Effect of dry heat on germination and viability of *Cryptostegia grandiflora* seeds

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Summary

The present study described the effect of six dry heat intensities (28, 40, 60, 80, 100, and 200°C) and eight heat durations (0.5, 1, 2, 3, 6, 12, 24, and 36 min) on germination, viability, and germination rate of one-year-old seeds of the invasive weed rubber vine (*Cryptostegia grandiflora* R.Br.). Heat induced seed mortality is of significance to land managers, especially in pastoral areas, where prescribed burning is used to control rubber vine. There was a highly significant interaction between heat intensity and heat duration on germination, viability and germination rate of rubber vine seed. Seed germination was reduced once temperatures reached 80°C for more than 24 min. Raising the temperature to 100°C completely inhibited germination at 6 min and induced a total kill of seeds at 24 min. Further increases in dry heat to 200°C advanced inhibition of germination to 1 min and induced total kill of seeds at 3 min. Germination rate at 80, 100, and 200°C slowed down by 42, 65 and 91% of the original rate respectively. Above 80°C a negative correlation was detected between (i) germination response and heat duration, (ii) seed viability and heat duration, and (iii) germination rate and heat duration whereas seed viability was positively correlated with seed germination. The apparent tolerance to high temperature in rubber vine seeds indicates that high-intensity fires may be a requirement for maintenance of an effective seed management strategy in rubber vine infested habitats.

Introduction

Rubber vine (*Cryptostegia grandiflora* R.Br.) is an invasive woody weed of the dry tropics of Queensland, Australia. It predominantly infests riparian habitats but can also infest open pastures where the water table is high (Grice 1996). It can set seed at least twice a year and produce more than 8 000 seeds per large shrub (3–4 m high) in a single reproductive episode (Grice 1996). Individual seeds of rubber vine weigh 9.7±0.7 mg and have a surface area of 8.7±0.2 mm^2 (mean±SE, n = 250; Bebawi unpublished data).

Dense infestations of rubber vine cause severe management problems at the grazing property level. These problems include a reduction in pasture productivity and increased costs through difficulties in mustering and cattle deaths due to poisoning (McGavin 1969). The cost to primary industry in the State of Queensland caused by rubber vine has been estimated at 18 million dollars per annum (Mackey 1996).

Grice (1996) suggested that most seeds of rubber vine have no dormancy and few, if any, survive for more than 12 months in the soil. Mechanical control methods used in management of rubber vine are likely to bury rubber vine seeds. Heavy-duty blade ploughs and bulldozers are common implements used in central Queensland for mechanical control of rubber vine infestations (Vitelli 1992a).

However, in the dry tropics of north Queensland where dense infestations of rubber vine occur, fire is the most common and most cost-effective management strategy. Fire can kill 19–96% of rubber vine plants depending on size and density of plants, fuel load and distribution, and location of the infested site (Dale 1980, Vitelli 1992b, Grice 1997). Rubber vine seeds buried in the topsoil or deposited on fuel-free gaps may escape the direct effect of fire but may not escape its indirect effects especially radiant heat and smoke.

Although literature on direct and indirect impacts of fire on seeds of rubber vine is very scarce it suggests that seeds buried at 0 cm and 2 cm depth may often survive the hottest grass fires (Brown *et al.* 1996). At 0 cm and at 2 cm below ground, temperatures of about 148–654°C and 36–50°C were recorded with five fuel loads ranging between 3500 and 15500 kg dry plant material less than 5 mm in diameter. The viability of unheated seed was 91%. Seeds left on the ground surface had 31% viability under the lowest fuel load. Seeds that were buried at a depth of 2 cm had viability similar to the controls (Brown *et al.* 1996).

Many studies have concentrated on determining whether heat is required to promote germination, however, studies on responses of species to heat beyond levels required to induce germination are scarce (Mbalo and Witkowski 1997). An understanding of the potential impact of heat on rubber vine seeds may provide an indication of the latent risk in those seeds as a source of new infestation. Heat is one component of fire. The present study examined the effects of different dry heat intensities and heat durations on germination behaviour of rubber vine seeds.

Materials and methods

Experimental design

A 6 × 8 factorial experiment replicated three times using a split-plot design was conducted at the Tropical Weeds Research Centre, Charters Towers, northern Queensland. Main plot treatment comprised six heat intensities (28, 40, 60, 80, 100 and 200°C) and sub-plot treatment comprised eight heat durations (0.5, 1, 2, 3, 6, 12, 24 and 36 min). All treatments for the one temperature were treated at once. The 28°C treatment was selected as the control temperature because optimal germination of rubber vine seed was reported to occur at this temperature (Grice 1996). The dry heat intensity and heat duration combinations used in this experiment were chosen to simulate the effects of heat that may occur during fire regimes in areas where rubber vine is present. They do not represent extremes in heat intensities but they are within the wider range of heat intensity types (40–800°C) that may be encountered across fire regimes in northern Australia (Brown *et al.* 1996, Lonsdale and Miller 1993).

However, it may be argued that under field conditions heat in the soil during a fire is not dry heat as it contains water that must be effectively boiled out of the soil before temperatures can reach over 100°C. This may be true but best fire practice used in the management of rubber vine in the dry tropics of north Queensland occurs during the dry season when soil moisture conditions are minimal. Soil moisture conditions at the soil surface (0–5 cm) during a late dry-season fire that was lit to control rubber vine infestations at 10 Mile Creek ranged between 1 and 3% (Bebawi unpublished data). This soil moisture content may not suggest absolute dry soil conditions but is indicative of reasonably dry soil conditions.

The rubber vine seeds used in the present study were collected from one-year-old ripe pods that had been stored under laboratory conditions (23±2°C) until needed. Moisture content of the seeds averaged 0.27%. Experimental units comprised 20 rubber vine seeds enclosed in perforated cubicles of (4 × 4 × 0.5 cm) aluminium wire mesh gauze. Seed samples were placed in the middle of a forced draught oven for the predetermined temperature / duration combinations. Because oven temperature fell by 6±1ºC for approximately four seconds due to opening of oven door at treatment initiation, treatment duration was extended for four seconds after treatment initiation to compensate for initial loss of heat intensity and heat duration interval. Treated seeds were placed in 9 cm petri dishes filled with 10 mL of distilled water. Petridishes were randomly stacked in containers across all heat intensity and duration treatments, 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 a controlled environment glasshouse set at a 12 h / 12 h temperature of 30±1°C and 20±1°C, respectively. Germinated 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 viability using the tetrazolium method (Moore 1985). Viable seeds were pink when cut longitudinally with a sharp scalpel. Seed viability was expressed as the total seed less non-viable seeds.

Data analysis

Cumulative germination percentages were calculated on the basis of total seed numbers. The germination rate index was calculated by dividing the number of germinated seeds obtained at each daily counting in the standard germination test by the number of days seeds had been in the petri dish. The values obtained at each count were then summed at the end of the germination test to obtain the germination rate index (Maguire 1962). Statistical analysis was performed on arc sine transformed data that was later back-transformed. Regression analysis was also used to relate both germination response and germination rate to heat intensity and heat duration values.

Results

There was a highly significant interaction (P>0.01) between heat intensity and heat duration on germination, viability and germination rate of rubber vine seed (Table 1). The effects of the heat intensity were not equally affected by heat duration. Seed germination was significantly reduced after six minutes at 80ºC, after only two minutes at 100ºC, and after 30 seconds at 200ºC (Table 2). Seed viability was not affected by up to 36 minutes at 80ºC, but was significantly reduced by three minutes at 100ºC and by one minute at 200ºC (Table 3). Germination rate was significantly reduced after three minutes at 80 or 100ºC, or after 30 seconds at 200ºC (Table 4). Seed germination was positively correlated (r^2 = 0.99) with seed viability over all heat intensity and duration treatments.

Discussion

The present results demonstrated that rubber vine seeds were vulnerable to heat intensities of 100°C and above. Similar findings have been reported for other species, although some exhibit a much greater tolerance to dry heat exposure (Auld and O'Connell 1991). For example, whilst all rubber vine seeds were killed from exposure to 100°C for 24 min, seeds of *Acacia myrtifolia, Sphaerolobium viminium* and several species of *Pultenaea* survived more than 2 h at 100°C (Auld and O'Connell 1991). Differences in response to heat between rubber vine seeds and those reported for these species may be attributed to differences in seed size and hard-seededness. Differences in tolerance to high temperatures (dry heat) between seeds of *Acacia karroo* and those of *Chromolaena odorata* (siam weed) were also attributed to seed size (Mbalo and Witkowski 1997).

Table 1. Statistical analysis of effect of dry heat on germination and viability of *C. grandiflora* seeds.

Source of variance	df	Mean squares		
		Germination	Viability	Germination rate
Replicates	2	0.016	0.035	0.396
Heat intensity (HI)	5	7.960**	7.99**	330.180**
Error (a)	10	0.018	0.019	0.722
Heat duration (HD)	7	0.926**	0.820**	29.700**
HI x HD	35	0.385**	0.360**	17.083**
Error (b)	84	0.028	0.014	0.438
Total	144			

** Significant at P<0.01 level of significance.

Table 2. Germination (%) of *C. grandiflora* seeds as affected by intensity and duration of dry heat.

Heat intensity (°C)[1]	Heat duration (min)[A]							
	0.5	1	2	3	6	12	24	36
28	99.4	100	97.8	100	99.4	99.4	99.4	99.4
40	100	99.4	100	97.8	100	99.4	98.9	99.4
60	100	99.4	100	100	100	100	100	98.9
80	100	100	99.4	100	95.5*	92.4*	82.8*	75.5*
100	100	100	99.4	5.0*	0*	0*	0*	0*
200	97.7*	0*	0*	0*	0*	0*	0*	0*

[A] Means followed by an asterix are significantly (P>0.05) affected by heat intensity and heat duration treatments.

Table 3. Viability (%) of *C. grandiflora* seeds as affected by intensity and duration of dry heat.

Heat intensity (°C)[1]	Heat duration (min)[A]							
	0.5	1	2	3	6	12	24	36
28	100	100	99	100	99	100	100	99
40	100	100	100	99	100	99	99	99
60	100	100	100	100	100	100	100	99
80	100	100	100	100	99	99	99	100
100	100	100	100	30*	8*	2*	0*	0*
200	99	29*	18*	0*	0*	0*	0*	0*

[A] Means followed by an asterix are significantly (P>0.05) affected by heat intensity and heat duration treatments.

Table 4. Germination rate index of *C. grandiflora* seeds as affected by intensity and duration of dry heat. The higher the index values the faster the germination rate.

Heat intensity (°C)[1]	Heat duration (min)[A]							
	0.5	1	2	3	6	12	24	36
28	9.8	9.8	9.6	10.0	9.7	9.8	9.8	9.8
40	9.0	9.0	9.0	9.7	10.0	9.8	9.7	9.8
60	9.0	9.0	9.0	10.0	9.9	9.8	9.8	9.6
80	9.1	9.0	9.1	7.5*	3.5*	2.6*	2.7*	2.4*
100	9.1	9.0	9.1	0.2*	0*	0*	0*	0*
200	7.1*	0*	0*	0*	0*	0*	0*	0*

[A] Means followed by an asterix are significantly (P>0.05) affected by heat intensity and heat duration treatments.

It is interesting to note that above 80°C, viability of rubber vine seeds declined with increasing heat duration whereas that of *A. karroo* seeds declined with increasing duration of exposure at 50°C (Mbalo and Witkowski 1997). Differences in seed weight and structure (hard seededness) of rubber vine (9.7±0.7 mg) and *A. karroo* (63.0±7.0 mg) do not give a rational explanation for differences in viability response of these two species to heat durations at these two heat intensity treatments. Factors other than seed weight and structure may give a rational explanation to differences in thermo-tolerance between seeds of different species. For example, chemical composition of seeds may play a predominant role in thermo-tolerance of seeds. It is possible that the latex component found in the seed structure of rubber vine may confer some kind of thermo-tolerance (chemical defence) to prolonged heat durations of high heat intensities. It is common knowledge that rubberized surfaces or structures serve as good insulators against heat (Choi *et al*. 2000, Ludowici 2000, Mimura and Fujioka 2000).

The location of rubber vine seed in the soil profile will largely determine what temperature seeds are exposed to and consequently how many seeds would remain viable following burning. Soil is an excellent insulator (Bradstock *et al*. 1992), with any seeds becoming buried having a much greater chance of survival (Daubenmire 1968). Bradstock *et al*. (1992) found that during burning of a hummock grassland containing a fuel load of 3850 kg ha^{-1}, at no soil depth did temperatures exceed 120°C. Similarly, Tothill and Shaw (1968) found temperatures at 1.25 cm seldom exceeded 65–75°C with near normal temperatures being restored within 4 min. Temperatures lower than that reported by Tothill and Shaw (1968) were recorded at 2 cm depth (36–50°C) for a shrub fire used to control rubber vine plants (Brown *et al*. 1996). Where rubber vine seed is located within greater depths other than that reported by Brown *et al*. (1996) at the time of burning has not been quantified to date.

Since the seed bank of rubber vine has not been quantified to date, buried seeds may not only escape exposure to high temperatures but may also keep surfacing as a result of the dynamic nature of physical events and ongoing mechanical clearing of rubber vine infestations. Eventually this process may contribute to repeated re-infestation of cleaned-up land in spite of the fact that rubber vine seeds are short-lived. Furthermore, the annual production of massive numbers of fresh seeds from rubber vine plants not killed by fire would still pose an on-going threat of a re-infestation problem.

Conclusion

The present study concludes that rubber vine seeds have the ability to tolerate dry heat temperatures up to 100°C for 2 min and up to 200°C for 0.5 min. This ability may be of survival value under fire and drought conditions that are likely to occur in the dry tropics of northern Australia particularly where a cool fire is used. However, before any further studies are undertaken on the exposure of rubber vine seeds to higher temperatures than those of the current studies and for shorter durations, studies on viable seed bank of rubber vine need to be determined. This will then provide a better indication of the type of fires necessary to reduce the viable seed bank. Where the seed is located within the soil profile at the time of burning also needs clarification, as this will dictate the temperature regimes that seeds are exposed to.

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References

Auld, T.A. and O'Connell, M.A. (1991). Predicting patterns of post-fire germination in 35 eastern Australian Fabaceae. *Australian Journal of Ecology* 16, 53-70.

Bradstock, R.A., Auld, T.D., Ellis, M.E. and Cohn, J.S. (1992). Soil temperatures during bushfires in semi-arid, mallee shrublands. *Australian Journal of Ecology* 17, 433-40.

Brown, J.R., Grice, A.C., Vitelli, J.S., Lindsay, A.M., Campbell, S.D., Jeffrey, P.L., Kheir, M. and Barker, M. (1996). Managing tropical woodlands to control exotic woody weeds. Final Report, MRC Project CS219, CSIRO Tropical Agriculture, Aitkenvale, Queensland, Australia.

Choi, H.J., Park, S.H., Kim, J.K. and Jun, J.I. (2000). Effects of acrylonitrile content on PC/ABS alloy systems with a flame retardant 2000. *Journal of Applied Polymer Science* 75, 417-23.

Dale, I.J. (1980). Factors affecting the distribution of rubber vine (*Cryptostegia grandiflora* R.Br.) in North Queensland. M.Agric.Sci. Thesis, University of Queensland.

Daubenmire, R. (1968). Ecology of fire in grasslands. *Advances in Ecological Research* 5, 209-66.

Grice, A.C. (1996). Seed production, dispersal and germination in *Cryptostegia grandiflora* and *Ziziphus mauritiana*, two invasive shrubs in tropical woodlands of northern Australia. *Australian Journal of Ecology* 21, 324-31.

Grice, A.C. (1997). Post-fire regrowth and survival of the invasive tropical shrubs *Cryptostegia grandiflora* and *Ziziphus mauritiana*. *Australian Journal of Ecology* 22, 49-55.

Lonsdale, W.M. and Miller, I.L. (1993). Fire as a management tool for a tropical woody weed: *Mimosa pigra* in northern Australia. *Journal of Environmental Management* 39, 77-87.

Ludowici Rubber and Plastics (2000). General properties of elastomers. Web site: http://www.Ludowicirubber.com/elastomer.html.

Mackey, A.P. (1996). Rubber vine (*Cryptostegia grandiflora*) in Queensland. Pest status review series, Land Protection Branch, Department of Natural Resources, Queensland.

Maguire, J.D. (1962). Speed of germination – aid in selection and evaluation for seedling emergence and vigour. *Crop Science* 2, 176-77.

Mbalo, B.A. and Witkowski, E.T.F. (1997). Tolerance to soil temperatures experienced during and after the passage of fire in seeds of *Acacia karroo*, *A. tortilis* and *Chromolaena odorata*: a laboratory study. *South African Journal of Botany* 63, 421-5.

McGavin, M.D. (1969). Rubber vine (*Cryptostegia grandiflora*) toxicity for ruminants. *Queensland Journal of Agriculture Animal Science* 26, 9-19.

Mimura, K.H.I., and Fujioka, H. (2000). Improvement of thermal and mechanical properties by control of morphologies in PES-modified epoxy resins. *Ploymer* 41, 4451-9.

Moore, R.P. (ed.) (1985). 'Handbook on tetrazolium testing'. (International Seed Testing Association, Zurich, Switzerland).

Sen, D.N. (1968). Ecology of desert plants and observations on their seedlings. II. Germination behaviour of seeds in Asclepiadaceae. *Osterreichische Botanische Zeitschrift* 115, 18-27.

Tothill, J.C. and Shaw, N.H. (1968). Temperatures under fires in bunch spear grass pastures of south-east Queensland. *Journal of Australian Institute of Agricultural Science* 34, 94-7.

Vitelli, J.S. (1992a). Mechanical. *In* 'The control and management of rubber vine', ed. B. Shepherd, pp. 57-9. (Queensland Department of Primary Industries, Brisbane).

Vitelli, J.S. (1992b). Fire. *In* 'The control and management of rubber vine', ed. B. Shepherd, pp. 15-17. (Queensland Department of Primary Industries, Brisbane).