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The response of *Nassella trichotoma* (serrated tussock) seeds and seedlings to different levels of fire intensity

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ABSTRACT

Context. Fire is an important disturbance regime in grassland communities, since it is responsible for stimulating the regeneration of many species and for maintaining levels of biodiversity. When invasive plants, such as *Nassella trichotoma*, establish and become widespread in a grassland community, these important fire events can be altered in intensity and frequency, which means that they are able to facilitate the establishment of the exotic species. Therefore, before fire can be recommended as a suitable control technique for invasive species, or alternatively to be integrated into grassland restoration programs, understanding the response of the seeds of exotic species to high temperatures, such as those experienced during a fire, should be well understood. **Aims.** Our aim was to identify their response to a gradient of temperatures associated with different levels of fire intensity. We examined how increased duration of exposure affects their response, and whether seed age or seed moisture content affect the germination response of this species. **Methods.** To gain a fuller understanding of the fire response of *N. trichotoma's* seedbank, seeds were collected in 2016, 2017, 2018 and 2019 and then stored until the commencement of the experiments in 2020. Selected seeds were first subjected to an increasing temperature gradient (80°C, 100°C, 120°C, 140°C, and a control), and an increasing duration of exposure (of 1, 3, 6, and 9 min). In the second experiment, one population was selected to test these same temperatures and duration of exposure after the seeds were hydrated to 15%, 50%, or 95%. Last, seedlings were grown for 3 months under glasshouse conditions and then exposed to increasing temperatures (20°C, 60°C, 80°C, 100°C, and 120°C), and an increasing duration of exposure (3, 6, and 9 min). The seedlings were assessed 2 weeks after the heat exposure for signs of damage. **Key results.** It was found that increased temperatures and duration of exposure had a subtle negative effect on germination parameters, including reduced total germination and increased time to 50% germination. The 140°C treatment was seen to be a significant threshold because it killed all the seeds at any duration of exposure. A significant difference among the ages of each seed lot was observed to be a factor on the tested germination metrics, with the oldest tested population (2016) demonstrating the highest germination percentage, uniformity, and rate. Seed germination percentage was significantly reduced for seeds hydrated to 95% compared with the control treatment, whereas no significant difference was observed for the seeds hydrated to 15% and 50%. For the heat treatment of the seedlings, damage to the leaves was observed in the 80°C, 100°C, and 120°C treatments, with some plants in the 120°C treatment experiencing extensive damage prior to resprouting. No seedlings were killed at the tested temperatures. **Conclusions.** Results of this study indicated that fire may be a useful tool for reducing seedbank density by killing a high proportion of the seeds on the soil surface, or located within the top 1 cm of the soil profile, but not for seeds buried more deeply. Efficacy of fire on surface and shallow-buried seeds is improved with high seed moisture content; however, these seeds buried below this depth are still protected by the soil from the lethal effects of temperature. **Implications.** Fire implemented before seed set could be used to effectively kill a large proportion of *N. trichotoma* seeds. However, for more comprehensive control, it is recommended that chemical treatment is integrated with the fire treatment to improve the overall control efficiency.

Keywords: climate warming, exotic species, fire ecology, grasses, *Nassella trichotoma*, seed germination, serrated tussock.

Introduction

Fire is an important ecosystem disturbance in grassland communities for removing excess biomass and stimulating the regeneration of vegetation (Price *et al.* 2019; Yan and Liu 2021). This mechanism also correlates to improved habitat structure, which maintains important diversity regarding higher trophic levels (Price *et al.* 2019; Beal-Neves, *et al.* 2020). Fire plays a key important role in breaking seed dormancy of many grassland species, through physical scarification of seed coats (Moreira and Pausas 2012), and chemical stimulation from exposure to smoke or charcoal (Franzese and Ghermandi 2011; Nelson *et al.* 2012; Hodges *et al.* 2022). Further, fire parameters, such as heat and smoke, are known to increase germination rate and germination uniformity, which both increase the vigour of seedling emergence (Zirondi *et al.* 2019; Hodges *et al.* 2022).

Increased urban and agricultural expansion into grassland communities has resulted in the reduction of burn frequencies (Florec *et al.* 2020). As a consequence, the biomass of the area accumulates, leading to increased fuel loads, which often results in fire intensities that are higher than those historically recorded (Price *et al.* 2021). The fire regimes in many grassland communities have also been altered through the introduction and spread of invasive plants, and once established, these invasive species are able to alter the fire regimes to facilitate their dominance (Fusco *et al.* 2019). Consequently, if the intensity and/or frequency of fire events are altered, either the germination responses for the native species will not be activated if the intensity and frequency of fire is reduced, or they may be killed by fires that are more frequent or of higher intensity (Fusco *et al.* 2019; Hodges *et al.* 2022). Therefore, understanding how a dominant weed responds to fire events can assist in more effective management control, through the breaking of their dominance cycle and reintroducing fire regimes that represent the ecosystems historical state (Price *et al.* 2021).

Nassella trichotoma (Nees) Hack. ex Arechav. is considered to be one of the most economically damaging invasive plant species in Australia (Campbell and Nicol 1999), New Zealand (Bourdôt and Saville 2019) and South Africa (Joubert 1984). This species has high reproductive output, with an individual plant being able to produce over 100 000 viable seeds per year, with up to 50 000 seeds per square metre being recorded in areas with heavy infestations (Joubert 1984; Campbell and Nicol 1999). Within its native range, a significant proportion of the seeds germinate between autumn and spring, with only approximately 25% of the seeds remaining in the seedbank for longer than 1 year (Garcia *et al.* 2021). Within its invasive range, the seeds have been observed to persist in the soil for up to 3 years (Osmond *et al.* 2008); however, the majority of the seedbank is considered transient and germination will occur within the first 12 months (Ruttledge *et al.* 2020: Humphries and Florentine 2022). Research into developing solutions to reduce *N. trichotoma's* seedbank density and longevity is therefore an important contribution for its long-term control.

One such method that merits exploration is incorporation of prescribed burning in areas invaded by *N. trichotoma*. Fire is an important ecosystem process that can selectively influence the recruitment of plant communities, and understanding how the seedbank of exotic plants responds to fire cues has important implications for their management (Mack *et al.* 2000; Franzese and Ghermandi 2011). If the required temperature to flush the seedbank or devitalise the seeds can be determined, managers can then strategically implement fire accordingly (Vermeire and Rinella 2009; Emery *et al.* 2011; Riveiro *et al.* 2019). It is noted that fire intensity is influenced by the interactions between fuel load and soil moisture, and these factors vary seasonally and across the landscape (Bradstock *et al.* 2010; Kreye *et al.* 2013).

High soil moisture often reduces the intensity of fire, whereas the intensity is significantly increased when dry matter burns (Kreye *et al.* 2013). Soil moisture also has a direct influence on the moisture content of seeds that lack physical dormancy structures (Tangney *et al.* 2018). Physiological processes within the seed become more active with increasing seed moisture levels (Walters *et al.* 2005) and, subsequently, seeds become more susceptible to devitalisation when exposed to high temperatures (Tangney *et al.* 2018). It has been observed that high seed moisture conditions can reduce the seed's ability to resist fire, demonstrating that lower-intensity burns can still provide effective control of invasive plants (Fer and Parker 2005). Therefore, understanding the interactions of seed moisture levels and fire intensity can be important for knowing when to implement strategic burns in weed-dominant areas.

It is known that seedling recruitment increases in volume and uniformity when *N. trichotoma* is exposed to fire (Joubert 1984; Hamilton 2012). Fire has also been observed to destroy approximately 18% of this species' seedbank (Wells and De Beer 1987). Another adaptive trait seen in fire-prone ecosystems is the ability for established plants to re-shoot rapidly after a fire event (Pausas and Keeley 2014). This trait has been observed in *N. trichotoma*, which is a result of the plant's base being well protected by its dense tussock growth form (Osmond *et al.* 2008). Once established, this weed alters fire regimes for its self-facilitation by producing higher than historic fuel loads through slow nutrient cycling and grazing avoidance (Moretto and Distel 2002). Because higher than historic fuel loads negatively affect native seeds and plants during fire events, fire has not been recommended for *N. trichotoma* control programs, despite it successfully flushing the persistent surface seedbank (Osmond *et al.* 2008).

The objective of this paper is to identify the response of *N. trichotoma* seeds of different (1) ages and (2) moisture content levels to an increased time of exposure to radiant heat. The ability of established plants to recover from these factors will also be explored. The findings of this research will provide important information leading to a better understanding of how *N. trichotoma* responds to fire and, consequently, what fire regimes might be useful in particular circumstances.

Materials and methods

Seed collection and preparation

Seeds were collected from mature *N. trichotoma* plants in Mambourin, Victoria, Australia (37°55′18.12″S, 144°32′43.079″E), located within the Greater Western Grasslands in central Victoria, Australia (Fig. 1), in December 2016, 2017, 2018 and 2019. The seeds were stored within their panicles in zip-locked bags at room temperature until use. When the study was about to commence, seeds were removed from the panicles and gently squeezed with forceps to test for seed fill. Seeds that collapsed easily were discarded. Seed mass was determined for each age class by weighing 100 randomly selected seeds from each age group.

Effect of radiant heat on seed germinability

For each treatment combination, 25 seeds were counted and placed into aluminium bowls (10 cm in diameter), and were then exposed to temperatures of either 80°C, 100°C, 120°C or 140°C for 1, 3, 6 or 9 min inside a temperature-controlled oven (Memmert, Type No. ULE500). This multifactor experiment contained four seed ages, four heat treatments and four exposure durations for a total of 64 treatment combinations, plus a control for each seed-collection year. Each treatment combination was replicated six times.

Immediately following heat exposure, seeds were placed into Petri dishes lined with sterilised filter paper and moistened with sterilised reverse osmosis (RO) water, with parafilm being used to prevent drying. The treatments were placed into incubation chambers set to alternating 25°C/15°C, 12-h light/12-h dark conditions, which have been pre-determined as the optimal growth conditions for *N. trichotoma* (Humphries *et al.* 2018). The treatments were checked twice a week for 35 days and germination was determined by protrusion of the radicle.

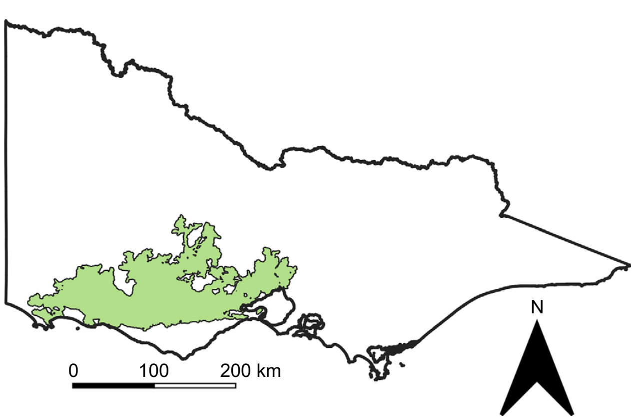

Fig. 1. Map showing seed-collection areas located within the Greater Western Grasslands in central Victoria, Australia.

Effect of moisture content and radiant heat on seed germinability

It was observed that the seed collected in 2016 provided the highest germination across all tested temperatures, and they were therefore preferentially selected for the rehydration treatments. To prepare for the experiments, nylon mesh bags containing filled seeds (determined by the squeeze test described previously) were secured within an airtight polycarbonate electrical enclosure box (28 × 28 × 14 cm) containing LiCl solutions adjusted to either 15% RH (740 g/L), 50% RH (364 g/L), or 95% RH (48 g/L). The relative humidity (RH) levels were selected because these moisture contents are associated with the three distinct seed hydration levels that have previously been shown to promote different physiological activity within the seeds (Walters *et al.* 2005). The boxes were stored at 20°C for 2 weeks, and seed hydration was assessed at the end of this period with a digital hygrometer. After the rehydration period, the seeds were exposed to the methods previously described for heat exposure and subsequent germination monitoring.

Effect of radiant heat on established plants

To assess the effect of radiant heat on established plants, 100 plastic garden pots (140 mm in diameter and 140 mm in height) were lined with absorbent towelling, filled with a fine layer of river sand and then topped with commercially purchased soil. Five viable *N. trichotoma* seeds were placed into each pot and lightly covered with soil. The pots were placed into watertight plastic trays (28 cm × 44 cm × 5.5 cm), and were placed into a glasshouse at ambient temperature on 24 February 2022. Pots were bottom-watered at least once a week and seedlings were thinned to one plant per pot after the first month of growth. The final plants were grown under glasshouse conditions for 3 months.

The heat treatments were conducted on 22 May 2022. To prepare the plants for the heat-exposure experiment, the plant and soil were together carefully removed from the pots and placed into heat-safe aluminium trays. Five plants were randomly selected and exposed to radiant heat levels of 20°C, 60°C, 80°C, 100°C or 120°C for 3, 6 or 9 min. The plants were then re-potted and monitored for a further 2 weeks under glasshouse conditions, when any signs of wilting or recovery were visually observed.

Data analysis

Effect of radiant heat on seed germinability

To determine whether the collection year, the temperature or the duration of exposure had a significant influence on total germination, the data were tested using a three-way ANOVA by using the statistical analysis program SPSS (IBM). This test accounted for all possible interactions of these factors, and the significance was set to 0.05.

The total germination percentage, the mean germination time (MGT), germination index (GI) and time to 50% germination (T_{50}), were calculated using the following formulae:

1. Mean germination time was calculated using the equation

$$\text{Mean germination time } = \Sigma D_n/\Sigma n$$

where n is the number of seeds, which were germinated on Day D_n, and n is the number of days counted from the beginning of germination (Sadeghi *et al.* 2011).

2. The germination index was calculated as described in the Association of Official Seed Analysis (AOSA 1993) by following the formula:

$$\text{No. of germinated seeds/day of first count } + \ldots + \text{ No. of germinated seeds/day of final count}$$

3. The time to reach 50% germination was calculated using the formula as described by Kader (2005), as follows:

$$\text{Time taken to reach 50\% germination } (T_{50}) = t_i \frac{(\frac{n}{2} - n_i)(t_j - t_i)}{n_j - n_i}$$

where n is the final number of germinated seeds, and n_j and n_i are the cumulative number of seeds germinated by adjacent counts at Times t_j (day) and t_i (day) respectively, when $n_i < n/2 < n_j$.

Effect of moisture content and radiant heat on seed germinability

The germination totals were tested for significant interactions by using a three-way ANOVA, by using the statistical analysis program SPSS (IBM®) to determine the effects of hydration level, temperature, duration of exposure and all possible interactions of these factors.

Effect of radiant heat on established plants

A mean damage score was calculated for each experiment to assess the condition of the plants. The damage scores were determined as follows: (1) 0 = no damage, (2) 1 = some dryness observed on only the tips of the leaves, or (3) 2 = extensive drying observed to the leaves.

Results

Climate conditions for each collection year

Variations in the seed mass were observed among the collections made across the different years, with the highest seed mass being recorded for the seeds collected in 2016, which correlated with optimal germination parameters (Table 1). In 2016, the average spring maximum monthly temperature was lower than in the following years, and the total rainfall was much higher during the same period, and these factors may have contributed to the higher seed mass than for the seeds collected in 2017–2019. The higher average spring temperature may have been a leading cause for the reduced seed mass for those collected in 2017, whereas the low total spring rainfall may have contributed to the low seed mass in the seeds collected in 2019.

Table 1. The total rainfall (mm) and average maximum temperatures (°C) for the spring months (September–November) during 2016–2019, together with the seed mass observed for each collection.

Collection year	Spring rainfall (mm)	Average spring temperature (°C)	Seed mass (g)
2016	178.8	19	0.0709
2017	95.2	21.7	0.0429
2018	76	20.9	0.0641
2019	43.7	20.4	0.0481

Note: data were sourced from Mount Rothwell weather station, which is located approximately 10 km from the seed-collection site (Bureau of Meteorology 2021).

Effect of radiant heat on seed germinability

The effect of temperature ($P < 0.001$), duration of exposure ($P < 0.001$), and seed-collection year ($P < 0.001$) had a significant impact on *N. trichotoma's* total seed germination (%), as did the interactions of these factors (Table 2). The control experiment (no heat) demonstrated that seeds collected in 2016 had a significantly higher total germination percentage than did the seeds collected in more recent years, and the germinability of the seeds appeared to increase with the age of collection, with the total germination percentage increasing with the age of the seeds (Table 3).

Total germination percentage and GI decreased as the temperature gradient increased, whereas the effect of the duration of the heat exposure had a lesser effect on these factors (Table 2). A similar observation was made for mean

Table 2. The effect of increased radiant-heat exposure on the germination of *N. trichotoma* seeds of increasing age.

Factor	d.f.	*F*	*P*-value
Population	3	65.41	<0.001
Temperature	3	395.12	<0.001
Time	3	6.49	<0.001
Population × temperature	9	17.27	<0.001
Population × time	9	2.58	0.007
Temperature × time	9	3.91	<0.001

Significance was set to 0.05.
d.f., degrees of freedom.

Table 3. Germination percentage (Ger %), germination index (GI), mean germination time (MGT), and time to 50% germination (T_{50}) after increased exposure to radiant heat for four populations of *N. trichotoma* seeds.

Population	Temperature (°C)	Time (min)	Ger (%)	GI	MGT	T_{50}
2016	Control	0	91.33	2.74	9.18	7.62
	80	1	100	3.50	7.36	6.52
		3	94.67	3.20	7.61	6.60
		6	74.67	2.38	9.82	6.76
		9	98.67	3.26	8.06	6.62
	100	1	100	3.08	8.80	7.58
		3	98.67	2.33	11.86	11.19
		6	99.33	2.61	10.74	10.03
		9	99.33	2.32	11.57	11.43
	120	1	84.00	1.33	17.06	16.29
		3	84.00	1.32	18.48	17.19
		6	86.00	1.48	15.14	13.63
		9	86.67	1.28	18.47	14.75
2017	Control	0	78.67	2.05	11.84	7.78
	80	1	78.67	2.32	9.31	7.91
		3	78.00	1.15	12.85	10.64
		6	46.00	2.31	10.28	9.16
		9	85.33	1.91	10.76	8.01
	100	1	68.00	2.06	9.94	8.89
		3	68.00	1.20	9.47	7.79
		6	39.33	0.86	16.16	9.52
		9	31.33	0.76	16.16	14.08
	120	1	34.00	1.23	17.78	16.33
		3	82.00	0.92	18.75	17.49
		6	66.67	1.28	16.80	16.13
		9	80.67	0.77	19.63	17.71
2018	Control	0	75.33	1.28	17.03	13.37
	80	1	59.33	1.15	14.62	13.88
		3	68.00	1.49	13.38	9.78
		6	50.67	0.88	16.84	13.97
		9	72.00	1.55	13.99	12.49
	100	1	68.67	1.32	15.35	14.48
		3	80.67	1.68	13.88	13.48
		6	66.00	1.39	15.15	12.52
		9	75.33	1.35	16.66	13.65
	120	1	74.00	1.12	18.19	16.64
		3	30.67	0.38	20.86	19.50
		6	40.00	0.44	22.27	21.86
		9	58.67	0.62	24.00	22.19
2019	Control	0	53.33	1.09	14.97	13.01
	80	1	62.67	1.23	13.79	11.64
		3	42.67	0.58	19.40	17.24
		6	16.67	0.21	22.14	20.55
		9	65.33	1.22	14.66	12.65
	100	1	44.00	0.48	25.05	22.68
		3	62.00	0.99	19.55	17.94
		6	64.00	1.28	14.42	11.85
		9	70.00	1.40	16.40	13.01
	120	1	68.00	0.85	21.12	18.42
		3	34.67	0.35	27.79	27.63
		6	38.00	0.45	25.20	21.69
		9	8.00	0.07	0	34.50

(*Continued on next column*)

Table 3. (*Continued*).

Note: the 140°C treatment was not included in this Table as germination was insufficient to conduct analysis.

germination time (MGT) and time to 50% germination (T_{50}), with these parameters increasing when exposed to the higher temperature gradients. The two older seed collections, namely from 2016 and 2017, reached 50% germination sooner than the more recently collected seeds in 2018 and 2019. In most cases, the heat treatments increased *N. trichotoma* germination percentage compared with the germination percentage recorded for the control treatment. For all the seed-collection times, the 140°C temperature was significant at reducing total seed germination percentage, with the exception of only 0.64% of the seeds germinating in the 6- and 9-min treatments for the seeds collected in 2018. The seeds collected in 2019 had significantly reduced viability when exposed to 120°C for 9 min; however, this decline was not observed for the other treatments. There are variations in total germination percentage for the 80°C, 100°C and 120°C treatments for all the collected seeds, with 2016 having the highest consistency.

Effect of moisture content and radiant heat on seed germinability

As the seeds collected in 2016 provided the highest total germination percentage under controlled testing, as well as providing the most uniformed germination percentage across all temperatures, these seeds were selected for the subsequent tests.

The seed moisture content ($P < 0.001$), temperature ($P = 0.002$), and their interaction ($P < 0.001$) were significant factors affecting *N. trichotoma* total seed germination percentage (Table 4). Seeds hydrated to 15% had no significant difference in germination percentage when exposed to 80°C, 100°C or 120°C, and the first two temperatures

Table 4. The effect of increased radiant-heat exposure on the germination of *N. trichotoma* seeds rehydrated to 15%, 50% and 95%.

Factor	d.f.	*F*	*P*-value
Seed hydration	2	5463.92	<0.001
Temperature	2	6.59	0.002
Time	3	0.38	0.761
Seed hydration × temperature	4	11.52	<0.001
RH% × time	6	0.65	0.687
Temperature × time	6	0.21	0.975
Seed hydration × temperature × time	12	0.83	0.614

Significance was set to 0.05.
d.f., degrees of freedom.

increased germination percentage when compared with the control (Table 5). The seeds hydrated to 50% experienced a significant decline in total germination percentage when the seeds were exposed to 120°C for 9 min ($P = 0.002$).

Table 5. The germination response of hydrated *N. trichotoma* seeds to radiant heat.

Hydration	Temperature (°C)	Duration (min)			
		1	3	6	9
15%	80	90.66	96	96	94
	100	99.33	100	98.66	97.33
	120	97.33	90.66	90.66	90.66
50%	80	100	96	90.66	98
	100	99.33	94.66	96	98.66
	120	88	88.66	88.66	81.33
95%	80	4.6	0	0	0
	100	0	0	0	0
	120	0	0	0	0

Note: the seeds were hydrated to either 15%, 50%, or 95% and then exposed to one of three temperatures, namely 80°C, 100°C, and 120°C, for 1, 3 6, or 9 min. The control treatment of no heat or hydration pre-treatment and recorded 91.33% total germination.

The exposure to heat was lethal to seeds with moisture contents of 90%, with only 4.6% of the seeds germinating after 1 min of exposure to 80°C. No further germination occurred for seeds hydrated to 90%.

Effect of radiant heat on seedlings

All the plants exposed to the temperatures of 20°C and 60°C were undamaged, and the leaves remained a vibrant green from the base to the tip. At the higher temperatures tested, namely 80°C, 100°C, and 120°C, notable drying of the leaves was observed, but the base of the plant remained undamaged. Four plants exposed to the 120°C treatment (two exposed for 6 min, and two exposed for 9 min) suffered extensive drying, but these plants resprouted from the base. Fig. 2 shows the visual differences among the damage scores of 1 (undamaged), 2 (leaf drying) and 3 (extensive leaf drying and resprouting). No plants were completely killed from the trialled heat treatments.

Discussion

Seed germinability

Our results observed a significant increase in germination percentage for the seeds collected in 2016 compared with those from the following collection years, with the most recent seeds, namely those collected in 2019, demonstrating the lowest germination percentage. In many cases, seed viability and seedling vigour decline as time in dry storage increases (De Vitis *et al.* 2020), making the results of the present study controversial. Studies into *N. tricotoma's* seed longevity under field conditions suggest that the majority of this species seedbank will germinate within the first year of seed set, with only small proportion (<10%) demonstrating short-term persistence of up to 3 years (Ruttledge *et al.* 2020; Humphries and Florentine 2022). However, under stored conditions, 20-year-old seeds of this species have been observed to germinate successfully (Taylor 1987). This suggests that dry storage does not have a significant impact on *N. trichotoma* seed viability.

Fig. 2. (L → R) Visual differences among the damage scores of 1 (undamaged), 2 (leaf drying) and 3 (extensive leaf drying and resprouting).

The complex interactions of various biotic and abiotic pressures experienced by the maternal environment during seed development can result in interannual variations in seed traits (Franzese and Ghermandi 2011; Pearse *et al*. 2017). Various environmental factors, such as temperature and rainfall that a plant experiences during its seed-development stage, can directly influence the progeny seeds dormancy and germination potential (Penfield and MacGregor 2017; Souza *et al*. 2019). For example, the combination of high temperature and osmotic stress reduce the production of viable seeds of the invasive annual grass *Avena fatua* (Peters 1982). Plants exposed to higher rainfall and soil nutrients will often allocate more resources to reproduction, resulting in higher seed output and seeds of greater mass and size (Salisbury 1943; Lazaro-Nogal *et al*. 2015; Souza *et al*. 2019). The substantially higher spring rainfall experienced in 2016 than that in the following years, may have been one of the factors contributing to the higher seed mass observed for this collection year. Increased seed mass can often result in more vigorous germination and seedling emergence, providing these seeds with a competitive advantage in this critical life stage (Vaughton and Ramsey 2001; Paz and Martinez-Ramos 2003).

The seeds collected in 2016 had greater average seed mass than did those collected in the subsequent years, which was a significant issue for the investigation because increased seed mass can improve the total germination percentage and germination time, together with improving the success of the seedling survival (Bonfil 1998; Cordazzo 2002). The results of this study support the contention that greater seed mass increases total gemination percentage and germination time compared with seeds with a reduced mass. Additionally, an inverse relationship in seed size and seed longevity has been observed for several grassland species, including *Ambrosia trifida* (Schutte *et al*. 2008), whereby the smaller seeds persist longer in the seedbank than do larger, denser seeds. It is possible that *N. trichotoma* has adopted a similar strategy in the present study, and the lighter seeds collected in 2019 may have a higher proportion of dormant seeds, therefore reducing the germinability of this sample.

Understanding the variations in the degree of dormancy a population displays has important implications on the populations ability to successfully establish and persist (Baloch *et al*. 2001). It is known that *N. trichotoma* experiences a primary dormancy period that extends for approximately 3 months, which prevents the seeds from germinating in the water-limited summer months (Osmond *et al*. 2008). This species has also demonstrated the capacity to undergo secondary dormancy, which is induced when the seeds are exposed to prolonged unfavourable conditions, even after the primary dormancy period has ended (Buijs 2020). Intraspecific variations in secondary dormancy are important for ensuring survival in disturbance-prone ecosystems, such as the grassland communities that *N. trichotoma* has successfully invaded, because this strategy protects the population from failed establishment in times where multiple disturbances are present, such as multiple fire events or prolonged drought (Liyanage and Ooi 2015).

Effect of radiant heat on seed age

N. trichotoma seeds demonstrated a high level of resistance to the tested radiant-heat treatments, with seeds collected in 3 of the 4 years maintaining a high total germination percentage at 120°C. The only exception was seen in the seeds collected in 2019, where the seed germination was significantly ($P = 0.002$) reduced when exposed for 9 min at 120°C treatment. For the seeds collected in 2016, exposure to 80°C and 100°C increased the total germination percentage compared with the control. The seeds collected in 2017–2019 also evidenced an increased total germination percentage compared with the control.

Mean germination time indicates the lag period between seed imbibition and radicle protrusion where the seed undergoes repairs from damages caused by the environment or seed aging (Mavi *et al*. 2010). In the present study, *N. trichotoma* seeds decreased their mean germination time (MGT) after exposure to the increasing temperature gradient, particularly when the exposure time was short. Longer duration of exposure to the temperature gradient resulted in an increase in MGT, indicating these seeds required time to repair any damage prior to germination.

The ability of a species to germinate with greater speed and uniformity increases its competitiveness following fire (Hodges *et al*. 2022). Mean germination time can be used as an indicator in determining field emergence, with an increased MGT being correlated to lower and less vigorous field emergence (Matthews and Khajeh-Hosseini 2006). Although this could indicate that a high-intensity fire that heats the soil to 120°C or above could damage the seeds enough to reduce competitive emergence in field, this would, however, be subject to the parallel fire response of the native species in the same corresponding seedbank area (Hodges *et al*. 2022). Time to 50% germination followed the same pattern as did MGT, whereby germination rate slowed as the temperature gradients and duration of exposure increased.

The 140°C treatment was shown to significantly devitalise the seeds, with the exception of one seed germinating in each of the 6- and 9-min-exposure treatments (0.64%) for the seeds collected in 2018. This temperature has been observed to be lethal in other awned grass seeds, such as *Piptochaetium napostaense* (Kin *et al*. 2016). Seed awns are an important adaptive trait for seed survival in fire-prone ecosystems, and increased fire intensity can be a selection mechanism for awns with an increased length, because this allows for the seed to burrow further into the soil to escape lethal temperatures (Garnier and Dajoz 2001). The hygroscopic awn on *N. trichotoma* seeds allows it to burrow into the soil profile, with the majority of this species seedbank found buried at an approximately 2.5-cm depth (Joubert 1984). Shallow seed burial can alleviate the severity of heat shock from fire events, and temperatures 2 cm below the soil surface rarely exceed 80°C (Penman and Towerton 2008). Indeed, a controlled study demonstrated that for surface temperatures that reached 500°C, these conditions were reduced to approximately 70°C at 2 cm depth (Girona-Garcia *et al.* 2015). Another study showed a significant reduction in temperature (approximately 60%) at a depth of 1 cm, and increased soil moisture further reduced the transfer of heat into the soil profile (Valette *et al.* 1994). It is therefore unlikely that the majority of *N. trichotoma* seeds that have burrowed into the soil will be exposed to temperatures that exceed their lethal temperature threshold.

Effect of radiant heat on hydrated seeds

The moisture levels of vegetation and the soil have been shown to influence the intensity of prescribed fire, with the highest-intensity fires thus occuring under low moisture levels. Soils with a high moisture content decrease the thermal energy of the fire (Valette *et al.* 1994), and this is due to (1) a reduced rate of fuel consumption (Marino *et al.* 2012), and (2) water in the soil dissipating heat as steam (Stoof *et al.* 2013). The soil moisture levels strongly influence the moisture level of the seeds that reside within the subsequent soil seedbank, particularly those lacking physical dormancy structures (Dasberg 1971; Josuah *et al.* 1994). However, *N. trichtoma* seeds possess no such structures, but and can readily take up water when it is available in the soil (Campbell and Nicol 1999).

Germination percentages for the seeds hydrated to 15% and 50% experienced no significant changes in germination compared with the control treatment. The seeds hydrated to 95% experienced a significant decline in germination percentage at all tested temperatures. The literature suggests that seeds hydrated to 95% are killed by much lower temperatures than those with lower moisture contents (Ruprecht *et al.* 2016; Tangney *et al.* 2018), and in the present study, this was confirmed by comparison with levels of 15% and 50%. As the seed moisture content increases, so too does the seeds physiological activity (Walters *et al.* 2005). When the seeds are hydrated above 90%, there is high free-water content within the seeds, and this can be heated to a temperature that damages cellular processes, subsequently stopping the physiological processes that initiate the germination process (Tangney *et al.* 2018). Also, proteins associated with protecting the seed while it is in a dormant state, including the synthesis of small heat-shock proteins, cease to provide adequate protection at high seed moisture levels as they do when the seed is in a dry and dormant state (Wehmeyer *et al.* 1996).

Effect of radiant heat on seedlings

It has been reported that prescribed burning has been implemented effectively for weed-control programs and ecological restoration of grasslands (Mainardis *et al.* 2020; Assis *et al.* 2021). However, it is known that adult *N. trichotoma* plants are able to resist high-intensity fire (Joubert 1984; CRC 2003), and our results suggested that this resistance is also demonstrated in 3-month-old seedlings. In our work, the *N. trichotoma* seedlings resisted all the temperatures and duration of exposure times tested in this study, and no plants were killed up to 2 weeks post-heat exposure. Exposure to the 120°C treatment did cause extensive drying to the leaves; however, in some cases, the plant was seen to resprout from the base. The ability to resprout rapidly following a fire is critical for maintaining competitive dominance, and ensuring survival of the population, particularly when the seedbank may have been depleted following a fire through either increased seed germination or devitalisation (Tangney *et al.* 2020; Hodges *et al.* 2022).

This study has thus demonstrated that *N. trichotoma* can withstand high temperatures related to fire. Because this species produces unpalatable, sclerophyllous leaves, it allows it to contribute a large volume of dry biomass to the grasslands that it invades (Distel 2013). This further allows for this species to contribute to increases in the intensity of fire regimes, which it can withstand, and thus successfully displace native plants, which cannot survive such high temperatures. This suggests that for control of *N. trichotoma*, it would be recommended that managers use fire-integrated techniques, using control actions such as target spraying this species with herbicide prior to burning, then broadcasting competing seeds and introducing controlled grazing post-fire (Distel 2013) in grassland ecosystems.

Conclusions

In this paper, we explored the response of *N. trchotoma* seeds and seedlings to fire through controlled radiant heat-exposure trials. Whereas the seeds were able to survive temperatures of 120°C, the majority of the seeds were killed when exposed to 140°C. This suggests that only seeds at the soil surface, or within the top 1 cm, could be killed by hot fire, because these temperatures are alleviated by the soil, producing favourable temperatures for spiking germination (approximately 80°C) below this depth. Both MGT and T_{50} increased under higher temperature gradients and duration of exposure, suggesting that these treatments caused intracellular damage, whereas the delayed germination was due to the seeds requiring time to repair. The seed mass for each collection year appeared to be a key indicator of germinability, with higher seed mass producing the highest germination percentage. Implementing fire with increased moisture levels may not be effective for managing this species, because no change in germination percentage was observed for seeds pre-treated to 50% hydration. Almost all the seeds were killed when hydrated to 95%; however, achieving effective heat penetration into the soil profile at this soil moisture level poses its own challenges. Last, whereas no seedlings were killed by the applied treatments, the 120°C treatment did result in extensive drying to the leaves, causing resprouting from the base of the plant.

Rapid seedling recruitment from the seedbank has been observed for *N. trichotoma* following fire events, and the results of the present study suggested that a moderate-heat fire will reduce the time to 50% germination, which supports observations in the field. For fire to be used effectively to kill *N. trichotoma*, it should be implemented in conjunction with other control actions.

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

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