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The role of soil temperature and seed dormancy in the creation and maintenance of persistent seed banks of *Nassella trichotoma* (serrated tussock) on the Northern Tablelands of New South Wales

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Abstract. A large and persistent soil seed bank characterises many important grass weeds, including *Nassella trichotoma* (Nees) Hack. ex Arechav. (serrated tussock), a major weed in Australia and other countries. In the present study we examined the effects of constant and alternating temperatures in regulating primary and secondary dormancy and the creation and maintenance of its soil seed bank in northern NSW, Australia. One-month-old seeds were stored at 4, 25°C, 40/10°C and 40°C, in a laboratory, and germination tests were conducted every two weeks. Few seeds germinated following storage at 4°C, compared with seeds stored at 25°C, 40/10°C and 40°C. Nylon bags containing freshly harvested seeds were buried among *N. trichotoma* stands in early summer, and germination tests conducted following exhumation after each season over the next 12 months. Seeds buried over summer and summer plus autumn had higher germination than seeds buried over summer plus autumn plus winter, but germination increased again in the subsequent spring. Seeds stored for zero, three, six and 12 months at laboratory temperatures were placed on a thermogradient plate with 81 temperature combinations, followed by incubation at constant 25°C of un-germinated seeds. Constant high or low temperatures prolonged primary dormancy or induced secondary dormancy whereas alternating temperatures tended to break dormancy. Few temperature combinations resulted in more than 80% germination.

Additional keywords: after-ripening, dormancy cycling, environmental sensing, seed burial.

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Introduction

A large and persistent soil seed bank is a characteristic of many important weeds of grasslands (Andrews *et al.* 1996; Gardener *et al.* 2003). In all cases that we have examined, when an invasive weed dominates the herbaceous vegetation, large numbers of seeds of this species are present in the soil seed bank. In most cases, this seed bank persists for many years even if further input of seed of the species in question is prevented (Andrews *et al.* 1996; Gardener *et al.* 2003). *Nassella trichotoma* (Nees) Hack. ex Arechav. (formerly *Stipa trichotoma*) produces large numbers of seeds; of the order of 100 000 in one year from a mature plant of ~250 mm basal diameter (Healey 1945; Lamoureaux *et al.* 2011). The literature relating to *N. trichotoma* seed characteristics and production (Joubert and Small 1982; Taylor 1987; Campbell and Nicol 1999; Casonato 2003; Trotter 2007) indicates that much of this seed is initially dormant but has not resolved how this dormancy leads to the formation of a persistent seed bank in Australian temperate pastures. The role of temperature in regulating seed dormancy and seed longevity in the soil of *N. trichotoma* has received little attention.

Dormancy is an innate seed property that is continuously changing with time and determines the environmental conditions under which seeds will germinate (Finch-Savage and Leubner-Metzger 2006). Dormancy present in freshly mature seed is termed primary dormancy and may break down in time in the dry state (after-ripening), or following dormancy release treatments in the imbibed state (Finch-Savage and Leubner-Metzger 2006). Baskin and Baskin (2004) have defined this type of dormancy as non-deep physiological dormancy (PD) and seeds in the state of primary dormancy (Sp) can go through a series of temperature-driven stages (Sc_1 to Sc_i) to the non-dormant state (Sn). If they do not germinate, seeds may then go back through the series (Sc_i to Sc_1) to a secondary dormancy state (Ss) (Baskin and Baskin 2004). These sequences of dormancy changes have been described by Baskin and Baskin

(2004) as Type 1. These changes are often associated with annual cycles of dormancy in un-germinated seed in the soil seed bank (Baskin and Baskin 1989, 2004; Finch-Savage and Leubner-Metzger 2006). These dormancy mechanisms can enable seeds to remain viable in the soil for many years, exhibiting dormancy/non dormancy cycles, leading to the formation of a persistent seed bank (Bewley 1997). This, in turn, increases the probability that some seeds will encounter conditions favourable for germination, seedling growth and survival (Chambers and MacMahon 1994). The seeds of many weedy species are shed with these inherent dormancy mechanisms that complicate the task of predicting the timing of emergence of seedlings (Benech-Arnold *et al.* 2000).

Temperature often regulates these seed dormancy stages (Finch-Savage and Leubner-Metzger 2006). Seeds of grasses may be released from dormancy by heat or chilling (Brown 1982; Clarke and French 2005), or exposure to alternating temperatures when imbibed (Burke *et al.* 2003; Lodge and Whalley 1981). Investigations of temperature and germination in some *Nassella* species have shown that seeds of *N. viridula* require pre-chilling for germination (Fulbright *et al.* 1983), whereas in *N. leucotricha*, low temperatures prolong seed dormancy and high temperatures break seed dormancy (Van Auken 1997). Aside from stimulating a breakdown of seed dormancy, the range of temperatures required for germination may change with the reduction of primary dormancy during after-ripening (Probert 2000). In some species of *Avena* (*A. fatua* and *A. ludoviciana*) the optimum temperature for germination of the few seeds which will germinate from a sample of dormant seeds (Sp) is close to 0°C, and shifts progressively to higher temperatures as dormancy goes through the five stages described above and, in a sample of non-dormant seeds (Sn), is between 18°C and 20°C (Simpson 1990).

The three experiments described in this paper were conducted to investigate the changes in dormancy of seeds of *N. trichotoma* from the Northern Tablelands of New South Wales (NSW), Australia under a range of storage conditions. This location is near the northern extremity of the distribution of this introduced grass and at an elevation of ~1000 m above sea level. In the first experiment, the germination and dormancy of seeds stored at cool, warm and alternating storage temperatures were tested. In a second experiment, seeds were buried at field sites and germination tests of exhumed seed were conducted every three months to identify changes in seed dormancy in relation to soil temperature and rainfall throughout one year. In a third experiment, a thermogradient plate and a constant temperature germination cabinet were used to study patterns in germination at different temperatures over the course of seed ageing.

Material and methods

Experiment 1: seed storage conditions

The seeds used in this experiment had been harvested in January 2009, from a field site near Armidale, in Northern NSW (30.56083°S, 151.6194°E) and dry-stored for one month in paper bags at ambient temperatures in the laboratory. Seed preparation involved removal of the awns but the careopses were still enclosed in the lemmas and paleas. The temperature in the laboratory was ~20–25°C. The same procedures for seed preparation were used in all the experiments described in this paper.

Several parcels, each of several hundred seeds, were prepared for storage for different times and under different conditions. For each parcel, seeds were wrapped in unbleached dry paper towel and then in aluminium foil (to exclude light). The parcels were then stored in four separate cabinets, three at constant temperatures (4, 25 and 40°C), and one alternating temperature regime 40/10°C, with 12 h at each temperature. Humidity was not measured in the cabinets but was probably low to very low. For cold-moist storage (stratification), the paper used to wrap the seeds was wetted with distilled water before being wrapped in aluminium foil and incubated at 4°C; this wrapping kept the seeds moist for the duration of the trial (maximum 10 weeks). Germination tests were conducted after exposure to these temperatures for two, four, six, eight and 10 weeks using seeds from a different parcel for each time/temperature combination. In these tests, seeds were placed on moist Whatman No. 42 Quantitative Filter Paper (70 mm diameter) inside transparent plastic Petri dishes (100 mm diameter and 15 mm height). The Petri dishes were sealed with Parafilm to prevent desiccation, and placed in a germination cabinet with a 12-h photoperiod (50–100 μmol m^{-2} s^{-1}) at 25°C, and 12 h of darkness at 15°C. If the filter paper became dry, it was wetted and the excess liquid drained before resealing the dishes with a new strip of Parafilm. Each test involved four replicate Petri dishes, each containing 25 seeds, for each storage treatment, and the position of the treatments on the shelves of the germination cabinet was randomised. Every two to five days for 14 days, Petri dishes were opened to allow gas exchange and germination was recorded and germinated seeds removed. Germination was defined as both the radicle and coleoptile being visible. At the end of the 14 days, all seeds that had not germinated were viability tested using the imbibed seed crush test (Borza *et al.* 2007). Those seeds that did not collapse under gentle pressure were judged viable in the crush test and were classed as dormant. Therefore, each sample of seeds was divided into germinable, dormant, and dead, with the germinable plus dormant classed as viable seeds.

Germination was calculated as a percentage of viable seeds. One-way ANOVA and Tukey's honest significant difference (HSD) test for pairwise multiple comparisons were used to analyse the data for differences among treatments at each germination test time. The Kruskal–Wallis one-way ANOVA and the Mann–Whitney U test for pairwise multiple comparisons were used to determine differences among treatments at Week eight, because transforming the data using the arcsine function failed to correct for the non-linear distribution of variance often associated with proportions (McDonald 2008). Throughout this paper, unless otherwise stated, data analyses were performed using IBM SPSS Statistics for Windows, ver. 20.0. (IBM Corp., St Leonards, NSW, Australia).

Experiment 2: seed burial

Four burial sites, one on each of four different farms, were selected within 20 km of Armidale in the Northern Tablelands of NSW. The sites were in semi-degraded native pastures on sheep and cattle farms. At each site, a 2 m high perimeter fence made of wire mesh (5 cm diameter) was erected around a 5 × 5 m area

enclosing a patch of *N. trichotoma*. This fence was to restrict large animals from disturbing the site and to minimise seed spread. Soil was collected from each site (to 10 cm depth) for inclusion with seed in nylon bags.

Nylon bags (0.4 mm mesh) were filled with 50 firm (i.e. filled) seeds, which had been mixed into 64 cm^3 of soil that had been sterilised in an oven at 80°C for 48 h and then sieved through a 0.710 mm sieve in order to remove any *N. trichotoma* seeds that might already be present within the soil. The bags were prepared using the soil collected from each site, so that the soils within and outside each bag were as similar as possible. So that burial site and season of retrieval were the only variables, the seeds used in this experiment were from a single site, collected from ~30 *N. trichotoma* plants two weeks before the start of the burial experiment.

In early December 2008, (the start of summer), nylon bags (16 per site) were buried at random inside the fenced enclosure at each site, in shallow holes (3–4 cm deep) created by driving a hoe down to the appropriate depth, placing the bag in the hole, and then gently pressing the sod back into place. A wooden stake was driven into the ground to mark the spot where each bag was buried. A Nylex '1000' Professional Rain Gauge (Melbourne, Vic, Australia) was erected in the middle of each enclosure, with the tops of the gauges well above the grass canopy, so that rainfall could be measured monthly throughout the experimental period. Soil temperature and volumetric water content were recorded at the start of each month throughout the experimental period, with recordings taken at all sites in a random order, between the hours of 10:00 and 14:00 hours. Soil temperature was measured using a temperature probe that extended into the top 5 cm of soil, and soil volumetric water content was determined using a TDR (time-domain reflectometer) probe that extended into the top 6 cm of soil. If the soil was hard and dry it was not always possible to extend the probes to their full depth. These readings were taken at three randomly chosen locations within the plot at each site, for the purpose of indicating the typical soil conditions to which seeds were exposed.

At the time the bags were buried, a germination test was conducted (25 seeds × four replicates), to indicate seed germinability and dormancy at time zero. Four nylon bags were exhumed from each site every three months in March (autumn), June (winter), September (spring) and December (summer) 2009, to coincide with the approximate end of the preceding season and start of the new season. To separate the seeds from the soil, the contents of each bag were washed through two sieves (4 mm and 0.710 mm). The material caught in the smallest sieve was retained and air-dried, while the material caught in the 4 mm sieve was also carefully inspected for *N. trichotoma* seeds before being discarded. Seeds were discarded that had germinated before recovery (i.e. while buried). For each season, and at T0 (start of the experiment), germination tests were performed according to the method described above for Experiment 1, except that germination was counted every three to four days for 14 days. Germination data (i.e. seeds that germinated during 14 days in a germination cabinet) were calculated as a percentage of recovered seeds. Seeds that did not germinate but were judged viable in the crush test (Borza *et al.* 2007) were classed as dormant. To test for differences in seed germination between sites and sampling times, univariate GLM and post-hoc contrasts using Tukey's HSD were conducted.

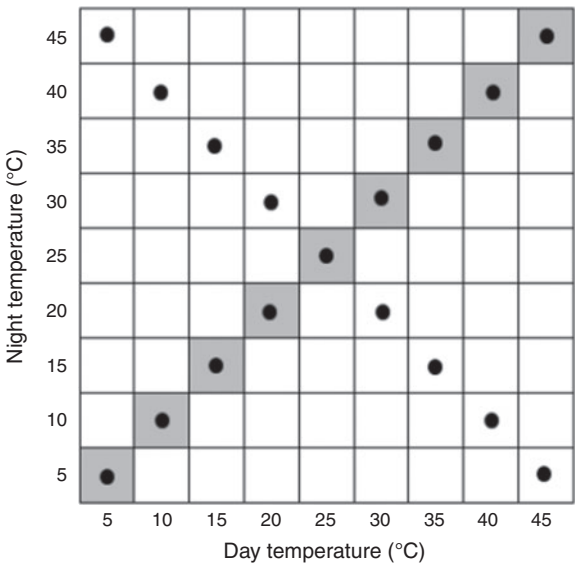

Fig. 1. Layout of the thermogradient plate used in Experiment 3. The locations of the thermocouples are shown by • and the cells with constant temperatures are shaded.

Experiment 3: thermogradient plate

The procedures used in this experiment were adapted from Andrews *et al.* (1997) and Chejara *et al.* (2008). A two-way thermogradient plate (760 × 760 mm) was used to provide a total of 81 day/night combinations of constant and alternating temperatures with a 12-h alternation (Fig. 1). The cells along one diagonal were at constant temperatures, and those at the corners on the other diagonal alternated between 5 and 45°C. Using thermocouples placed near the centre of 17 of the cells, temperatures were logged at 1-h intervals. The data from the thermocouples showed that the temperature gradients were linear across the plate. Weak fluorescent light (irradiance ~25 μmol m^{-2} s^{-1}) was provided for 12 h each day in phase with the day temperatures on the thermogradient plate.

The seed used was harvested from a wild population in January 2008 and January 2009, and kept in dry storage at ambient temperatures (see above for laboratory storage conditions) until use. The 2008 harvest was tested after three, six and 12 months of storage, and the 2009 harvest was tested after zero and six months of storage. Aluminium dishes (3.5 × 3.5 cm) were lined with 'Kimpak' padding under a square of blue germination paper. This blue germination paper is commercially available in squares with each side 75 mm and with 100 indentations in each square. When cut into four, each square then had 25 indentations and fitted neatly into the aluminium dishes. The emerging radicles and coleoptiles of germinating seeds were very easy to see against the blue background.

The dishes were constructed from aluminium foil (0.11 mm thick) and the area in which the seeds were placed was ~3 × 3 cm. The dishes were surrounded by moistened fine

sand to stabilise the temperatures and pierced at one corner to allow drainage of excess water. Into each dish, 25 firm seeds were placed flat on the germination paper, and the dish was covered with a numbered transparent perspex lid. There was about a 2°C temperature variation in each direction in the area over which the seeds were spread in each dish. For instance, the 25 seeds in the 5°C constant temperature dish were exposed to temperatures of 4–6°C during each 24-h period. The same variation would have occurred in the temperatures within all of the cells. Further variations in temperatures would have occurred during the change-over periods (which lasted for about half an hour) twice in every 24-h period. The entire thermogradient plate was covered with another transparent perspex lid to maintain temperature control. Seeds were on the thermogradient plate for 14 days and germinated seeds were removed each morning. After this period, the dishes containing any remaining seeds that had not germinated were transferred to a germination cabinet set at constant 25°C, with a 12-h photoperiod (50–100 μmol m^{-2} s^{-1}) and 12 h of darkness, for another 14 days. This was done to determine whether seeds that did not germinate on the thermogradient plate were germinable when exposed to a generally favourable temperature for germination (Lodge and Whalley 1981). Germination was recorded daily over the 28-day period (14 days on the thermogradient plate + 14 days in the germination cabinet). Any remaining seeds that had not germinated were viability tested using the imbibed seed crush test (Borza *et al.* 2007) and if they were judged viable, were classed as dormant. This process was repeated with each seed age, except that the crush test was not performed on the three-month-old seeds harvested in 2008. This was because this was the first time we had tested viability on the thermogradient plate and at that time we had no method for distinguishing dead from dormant seeds.

The thermogradient plate was constructed about 25 years ago and the temperature response surfaces of percentage germination of seeds of different species computed in various ways, for example, see Andrews *et al.* (1997) and Chejara *et al.* (2008). These response surfaces illustrated major differences in percentage germination at some temperatures but there was no way at the time to apply confidence limits to the data at different temperatures. A universal criticism of these studies has been the lack of suitable methods for the statistical analysis of the results, particularly where there are small differences in the percentage germination at different temperatures. A further problem is that occasional errors occurred in counting out 81 lots of 25 small seeds when setting up the gradient plate for a particular seed sample.

Lodge and Whalley (2002) provided the first attempt at the statistical analysis of data from the gradient plate. They provided light for 24 h and so the two halves of the gradient plate provided two replications of the alternating temperatures but only one replication of the constant temperatures. They analysed the transformed data (square root +1) using an analysis of variance but this approach was not satisfactory because the germinating seeds were exposed to continuous light and the constant temperature treatments were not replicated.

A different approach was used by Tarasoff *et al.* (2005) who used geographical information systems (GIS) based techniques to model the germination prediction surfaces from gradient plate data. Macdonald (2008) used this approach to produce prediction surfaces for the seed germination of the weed Lippia (*Phyla canescens*). These diagrams clearly showed the complete lack of germination at constant temperatures and at temperatures <24°C or >40°C. Again, there were no statistical comparisons between the different prediction surfaces. We have now developed a valid statistical procedure for handling gradient plate germination data as described below.

Thermogradient plate data were used to estimate the number of germinations that occur across a range of day/night temperatures. These 'prediction surface' models were created for each experiment (storage duration, zero, three, six and 12 months) and treatment (thermogradient, thermogradient + germination cabinet). These prediction surface models allowed visualisation of trends in germination numbers across the day/night temperature combinations in each experiment. Statistical tests were performed to determine which day/night time temperature observations exceeded the number of germinations produced by random chance.

To explore the responses of fresh and stored seeds to temperature during germination tests (i.e. on the thermogradient plate and afterwards in the 25°C germination cabinet), germination and viability prediction surfaces were modelled using R 3.0.2 (R Core Team 2013). Analysis involved a binomial test per cell (day/night temperature combination) assuming a random germination probability of 0.05 (one in 20), based on the number of germinations per 25 seeds ($n = 25$). The resulting P-values were extracted and adjusted for multiple comparisons using the false discovery rate procedure (Benjamini and Hochberg 1995). A prediction surface was also created to visualise any general trends in germination numbers across the temperature combinations using a multi-level B-spline (Lee *et al.* 1997). The prediction surface reports on the mean number (estimated Poisson rate parameter) of germinations across temperature combinations per standard cell (25 seeds). The symbol 'X' was placed at those day/night temperature combinations where germination was found to be significantly ($P \leq 0.01$) different from the expected value.

To ensure that the results were not confounded by seed viability, a similar approach was used to test for differences in viability of the seeds used in this experiment, harvested from the same field site in January 2008 and January 2009. The proportion of viable seeds (germinated + firm per 25 seeds) at each day/night temperature combination was determined for both harvest times after six months in storage – a period of six months dry storage at ambient temperatures has been shown to relieve dormancy in this species (Taylor 1987; Campbell and Nicol 1999; Trotter 2007). A difference in proportions test was used to statistically assess for differences in these two proportions (Newcombe 1998). Multiple comparison corrections were then used to adjust P-values before assessment for statistical significance. For those temperature combinations with statistically significantly difference ($P \leq 0.01$) in the two proportions, a symbol 'X' was marked on the plot. A multi-level B-spline was also used to estimate the 'trend' surface of the difference in means (expected difference in Poisson rate parameters) of the two storage times.

Survival of the seeds during the time on the thermogradient plate followed by the time in the germination cabinet involved a binomial test per cell (day/night temperature combination)

Table 1. Germination (% of viable) of one-month-old *Nassella trichotoma* seeds exposed to different storage treatments (4°C moist, 4°C dry, 25°C dry, 40°C dry, and 40/10°C dry) for 10 weeks

Within each row, values followed by different letters are significantly different ($P < 0.05$)

Week	% Germination of viable seeds (± s.e.)				
	4°C Moist	4°C Dry	25°C Dry	40/10°C Dry	40°C Dry
2	14.5a ± 1.3	31.7ab ± 6.7	31.3ab ± 2.3	50.9b ± 5.6	47.3b ± 3.5
4	12.7a ± 2.7	25.8ab ± 11.4	43.7b ± 6.5	47.1b ± 5.7	39.3ab ± 2.1
6	23.1a ± 5.9	42.6ab ± 2.5	54.0ab ± 11.2	62.2b ± 6.9	68.3b ± 9.3
8	17.8a ± 6.7	31.3a ± 3.5	54.9ab ± 7.8	60.0ab ± 12.3	84.6b ± 3.0
10	22.4a ± 8.8	45.0ab ± 5.3	57.0b ± 9.5	56.6b ± 5.2	61.1b ± 8.3

Rows represent germination tests conducted after two, four, six, eight and 10 week's storage at different temperatures.

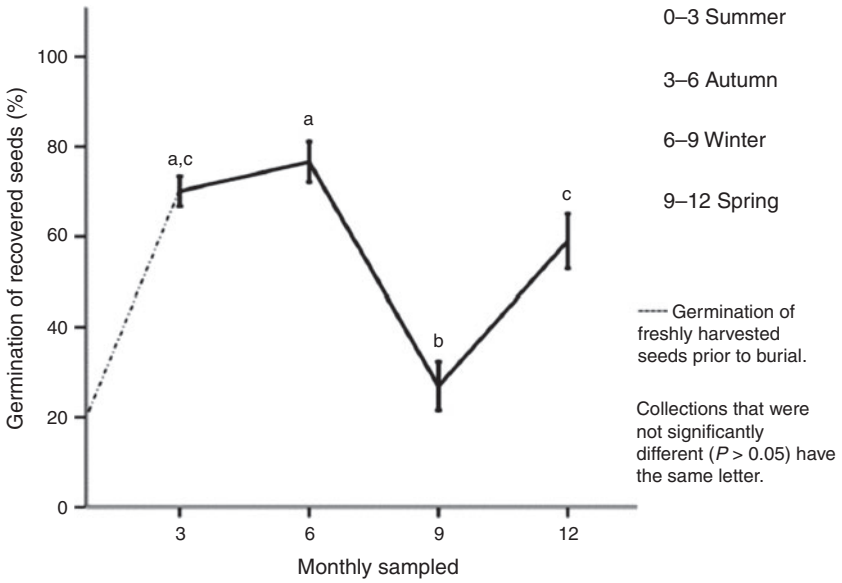

Fig. 2. Percentage (mean ± s.e.) germination of recovered seeds in Experiment 2 (there was no evidence of a site × time interaction ($P > 0.05$), and therefore the germination results were averaged across the four sites).

assuming a random viability probability of 96%, based on the number of viable seeds per 25 (i.e. 24 out of 25 seeds are viable). The same procedure was used as for the germination data above, to visualise the trends in viability across the temperature combinations. Again, the symbol 'X' was used to indicate day/night temperature combinations found to be significantly ($P \leq 0.01$) different from the expected value.

Results

Experiment 1: seed storage conditions

There were significant differences in germination among the storage treatments (Table 1). The 4°C moist storage treatment consistently resulted in the lowest germination at each testing period. After 10 weeks, percentage germination of the seeds in the 4°C moist treatment (22.4 ± 8.8%) did not differ significantly from the 4°C dry treatment (45.0 ± 5.3%), but was significantly lower than for all the 'warm-dry' treatments (57.0 ± 9.5% germination at 25°C, 56.6 ± 5.2% at 40/10°C, 61.1 ± 8.3% at 40°C).

Experiment 2: seed burial

Univariate GLM revealed that there was a significant difference in germination among the sampling periods ($F_{3,63} = 24.40$, $P < 0.001$, Fig. 2), but not between sites (data not shown). After burial for three months over summer, germination of recovered seeds had increased to ~70%, starting from 21% at the start of the experiment. Therefore, during burial, the percentage of dormant seeds had decreased from ~80 to ~30%. There was little change over the second three months (autumn), but over the next three months through winter, the percentage germination dropped significantly to ~27% and the percentage of dormant seeds had increased to ~70%. Over the following three months (spring), seed germination increased significantly to ~60%; a level similar to the early autumn results and the percentage of dormant seeds had decreased to ~40%.

These changes in germination and dormancy (Fig. 2) reflected the soil temperature changes measured at the sites (Fig. 3*a*). The seeds that were exposed to soil temperatures, initially ~20–30°C, then dropping from 20 to 10°C and then increasing again to ~20°C over the three months preceding

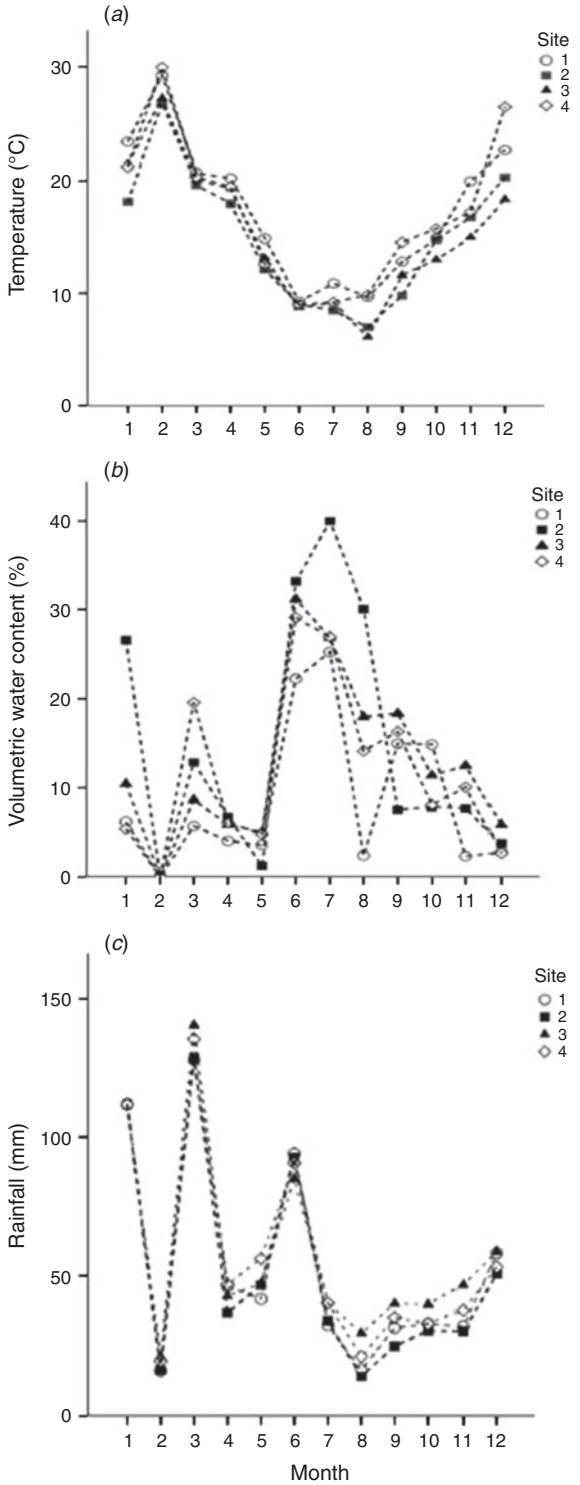

Fig. 3. Soil temperature (°C at 3–5 cm), volumetric water content of the soil (% at 3–6 cm) and rainfall (mm) measured at the four field sites over the 12-month seed burial period in Experiment 2.

exhumation, had similar levels of germination and dormancy (i.e. germination was similar in months three, six and 12). Seeds exhumed in September (at nine months), which had been exposed to the coldest soil temperatures over the winter months (June, July and August, 8–12°C) had significantly lower germination and a higher percentage of dormancy (except for the initial level) than at any of the other sampling periods. Soil moisture (volumetric water content) (Fig. 3*b*) was highest over the winter months.

Experiment 3: thermogradient plate

Within each test where viability was determined (all except for three-month-old 2008), occasional significant differences in seed viability ($P \leq 0.01$) were observed in a few cells but these differences were randomly distributed across the cells (data not shown). The lack of a consistent pattern of significant differences between the observed and assumed value of viability (96%) across the five separate tests, indicates that none of the temperature combinations was associated with seed mortality. The only exception was that in two tests, (zero months 2009 and 12 months 2008), viability was significantly lower than 96% at constant 45°C. Seeds stored for six months from harvest in 2008 and 2009 had no significant differences in seed viability in cells exposed to 45°C during either the day or night phase. These results indicate that the very low germination of seeds exposed to high temperatures over the 14 days on the thermogradient plate, followed by 14 days in the germination cabinet at 25°C, cannot be readily attributed to the death of these seeds.

Seeds freshly harvested in January 2009 were strongly dormant, with no significant germination occurring by the end of 14 days on the thermogradient plate (Fig. 4*a*). Transfer of these seeds to 25°C resulted in the germination of seeds exposed to 5°C in either the day or night phase on the gradient plate, peaking at 48% with the alternating temperatures 20/5°C and 25/5°C (day/night) at the end of the 14 days (Fig. 4*b*) indicating that these alternating temperatures had broken the primary dormancy of some of the seeds. However, there had been no break of dormancy of seeds that had been at or near the constant temperature diagonal for 14 days on the thermogradient plate.

Seeds from the same harvest which had been stored for six months in the laboratory gave greatest germination on the gradient plate (84%) at 30/15°C and there was some germination, up to 72%, on the constant temperature diagonal at 30°C (Fig. 4*c*). However, exposure to low temperatures on the gradient plate resulted in no germination in 14 days but germination of up to 80% at 25°C in the germination cabinet in the subsequent 14 days (Fig. 4*d*) indicating that the exposure to low temperatures had broken the primary dormancy of most of the seeds.

Seeds harvested in January 2008 and stored for three months in the laboratory experienced significant germination on the thermogradient plate (Fig. 5*a*) at alternating temperatures but not at constant temperatures, with up to 48% germination at 35/10°C day/night. However, at constant 25°C, those seeds that had been at the lower temperatures (both constant and alternating) on the thermogradient plate also gave up to 48% germination (Fig. 5*b*) whereas seeds were still dormant after 14 days at higher constant temperatures on the thermogradient plate and did not germinate during 14 days at 25°C in the germination cabinet (Fig. 5*b*).

Seeds that had been stored for six months had significant germination at a wide range of both constant and alternating temperatures on the thermogradient plate (Figs 4*c*, 5*c*), and there

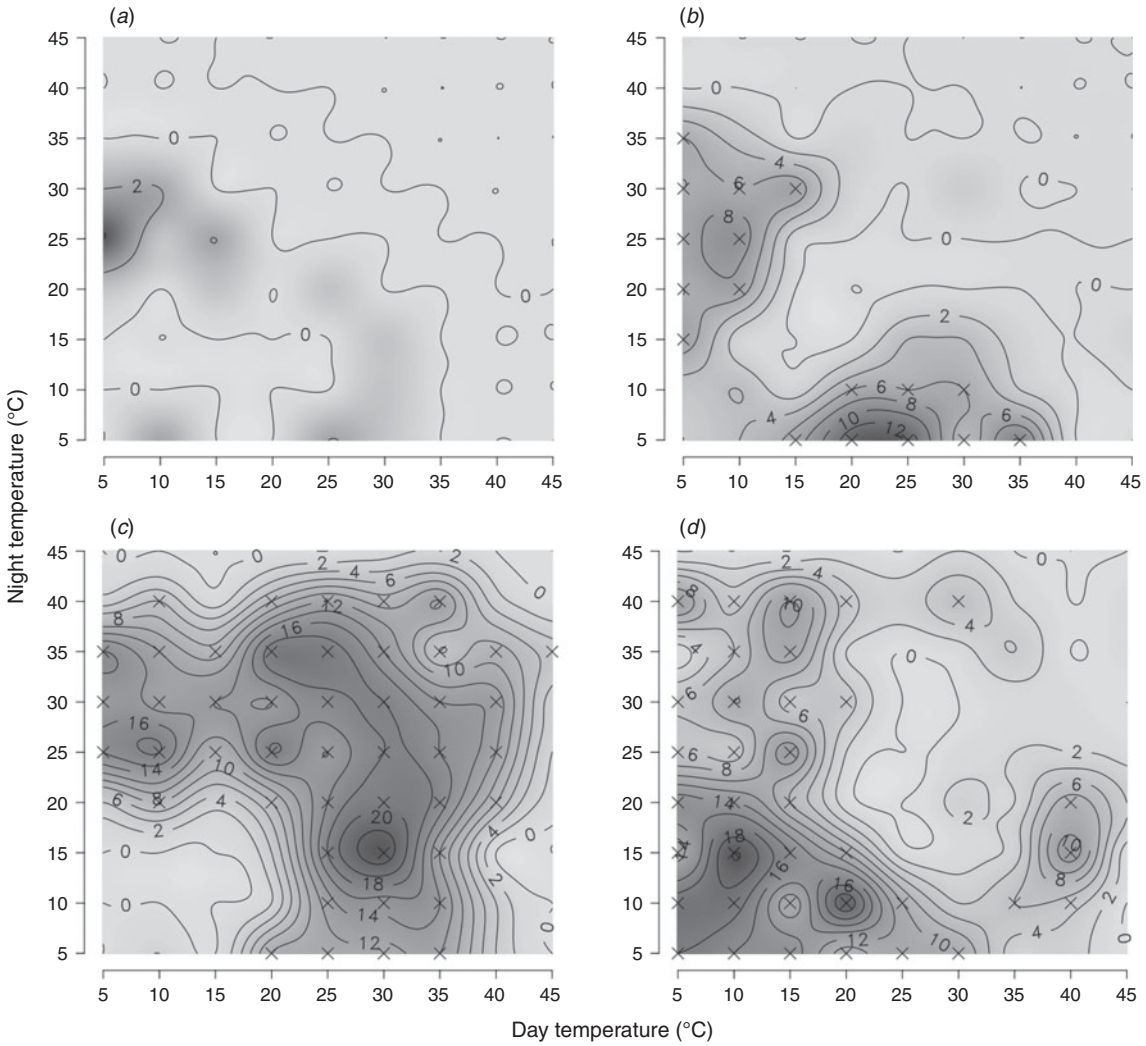

Fig. 4. Germination of imbibed seeds harvested in January 2009, after storage for zero months (*a*, *b*) and six months (*c*, *d*). Charts on the left hand side show germination on the thermogradient plate, and charts on the right show germination of remaining seeds that were subsequently transferred to a germination cabinet set at 25°C. The day (*x*-axis) and night (*y*-axis) temperatures represent the conditions that each cell was exposed to while on the thermogradient plate. Numbers marked on contour lines refer to the unscaled rate parameters for a truncated Poisson distribution based on observed germination of $n = 25$ seeds. Colours shift from light to dark grey with increasing germination. The symbol 'X' occurs at day/night temperature combinations found to be statistically significant ($P \leq 0.01$), from a random germination probability of 0.05 (one in 20).

was no sign of dormancy at constant temperatures. Both lots of seeds exhibited in excess of 80% germination on the thermogradient plate at the alternating day/night temperatures 30/15°C, 30/20°C, 25/15°C and 35/25°C with the highest percentage (88%) for the 2009 harvest. However, there were different levels of dormancy in the seeds harvested from the same stand of plants in the two consecutive years. The range of temperatures at which significant germination occurred was greater for the 2009 harvest than for the 2008 harvest with significant germination ($P < 0.01$) at 46 cells on the plate whereas there were only 41 such cells for the seed harvested in 2008 (Figs 4*c*, 5*c*). Germination in the germination cabinet again occurred at the lower temperatures with a maximum of 80% germination at 20/10°C for the 2009 harvest and only 48% for the same temperatures for the 2008 harvest (Figs 4*d*, 5*d*). These differences indicate a higher degree of dormancy in the 2008 harvest compared with the 2009 harvest. However, there were no differences in viability after six months of storage between the 2008 and 2009 seed collections, no matter what temperatures the seeds were exposed to on the gradient plate (Fig. 6).

In seed tested after 12 months in storage, the range of temperatures conducive for germination had increased again relative to the six-month-old and three-month-old seed, shifting towards the higher temperatures (40°C, Fig. 5*e*). However, the level of dormancy was consistently higher for the 12-month-old seed compared with both samples of six-month-old seed. Similar to the tests at six months (Figs 4*d*, 5*d*), 12-month-old seed exposed to lower temperatures on the thermogradient plate did

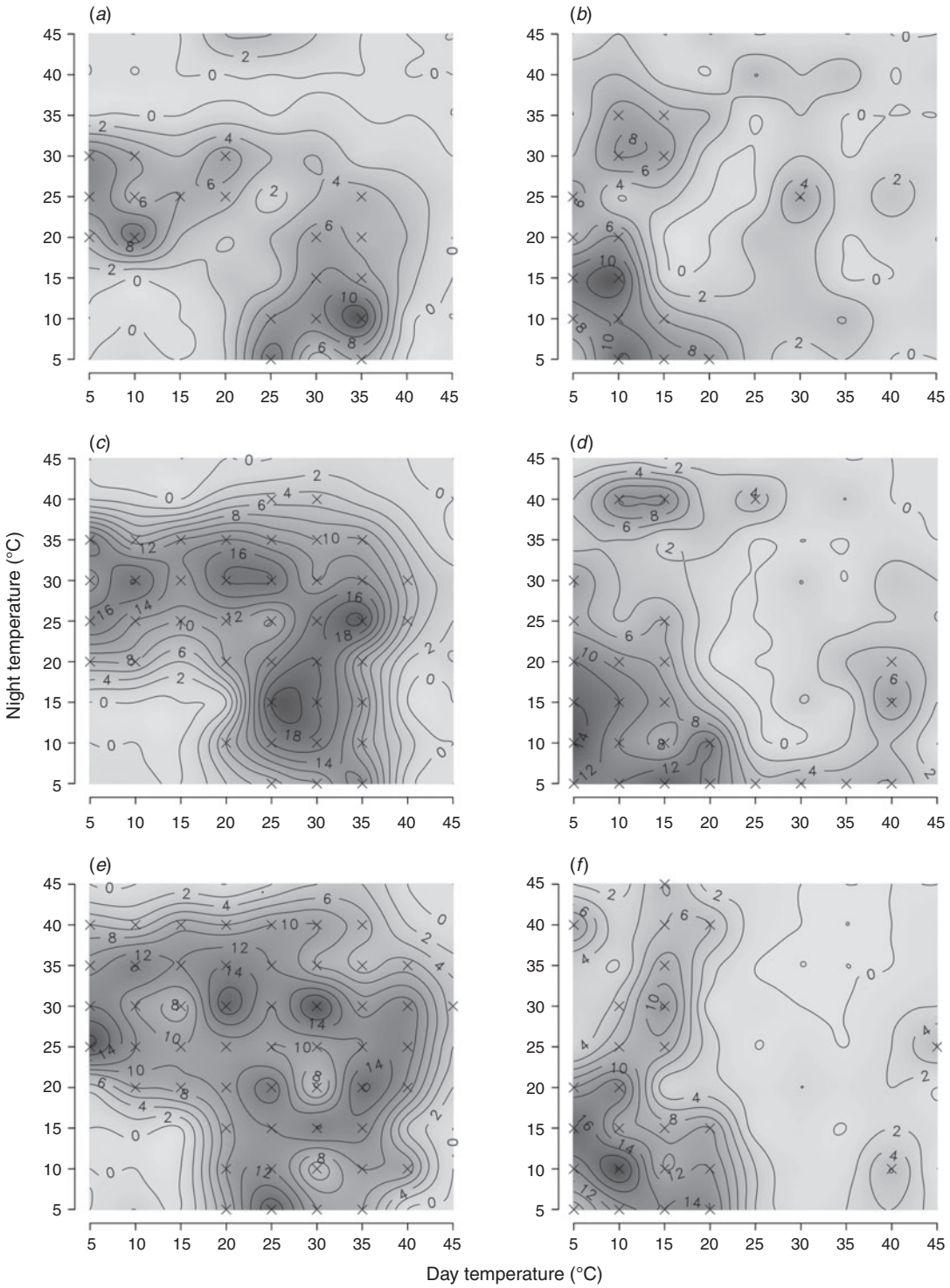

Fig. 5. Germination of imbibed seeds harvested in January 2008, after laboratory storage for three months (*a*, *b*), six months (*c*, *d*), and 12 months (*e*, *f*). Charts on the left hand side show germination on the thermogradient plate, and charts on the right show subsequent germination of remaining seeds that were subsequently transferred to a germination cabinet set at 25°C. The day and night temperatures represent the environment of the cell while on the thermogradient plate. Numbers marked on contour lines refer to the unscaled rate parameters for a truncated Poisson distribution based on observed germination of $n = 25$ seeds. Colours shift from light to dark grey with increasing germination. The symbol 'X' occurs at day/night temperature combinations found to be statistically significant ($P \leq 0.01$), from a random germination probability of 0.05 (one in 20).

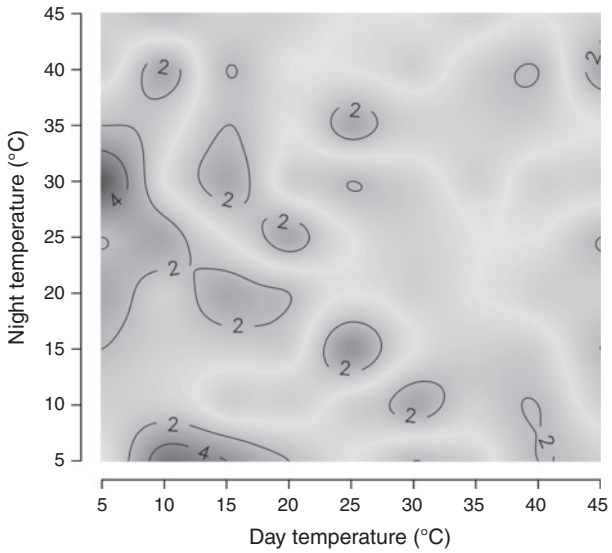

Fig. 6. Differences in viability of the two harvest periods January 2008 and January 2009, after six months dry storage. Viability = germination on the thermogradient plate + germination in the germination cabinet + remaining viable non-germinated seeds. The day (*x*-axis) and night (*y*-axis) temperatures represent the conditions that each cell was exposed to while on the thermogradient plate. Numbers marked on contour lines refer to the unscaled rate parameters for a truncated Poisson distribution based on observed viability of $n = 25$ seeds. Colours shift from light to dark grey with increasing differences in viability between the two seed lots. Absence of cells marked with an 'X' indicates that viability was not significantly different ($P > 0.01$) between the seed from the two harvests (2009 and 2008) at any temperature combination.

germinate after transfer to the germination cabinet (Fig. 5*f*). However, the highest germination was only 72% (28% dormancy) after exposure to constant 10°C, and the level of dormancy of seeds exposed to the other low temperatures on the gradient plate was much higher (Fig. 5*e*, *f*).

Discussion

These results seem to indicate that freshly mature seeds of *N. trichotoma* exhibit Type 1 non-deep physiological dormancy as per the classification scheme for seed dormancy proposed by Baskin and Baskin (2004). This classification is supported by other observations for this species which also showed that dormancy can be broken by after-ripening in dry storage (Campbell and Nicol 1999; Trotter 2007), and seeds often exhibit increased sensitivity to light and gibberellin (GA) as dormancy is released (Campbell and Nicol 1999; Casonato 2003). These results suggest that storage temperature does affect seed dormancy in this species and that even under ideal conditions for breaking dormancy, ~20% of the seed bank is still dormant (Fig. 2). This pattern is quite different from that of *Hyparrhenia hirta*, which has relatively recently become a weed in Australia. Stands of this species on the Northern Tablelands of New South Wales produced a large number of seeds each year but few were dormant. Therefore, it is possible to run down this large seed bank if seed production is prevented for one or two years (Chejara *et al.* 2019).

The fresh seed buried in the nylon bags in early December had ~80% primary dormancy but after three-month's burial with soil temperatures of the order of 20–30°C, the dormancy level had dropped to below 30%. However, after nine months in the soil with temperatures in the winter generally below 10°C, the dormancy level had increased again to ~70%. This dormancy could partially consist of residual primary dormancy, but would mostly be secondary dormancy induced by the low winter temperatures (Baskin and Baskin 2004; Finch-Savage and Leubner-Metzger 2006). After increasing soil temperatures in the spring, the secondary dormancy was partially broken. These results are also consistent with those in this study where moist storage of seeds at 4°C led to little change in dormancy. Conversely, dry storage, particularly at higher and/or alternating temperatures resulted in partial release of primary dormancy, more or less independent of temperature. The seasonal patterns of dormancy and non-dormancy observed in the exhumed seeds are similar to those in field studies of emergence in *N. trichotoma* (Healey 1945; Badgery 2004; Trotter 2007), in which germination was lower in summer and winter, with peaks in autumn and spring. These results suggest that the proportion of the buried seeds that were dormant changed during the year. Baskin and Baskin (1985) observed annual weed seeds in the soil going through annual fluctuations in dormancy level and exhibiting a continuum of changes in physiological responses.

Soil temperatures measured at the field sites over the 12-month seed burial experiment illustrate how temperatures in the soil at 3–5 cm depth varied over time with quite substantial changes over some 28-day periods. The gradient plate data illustrate a progressive loss of primary dormancy of *N. trichotoma* seeds with dry storage under ambient temperatures (Figs 4, 5) as well as the effects of different constant and alternating temperatures on imbibed seed over a 14-day period on the breaking of primary dormancy and the imposition of secondary dormancy. The primary dormancy of the freshly harvested seed appeared to be partially broken in 14 days by exposure to alternating temperatures in a range from 15–35°C but only if the other 12-h period had temperatures between 5 and 15°C in the light, or temperatures between 5 and 10°C in the dark. The degree of primary dormancy was not reduced at all at any constant temperature between 5 and 45°C (Fig. 4*b*).

After six-month's storage, the primary dormancy had largely been broken and substantial germination occurred over a broad range of constant and alternating temperatures on the gradient plate (Figs 4*c*, 5*c*). These results are consistent with those of Taylor (1987), Campbell and Nicol (1999) and Trotter (2007), but the dormancy was not completely broken except in a few cells on the gradient plate. This result is in accordance with those of Joubert and Small (1982), and Casonato (2003), who observed that *N. trichotoma* seed dormancy can last much longer than 12 months. However, when the ungerminated seeds were placed in the germination cabinet for a further 14 days, these seeds displayed substantial secondary dormancy if they had experienced either very cold (<10°C) or very hot (>40°C) conditions on the gradient plate. These results perhaps explain the annual dormancy cycling of *N. trichotoma* seeds in the soil seed bank apparent in our data and indicated by other research (Healey 1945; Badgery 2004; Trotter 2007). This information is helpful in explaining why *N. trichotoma* seed dormancy increases with burial depth (Aarssen and Irwin 1991; Trotter 2007); because the diurnal soil temperature changes would be smaller (i.e. closer

to constant temperatures) as the depth in the soil increases. Soil disturbance is known to promote emergence of *N. trichotoma* (Osmond *et al.* 2008), which may be the result of some seed being moved closer to the soil surface where it is subjected to fluctuating temperatures that break dormancy and stimulate germination.

These results suggest that the age of seeds interacted with temperature such that germination patterns in the field can be explained by these temperature responses. Furthermore, few if any storage times (up to 12 months) followed by 14 days on the gradient plate plus 14 days incubation at 25°C, resulted in 100% germination. Most of these totals were below 80%. Therefore, even cultivation in the field which will bring *N. trichotoma* seeds to the surface and allow dormancy to be broken by exposure to alternating temperatures will probably not result in 100% of these seeds germinating. This mechanism probably contributes to the long-lived seed bank which is a feature of this important weed and the data presented in this study contribute to our understanding of the mechanisms involved.

Casonato (2003) reported that seed provenance influenced germination responses of stored *N. trichotoma* seeds suggesting that different ecotypes may have different levels and duration of dormancy. All the seeds used in this experiment were harvested from the same small population of *N. trichotoma* and so any differences in dormancy of the seeds collected in different years would not be the result of genetic differences in the material collected. The two *N. trichotoma* seed lots from the same site collected in 2008 and 2009 had similar levels of seed viability, and this suggests that patterns in germination were not confounded by discrepancies in viability (i.e. the seed crush test method of determining viability produced consistent results). Similar results have been observed in *N. leucotricha*. Freshly harvested, mature seeds of *N. leucotricha* exhibit primary dormancy and require three to six months after-ripening at 25 to 35°C to break dormancy, but seeds stored at 4°C will not germinate (Van Auken 1997).

In addition to the cold temperatures, the slow rate of change in the state of dormancy of seeds subjected to the 4°C moist treatment could be due to an anaerobic environment surrounding the seeds. Holm (1972) showed that as oxygen levels in the microenvironment decreased, acetaldehyde, ethanol, and acetone accumulated in buried seeds in amounts sufficient to inhibit germination. In certain genetic lines of *Avena fatua*, after-ripened caryopses were induced into secondary dormancy by anoxia at temperatures from 5 to 35°C, probably because after-ripening involves some oxidative changes in the embryo; so keeping seeds in anaerobic conditions slows the loss of dormancy (Symons *et al.* 1987).

Conclusions

These results suggest that seeds of *N. trichotoma* initially have a high level of Type 1 non-deep physiological dormancy at maturity. This primary dormancy may then be broken depending on the temperature conditions to which the seed is exposed. If the seed is buried and enters the soil seed bank, it may enter secondary dormancy and can remain viable for many years. Seeds produced by the same stand of plants in different years may have different levels of primary dormancy and different rates of the release of primary and the imposition of secondary dormancy.

A key finding of this research is that *N. trichotoma* seeds exhibit annual dormancy/non-dormancy cycles induced by seasonal temperature changes in the soil in which they reside. Seeds deeper in the soil profile, with lower diurnal fluctuations in temperature, would probably exhibit deeper and more prolonged dormancy than seeds closer to the soil surface with greater temperature fluctuations. In addition, these results perhaps explain field observations of spring and autumn flushes of seedlings of this species, provided rain occurs at appropriate times. They also suggest that the soil seed bank of *N. trichotoma* would be long-lived so that prevention of further seed input must be continued for a long period of time if it is to be an effective aid in the control of this invasive weed.

Conflicts of interest

The authors declare no conflicts of interest.

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