to the threat of any seed as a source of new infestation.

Preliminary research conducted by Grice (1996) and Dale (1980) suggests that rubber vine seeds are short-lived, with viability under field conditions not exceeding 12 months. Grice studied germinability of rubber vine seeds in growth cabinets and under pot conditions in a glasshouse. He showed that more than 90% of seeds germinated within 10 days of moisture becoming available. In this study seed longevity was equated to germinability; a viability test was not made on seed that had not germinated (Moore 1985).

The high germinability of rubber vine seeds under warm moist conditions suggests that they have little or no innate dormancy. In the field, such conditions will normally be encountered at least once in any year and so most seeds will probably germinate, or rot. Nonetheless, experiments with seeds of plants other than rubber vine have shown that dormancy can be induced (e.g. by light, Sen 1968) or enforced by conditions that inhibit germination. Enforced dormancy is produced by precisely those conditions used to store seeds, low temperatures and the absence of water. Absence of water alone, as can occur under conditions of drought or seed storage, may also prolong the life of rubber vine seed beyond 12 months.

The present study provides information on the viability of seeds that had been buried at different depths under both natural and rain-shelter conditions for a period between 1 and 4 years and on seeds that had been under dry storage for periods between 1 and 20 years.

Materials and methods

Longevity of buried seeds

A 2 × 6 factorial experiment compared rubber vine seed longevity following exposure to two levels of moisture availability and burial at six locations on or in the soil profile. Each treatment was replicated three times.

The first factor involved either exposing seeds to natural rainfall (hereafter referred to as natural), or exclusion of rainfall through construction of rain-shelters (hereafter referred to as rain-shelter). The second factor involved placement of seed lots on the surface (0 cm on bare ground and 0 cm ground level with seeds covered with 10 cm thick, slightly pressed, dry vegetation mulch) or burial under bare ground at 5, 10, 20 and 40 cm depth.

The field site was located at 10 Mile Creek (19°58'S, 146°29'E), approximately 30 km east of Charters Towers, northern Queensland. Plots where seeds were buried were approximately 1 × 2.4 m in size. Those exposed to natural rainfall conditions were left uncovered, whilst rain-shelter plots were surrounded by a rectangular wall constructed of railway timber sleepers to a height of 23 cm and covered with a hinged, polycarbonate 'Laserlite' roofing (10 cm above the railway sleepers frame), that minimized ambient temperature differences within the shelter space and allowed daylight to diffuse through but not rain.

Fresh seeds for the experiment were derived from bulked collections (October 1997) of a large number of ripe pods (about 300 pods) gathered from plants growing close to Charters Towers. Sub-samples of fifty filled, large (seed that did not pass through a 2.00 mm diameter sieve), fresh rubber vine seeds were then placed into each of 360 nylon-mesh bags (mesh size approximately 250 mm, 4 × 4 cm in size), sealed with nylon fishing line and buried in October 1997.

Pre-fabricated PVC cylindrical water pipes (50 cm long × 10 cm diameter) were used to place seed lots at the designated depths. The cylinders were filled with sandy soil collected from the field site (Table 1). Cylinders had eight circular perforations (1 cm diameter) equally spaced at 5, 10, 20 and 40 cm depth and four circular perforations at the capped base to allow for water drainage. A rectangular sheet of blotting paper (50 × 45 cm) was rolled into a cylinder shape and inserted along the inner wall of each cylinder, in addition to 9 cm diameter blotting paper placed on the perforated capped base, to prevent loss of soil through the lateral and basal perforations of the cylinder. Within cylinders, bags were buried horizontally at the respective depths, except for the 0 cm vegetation mulch treatment, which was placed in separate open ended, 5 cm long × 10 cm diameter, PVC cylinder off-cuts, filled with dry vegetation mulch collected from the vicinity of the burial site.

Within plots, 10 cylinders containing seeds and one blank containing thermocouples only were placed into a centrally located dug out trench (50 cm long × 20 cm wide × 50 cm deep) which was then refilled with the excavated soil leaving the open end of each buried cylinder level with the surrounding ground. The open end of the buried cylinder was the position where the 0 cm on bare ground treatment was located. The 10 open-ended cylinders, containing the 0 cm vegetation mulched treatment, were then placed on the soil surface adjacent to the buried cylinders.

Placement of thermocouples at the depths where seeds were located was undertaken to clarify whether seed lots housed under rain-shelter conditions were exposed to similar temperatures to those under natural conditions. A data logger (Data Electronics Australia Pty. Ltd.) connected to the thermocouples recorded temperatures for the first 12 months of the experiment. Rainfall data was obtained from a weather station installed in 1997 by the Department of Natural Resources at 10 Mile Creek.

The initial intention was to retrieve one of the buried and surface located cylinders from within each plot on a yearly basis, for a total of ten years. However, seed lots were exhumed for only four years after no viable seeds were retrieved from any treatments for two consecutive years.

Viability (number of germinable plus dormant seeds) of fresh seeds was determined at commencement of the trial in October 1997 and at each recovery interval. Retrieved bags of older seeds were transferred to the laboratory, opened, their contents examined and all intact seeds removed.

To determine 'germinability', the seeds were washed in water, surface sterilized in a sodium hypochlorite solution (1% v/v) for 1 min, washed in distilled water, then placed in 9 cm Petri-dishes filled with 10 mL distilled water. Petri-dishes were randomized within stacks in plastic containers (9 × 10.7 cm diameter) with the lid sealed to reduce evaporation, then stored in black plastic bags as rubber vine seeds have a definite preference for germination in the dark (Sen 1968). The bags were placed in an incubator set at 28°C because optimal germination of rubber vine seed was reported to occur at this temperature (Grice 1996). Germinable seeds (identified by radicle emergence) were counted in a dark room under green light and removed daily for 10 days. Seeds that did not germinate were checked for dormancy using the tetrazolium method (Moore 1985). Viable but dormant seeds were pink when cut longitudinally with a sharp scalpel. Seed viability was expressed as germinable plus dormant seeds.

Longevity of aged-seeds

To assess the longevity of rubber vine seeds kept under dry storage, six replicates of 50 seeds of five age groups (0, 1, 9, 11 and 20 year old) were tested for both germinability and viability. The 0 year old seeds came from the same seed lot used in the experiment previously described. The 1, 9, 11 and 20 year old seeds came from seed lots that had been collected in the vicinity of Charters Towers for various research purposes during 1996, 1988, 1986 and 1977, respectively. These seed lots were all kept in dry storage (7 ± 1°C) following collection. The experiment was conducted in October 1997.

Seed lots were exposed to the same germination and viability tests as described in the previous experiment, with Petri dishes arranged within the incubator utilizing a completely randomized design. Because of the slowness of some of the older seed lots to germinate, daily measurements were undertaken for 57 days. A germination rate index was used to compare the rate at which the different aged seed lots germinated. The index was calculated as the time taken to reach 50% of maximum germination for individual seed lots (Gramshaw 1972).

To determine whether aged seeds had sufficient vigour to develop into seedlings, six replicates of 50 seeds of the five age groups were sown 1 cm deep into 285 mL plastic tumblers filled with sandy loam soil (52% coarse sand, 39% fine sand, 1% silt, and 8% clay). Before soil was added, the base of each tumbler was perforated to provide drainage and filter paper (42.5 mm diameter) was inserted over the base of each tumbler to prevent soil loss. Following sowing, the tumblers were placed in a controlled environment glasshouse set at a day and night temperature of 28 ± 1°C and 20 ± 1°C, respectively. All tumblers were watered daily to maintain soil at, or slightly above, field capacity.

Emerged seedlings (identified when the cotyledons became visible) were counted and removed daily for 57 days to ensure that seedlings from all seed age groups had adequate time to emerge. Ungerminated seeds were retrieved at the end of the experiment by washing away soil through a 1.0 mm mesh sieve. The seeds were then checked for viability using the tetrazolium salt test as described previously.

Data analysis

For both experiments, percentage data were arcsine transformed to normalize the distribution of experimental errors before being analyzed, but were later presented as back-transformed data. Analysis of variance was used to identify whether significant treatment differences were

Table 1. Soil properties where rubber vine seeds were buried at 10 Mile Creek (Agricultural Chemistry Laboratory Analytical Information Management System, Department of Natural Resources and Mines, Indooroopilly, Queensland).

Soil properties	
pH	8.8
EC (mS cm^{-1})	0.05
Cl (mg kg^{-1})	11.6
NO_3-N (mg kg^{-1})	2.2
P (mg kg^{-1})	5.7
K (meq 100 g^{-1})	0.62
Air Dry Moisture Content (%)	0.41
Coarse sand (%)	83
Fine sand (%)	11
Silt (%)	2
Clay (%)	3
Organic C (%)	0.55

present. Fisher's protected l.s.d. test was used to identify differences between treatments.

Results

Rainfall

Total annual rainfall during 1997, 1998, 1999 and 2000 was 523 mm, 1143 mm, 807 mm and 1248 mm, respectively. The 1998, 1999 and 2000 annual rainfall was well above the 657 mm average for the area (Figure 1). The first wet season following commencement of the field trial in October 1997 provided many opportunities for germination to occur. There were seven periods where rainfall exceeded 25 mm, some of which were of several days duration. For example, during December 1997, 90 mm fell over a five day period and in the following January the site received some 312 mm over a 12 day period. Similar trends occurred for the 1998/99 and the 1999/2000 wet seasons, with 9 and 11 rainfall events greater than 25 mm recorded, respectively.

Longevity of buried seeds

Germinability and viability of freshly collected rubber vine seed averaged 96% and 97%, respectively. Following placement in the field, viability of all seed lots declined significantly (P <0.05) over time. The most rapid loss in viability occurred under natural rainfall conditions, where no viable rubber vine seeds remained in the soil seed bank after one year, irrespective of burial depth. In contrast, viability of seed lots under rain-shelter conditions averaged 68, 29 and 0% after 1, 2 and 3 years, respectively (Figure 2).

Significant differences (P <0.05) occurred between seed lots located at different depths within the rain-shelter plots. Generally, viability remained highest for longer if seeds were positioned either on the soil surface (bare ground) or buried at 40 cm below ground (Figure 2).

Despite the presence of structures over the rain-shelter plots, soil temperatures were not significantly different (P <0.05) between natural and rain-shelter treatments, averaging 25.1 ± 1.2°C and 23.4 ± 1.3°C, respectively. Whilst the shelters were designed to exclude rainfall, a small number of seeds buried at 0 cm on bare ground, 0 cm below mulch and 5 cm below ground germinated, through subsoil moisture movement. Damaged seeds recovered under rain-shelter conditions were either predated or decomposed by soil micro-organisms. In contrast, under natural conditions all seeds located on the soil surface or buried to a depth of 5 cm germinated. Those buried at greater depths were either predated or decomposed by micro-organisms. Empty seed shells indicated that seeds germinated, whereas seeds that were partially damaged or completely transformed to dirt indicated that they were either predated or decomposed by micro-organisms.

Longevity of aged-seeds

Seed age significantly (P <0.01) affected germination and viability of rubber vine seeds kept under dry storage. Germination of 1 year old seed was extremely high (99%) and not significantly different to that of freshly collected seed (Figure 3). In comparison, germination of 9 and 11 year old seed averaged 73%, and only 16% of 20 year old seeds germinated. Similar trends occurred for viability, but not all age groups exhibited similar seed dormancy. Fresh seeds were least dormant (1%) followed by 9 and 11 year old seeds (3%) with 20 year old seeds exhibiting

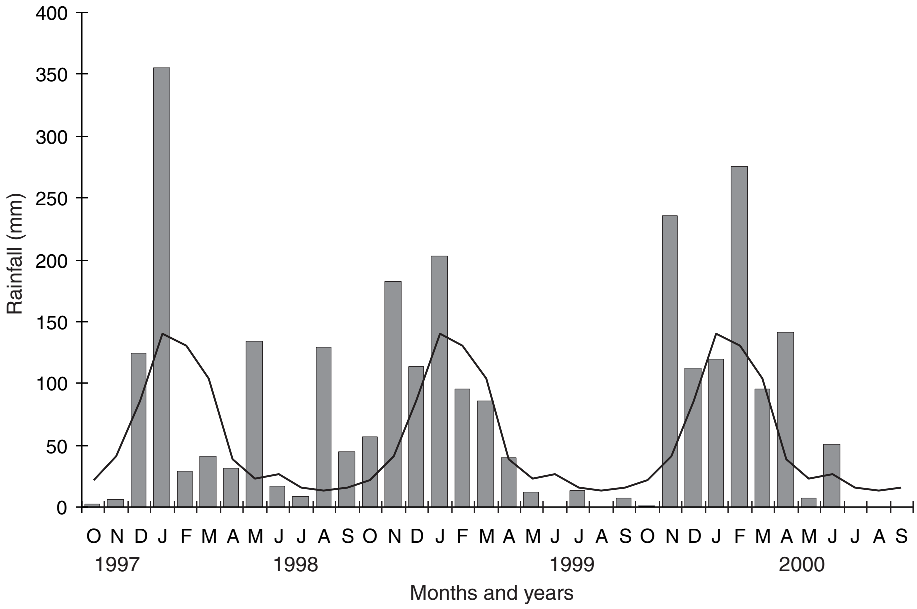

Figure 1. Monthly rainfall from October 1997 – September 2000 recorded at 10 Mile Creek and long-term mean monthly rainfall (104 years) at Charters Towers.

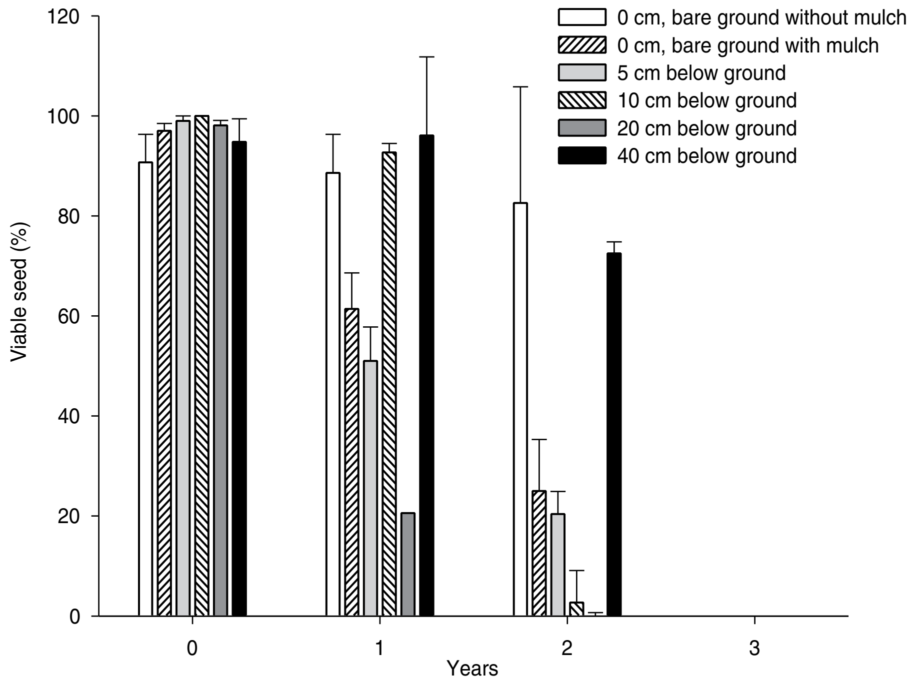

Figure 2. Viability of rubber vine seeds when tested fresh (0 years) and after exposure for 1 or 2 years on bare ground, at the soil surface on mulched ground, or buried 5, 10, 20 or 40 cm below ground, under rain-shelter. Bars indicate the s.e. of the mean.

maximum dormancy (4%). Germination rates of fresh and 1 year old seeds were, on average, 3.2-fold faster than 9 and 11 year old seeds and 55-fold faster than 20 year old seeds, respectively (Figure 4).

On average, 99% of all viable seed had sufficient vigour to develop into seedlings, irrespective of age.

Discussion and conclusions

The results from this study suggest that the soil seed bank of rubber vine may be short-lived (<12 months) when environmental conditions are conducive for seed germination. In contrast, under moisture limiting conditions, where germination is prevented, longevity of the seed bank could be extended to in excess of two years. The findings under natural conditions concur with those reported by Grice (1996) and Dale (1980) who both suggested that the existing seed bank would be depleted after 12 months. High germinability of fresh seeds inevitably leads to the majority of seeds germinating following rainfall. Anecdotal evidence indicates that for many exotic woody weeds at least 25 mm of rainfall is necessary before germination will occur in the rangelands of northern Queensland. During the trial period there was numerous opportunities for seeds to germinate within the natural rainfall treatment, with seven rainfall events exceeding 25 mm in the first wet season alone.

Under rain-shelter conditions it took three years before the seed bank was totally depleted, even though some germination occurred under the shelters. Plots were prevented from receiving direct rainfall but some buried seed lots received adequate moisture through sub-soil movement to allow a small percentage of seeds to germinate. It is possible that seeds could survive for even longer under drier conditions in the field, as occurs during extreme droughts. If further studies were considered warranted, an improvement in the rain shelter methodology would be necessary, and could involve increasing the size of the rain-shelters and establishment of a border drain around the perimeter.

While the current study exposed rubber vine seeds to just two of many possible scenarios that they could face under natural conditions, some management implications can be derived. The highly germinable nature of fresh rubber vine seed suggests that in order for rubber vine to consistently maintain a viable soil seed bank under conditions of average rainfall or above, it would need a continual input of seeds into the soil from fruiting of mature plants. This lack of a persistent seed bank is potentially a major weakness of rubber vine and provides an opportunity to focus control and research efforts into techniques that reduce the input of seed into the soil seed bank.

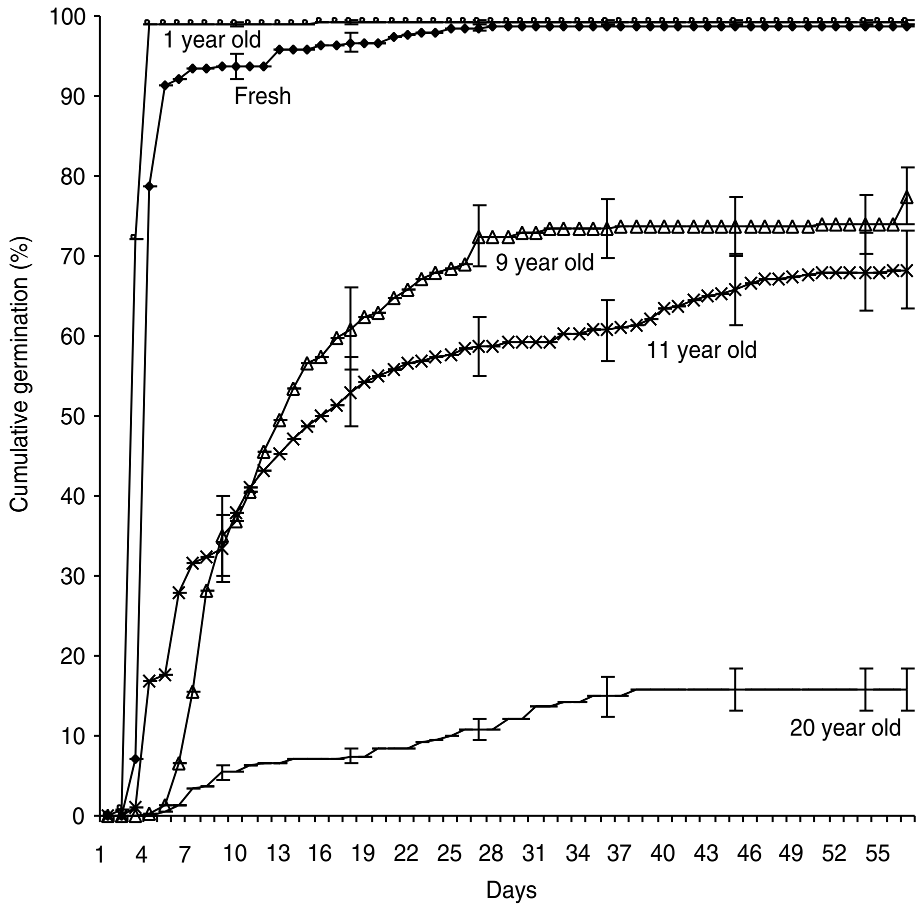

Figure 3. Cumulative germination of rubber vine seeds when tested fresh or after 1, 9, 11 or 20 years of dry storage. Bars indicate the s.e. of the mean.

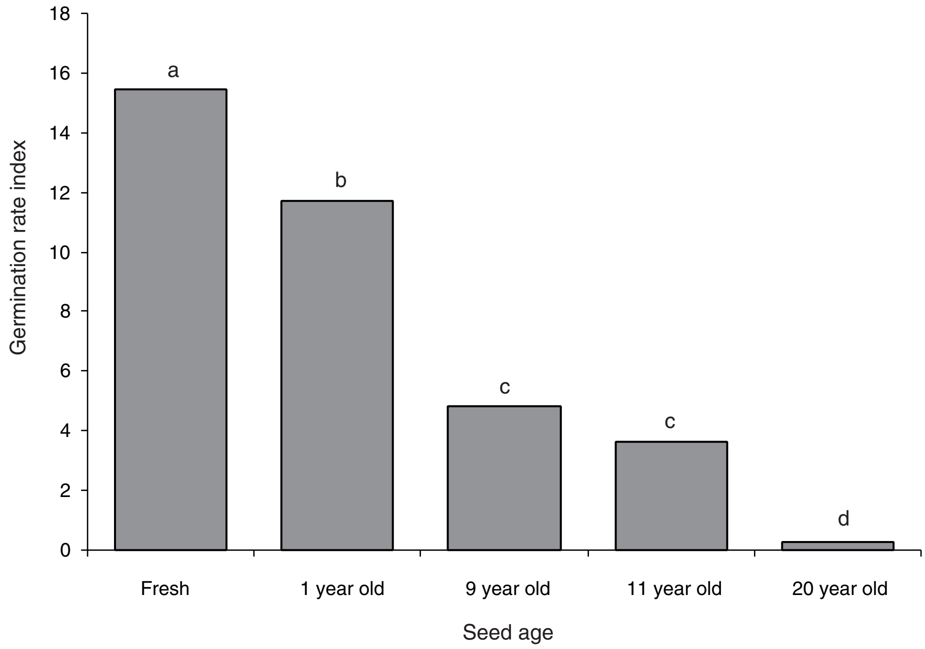

Figure 4. Germination rate of rubber vine seeds tested when fresh or after 1, 9, 11 or 20 years of dry storage.

In recent years, a rust (*Maravalia cryptostegiae*) introduced from Madagascar has proven very effective in reducing the reproductive output of rubber vine plants in most areas of Queensland (Vogler *et al.* 2002). Anecdotal evidence suggests that the only areas where it does not appear to be particularly effective are the drier regions of north-western Queensland. Some preliminary results from sites located within central and northern Queensland have found that since the release of the rust in 1995, seedling recruitment has depleted markedly (Vogler *et al*. 2002). This is encouraging news for land managers, as it means that provided initial control of infestations achieves high mortality, subsequent seedling establishment should be minimal.

In areas where biological control is not effective, it is highly likely that large numbers of viable rubber vine seed will be present in the soil seed bank. Eradicating rubber vine under these situations will require diligence in controlling seedlings following treatment of the initial infestation.

Previous research has found that it takes between 200 and 500 days, depending on rainfall, before a newly emerged seedling is capable of producing pods (J.S. Vitelli unpublished data). Therefore, under average rainfall conditions, delaying follow-up control of seedling regrowth to around 1.5 years after initial control should be an effective strategy for achieving long- term management of rubber vine. By this time the seed bank should have declined to zero, and seedling regrowth would not yet have commenced producing pods. If extremely wet conditions prevail after implementation of control treatments, follow-up control may need to be brought forward. Conversely, if extremely dry conditions prevail, land managers need to be aware that the seed bank will persist for longer.

The extended longevity of rubber vine when held in dry storage has implications for research facilities and nurseries that may have old stores of seeds. In the current study, high viability was still evident after 11 years and a small percentage of viable seeds were present 20 years after storage. The majority of these seeds were germinable and capable of developing into seedlings, irrespective of age. Some of the variation between seed ages could possibly be attributable to the environmental conditions under which the seed lots matured and not just the length of storage. Whilst all seed lots were collected in close proximity to each other and held once collected under similar storage conditions, because of differences in time of collection they could have experienced different environmental conditions. The literature reports instances where studies have found that the conditions under which seeds mature can affect germination (Roberts and Boddrell 1984). Nevertheless, procedures that ensure that seeds are destroyed at the end of their time in storage should be implemented.

In conclusion, this study has identified a weakness in the life cycle of rubber vine that land managers can exploit if they wish to effectively manage the threat imposed by this exotic weed. Commitment to follow-up control for the first few years following control of original infestations should be sufficient to run down the seed bank to a negligible level. However, eradication of a localized infestation does not necessarily mean that the threat of rubber vine is over. Rubber vine is capable of long-distance dispersal of its seed (Parsons and Cuthbertson 2001), so there is a risk of re-establishment even if the local seed reserves are depleted and all plants killed.

Acknowledgments

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