



**HAL**  
open science

## **Breaking hard seed dormancy in the perennial legume *Lebeckia ambigua* E. Mey. to enhance sustainable agricultural production**

Robert J. Harrison, Tom J. Edwards, Emma Steel, Ron J. Yates, Brad J. Nutt, John G.  
Howieson

### **► To cite this version:**

Robert J. Harrison, Tom J. Edwards, Emma Steel, Ron J. Yates, Brad J. Nutt, et al.. Breaking hard seed dormancy in the perennial legume *Lebeckia ambigua* E. Mey. to enhance sustainable agricultural production. *Agronomy for Sustainable Development*, 2021, 41 (4), pp.45. <10.1007/s13593-021-00704-0>. <hal-03699043>

**HAL Id: hal-03699043**

**<https://hal.science/hal-03699043v1>**

Submitted on 20 Jun 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization



# Breaking hard seed dormancy in the perennial legume *Lebeckia ambigua* E. Mey. to enhance sustainable agricultural production

Robert J. Harrison<sup>1</sup> · Tom J. Edwards<sup>1,2</sup> · Emma Steel<sup>1</sup> · Ron J. Yates<sup>1,2</sup> · Brad J. Nutt<sup>1</sup> · John G. Howieson<sup>1</sup>

Accepted: 19 May 2021 / Published online: 16 June 2021  
© INRAE and Springer-Verlag France SAS, part of Springer Nature 2021

## Abstract

In this study, seeds of *Lebeckia ambigua* E. Mey., a South African perennial legume, displayed a high level of physical dormancy, commonly termed ‘hardseededness’. In Australian agricultural systems, this trait is exploited in annual legumes to ensure their regeneration after a cropping phase. While hardseededness in annual legumes has been studied extensively, there have been fewer studies of this feature in perennial legumes. Here, for the first time, we examine the conditions required for hard seed breakdown in *L. ambigua*, with experiments undertaken in both the field and laboratory. The annual legumes *Ornithopus sativus* Brot. and *Biserrula pelecinus* L. were included for comparison. More than 50% of the hard seed of *L. ambigua*, when buried at 0.5 cm for 87 weeks, remained hard, and 25% were still hard after 188 weeks. We are also the first to demonstrate genetic variation in hard seed breakdown patterns of *L. ambigua* when buried at 0.5 cm. In the laboratory, *L. ambigua* seed softened after exposure to a temperature of 80°C for 2–8 days in a dry oven and also after reaching 60°C in a cycling temperature oven, with fluctuating humidity. Seed of *L. ambigua* produced in two different geographic regions of Western Australia and then buried at 0.5 or 4 cm in the soil, at two softening locations, became differentially soft over 188 weeks. There was a significant three-way interaction between burial depth, site and maternal influences ( $P < 0.01$ ) on hard seed breakdown. The pattern of hard seed breakdown revealed in this perennial legume reflects that described for pyrogenic species and does not fit the models developed by agricultural researchers for annual legumes. Understanding the ecological triggers for release of seeds of *L. ambigua* from dormancy has provided opportunities for exploitation of this trait in future sustainable agricultural development.

**Keywords** Hard seed breakdown · Ecology · Perennial legume · Sustainable agriculture · Seed dormancy · Perennial ley

## 1 Introduction

Seed production is an essential reproductive trait in angiosperms. Physical seed dormancy is just one of a number of mechanisms that protect seeds from untimely germination, and is of substantial ecological significance in many species of Fabaceae (Lindl.) (Baskin and Baskin 2004). Physical dormancy develops via an impermeable “hard” seed coat that prevents the imbibition of water and therefore the immediate germination of the seeds when exposed to moisture (Gama-Arachchige et al. 2013; Baskin and Baskin 1998; Baskin

2003; Baskin et al. 2000). A second adaptive mechanism to ensure longevity that is common in perennial plants from fire-prone environments is resprouting (Fig. 1). New growth arises from growing points below the ground that are protected from the direct effects of fire (Keeley and Zedler 1978; van Wilgen 2013). In natural settings, there is a complex interplay between resprouting, reseeding and patterns of hard seed breakdown that impacts the long-term persistence of perennial plants. These interactions must be understood if perennial plants are to be developed for the advancement of sustainable agriculture and agroecology (Crews et al. 2016; Edwards et al. 2019; Wezel et al. 2009; Malézieux 2012).

*Lebeckia ambigua* E. Mey. is a perennial, suffrutescent legume found narrowly distributed in the fynbos biome of the Western Cape floristic region of South Africa (Le Roux and Van Wyk 2007). It grows on sandstone derived soils of inherent low fertility, with an acid reaction trend ( $\text{pH}_{\text{CaCl}_2}$  4.5–6.5), in regions that receive an annual rainfall of between 250 and 450 mm (Le Roux and Van Wyk 2007; Howieson et al.

✉ Robert J. Harrison  
r.harrison@murdoch.edu.au

<sup>1</sup> Centre for Rhizobium Studies, Murdoch University, 90 South St, Murdoch, Western Australia 6150, Australia

<sup>2</sup> Department of Primary Industries and Regional Development, 3 Baron-Hay Ct, South Perth, Western Australia 6151, Australia



**Fig. 1** *Lebeckia ambigua* reseeding under the mother plant (left), and resprouting after severe grazing (right) on a coarse sandy soil in Western Australia.

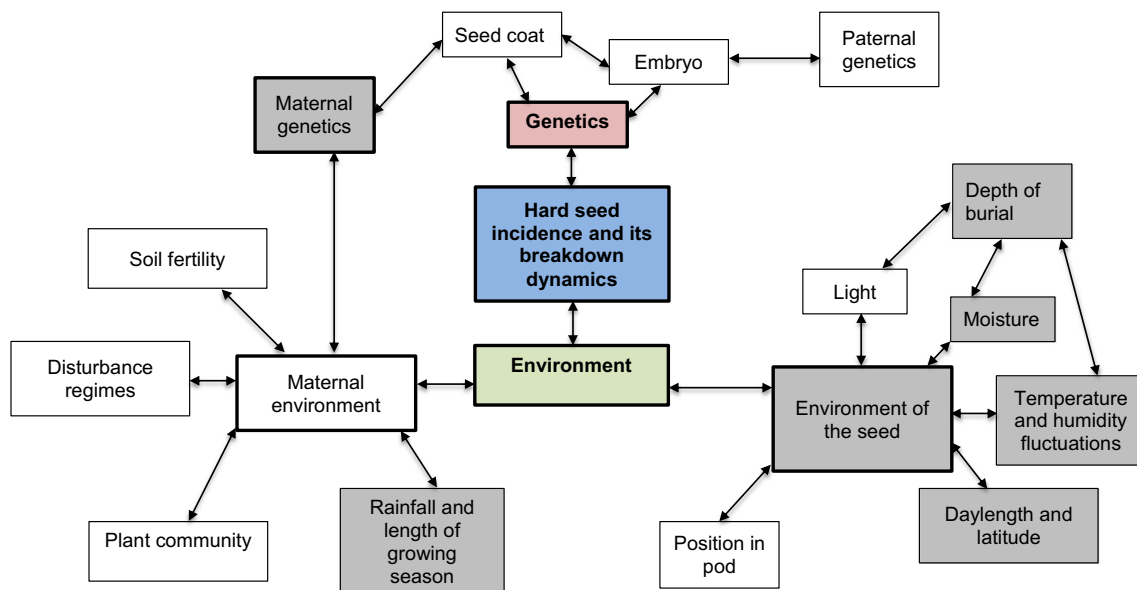
2008). These challenging edaphic constraints for legume cultivation are analogous to some of the agricultural zones of Western Australia (WA), a major agricultural region of some 16 million hectares. Globally, Huang and Hartemink (2020) estimate that coarse-textured sandy soils account for 199 million hectares of agricultural soils and reliance on these soils to increase food and fibre production is increasing. Africa and WA comprise the greatest proportion of sand soils under agriculture, and production on these soils is typically challenged by physical and chemical constraints such as soil water repellence, low inherent fertility, susceptibility to erosion, poor water holding capacity and low pH (Hall et al. 2020). Additionally, due to the inherent fragility of these soils, they are prone to degradation when regularly disturbed for agriculture based upon annual plants (Lal 1988). Breeding a robust perennial legume suited for acid sandy soils offers a solution to combat unsustainable management of such soils.

*Lebeckia ambigua* was identified as a candidate legume for domestication after observations of it surviving and regenerating after being grazed by sheep in the fynbos biome (Howieson et al. 2008). This perennial legume is highly fecund and can produce up to 25,000 highly dormant seeds per plant (Edwards et al. 2019; Howieson et al. 2008). *Lebeckia ambigua* is also considered a resprouter (Goldblatt and Manning 2000), and thus there are likely to be complex interactions between fire, resprouting and seed germination that ultimately determine its reproductive dynamics and eventual success.

In both ecological and agricultural settings, dormant legume seeds can form a reserve “bank” of viable seeds in the soil, a portion of which are released from dormancy when the seed coat eventually becomes permeable (hereafter termed hard seed breakdown) (Ehrman and Cocks 1996). The regulation of hard seed breakdown encourages the seeds to germinate at a time that provides them with

the greatest chance of survival and subsequent reproductive success. This is often in the context of favourable rainfall events in Mediterranean-type systems dominated by annual legumes (Baskin and Baskin 1998), or following fire events in more complex biomes dominated by perennial legumes such as the Cape Floristic region (CFR) in South Africa (Goldblatt and Manning 2000) and the Kwongan biome of South-west Australia (Beard et al. 2000).

There are numerous genetic and environmental factors that interact and contribute to hard seed formation in plants and subsequent seed softening (summarised in Fig. 2). Taylor (1981) proposed a “two-stage” model to explain hard seed breakdown in annual Mediterranean legumes of agricultural relevance. The first stage requires a sustained temperature of > 50°C that “preconditions” the seeds, followed by a second stage of diurnal fluctuating soil temperatures (60/15°C; after Quinlivan 1961) that renders them permeable. The impact of the maternal environment of seed formation, and influences of light, humidity, burial and storage on hard seed breakdown have received increasing attention in recent years. Storage of seed at lower temperatures for over 10 years may have the same effect on Fabaceae hard seeds as high temperatures during Taylor’s preconditioning stage (Harrison et al. 2020). Temperature aside, Taylor (2005) reported moisture to be an important factor that advanced the first step of hard seed breakdown in the second stage (Taylor 1981, 2005). The effect of cycling of temperature and humidity to break physical dormancy has only been reported in the seeds of Fabaceae and Convolvulaceae (Jayasuriya et al. 2009a; Rodrigues-Junior et al. 2018; Baskin and Baskin 2014; Jayasuriya et al. 2009b). This knowledge has recently been exploited to select and breed annual legumes that potentiate the development of a less disruptive and more sustainable form of legume-based farming system (Nutt et al. 2021b; Howieson et al. 2021).



**Fig. 2** An interactive flowchart of factors that may influence hard seed formation in Fabaceae, and its breakdown dynamics. Grey shading indicates factors examined in this study.

Unfortunately, the trait of hard seed dormancy presents two substantial challenges in the domestication of legumes for agriculture: firstly, how to ensure synchronous germination of seed when the legume is first sown, and secondly, how to manage patterns of self-regeneration of the legume in any given farming system. The former challenge has historically been overcome by crude methods of physical scarification such as heating, freezing, mechanical abrasion, or by treating seeds with acid (Kimura and Islam 2012), and by applying these measures to more intractable legume seed bracts (Carruggio et al. 2020). However, handling and processing of seed becomes a substantial barrier to adoption of legumes in farming as it increases their cost of establishment (Nutt et al. 2021b). Howieson et al. (2021) describe a more elegant and economically viable approach, whereby untreated hard seeds are sown in the hot, dry months preceding rainfall to allow the environment to release them from dormancy. The second challenge (of managing hard seed banks) has been overcome by developing farming systems with particular sequences of crop and forage pasture rotations to suit the dormancy patterns of the (forage) legume (Taylor et al. 1991; Loi et al. 1999).

Edwards et al. (2019) proposed the term “perennial ley” to describe the potential utilisation of the hard seed formed by *L. ambigua* in agriculture. However, field trials designed to investigate the agronomy of *L. ambigua* recorded low levels of seedling recruitment up to 4 years after the hard seed was formed and released onto the soil (Fig. 1). An understanding of the specific ecological requirements for hard seed breakdown of seed of *L. ambigua* is required to exploit its seed bank in agriculture. Previous studies into the ecological triggers required for hard seed breakdown in *Trifolium* (*L.*) spp. and *Ornithopus* (*L.*) spp. have identified ex situ methodology to

predict release from dormancy in these annual species originating from the Mediterranean basin (Revell et al. 2012; Taylor and Revell 1999, 2002; Howieson et al. 2021). However, these legumes evolved under different ecological conditions from those of perennial legumes in the fynbos biome of South Africa, which is prone to fire events, and so the methodology may not be applicable.

Based on our field observations, we hypothesised that, as a consequence of the evolutionary origins of *L. ambigua*, the temperature regimes required to reliably break hard seed dormancy would differ from those of the annual legumes previously studied. Furthermore, we hypothesised that exposure to temperatures reflecting those at the soil surface in fire-prone environments would impact the hard seed breakdown. Therefore, we designed experiments to investigate triggers that might break the dormancy of *L. ambigua* seed to assist in the commercial utilisation of the species in the proposed perennial ley farming system. The grey boxes shaded in Fig. 2 (depth of burial, moisture, temperature and humidity fluctuations, day length and altitude, growing season and maternal genetics) indicate the factors affecting hard seed formation and breakdown that were examined in this study.

## 2 Materials and methods

Three experiments were undertaken. The first two examined effects of environment on hard seed breakdown when seed was buried in situ at different sites in WA for up to 188 weeks. The third experiment evaluated whether the ex situ methodology developed for annual legumes from the Mediterranean

basin could be applied to *L. ambigua* to predict its hard seed breakdown.

## 2.1 Acquisition of seed for study of hard seed breakdown

Seed of *L. ambigua* was collected from four sites within South Africa (RSA) between latitude 31°26'47" S and 33°29'07" W (Table 1). Soil pH at the sites was between 5.5 and 6.5 (CSIRO field kit) and the average annual rainfall was between 300 and 450 mm (Howieson et al. 2013). Collected seed was then germinated and grown to maturity at Murdoch University, Perth, WA (32°04'04.1" S 115°50'24.6" E), over several years (where there was potential for some cross pollination between plants from the different collection sites). Seeds from plants from each of the four original sites in RSA were bulked for each site to give a population genotype and this provided material for an initial study of hard seed breakdown at Murdoch University. Based upon seed production and growth habit, seeds from plants originally from site 32 in RSA were then harvested for a second set of field experiments in the wheatbelt of WA.

## 2.2 Hard seed breakdown of *L. ambigua* in situ

### 2.2.1 The effect of genotype on the hard seed breakdown pattern of buried seed of *L. ambigua*

Seeds produced from the four genotypes (Table 1) were harvested at Murdoch University in December 2014 then stored for 8 weeks at room temperature before the experiment commenced in February 2015. Seed from each genotype was passed three times through an Encott two-way seed divider before being subsampled into 30 groups of 100 seeds. These subsamples were randomly assigned a replicate and treatment number then sewn securely into pockets of flywire micromesh (Howieson et al. 2021). The mesh pockets were then buried at Murdoch University (coarse textured sand) at a depth of 0.5 cm (Fig. 3). The pockets remained buried for up to 87 weeks, with samples exhumed for assessment of remaining hard seeds at 8, 24, 38, 57 and 87 weeks post-burial. There were four replicates from each genotype exhumed at each



**Fig. 3** *Lebeckia ambigua* hard seed enclosed in micro-mesh before being buried at 0.5 or 4 cm at Murdoch, WA.

sampling date. Seeds were placed onto moist filter paper in a tray with excess water, in a room held at 16°C, to provide conditions for germination for those seeds whose dormancy had been broken during the burial period. The seeds were checked for germination at regular intervals over 3 weeks (Howieson et al. 2021). The criterion for germination was an emergence of a radicle. Any seed that had not imbibed after 21 days was considered to be “hard” (i.e. impermeable to water). A separate subsample was assessed for initial hard seed level at the commencement of the experiment, prior to burial.

### 2.2.2 The effect of maternal growing environment, seed burial depth and location on the hard seed breakdown pattern of *L. ambigua*

Seed from “site 32” genotype was harvested from Murdoch University then hand scarified and sown to establish two field stands in September 2014 on soils which are both classified as coarse-textured sands. The first stand was established near Brookton (32°22'4.8" S, 117°0'21.6" E), considered to be a

**Table 1** The location, soil pH, annual rainfall and altitude of collection sites in South Africa of *Lebeckia ambigua* genotypes.

Collection site	Latitude	Longitude	pH (BaSO <sub>4</sub> -CSIRO field kit)	Annual rainfall (mm)	Altitude (m)
5	33°29'21"	18°19'36"	6.5	450	50
11	31°47'59"	18°37'16"	5.5	200	100
14	31°26'47"	19°8'41"	5.5	350	700
32	32°50'36"	18°37'99"	6	400	300

medium to high annual rainfall environment (600 mm annual rainfall), and the second stand was established at Harrismith (32°52'21.4" S 117°51'11.4" E), considered to be a low to medium rainfall environment (400 mm). Fifteen months later, the seeds produced at both sites were hand harvested, on the same day (10th of December 2015). The researchers who hand harvested the seeds visually selected the highest density areas, with ripe seed held in pods that had not shattered, yielding a high percentage of viable seed. Seed produced from Brookton and Harrismith was stored in airtight containers at  $16 \pm 4^\circ\text{C}$  (<20% relative humidity (RH)) for 2 months prior to the start of the seed burial experiments in February 2016.

Seeds from each maternal source were initially divided into 200 subsamples, each containing 40 seeds. These subsamples were randomly assigned a treatment number and placed securely into pockets of flywire micromesh as described in Section 2.2.1. Seeds (enclosed in pockets) were exposed to the environment at two locations: Murdoch (32°04'04.1" S 115°50'24.6" E) and Harrismith (32°56'23.7" S, 117°50'59.7" E), beginning on the 4th of February 2016, by placing them at depths of 0.5 and 4 cm below the soil surface in four replicated blocks and then covering with topsoil (Fig. 3). The pockets were protected from disturbance by placing chicken wire on the surface. Seeds were exposed to the ambient field conditions for up to 188 weeks and sampled nine times at intervals of approximately 26 weeks during that period. Temperature loggers (Thermochron TC) were placed adjacent to the seed pockets and retrieved and replaced after every 6 months to obtain temperature fluctuations in the soil. Further temperature data was collected nearby from Stephenson screen apparatus (Table 2).

## 2.3 Ex situ methodology for study of release of legume seeds from physical dormancy

### 2.3.1 Acquisition of legume seeds

*Biserrula pelecinus* (L.) cv. Casbah is an annual legume from the Mediterranean basin (Howieson et al. 1995). It was selected as a comparator in this study because it has a high hard seed content with a greater percentage of seed germinating in the second year after seed set, relative to the first (Loi et al. 1999). *Ornithopus sativus* (Brot.) cv. Margarita is an annual legume bred for the ley-farming system of WA (Nutt et al. 2021a). It was selected as the second comparator in this study as its pattern of hard seed breakdown differs from *B. pelecinus* in that it has a greater percentage of hard seed breakdown in the first year after seed production than the second (Loi et al. 1999). Seeds of both comparators were collected from neighbouring 50-ha stands growing in the wheatbelt of WA at Naremben (32°05'49.1" S 118°19'17.6" E). Seeds of *B. pelecinus* were gently separated from pods by rubber beaters in a Kimseed/ L & T Venebles thresher equipped with

a 3-mm sieve. Pods of *O. sativus* were hand harvested and aspirated to remove the light organic matter. In contrast to *B. pelecinus* (and *L. ambigua*), *O. sativus* seed was left inside their woody pods, as removal of seed leads to unavoidable scarification. Seeds of *B. pelecinus* and pods of *O. sativus* were then stored in paper bags at  $16 \pm 4^\circ\text{C}$  (<20% RH) until use in the experiments described in Section 2.3.2 below. Pods of *L. ambigua* were hand harvested from Harrismith in December 2019 and seeds were gently removed from the pods, then stored as described for *B. pelecinus*.

### 2.3.2 Seed drying and exposure to subsequent cycling of temperature and humidity

Seeds of *L. ambigua* and *B. pelecinus* were screened for uniformity by passing through a sieve, then through an Encott two-way seed divider and subsampled. One hundred seeds of *L. ambigua* and 100 seeds of *O. sativus* (in pods) were sorted into eight replicates and allocated to each of three temperature regimes in laboratory ovens set to 40, 60 or 80 °C (treatments B–M, Table 3). The ovens all had a mercury glass thermometer placed next to the seeds and were inspected daily to monitor temperature. Seeds “dried” at 20°C were considered to be a room temperature control (Table 3). *B. pelecinus* (100 seeds) was subject to dry constant temperatures of 20°C and 80°C, as a preliminary study indicated that this species did not release from dormancy when exposed to temperatures below 75°C (data not shown). The oven temperatures of 40°C and above were selected as they are commonly achieved on the soil surface in WA (Quinlivan 1961, 1966). The temperature of 80°C can occasionally be reached on certain soil types in Australia (Ooi et al. 2009; Taylor 2005). Additionally, Ooi et al. (2014) described 80°C cues for conditions that break dormancy in legumes that persist in fire-prone environments.

Following the initial oven-drying phase, seeds/pods of four replicates of each species were placed onto moist filter paper and left to germinate for 21 days, with germination counts every 3 days as described in Section 2.2.1. The remaining four replicates were placed into a Thermoline Scientific temperature and humidity chamber (Model: TRH-150) and exposed to two temperature fluctuations per 24 h for 10 days (i.e. 20 fluctuation cycles). The fluctuations consisted of 60°C (20% RH) for 2 h then 15°C (50% RH) for 2 h. The remaining 8 h were spent reaching the minimum and maximum temperature and humidity (i.e. 4 h from 60°C (20% RH) to 15°C (50% RH) then 2 h of stable temperature and RH, then 4 h returning to 60°C (20% RH)), referred to as the cooling and heating phases.

After exposure to 20 cycles, seeds/pods were assessed for germination as described above (Section 2.2.1). Seed was defined as germinated when a radicle measuring at least 2 mm in length was evident and as “hard” if there was absence of imbibition, hence remaining dormant.

**Table 2** The cumulative number of days where soil temperature was >30°C, and soil temperature fluctuations >25°C, in two different agro-ecological zones (Murdoch and Harrismith) in Western Australia. Temperature fluctuations were recorded 0.5 cm under the soil surface. Annual rainfall, and maximum and minimum temperatures are included for general site description.

Location	Measurements	2016	2017	2018	2019
Murdoch	Cumulative summer days >30°C	63	149	169	263
	Annual rainfall (mm)	745.0	890.8	836.8	613.2
	Cumulative days >25°C fluctuation	0	0	0	0
	Maximum summer temperature (°C)	43.5	42.4	41.1	42.4
	Minimum summer temperature (°C)	10.3	8.8	10.1	10.7
Harrismith	Cumulative summer days >30°C	53	125	152	263
	Annual rainfall (mm)	378.4	470.6	294.2	238.0
	Cumulative days >25°C fluctuation	9	30	49	62
	Maximum summer temperature (°C)	43.2	40.6	40.2	41.9
	Minimum summer temperature (°C)	8.5	6.7	6.6	8.1

## 2.4 Statistical analysis and calculations

A generalised mixed model (function lmer in package lme4) (Bates et al. 2019) with family binomial (logit link) was performed. The percentage hard seed breakdown was the response variable, treatments were fixed effects and block effect was a random effect to account for dependency in the data. Residuals were visually assessed to ensure

**Table 3** Temperature regimes that were imposed on each of the seed sources in the cabinet experiment (ex situ). Treatments in the left column (A–M) were exposed to a constant dry temperature for a number of days only. The treatments in the second column (N–Z) were exposed to 20 cabinet cycles consisting of 60°C (20% relative humidity (RH)) for 2 h then 15°C (50% RH) for 2 h, with 4 h of heating or cooling stages between cycles after receiving an initial constant dry temperature. \**Biserrula pelecinus* was only subject to 20°C and 80°C constant dry temperature treatments.

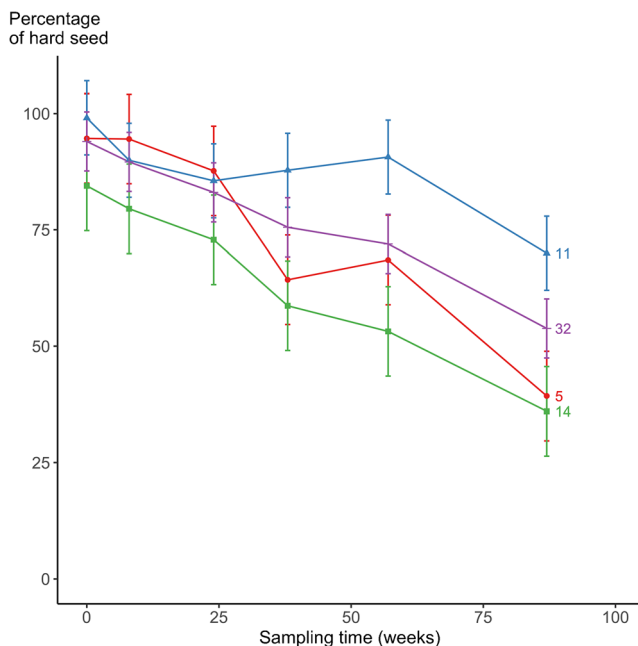
Treatments		Constant dry temperature (°C)	Days of exposure to dry heat
Constant dry temperature	Constant dry temperature followed by fluctuating temperature (60/15°C) and humidity (20/50% RH)		
A*	N*	20 (control)	-
B	O	40	1
C	P	40	2
D	Q	40	4
E	R	40	8
F	S	60	1
G	T	60	2
H	U	60	4
I	V	60	8
J*	W*	80	1
K*	X*	80	2
L*	Y*	80	4
M*	Z*	80	8

the model assumptions were met. The Akaike Information Criterion (AIC) was used in the model selection where appropriate. The function emmeans from package emmeans (Lenth 2016) was used for post hoc testing using the Tukey method of *P* value adjustment and the Kenward-Roger method of calculating degrees of freedom. Fitted values, 95% confidence intervals and comparison arrows (based on Tukey method) were extracted from models using the emmeans function from the package emmeans (Lenth 2016) and plotted with package ggplot2 (Wickham et al. 2016). Comparison arrows represent Tukey pairwise comparisons between treatments and where the arrows overlap there is no significant difference between treatments.

## 3 Results

### 3.1 Hard seed breakdown of *L. ambigua* seed in situ

**Effect of genotype on the hard seed breakdown pattern of *L. ambigua*** Hard seed of *L. ambigua* from the four genotypes originally sourced in RSA differed ( $P>0.05$ ) with respect to their pattern of hard seed breakdown over 87 weeks of burial at 0.5 cm (Fig. 4). The initial hard seed content varied between 84% (genotype 14) and 99% (genotype 11) ( $P>0.05$ ). After burial, the hard seed from the four genotypes had a similar breakdown pattern in the first 24 weeks with approximately 5–10% of the seeds softening ( $P>0.05$ ). After this, two patterns emerged, with genotypes 14 and 32 softening linearly, while 5 and 11 softened less between weeks 37 and 54 and more rapidly thereafter. Genotype 11 seed remained relatively hard at 87 weeks, while genotype 14 continued to soften with only 38% remaining hard at the completion of the experiment ( $P<0.0001$ ). The largest decline in hard seed between sampling times occurred between 57 and 87 weeks for genotypes 5 and 11 ( $P<0.05$ ).



**Fig. 4** The hard seed breakdown of four genotypes of *Lebeckia ambigua* from the western cape of South Africa when buried at a depth of 0.5 cm at Murdoch University for 87 weeks. Error bars represent 95% confidence intervals.

**The effect of maternal growing environment, burial depth and location on the hard seed breakdown pattern of *L. ambigua* genotype 32** Both Harrismith and Murdoch sites experienced 263 summer days where the temperature reached  $>30^{\circ}\text{C}$  over the 4 years of the experiment. However, Harrismith also experienced 62 days where the diurnal temperature fluctuation was  $>25^{\circ}\text{C}$ , while the latter site recorded 0 days (Table 2). The maximum summer air temperature recorded during the 4 years was similar at both sites ( $43.2^{\circ}\text{C}$  at Harrismith, and  $43.5^{\circ}\text{C}$  at Murdoch), while the mean rainfall during this period was 345.3 mm for Harrismith and 771.5 mm at Murdoch.

*L. ambigua* seed from the maternal sites was initially hard (92% Brookton; 85% Harrismith) before burial. No release from dormancy of this hard seed when buried at 0.5 cm was detected at either site after 11 weeks of burial ( $P>0.05$ ), nor when sampled again after 33 weeks (Fig. 5). However, after 55 weeks of burial at 0.5 cm at Harrismith, both maternal sources softened to around 70% hard seed remaining, which was less than that at Murdoch (80%,  $P<0.01$ , Fig. 5). After 188 weeks,  $<26\%$  of seed buried at 0.5 cm remained hard at both sites when the mesh pockets were finally exhumed. The level of dormancy in seed buried at 0.5 cm did not differ between the two burial sites and maternal seed sources except at the 110-week sampling time, where seed produced at Harrismith had softened to 59% and 36% at Murdoch and Brookton, respectively ( $P<0.05$ ).

However, the effect of maternal source on hard seed breakdown pattern was highly significant ( $P<0.01$ ) when seed was

buried at 4 cm. Harrismith maternal seed remained greater than 60% hard when buried at this depth at both Harrismith and Murdoch ( $P<0.01$ ), whereas Brookton maternal seed had only 25 and 30% hard seeds at the completion of the experiment. Furthermore, the pattern of hard seed breakdown of maternal seed from Harrismith differed for burial depth, irrespective of burial location ( $P<0.05$ ).

In a further significant departure from the patterns established by burial at 0.5 cm, maternal seed from Brookton did not continue to soften after 50 weeks when buried at 4 cm at Murdoch, whereas it did when buried at this depth at Harrismith ( $P<0.05$ ). There was thus a significant three-way interaction between burial depth, site and maternal influences ( $P<0.01$ ) on hard seed breakdown over the 188 weeks of the experiment.

### 3.2 Release from dormancy of *L. ambigua*, *O. sativus* and *B. pelecinus* seed ex situ

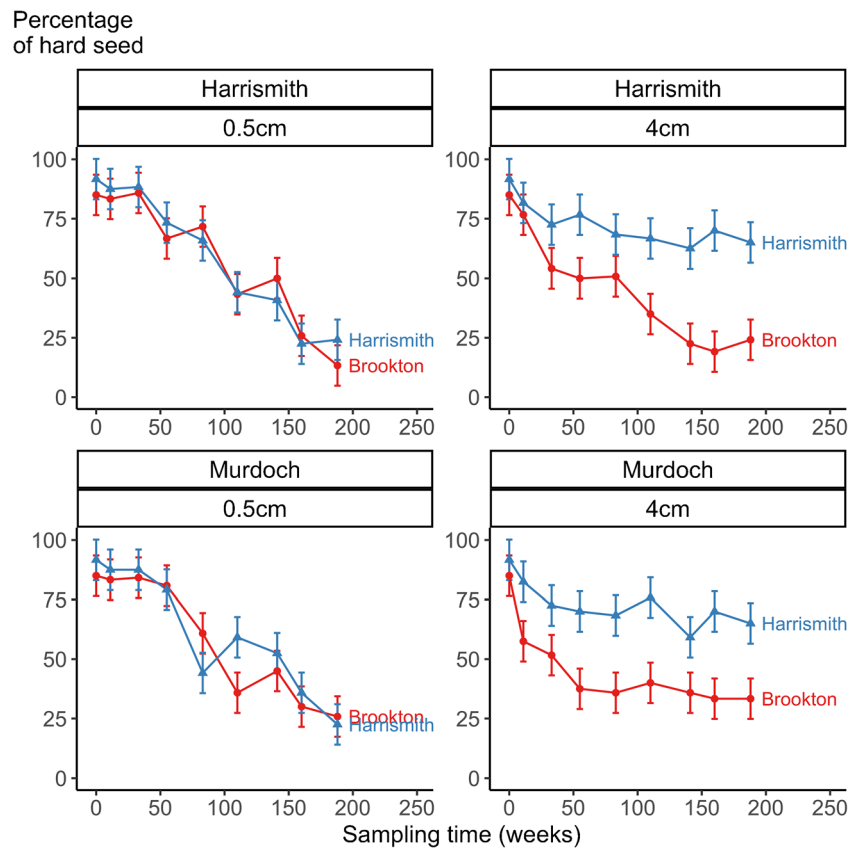
Eighty-nine per cent of seeds of *L. ambigua* produced for the oven and cabinet experiments were initially hard (Fig. 6, treatment A). Exposing these hard seeds to  $40$  or  $60^{\circ}\text{C}$  in a dry oven for up to 8 days did not influence hard seed breakdown ( $P>0.05$ ; treatments A–H, Fig. 6); however, exposure of seeds to  $80^{\circ}\text{C}$  reduced the proportion of hard seed to between 65 and 74% ( $P<0.05$ ). This release from dormancy by exposure to  $80^{\circ}\text{C}$  was not increased by subsequently exposing oven-dried seeds to cycling of temperature and humidity ( $P>0.05$ ; treatments W–Z, Fig. 6). In contrast, cycling oven-dried seed through the same series of temperature and humidity changes reduced hard seed from the initial 89% to approximately 75% ( $P<0.05$ ), whether the constant dry temperature was at 20, 40 or  $60^{\circ}\text{C}$  (N–V).

*O. sativus* seed (in pod) had an initial hard seed content of 90% (Fig. 7, treatment A). Hard seeds of this species were not released from dormancy by exposure to any temperature in the dry ovens ( $P>0.05$ , left panel). However, when this seed was then exposed to alternating cycles of temperature and humidity, only 50–65% remained hard ( $P<0.05$ ; treatments N–Z, Fig. 7). The effect of fluctuating temperature and humidity on seed softening appeared additive to the effect of oven drying, with the greatest release from dormancy being recorded from seeds initially dried at  $80^{\circ}\text{C}$  ( $P<0.05$ ; treatments W–Z).

Seed of *B. pelecinus* was initially 92% hard (Fig. 7, treatment A). There was no release from dormancy of this hard seed sample by exposure to any temperature in the dry oven, nor by combining this drying with subsequent fluctuations of temperature and humidity ( $P<0.05$ ). Hard seed of *B. pelecinus* increased from 92 to 97% after being in the dry oven for 4 days at  $80^{\circ}\text{C}$  (treatment L, Fig. 8) ( $P<0.05$ ).

Thus, all three legumes behaved significantly differently in their patterns of hard seed breakdown under the ex situ conditions.

**Fig. 5** The hard seed breakdown of *Lebeckia ambigua* seeds grown at two different maternal locations (Brookton and Harrismith) before being buried at 0.5 cm or 4 cm in two softening locations, Harrismith and Murdoch, for 188 weeks. Error bars represent 95% confidence intervals.

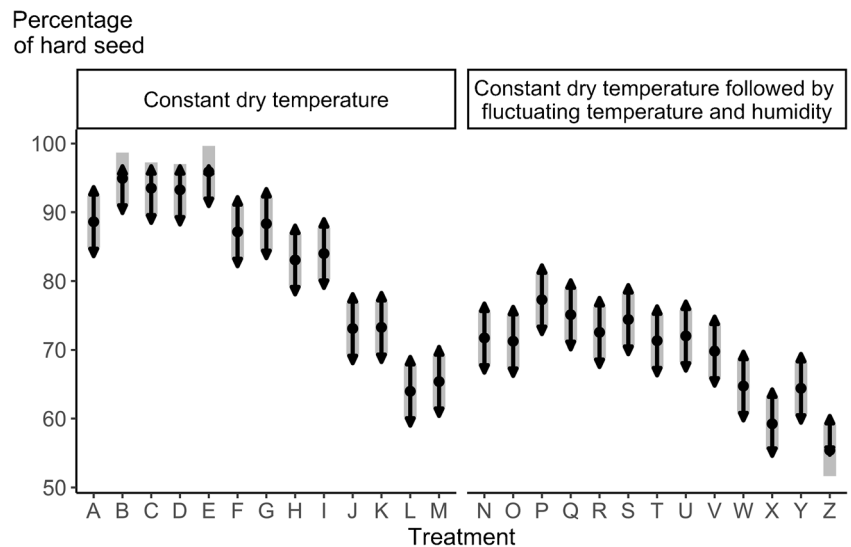


### 4 Discussion

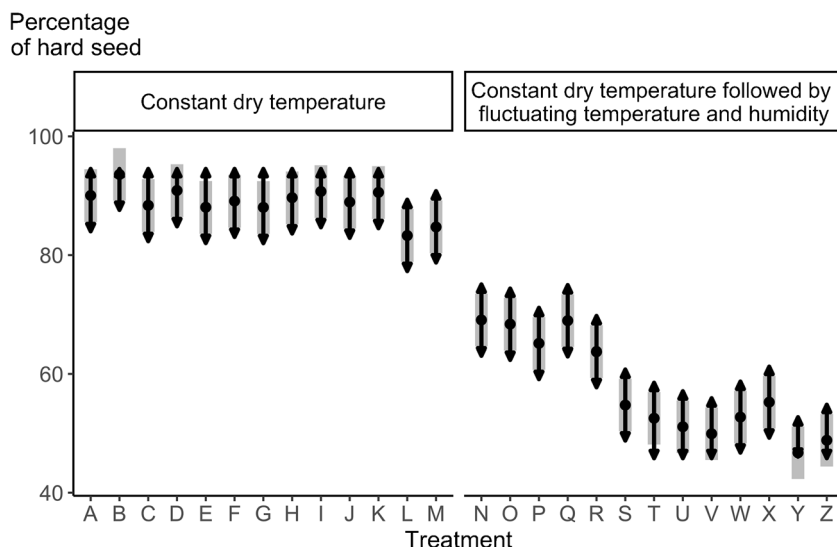
Hard seed dormancy is considered to have substantial ecological value, in both natural and agricultural ecosystems, and is a common trait in legumes inhabiting Mediterranean-type climates (Enright et al. 2014; Edwards et al. 2019; Rundel 1989; Rolston 1978). Understanding the pattern of hard seed breakdown of forage legumes can promote optimisation of farm

management practices, leading to a more profitable and sustainable forage pasture production system (Nutt et al. 2021b). Contemporary domestication programmes for perennial forage legumes should also consider this to be relevant (Beukes et al. 2021; Chimphango et al. 2020). *Lebeckia ambigua* has potential benefits to both cereal cropping and animal production in agricultural systems, particularly on infertile soils which have proven intractable to establishment of perennial

**Fig. 6** Hard seed of *Lebeckia ambigua* following different temperature treatments in a controlled environment (A–Z) (x-axis). The centre points represent means, shading represents 95% confidence intervals and the arrows indicate comparison values of significance.



**Fig. 7** Hard seed of *Ornithopus sativus* cv. Margurita following different temperature treatments in a controlled environment (A–Z) (x-axis). The centre points represent means, shading represents 95% confidence intervals and the arrows indicate comparison values of significance.



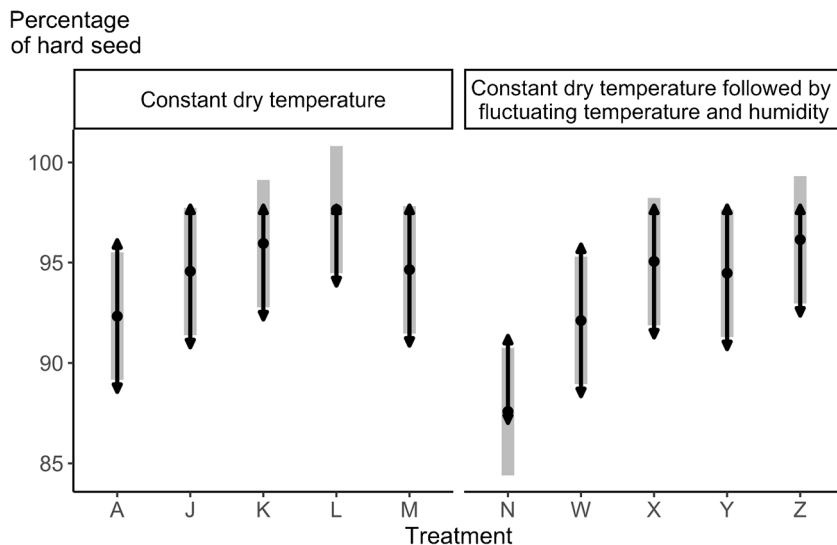
forage plants (Edwards et al. 2019). Hence, understanding the pattern of hard seed breakdown of this legume may add considerable value to farming enterprises in both southern Australia and global regions with similar edaphic constraints.

Our experiments demonstrated that a large proportion (80–90%) of seeds produced by *L. ambigua* has physical hard seed dormancy and that genotypes differed in the extent and patterns of breakdown of this dormancy when buried in soil. All four genotypes produced an initial high level of hard seeds; however, after 87 weeks of burial at 0.5 cm this hard seed content had reduced to < 40% for genotype 14 but remained close to 75% for genotype 11. When genotype 32 was examined in greater detail, the rate and pattern of this breakdown were consistent when buried at 0.5 cm, irrespective of the site of its seed production (the maternal effect) or the site of its burial (location effect). However, if burial was at 4 cm, then patterns of hard seed breakdown were greatly conditioned by both maternal and location effects.

After burial in the soil at 0.5 cm for approximately 90 weeks, half of the hard seed of genotype 32 had been released from dormancy in both field experiments. This seed would be expected to germinate given appropriate conditions of moisture and temperature. Importantly, for this genotype, even after shallow (0.5 cm) burial for 188 weeks, up to 25% of its seed remained hard. Combined, these data have great significance for those attempting to domesticate a hard seeded perennial legume, and developing a sustainable farming system around it, to achieve long-term environmental benefits within an agricultural context.

Although both softening locations in experiment 2 experienced the same number of summer days >30°C, the Harrismith site accumulated 62 days where diurnal temperature fluctuations were >25°C at the soil surface, while there was zero at the Murdoch site. The contrast in exposure to diurnal fluctuations of temperature at ambient humidity at the two sites did not alter the patterns, or extent, of breakdown of hard seed buried at 0.5 cm. It could be argued that the lack

**Fig. 8** Hard seed of *Biserrula pelecinus* cv. Casbah following different temperature treatments in a controlled environment (A–Z) (x-axis). The centre points represent means, shading represents 95% confidence intervals and the arrows indicate comparison values of significance.



of response to diurnal fluctuations in temperature in situ was in conflict with our laboratory data. In the third experiment, fluctuations of temperature, (60/15°C) combined with variable humidity (50/20%), appeared to stimulate the hard seed breakdown of *L. ambigua*, independent of any impact of the initial constant dry temperature. However, the release from dormancy by diurnal fluctuations might simply be the result of an accumulation of hours of exposure to 60°C, which was reached at the peak temperature of these cycles, rather than temperature fluctuations per se. For example, twenty oscillations of temperature in the cabinets exposed seeds to a minimum of 40 accumulated hours at 60°C and this released seeds from dormancy (treatments N–Q, Fig. 5). The greater release of dormancy in treatments S–V cf. F–I may be explained by longer exposure of the former to 60°C (oven drying at 60°C + cycling to 60°C). Exposure of seeds for 4–8 days to 80°C without fluctuations produced a similar result (treatments L and M, Fig. 5). It is also possible that the moisture present in the diurnal cycling treatments accelerated the breakdown of hard seed. Exposure to moisture is considered to degrade the lens region of hard, dry legume seeds, allowing them to become permeable to water (Gama-Arachchige et al. 2013; Baskin 2003; Baskin et al. 2000).

It is uncommon for small seeds (<5 mg) in either an ecological or agricultural system to be buried below 2 cm, especially if farmers in the latter utilise a zero-tillage (conservation agriculture) approach when sowing crops (Taylor 1985; Taylor and Ewing 1988). However, if seed of *L. ambigua* is buried deeply, perhaps by the action of insects, sand blow or by deep tillage, the release of these seeds from dormancy is likely to be substantially lessened depending upon maternal influences on hard seed development. *L. ambigua* seed grown at the maternal site of Brookton in 2015 received 289 mm with 51.3 mm falling in the seed-forming months of October–December, while Harrismith received 341 mm with 74.8 mm falling in the seed-forming months. It is feasible that the hard seed coat may not have fully formed at the time of harvest at the latter site or the lens did not fully seal. Nonetheless, seed from both sites softened equally when buried at 0.5 cm, presumably after receiving the temperature triggers necessary for hard seed breakdown. Further research is necessary to understand the effect of depth of burial and moisture on hard seeds of this species.

The hard seed breakdown pattern revealed for *L. ambigua* ex situ was dissimilar to that recorded for the annual legume *O. sativus*, which behaved in accordance with the two-stage model proposed by Taylor (1981). That study linked a pre-exposure of dry, hot conditions with subsequent cycling of temperature and humidity and their (combined) impacts on release of forage legume seeds from dormancy. Neither *L. ambigua* nor *B. pelecinus* conformed to that model in our experiments. Aberrant behaviour in this respect has previously been reported for other legumes. For example, the triggers for release of hard seed from dormancy for annual forage legume

species *Trifolium dasyurum* C. Presl, *T. glomeratum* L. and *O. compressus* L. (cv. Santorini) eluded agricultural seed ecologists when they studied the conformity of these species to the two-stage model (Taylor 2005; Smith et al. 1998; Taylor and Revell 2002; Taylor 1996; Loi et al. 1999).

Furthermore, in our field experiments, both sites recorded 263 days with an air temperature above 30°C, which can equate to a soil surface temperature of approximately 60°C (Ooi et al. 2009). Quinlivan (1961), in his formative studies of legume seed dormancy, measured soil temperatures over summer of this magnitude in WA. Quinlivan then developed an approach to investigating hard seed dormancy ex situ in a fluctuating temperature cabinet that reached 60°C. Cumulative exposure of hard seeds of *L. ambigua* to 60°C and above to break dormancy conforms to the outcomes of Quinlivan (1961, 1966). However, the relative lack of hard seed breakdown during 33–87 weeks buried in situ (with an accumulation over 120 days exposed to this temperature) is difficult to explain. A recent report by Harrison et al. (2020) of long-term storage (>10 years) changing the pattern of hard seed breakdown in annual legumes raises the possibility that the 33+ weeks of burial might be considered a period of “storage” that potentiated the subsequent release of hard seeds of *L. ambigua* from dormancy when buried for 188 weeks.

Our understanding of factors that release hard seeds from dormancy might be deficient to explain patterns of behaviour for legume seed that evolved in biomes other than the Mediterranean basin. *Lebeckia ambigua* hard seeds were released from dormancy in our experiments by exposure to 60°C and 80°C for 1–8 days in a dry oven, and this effect has not previously been reported for any perennial legume of Mediterranean origin in agriculture. Temperatures on the soil surface during fires can be in excess of 80°C for short periods of time in the fynbos and similar biomes, albeit dependent upon the aboveground biomass (Auld and Bradstock 1996; Ooi et al. 2014; Hall et al. 2017; Jeffery et al. 1988; Cocks and Stock 1997; Ooi et al. 2009; Bell et al. 1993; Gresta et al. 2011; Arianoutsou and Thanos 1996). Exposure to fire events in its evolutionary environment could well explain why the hard seed of *L. ambigua* softened at 80°C in our experiments. In support of this, reliance on a high temperature threshold to break seed dormancy has recently been demonstrated for a group of facultative pyrogenic perennial legumes (Ooi et al. 2014; Santana et al. 2010). Fire has also been demonstrated to break the dormancy of seeds of several annual legumes in the Mediterranean basin (Arianoutsou and Thanos 1996; Gresta et al. 2011). Smoke is often associated with fire and can play a role in breaking dormancy in fynbos species (Brown 1993; Waller et al. 2015; Brown et al. 2004) and other plants (Dixon et al. 1995). Although Brown et al. (2004) reported an increase in germination of fynbos legumes exposed to smoke, our preliminary research for a smoke effect on hard seed breakdown in *L. ambigua* was equivocal

(Edwards 2015). Further experiments combining smoke, high temperatures and subsequent fluctuating temperatures and humidity may be required to fully understand the hard seed dormancy of *L. ambigua*.

Facultative pyrogenic legumes differ in obligating pyrogenic functional groups in that some hard seed breakdown is recorded in response to high temperatures in the former, rather than to fire. *Lebeckia ambigua* seed germinated when provided with moisture in our laboratory experiments after exposure to typical summer soil temperatures. Nevertheless, Ooi et al. (2014) did not impose fluctuating temperatures or humidity on the species they studied. However, from the low percentage of germination reported, and the slow time to achieve 50% germination (maximum 42 days), we speculate that imposing temperature fluctuations post fire might have increased germination in their studies. Thus, without discarding factors such as the action of soil microbiota and sudden humidity changes (Quinlivan 1968; Taylor 1981, 2005; Quinlivan 1961; Auld and Bradstock 1996; Ooi et al. 2014; Hall et al. 2017; Jeffery et al. 1988; Cocks and Stock 1997), we postulate that exposure to very high temperature (e.g. 80°C) is necessary for the rapid hard seed breakdown of *L. ambigua*, and that this reflects the seed ecology of facultatively pyrogenic species.

The fynbos biome from which *L. ambigua* originated is considered a Mediterranean-type climate; however, the vegetation therein is dominated by sclerophyllous shrubs. These burn far more readily than those of the Mediterranean basin, leading to more regular fire disturbances, perhaps as often as every 10 years (Wisheu et al. 2000). The two most common reproductive/survival traits of plants from fire-prone environments are resprouting and reseeding (Keeley 1986). The population dominance of either resprouter or reseeders has been strongly linked to the frequency of fires (Keeley and Zedler 1978) moderated by the subsequent climatic events (Ojeda 2008). Both fire-defensive traits have been examined in the fynbos region (le Maitre 1992; Smith et al. 1992) where it appears the reseeders attribute is dominant among woody species like *Lebeckia* spp. (le Maitre and Midgley 1992). For these reasons, and the data produced in the experiments reported here, it may be better to consider patterns for hard seed breakdown generated by environmental researchers of legume ecology, rather than those produced by agricultural researchers, to understand triggers for hard seed breakdown in legumes found outside the Mediterranean basin.

## 5 Conclusion

An accumulation of hours exposed to 60°C and above may explain the release from dormancy of the hard seeds of *L. ambigua*. Edwards et al. (2019) postulated that the ancestral growing conditions of *L. ambigua* encouraged the production of high levels of hard seed that require a fire event to release

from dormancy. This is different from the current understanding of hard seed breakdown triggers in annual legumes of Mediterranean origin. In both ecological and agricultural contexts, it would be disadvantageous to have a distinct germination event if the seedlings are to compete against their mother plant for light, moisture and essential nutrients. If *L. ambigua* is to be utilised in agriculture, knowledge of hard seed breakdown triggers must be exploited to ensure its long-term persistence. For example, after several years of a forage pasture phase of *L. ambigua*, a crop could be established through zero tillage, and its stubble burnt to provide a seed softening event to allow fresh germination of *L. ambigua* without further intervention.

The extensive body of work on hard seed breakdown ecology by agricultural scientists could be augmented by studies on this phenomenon in natural settings, as new legume species adapted to a changing climate are further explored and domesticated to increase the sustainability of agriculture.

**Acknowledgements** The authors thank Mrs Jordyn House, Mr Joel Kidd, Ms Sam Richards and Dr Neil Ballard for their skilled technical assistance and expertise. The authors would also like to thank the farmers on whose property the seed was produced: the Butcher, Butler, Astbury and the Quartermaine families.

**Authors' contributions** Robert J. Harrison: conceptualisation, methodology, investigation, resources, data curation, writing- original, writing-review and editing, project administration

Thomas J. Edwards: conceptualisation, methodology, investigation, data curation, writing- original, writing- review and editing.

Emma Steel: formal analysis, visualisation

Ron J. Yates: writing- original, supervision

Bradley J. Nutt: conceptualisation, methodology

John G. Howieson: writing- original, writing- review and editing, supervision, visualisation, conceptualisation

**Data availability** The authors confirm that the data supporting the findings of this study are available within the article. Further raw data is available from the corresponding author, upon reasonable request.

## Declarations

**Ethics approval** Not applicable

**Consent for publication and participation** Not applicable

**Conflict of interest** The authors declare no competing interests.

## References

- Arianoutsou M, Thanos CA (1996) Legumes in the fire-prone Mediterranean regions: an example from Greece. *Int J Wildland Fire* 6(2):77–82. <https://doi.org/10.1071/WF9960077>
- Auld TD, Bradstock RA (1996) Soil temperatures after the passage of a fire: do they influence the germination of buried seeds? *Aust J Ecol* 21(1):106–109. <https://doi.org/10.1111/j.1442-9993.1996.tb00589.x>

- Baskin CC (2003) Breaking physical dormancy in seeds – focussing on the lens. *New Phytol* 158(2):229–232. <https://doi.org/10.1046/j.1469-8137.2003.00751.x>
- Baskin CC, Baskin JM (1998) Seeds: ecology, biogeography and evolution of dormancy and germination, 1st edn. Academic Press, San Diego
- Baskin JM, Baskin CC (2004) A classification system for seed dormancy. *Seed Sci Res* 14(1):1–16. <https://doi.org/10.1079/SSR2003150>
- Baskin CC, Baskin JM (2014) Seeds: ecology, biogeography and evolution of dormancy and germination, 2nd edn. Academic Press, San Diego
- Baskin JM, Baskin CC, Li X (2000) Taxonomy, anatomy and evolution of physical dormancy in seeds. *Plant Species Biol* 15(2):139–152. <https://doi.org/10.1046/j.1442-1984.2000.00034.x>
- Bates D, Maechler M, Bolker B, Walker S, Christensen RHB, Singmann H (2017) lme4: Linear mixed-effects models using Eigen and S4, 2014. R package version 1(4)
- Beard JS, Chapman AR, Gioia P (2000) Species richness and endemism in the Western Australian flora. *J Biogeogr* 27(6):1257–1268. <https://doi.org/10.1046/j.1365-2699.2000.00509.x>
- Bell DT, Plummer JA, Taylor SK (1993) Seed germination ecology in southwestern Western Australia. *Bot Rev* 59(1):24–73. <https://doi.org/10.1007/BF02856612>
- Beukes CW, Venter SN, Steenkamp ET (2021) The history and distribution of nodulating *Paraburkholderia*, a potential inoculum for Fynbos forage species. *Grass Forage Sci* 76(1):10–32. <https://doi.org/10.1111/gfs.12522>
- Brown NAC (1993) Promotion of germination of fynbos seeds by plant-derived smoke. *New Phytol* 123(3):575–583. <https://doi.org/10.1111/j.1469-8137.1993.tb03770.x>
- Brown NAC, Botha PA, van Staden J (2004) Smoke seed germination studies and a guide to seed propagation of plants from the major families of the Cape Floristic Region, South Africa. *S Afr J Bot* 70(4):559–581. [https://doi.org/10.1016/S0254-6299\(15\)30194-0](https://doi.org/10.1016/S0254-6299(15)30194-0)
- Carruggio F, Onofri A, Impelluso C, Giusso del Galdo G, Scopece G, Cristaudo A (2020) Seed dormancy breaking and germination in *Bituminaria basaltica* and *B. bituminosa* (Fabaceae). *Plants* 9(9):1110. <https://doi.org/10.3390/plants9091110>
- Chimphango SBM, Gallant LH, Poulsen ZC, Samuels MI, Hattas D, Curtis OE, Muasya AM, Cupido C, Boatwright JS, Howieson JG (2020) Native legume species as potential fodder crops in the Mediterranean renosterveld shrubland, South Africa. *J Arid Environ* 173:104015. <https://doi.org/10.1016/j.jaridenv.2019.104015>
- Cocks MP, Stock WD (1997) Heat stimulated germination in relation to seed characteristics in fynbos legumes of the Western Cape Province, South Africa. *S Afr J Bot* 63(3):129–132. [https://doi.org/10.1016/S0254-6299\(15\)30724-9](https://doi.org/10.1016/S0254-6299(15)30724-9)
- Crews TE, Blesh J, Culman SW, Hayes RC, Jensen ES, Mack MC, Peoples MB, Schipanski ME (2016) Going where no grains have gone before: from early to mid-succession. *Agric Ecosyst Environ* 223:223–238. <https://doi.org/10.1016/j.agee.2016.03.012>
- Dixon KW, Roche S, Pate JS (1995) The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. *Oecologia* 101(2):185–192. <https://doi.org/10.1007/BF00317282>
- Edwards T (2015) The seed ecology of *Lebeckia ambigua*. Unpublished honours thesis, vol 1. Murdoch University
- Edwards TJ, Howieson JG, Nutt BJ, Yates RJ, O'Hara GW, Van Wyk BE (2019) A ley-farming system for marginal lands based upon a self-regenerating perennial pasture legume. *Agron Sustain Dev* 39(13):1–19. <https://doi.org/10.1007/s13593-019-0558-2>
- Ehrman T, Cocks P (1996) Reproductive patterns in annual legume species on an aridity gradient. *Vegetation* 122:47–59. <https://doi.org/10.1007/BF00052815>
- Enright NJ, Fontaine JB, Lamont BB, Miller BP, Westcott VC (2014) Resistance and resilience to changing climate and fire regime depend on plant functional traits. *J Ecol* 102(6):1572–1581. <https://doi.org/10.1111/1365-2745.12306>
- Gama-Arachchige NS, Baskin JM, Geneve RL, Baskin CC (2013) Identification and characterization of ten new water gaps in seeds and fruits with physical dormancy and classification of water-gap complexes. *Ann Bot* 112:69–84. <https://doi.org/10.1093/aob/mct094>
- Goldblatt P, Manning J (2000) Cape plants: a conspectus of the Cape flora of South Africa. National Botanical Institute, Strelitzia
- Gresta F, Avola G, Tuttobene R, Onofri A, Barrile V, Cristaudo A, Abbate V (2011) The effect of fire on the dormancy break of three annual legume seeds. *Ital J Agron* 6(3):e23. <https://doi.org/10.4081/ija.2011.e23>
- Hall SA, Newton RJ, Holmes PM, Gaertner M, Esler KJ (2017) Heat and smoke pre-treatment of seeds to improve restoration of an endangered Mediterranean climate vegetation type. *Austral Ecol* 42(3):354–366. <https://doi.org/10.1111/aec.12449>
- Hall DJM, Davies SL, Bell RW, Edwards TJ (2020) Soil management systems to overcome multiple constraints for dryland crops on deep sands in a water limited environment on the south coast of Western Australia. *Agron* 10(12):1881. <https://doi.org/10.3390/agronomy10121881>
- Harrison RJ, Howieson JG, Yates RJ, Nutt BJ (2020) Long-term storage of forage legumes greatly alters the hardseed breakdown pattern in situ. *Grass Forage Sci* 76:72–81. <https://doi.org/10.1111/gfs.12490>
- Howieson JG, Loi A, Carr SJ (1995) *Biserrula pelecinus* L.-a legume pasture species with potential for acid, duplex soils which is nodulated by unique root-nodule bacteria. *Aust J Agric Res* 46(5):997–1009. <https://doi.org/10.1071/AR950997>
- Howieson JG, Yates RJ, Foster KJ, Real D, Besier RB (2008) Prospects for the future use of legumes. In: Dilworth MJ, James EK, Sprent JI, Newton WE (eds) Nitrogen-fixing leguminous symbioses. Springer, Dordrecht, pp 363–394. [https://doi.org/10.1007/978-1-4020-3548-7\\_12](https://doi.org/10.1007/978-1-4020-3548-7_12)
- Howieson JG, De Meyer SE, Vivas-Marfisi A, Ratnayakeb S, Ardley JK, Yates RJ (2013) Novel *Burkholderia* bacteria isolated from *Lebeckia ambigua* – a perennial suffrutescent legume of the fynbos. *Soil Biol Biochem* 60:55–64. <https://doi.org/10.1016/j.soilbio.2013.01.009>
- Howieson JG, Harrison RJ, Yates RJ, Hackney B, Loi A, Nutt BJ (2021) Hard seed breakdown patterns of unprocessed forage legume seed sown into dry soil in summer in southern Australia. *Grass Forage Sci* 76(1):82–92. <https://doi.org/10.1111/gfs.12526>
- Huang J, Hartemink AE (2020) Soil and environmental issues in sandy soils. *Earth Sci Rev* 208:1–22. <https://doi.org/10.1016/j.earscirev.2020.103295>
- Jayasuriya KMGG, Baskin JM, Baskin CC (2009a) Sensitivity cycling and its ecological role in seeds with physical dormancy. *Seed Sci Res* 19(1):3. <https://doi.org/10.1017/S096025850818730X>
- Jayasuriya KMGG, Baskin JM, Geneve RL, Baskin CC (2009b) Sensitivity cycling and mechanism of physical dormancy break in seeds of *Ipomoea hederacea* (Convolvulaceae). *Int J Plant Sci* 170(4):429–443. <https://doi.org/10.1086/597270>
- Jeffery DJ, Holmes PM, Rebelo AG (1988) Effects of dry heat on seed germination in selected indigenous and alien legume species in South Africa. *S Afr J Bot* 54(1):28–34. [https://doi.org/10.1016/S0254-6299\(16\)31358-8](https://doi.org/10.1016/S0254-6299(16)31358-8)
- Keeley JE (1986) Resilience of Mediterranean shrub communities to fires. In *Resilience in Mediterranean-type ecosystems* (pp. 95–112). Springer, Dordrecht
- Keeley JE, Zedler PH (1978) Reproduction of chaparral shrubs after fire: a comparison of sprouting and seeding strategies. *Am Midl Nat* 99:142–161

- Kimura E, Islam MA (2012) Seed scarification methods and their use in forage legumes. *J Res Seed Sci* 5:38–50. <https://doi.org/10.3923/rjss.2012.38.50>
- Lal R (1988) Soil degradation and the future of agriculture in sub-Saharan Africa. *J Soil Water Conserv* 43(6):444–451
- Le Maitre DC (1992) The relative advantages of seeding and sprouting in fire-prone environments: a comparison of life histories of *Protea neriifolia* and *Protea nitida*. In *Fire in South African mountain fynbos* (pp. 123–144). Springer, Berlin, Heidelberg. [https://doi.org/10.1007/978-3-642-76174-4\\_7](https://doi.org/10.1007/978-3-642-76174-4_7)
- le Maitre DC, Midgley JJ (1992) Plant reproductive ecology. In: Cowling RM (ed) *The ecology of fynbos: nutrients, fire and diversity*. Oxford University Press, Cape Town, pp 135–174
- Le Roux MM, Van Wyk B-E (2007) A revision of *Lebeckia* sect. *Lebeckia*: the *L. sepiaria* group. *S Afr J Bot* 73(1):118–130. <https://doi.org/10.1016/j.sajb.2006.09.005>
- Lenth RV (2016) Least-squares means: the R package lsmmeans. *J Stat Softw* 69(1):1–33
- Loi A, Cocks PS, Howieson JG, Carr SJ (1999) Hardseededness and the pattern of softening in *Biserrula pelecinus* L., *Ornithopus compressus* L., and *Trifolium subterraneum* L. seeds. *Aust J Agric Res* 50(6):1073–1082. <https://doi.org/10.1071/AR98061>
- Malézieux E (2012) Designing cropping systems from nature. *Agron Sustain Dev* 32(1):15–29. <https://doi.org/10.1007/s13593-011-0027-z>
- Nutt BJ, Harrison RJ, McComb JA, Howieson JG (2021a) The breeding system of *Ornithopus sativus* Brot. subsp. *sativus*. *Grass Forage Sci* 76(1):3–9
- Nutt BJ, Loi A, Hackney B, Yates RJ, D’Antuono M, Harrison RJ, Howieson JG (2021b) “Summer sowing”: a successful innovation to increase the adoption of key species of annual forage legumes for agriculture in Mediterranean and temperate environments. *Grass Forage Sci* 76(1):93–104. <https://doi.org/10.1111/gfs.12516>
- Ojeda F (2008) Biogeography of seeder and resprouter *Erica* species in the Cape Floristic Region—where are the resprouters? *Biol J Linn Soc* 63(3):331–347. <https://doi.org/10.1111/j.1095-8312.1998.tb01521.x>
- Ooi MKJ, Auld TD, Denham AJ (2009) Climate change and bet-hedging: interactions between increased soil temperatures and seed bank persistence. *Glob Chang Biol* 15(10):2375–2386. <https://doi.org/10.1111/j.1365-2486.2009.01887.x>
- Ooi MKJ, Denham AJ, Santana VM, Auld TD (2014) Temperature thresholds of physically dormant seeds and plant functional response to fire: variation among species and relative impact of climate change. *Ecol Evol* 4(5):656–671. <https://doi.org/10.1002/ece3.973>
- Quinlivan BJ (1961) The effect of constant and fluctuating temperatures on the permeability of the hard seeds of some legume species. *Aust J Agric Res* 12(6):1009–1022. <https://doi.org/10.1071/AR9611009>
- Quinlivan BJ (1966) The relationship between temperature fluctuations and the softening of hard seeds of some legume species. *Aust J Agric Res* 17(5):625–631. <https://doi.org/10.1071/AR9660625>
- Quinlivan BJ (1968) Seed coat impermeability in the common annual legume pasture species of Western Australia. *Aust J Exp Agric* 8(35):695–701. <https://doi.org/10.1071/EA9680695>
- Revell CK, Ewing MA, Nutt BJ (2012) Breeding and farming system opportunities for pasture legumes facing increasing climate variability in the south-west of Western Australia. *Crop Pasture Sci* 63(9):840–847. <https://doi.org/10.1071/CP12160>
- Rodrigues-Junior AG, Baskin CC, Baskin JM, Garcia QS (2018) Sensitivity cycling in physically dormant seeds of the Neotropical tree *Senna multijuga* (Fabaceae). *Plant Biol* 20(4):698–706. <https://doi.org/10.1111/plb.12719>
- Rolston MP (1978) Water impermeable seed dormancy. *Bot Rev* 44(3):365–396. <https://doi.org/10.1007/BF02957854>
- Rundel PW (1989) Ecological success in relation to plant form and function in woody legumes. Monographs in Systematic Botany. MO Bot Gard Press 29:377–398
- Santana VM, Bradstock RA, Ooi MKJ, Denham AJ, Auld TD, Baeza MJ (2010) Effects of soil temperature regimes after fire on seed dormancy and germination in six Australian Fabaceae species. *Aust J Bot* 58(7):539–545. <https://doi.org/10.1071/BT10144>
- Smith RE, Van Wilgen BW, Forsyth GG, Richardson DM (1992) Coexistence of seeders and sprouters in a fire-prone environment: the role of ecophysiology and soil moisture. In *Fire in South African mountain fynbos* (pp. 108–122). Springer, Berlin, Heidelberg. [https://doi.org/10.1007/978-3-642-76174-4\\_6](https://doi.org/10.1007/978-3-642-76174-4_6)
- Smith FP, Cocks PS, Ewing MA (1998) Seed production in cluster clover (*Trifolium glomeratum* L.). 2 Effect of sowing time and sowing rate on flowering time, abortion, seed size, and hardseededness. *Aust J Agric Res* 49(6):965–972. <https://doi.org/10.1071/A97116>
- Taylor GB (1981) Effect of constant temperature treatments followed by fluctuating temperatures on the softening of hard seeds of *Trifolium subterraneum* L. *Funct Plant Biol* 8(6):547–558. <https://doi.org/10.1071/PP9810547>
- Taylor GB (1985) Effect of tillage practices on the fate of hard seeds of subterranean clover in a ley farming system. *Aust J Exp Agric* 25(3):568–573. <https://doi.org/10.1071/EA9850568>
- Taylor GB (1996) Incidence and measurement of autumn seed softening within *Medicago polymorpha* L. *Aust J Agric Res* 47(4):575–586. <https://doi.org/10.1071/AR9960575>
- Taylor GB (2005) Hardseededness in Mediterranean annual pasture legumes in Australia: a review. *Aust J Agric Res* 56(7):645–661. <https://doi.org/10.1071/AR04284>
- Taylor GB, Ewing MA (1988) Effect of depth of burial on the longevity of hard seeds of subterranean clover and annual medics. *Aust J Exp Agric* 28(1):77–81. <https://doi.org/10.1071/EA9880077>
- Taylor GB, Revell CK (1999) Effect of pod burial, light, and temperature on seed softening in yellow serradella. *Aust J Agric Res* 50(7):1203–1209. <https://doi.org/10.1071/AR98206>
- Taylor GB, Revell CK (2002) Seed softening, imbibition time, and seedling establishment in yellow serradella. *Aust J Agric Res* 53(9):1011–1018. <https://doi.org/10.1071/AR01201>
- Taylor GB, Maller RA, Rossiter RC (1991) A model describing the influence of hard seededness on the persistence of an annual forage legume, in a ley farming system, in a Mediterranean-type environment. *Agric Ecosyst Environ* 37(4):275–301. [https://doi.org/10.1016/0167-8809\(91\)90157-S](https://doi.org/10.1016/0167-8809(91)90157-S)
- van Wilgen BW (2013) Fire management in species-rich Cape fynbos shrublands. *Front Ecol Environ* 11(1):35–e44. <https://doi.org/10.1890/120137>
- Waller PA, Anderson PM, Holmes PM, Newton RJ (2015) Developing a species selection index for seed-based ecological restoration in Peninsula Shale Renosterveld, Cape Town. *S Afr J Bot* 99:62–68. <https://doi.org/10.1016/j.sajb.2015.03.189>
- Wezel A, Bellon S, Doré T, Francis C, Vallod D, David C (2009) Agroecology as a science, a movement and a practice. A review. *Agron Sustain Dev* 29(4):503–515. <https://doi.org/10.1051/agro/2009004>
- Wickham H, Chang W, Henry L, Pedersen TL, Takahashi K, Wilke C, Yutani H (2016) ggplot2: create elegant data visualisations using the grammar of graphics. R package version 2(1)
- Wisheu IC, Rosenzweig ML, Olsvig-Whittaker L, Shmida A (2000) What makes nutrient-poor mediterranean heathlands so rich in plant diversity? *Evol Ecol Res* 2(7):935–955

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.