

Influence of selected environmental factors on seed germination and seedling survival of the arid zone invasive species tobacco bush (*Nicotiana glauca* R. Graham)

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Abstract. Tobacco bush (*Nicotiana glauca* R. Graham) is an aggressive invading species, which is active after disturbances such as high rainfall events and flooding. Past studies have focussed on population dynamics and allelopathic effects associated with the species, but little is known about its seed ecology. To address this dearth of information, this study aimed to investigate: (i) the effect of various stress factors (temperature, light, osmotic potential, salt stress, heat-shock, a combination of heat-shock and smoke, pH buffer, and burial depth of seed) on seed germination and seedling emergence, and (ii) factors affecting the fate of seedlings. The results show that *N. glauca* was able to germinate over a broad range of temperatures with highest seed germination occurring at 30/20°C with 12 h of light and 12 h of dark conditions. Seed germination was greatest (89%) when seeds were placed on the soil surface and emergence decreased considerably as planting depth increased from 0.5 to 1.5 cm. Water stress greatly reduced seed germination (58% germination at osmotic potentials below –0.2 MPa) and germination was completely inhibited at water potentials of –0.4 to –0.6 MPa. Although increasing salinity reduced the seed germination of this invasive species, *N. glauca* seed was able to germinate in both alkaline (81% at pH 10) and acidic (80% at pH 4) conditions. The trial on the effect of seed age and field seed burial on seed germination showed a slight decline in seed germination after 120 days of burial compared with non-buried seeds. Further, the combined effect of heat-shock and smoke effectively inhibited the germination of *N. glauca* seeds; however, our study shows that seedlings of *N. glauca* can withstand heat-shock of up to 130°C. Studies such as this will assist in the development of control strategies to prevent the spread of this invasive species into arid landscapes.

Additional keywords: germination, heat-shock, light, osmotic potential, salt stress, temperature.

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Introduction

Tobacco bush (*Nicotiana glauca* R. Graham), belongs to the family Solanaceae and is native to Argentina and Bolivia (Goodspeed 1954). Studies have demonstrated that *N. glauca* is toxic to humans and animals (Panter *et al.* 2000). *N. glauca* contains the alkaloid anabasine (Mizrachi *et al.* 2000), which is more toxic than nicotine (Sims *et al.* 1999). Grazing animals generally avoid it, as it is unpalatable. However, during drought and high grazing pressure, livestock may consume *N. glauca* and die (Cunningham *et al.* 1981). Panter *et al.* (2000) also reported that *N. glauca* causes defects in fetal goats and sheep if the mother does not die from consuming the plant. According to the Global Invasive Species Database (2016), *N. glauca* has spread from its original distribution into California (Schueller 2004), Mexico

(Hernández 1981), northern parts of the Mediterranean region (Tadmor-Melamed *et al.* 2004), and southern Africa (Geerts and Pauw 2009).

Nicotiana glauca is found in disturbed areas, including areas of high soil moisture, near earth tanks, roadsides and creek lines (Boylard *et al.* 1985). Cunningham *et al.* (1981) reported that in Australian arid and semi-arid landscapes, extensive stands of *N. glauca* may persist for some years on stream floodplains and drainage channels after summer floods. This species has also now naturalised in Australia, and has become common in many parts of the country including conservation areas in Victoria (e.g. Barkindji Biosphere Reserve and You Yangs Regional Park) and western New South Wales (e.g. Kinchega National Park, Tarawi Nature Reserve, and Sturt National Park). It is a fast growing

shrub/tree species, and can grow vigorously up to 3 m in height. Plants start to produce flowers ~1 year after emergence and may produce 10 000–1 000 000 seeds per year at maturity (Mizrachi *et al.* 2000).

Seed germination is a critical element of any plant's lifecycle, and is one of the key processes in determining the success of an invasive species (Wu and Du 2007). The rate of seed germination is influenced by numerous biotic and abiotic factors. An important variable is how long seeds can remain viable. Detailed information on how environmental factors influence seed germination of this species can help with the development of control measures in both on agricultural production areas and natural ecosystems. Four principal external factors have generally been identified in relation to seed germination; the availability of water, atmospheric gas composition, external soil and air temperature, and photo period. It is understood that external temperature and available light affect germination by regulating the enzymes which are directly involved in germination (Baskin and Baskin 1998).

As Florentine and Westbrooke (2005) and Florentine *et al.* (2006) have pointed out, lack of information regarding environmental factors affecting *N. glauca* seed germination makes it hard to develop systematic control strategies for this invasive species. The authors focussed their studies on understanding the overall effect of invasion by this species as a function of an episodic flooding event, which affected native species, soil seed bank dynamics, seed germination patterns, and seedling response to flood and drought. Although Florentine *et al.* (2006) examined seed germination using constant temperature regimes, there is relatively little known about the environmental factors identified above. A better understanding of seed germination, including the combined effect of factors such as alternating temperature and light regimes, and fire-related parameters of smoke exposure and heat-shock, is essential. Previous studies have shown that this species tends to colonise along creek lines and water bodies such as earth tanks after high rainfall (Florentine and Westbrooke 2005; Florentine *et al.* 2006), indicating that soil moisture is a key factor for *N. glauca* seed germination. Notwithstanding this observation, details of how low soil moisture level can affect seed germination of *N. glauca* have not yet been determined.

In view of the dearth of information on the species, this study aimed to investigate (i) the effect of temperature, light, osmotic potential, salt stress, heat-shock, a combination of heat-shock and smoke, pH buffer, and burial depth of seed on seed germination and seedling emergence, and (ii) the effect of radiant heat on the survival and development of emergent seedlings.

Materials and methods

Seed collection and storage

Capsules from more than 50 *N. glauca* plants were collected at maturity (when the seed capsule turned light brown) at the Federation University Australia's Nanya Research Station (33.12°09'8.37"S, 141.3°17'30.2"E), located in the Scotia Mallee region of south-west New South Wales, Australia, during May 2015. The capsules were placed in labelled paper bags and taken to the university's seed ecology laboratory at the Mt Helen Campus, where they were air-dried for 3 days and

cleaned. The seeds were then removed and placed in a labelled airtight clean glass bottle and kept at room temperature (20°C) until use.

The climate at Nanya Station is semi-arid, classified as hot (summer drought) grassland (Köppen 1900; BOM 2005). As such, the region is subject to a severe moisture deficit. Annual rainfall averages are between 200 and 300 mm, and the potential annual evaporation is ~2000–2400 mm (BOM 2013). The seasonal distribution of rainfall is fairly even, but annual variation between seasons is high. Highest summer temperatures occur during February (mean daily maximum = 32°C; minimum = 16°C) and mild winter temperatures are experienced during July (mean daily maximum = 15°C; minimum = 5°C) (BOM 2013).

General seed germination protocol

Seeds were surface-sterilised by rinsing in 1% sodium hypochlorite for 2 min then washed clean with double-distilled water before the start of each germination trial. Within the replicates of all seed germination trials, seeds were evenly spaced in 9-cm-diameter Petri dishes lined with Whatman® No. 11 filter papers, and moistened with 8-mL sterile distilled water or an appropriate treatment solution to ensure adequate moisture for the seeds. The Petri dishes containing the seeds were then placed in incubators (Thermoline Scientific, temperature and humidity cabinet, Model: TRISLH-495-1-s.d., Vol. 240, Sydney, Australia), fitted with cool-white fluorescent lamps that provided a photosynthetic photon flux of 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$. All the Petri dishes were sealed with parafilm. For trials involving treatments where seeds were exposed to 24 h of dark conditions, Petri dishes were wrapped in a double layer of aluminium foil. Subsequent germination monitoring of these replicates was undertaken under green safe light. For all trials, replicates were monitored for germination for a period of 30 days from the date they were placed in incubator. Seeds were considered germinated when the radicle was ~2 mm long and cotyledons had emerged from the seed coat (Ferrari and Parera 2015). At the conclusion of the germination test, non-germinated seeds were checked for viability using the 2,3,5-triphenyltetrazolium chloride test using the procedures described for the genus *Nicotiana* (F. Solanaceae) by Moore (1985). The seed coat of each seed was punctured at the distal end and the seeds were stained with a 1% solution of 2,3,5-triphenyltetrazolium chloride for 24 h, after which they were examined to assess viability. If the embryo and surrounding nutritive tissue were stained a dark pink colour, the seed was considered as viable. If the embryo did not stain or was poorly stained it was classed as unviable.

Temperatures and light

To examine the effect of temperatures and light on *N. glauca* seeds, surfaced sterilised seeds were exposed to day/night temperatures of 35/30, 30/20, and 17/7°C under 12/12 h light/dark and 24 h of continuous darkness. Three replicates of 25 seeds were used for the temperature and light studies. These temperature regimes were selected to approximate temperature variations during the period of spring to winter in New South Wales, Australia. As shown in the results, the combination 30/20°C with 12h/12 h light/dark temperature and light regime,

showed highest germination rates. For the remainder of the experiments, unless otherwise indicated, Petri dishes were incubated under these conditions to test the effect of the other factors.

Effect of osmotic potential and salt stress on germination

To examine different osmotic potentials, *N. glauca* seeds were placed in solutions with osmotic potential of 0, -0.1, -0.2, -0.4, and -0.6 MPa. Three replicates of 20 seeds were used for the temperature and light study. Different concentrations of osmotic potential solutions were prepared by dissolving polyethylene glycol 8000 (Sigma-Aldrich Co., MO, USA) in sterilised distilled water. For the effect of salt stress on *N. glauca*, a study was conducted using sodium chloride (NaCl) concentrations of 0, 25, 50, 100, 150, 200, and 250 mM (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA). Seeds were placed in Petri dishes containing 7 mL of the different osmotic or NaCl solutions.

Effect of pH on seed germination

The effect of pH on seed germination was examined by using buffer solutions with pH values of 4, 5, 6, 7, 8, 9, and 10 prepared according to the procedures described by Chachalis and Reddy (2000). Unbuffered deionised water (pH 6.4) was used as Control. Three replicates of 20 seeds were used for the temperature and light study.

Effect of burial depth on seedling emergence

Red sandy soil was collected from the Nanya Station and taken to a Federation University Australia glasshouse in Ballarat (Victoria). The soil was autoclaved to kill all seeds and other propagules. The sterilised soil was then air-dried in the laboratory before use. For the trial, plastic trays (10 cm × 6 cm × 6 cm) were lined with paper towels and the air-dried soil was added. Three replicates of 20 *N. glauca* seeds were placed on the top of the soil and covered with different volumes of soil to depths of 0, 0.2, 0.5, 1.0, 1.5, and 2.0 cm. Seeds covered with 2 mm of soil may not have covered uniformly due to the small size of the seed. Some of the seeds might therefore have been covered only lightly. Six plastic trays were placed inside three larger white 'butcher's' trays (28 cm × 44 cm × 5.5 cm) and water was added to the larger tray to facilitate watering from below and to ensure minimal disturbance of seed samples. The trays were placed in the 30/20°C cabinet and monitored regularly for seedling emergence.

Seed longevity in the field

The seed burial experiment was established in the field at Nanya Research Station, New South Wales, Australia. Twenty cleaned seeds were placed in each of 12, 5 cm × 5-cm envelopes made of 0.5-mm aluminium mesh. The envelopes were placed in the soil at 1 cm depth. Three envelopes were removed after 20, 44, 77, and 121 days. Control seeds were kept in an airtight glass jar at room temperature in the Seed Ecology Laboratory of Federation University Australia, in Ballarat. Seeds from individual envelopes were counted and tested with the standard germination and viability test protocols mentioned previously.

Combined effect of heat-shock and smoke on germination

To examine the combined effect of temperature regimes and smoke, seeds were exposed to different temperatures and a 10% smoke water solution. Approximately 30 *N. glauca* seeds were placed in stainless steel mesh envelopes (2 × 2 cm). Red soil (collected from Nanya Station) was placed in an aluminium container (15 cm × 9 cm × 6 cm) and placed in an oven at the selected temperature (Memmert, Type No. ULE500). A thermo probe (Thermocouple data logger: EL-USB-TC-LCD) was connected to an LED display and placed in the soil. The selected temperatures were 40°C, 60°C, 80°C, 100°C, 120°C and 140°C. At the selected temperature, three metal mesh envelopes containing seeds and the thermo probe were buried in the soil for 3 min. The envelopes were then extracted, the seeds removed and placed in a plastic tray and kept at room temperature for 24 h. The heat-treated seeds, as well as a Control sample of non-treated seeds were then placed in Petri dishes and watered using water or smoke water (Regen[®] 2000 smoke water). Three replicates of 20 seeds were used for this study.

Effects of radiant heat on seedlings

Nicotiana glauca seeds were germinated in the 30/20°C cabinet and individually transplanted, on 7 July 2015, into black plastic pots (140 mm height and 130 mm wide) containing commercially available potting mix. The pots were moistened and placed in a glasshouse at the Seed Ecology Laboratory of the University in Ballarat. All pots were watered daily with an automatic sprinkler system. Twenty-five uniform-sized seedlings (~18 cm height and 1 cm root collar diameter) were selected and divided into five groups of five seedlings. To acclimatise the seedlings to natural conditions, 10 days before the radiant heat treatment, all the selected seedlings were removed from the glasshouse and placed on a bench, where they were hand watered. Seedlings, with the soil retained around their roots, were carefully removed from the plastic pots and placed in an aluminium tray (38 cm × 27 cm × 14 cm). Trays were then placed in an oven at one of five temperatures (room temperature (20), 80, 110, 130, and 160°C) for 5 min (after Moore *et al.* 1977; Gleadow and Narayan 2007). These treatments were applied to approximate temperature-time curve that have been used in previous studies (Trabaud 1979; Moreno *et al.* 2004; Luna *et al.* 2007). Immediately after exposure to the heat-shock, the seedlings were returned to the pots in the glasshouse and watered twice a day, and monitored 6 weeks.

Data analyses

The study considered the effect of the various treatments on the final germination percentage (FG%) calculated as:

$$FG = \frac{SG}{TS} \times 100$$

– where SG is the total number of seeds germinated and TS is the total number of seeds placed in Petri dishes.

Two-way ANOVA was used to assess the effect of the various factors on FG%. FG% were tested for normality using the Shapiro–Wilk test ($\alpha=0.05$) before the ANOVA, whereas homogeneity of variance was assessed using the Brown–Forsythe

test. Data were transformed where necessary (see results). Data were analysed using SigmaPlot13 (Systat Software, Inc., Point Richmond, CA, USA).

The FG% values at different concentrations of osmotic potential, NaCl, burial depth, heat, heat+smoke seed germination data were fitted to a three-parameter sigmoid model using SigmaPlot13.

$$G(\%) = \frac{G_{\max}}{1 - e^{(-x-x_{50})/b}}$$

– where, G is the total germination (%) at concentration x , G_{\max} is the maximum germination (%), x_{50} is the concentration or osmotic potential for 50% inhibition of the maximum germination, and b indicates the slope.

A linear model was fitted to the 24D osmotic potential data.

$$G(\%) = G + a * x$$

An exponential decay curve was fitted to NaCl (light/dark regime), and temperature and smoke germination data.

$$G(\%) = a * e(-G * x)$$

$$E(\%) = a * e(-E * x)$$

– where, G or E represent cumulative germination or emergence (%), respectively, at time x , E_{\max}/G_{\max} is the maximum germination or emergence, and a indicates the intercept.

Results

Effect of temperature and light

The results show a significant interaction between the effects of light and temperature ($P < 0.05$) indicating that the effect of photoperiod depends on the prevailing temperature regime. The highest FG% percentages were obtained for seeds germinated under day/night temperatures of 30/20°C and 12/12 h light/dark. Lower FG% was observed when temperatures were either increased or decreased. The temperatures 35/30°C resulted in the lowest FG%, whereas FG% for the 17/7°C temperature regime was just over 80% and 60% for the 12/12 h light/dark and 24 h continuous dark, respectively (Fig. 1).

Osmotic potential

A two-way ANOVA showed an important significant interaction between the effects of osmotic potential of the growing medium and photoperiod ($F = 5.041$, $P = 0.026$, $R^2 = 0.83$). Separate sigmoid models were fitted to the data of the two light regimes, and both models showed an extremely good fit, with $R^2 = 0.99$ and $R^2 = 0.99$ for the 12/12 h light/dark and 24 h continuous dark, respectively (Fig. 2). Final germination decreased with increasing osmotic stress. Values decreased from 85% to 58% as the osmotic potential decreased from 0 to -0.2 MPa, and was completely inhibited at values lower than -0.4 MPa. The results show that the osmotic concentration required for 50% reduction in germination was -0.22 MPa (Fig. 2a and b). When the non-germinated seeds were transferred from the osmotic potential -0.6 MPa to sterilised RO water, the FG% reached $89 \pm 2.1\%$.

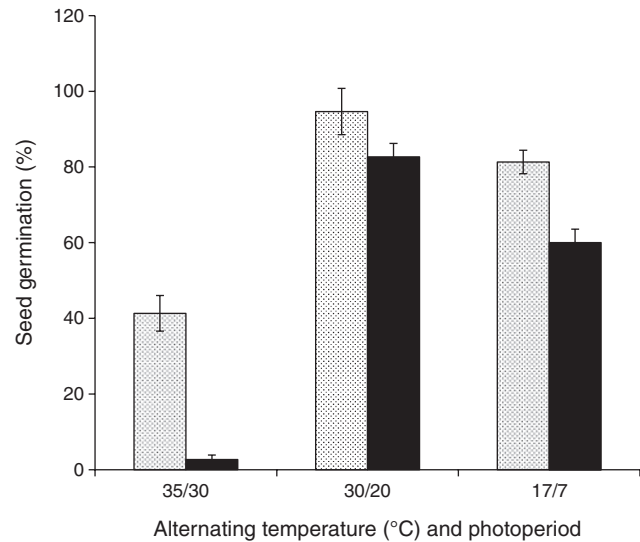


Fig. 1. The effects of alternating temperature and photoperiod regimes on the germination of *Nicotiana glauca* seeds. Vertical bars represent \pm standard error of the mean. 12 Light:12 Dark (▨); 24 Dark (■).

Effect of salt concentration and pH on germination

A two-way ANOVA showed an important significant interaction between the NaCl concentration and photoperiod ($F = 5.383$, $P = 0.009$, $R^2 = 0.92$). To further investigate the effect of salt, an exponential decay curve was fitted to the NaCl (12/12 h light/dark) data (Fig. 3a). The highest germination (87%) was observed when the seeds were exposed to no NaCl with germination declining until none was recorded at 200 mM NaCl, with germination being reduced with increasing NaCl concentration until no germination occurred at 200 mM NaCl. A sigmoid model best illustrated the effect of salt concentration on germination of the seeds exposed to a 24-h continuous dark. The salt concentration required for 50% reduction in germination was 119 mM NaCl. The lowest (37%) germination was obtained at 150 mM NaCl (Fig. 3b). No statistically significant effect of the variation of pH on germination was recorded for the pH range 7–10, although the final germination percent was generally lower for pH values less than 7 (Fig. 4).

Effect of seed burial depth on seedling emergence

A sigmoid model of seedling emergence against burial depth was fitted to the data ($R^2 = 0.97$) as shown in Fig. 5. Seed germination was highest when seeds were placed on the surface (80%) or buried at only 0.2 cm depth (78%). Increasing seed burial depth beyond 0.5 cm reduced emergence sharply, with no emergence recorded for seeds buried at depths of 1.5 and 2.0 cm. Using the fitted model, it is estimated that a burial depth of 0.42 cm would reduce seedling emergence by 50% (Fig. 5).

Seed longevity

The overall germination percentage of *N. glauca* seeds declined with duration of burial compared with (control) seeds that were not buried (Fig. 6). The average germination of *N. glauca* seeds kept in the room temperature remained at over 80%. FG% started to decline slowly over the period of burial. In just

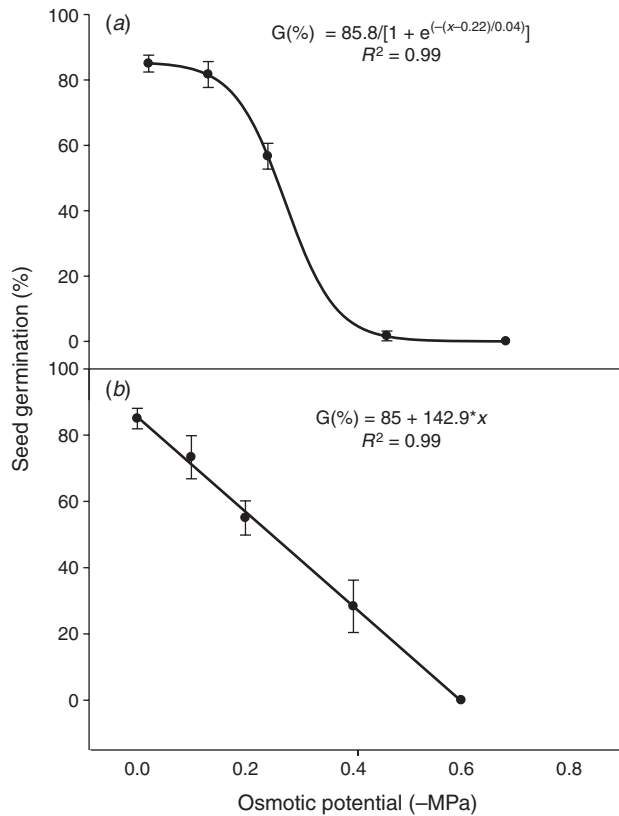


Fig. 2. Seed germination of *Nicotiana glauca* in response to osmotic potential (MPa) when incubated in a growth chamber at 30/20°C day/night temperature over a (a) 12 Light:12 Dark and (b) 24 Dark for 30 days. Vertical bars represent standard errors.

over 120 days, the germination percentage was reduced to 64%. Subsequent tetrazolium testing demonstrated that the non-germinated seeds had lost their viability.

Combined effect of heat-shock and smoke on germination

Germination patterns after the seeds were exposed to heat-shock and combined heat-shock and smoke are shown in Figs 7 and 8. The highest germination (100%) was obtained for the seeds kept at room temperature, whereas germination percentages remained high (exceeding 90%) for temperatures of up to 80°C. Germination percentages decreased sharply for seeds exposed to 100°C, and no germination was observed when the seeds were exposed to 130°C. The temperature required for 50% reduction in germination was 100.9 and 106.8 (°C) for germination conditions of 12/12 h light/dark and 24-h continuous dark, respectively. In contrast, when the seeds were exposed to heat-shock and smoke water, the overall germination decreased sharply. Subsequent tetrazolium testing demonstrated that the non-germinated seeds had lost their viability.

Effect of radiant heat on seedlings

The leaves and above ground stems of all *N. glauca* seedlings exposed to heat were killed by all radiant heat treatments. However, seedlings exposed to 80°C and 110°C heat resprouted after 9 days, whereas those exposed to 130°C and 160°C took

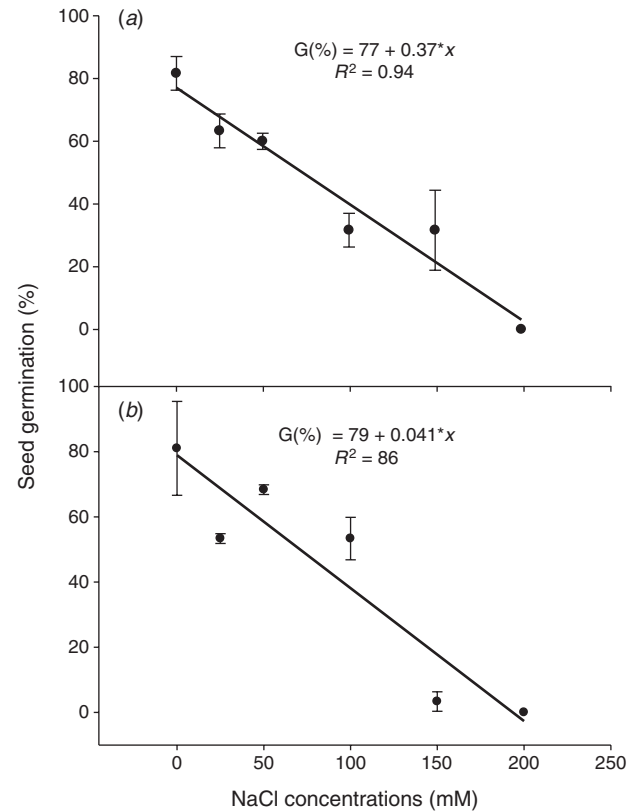


Fig. 3. Effect of salt concentration on germination of *Nicotiana glauca* seeds incubated in a growth chamber at 30/20°C day/night temperature over (a) a 12 Light:12 Dark and (b) 24 Dark for 30 days. Vertical bars represent standard errors of the means.

14 days to resprout. All the seedlings resprouted just below the root collar.

Discussion

Effect of temperature and light on germination

Our study shows that germination of *N. glauca* seeds is influenced by an interaction of exposure to temperature and light. Temperature has generally been found to be one of the key factors affecting the germination of seeds of many weedy species (Baskin and Baskin 1998). Although a lower proportion of the seeds germinated when exposed to continuously high temperatures (35/30°C), it is clear that *N. glauca* has the ability to germinate over a considerable range. This suggests that the overall regeneration and potential spread of the species is not significantly limited by seasonal temperature, and may readily germinate during late winter/early spring and early summer months. Light also seems to be a key environmental factor for *N. glauca* germination, with germination lower at high temperatures under continuous darkness. In part, this finding is supported by the rate of seedling emergence in relation to burial depth, discussed in more detail below.

Effects of osmotic potential, salt stress and pH on germination

Increased salinity was also found to cause a reduction in germination of *N. glauca* seeds in this study, although a

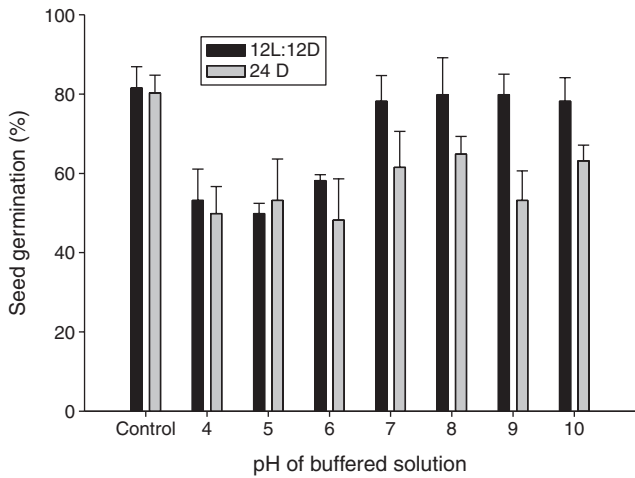


Fig. 4. Effect of buffered pH solutions on germination of *Nicotiana glauca* seeds incubated in a growth chamber at 30/20°C day/night temperature over a 24 dark for 30 days. Vertical bars represent standard errors of the means.

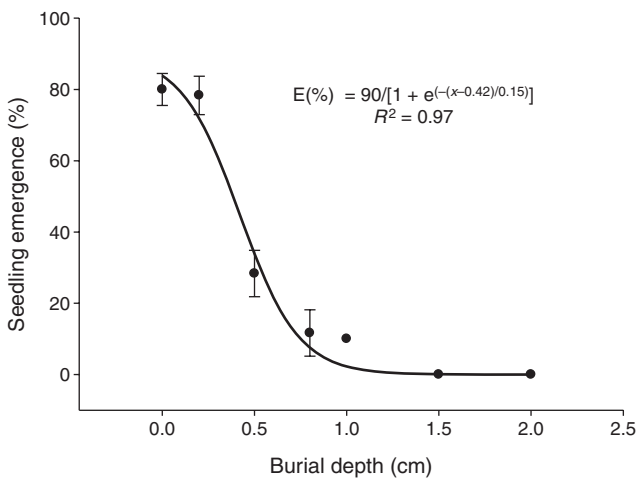


Fig. 5. Seedling emergence of *Nicotiana glauca* in response to seed burial depth in a growth chamber at 30/20°C day/night temperature. Vertical bars represent standard errors of the means.

proportion still germinated under high levels of salinity and at a wide range of pH levels. Our study shows that germination decreased from 85% to 58% as the osmotic potential decreased from 0 to -0.2 MPa, and was completely inhibited at values lower than -0.4 MPa. The findings indicate that osmotic stress leads to decreased germination, confirming that this invasive species requires high soil moisture for its germination. This is in line with the findings reported by Cunningham *et al.* (1981) that *N. glauca* stands were found mainly on stream floodplains and drainage channels after summer floods in Australian arid and semi-arid landscapes.

Our studies show that increasing concentration of NaCl reduced the seed germination percentage under the both 12 light:12dark and 24 dark conditions. Germination at 50–150 mM NaCl concentration was high, but was completely inhibited at 200 mM NaCl. This clearly shows that this species might occur

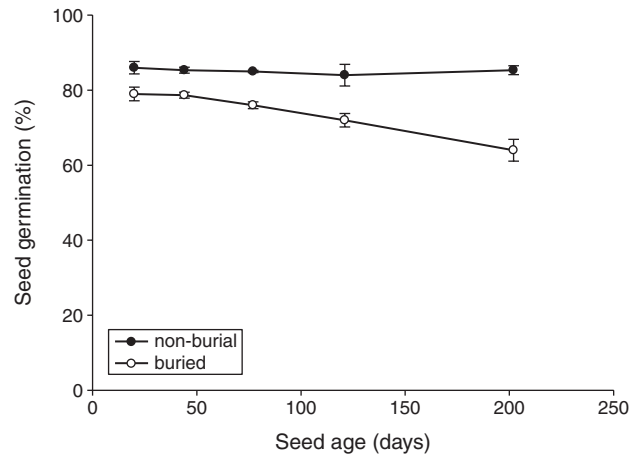


Fig. 6. Effects of dry storage and field burial on germination of *Nicotiana glauca* seeds. Vertical bars represent ± standard error of the mean.

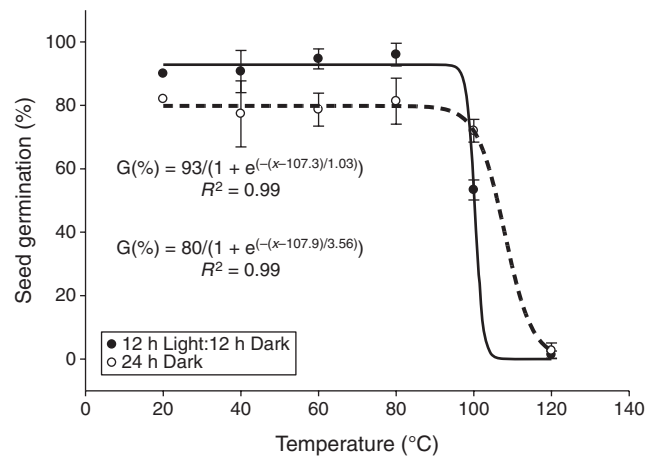


Fig. 7. Effect of heat on seed germination of *Nicotiana glauca* incubated at 30/20°C day/night temperature over a 24 dark for 30 days. Vertical bars represent standard errors of the means.

in areas with high soil salinity although its invasive potential is likely to be limited. Previous studies have shown that high concentration of salt can inhibit seed germination (Kim and Park 2008). The authors pointed out that high concentration of salt can directly affect the biosynthesis of gibberellic acid, which is essential for breaking dormancy (Kim and Park 2008). This trait gives *N. glauca* an advantage over native plants in more saline conditions in the arid and semi-arid areas.

Hassan *et al.* (2014) conducted a study on the ecological and phytochemical factors influencing *N. glauca* in Egypt, examining levels of soil pH under *N. glauca* stands. They found pH values ranging from 7.5 to 7.9. Although it appears that this species prefers slightly alkaline conditions, the high germination rates over broad pH ranges indicate that pH may not be a limiting factor for germination in most soils. Other studies on invasive species such as *Galenia pubescens* (Mahmood *et al.* 2016) and *Mimosa diplotricha* (Chauhan and Johnson 2008) were also able to germinate under a wide range of pH. This is

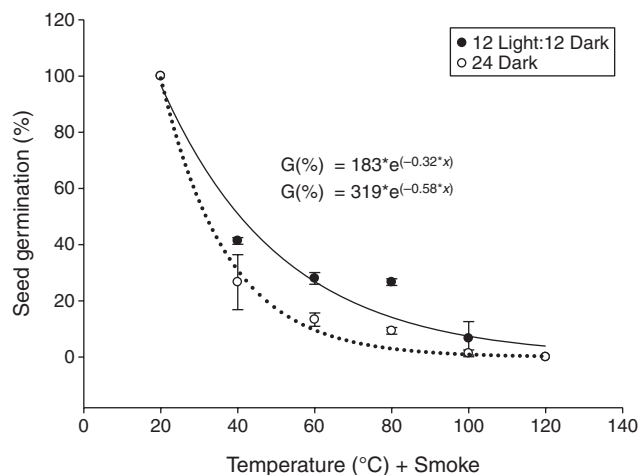


Fig. 8. Effect of heat and smoke on seed germination of *Nicotiana glauca* incubated at 30/20°C day/night temperature for 30 days. Vertical bars represent standard errors of the means.

a trait that provides invasive species with a competitive advantage during drought conditions.

Effect of depth of seed burial on seedling emergence

Our study showed that seedling emergence of *N. glauca* was inversely related to the seed burial depth. Significantly larger numbers of seeds germinated when they were placed on the surface, but seedling emergence declined to 10% when seeds were placed at a depth of 1 cm. Previous studies on seed germination and light have shown that light has varied effects on different plant species (Baskin and Baskin 1998; Milberg *et al.* 2000). The results obtained from the effect of depth of seed burial on seedling emergence are consistent with the light and temperature study. Seeds buried at depths of more than 0.2 cm below the soil surface receive only ~1% of the incident light (Woolley and Stoller 1978). However, lack of emergence of seeds buried deeper than 1 cm is likely due to the small seed size of *N. glauca*, and it is suspected that the nutritional content of the seed may be inadequate to facilitate emergence from lower depths (Bewley and Black 1994; Chauhan 2013). In addition to seed size, hypoxia and low rates of gaseous diffusion in soil may act as a barrier for seed germination and subsequent seedling emergence (Benvenuti *et al.* 2001).

Combined effect of heat-shock and smoke on germination

Fire is a common phenomenon in the arid zones, and has been used as a control technique to minimise the impacts of some native weeds (see for example Gavel *et al.* 2010). The role of fire and smoke are considered to be two major factors in seed germination. Despite its small seed size, results in this study have shown that *N. glauca* seeds may withstand a certain amount of heat-shock, although its seeds were more sensitive when they were exposed to temperatures higher than 80°C. In addition, this study has shown that the combined effect of heat-shock and smoke inhibits the germination of this invasive species, causing seeds to lose their viability. Similarly Read and Bellairs (1999) conducted a study on the germination of native grass species in

response to plant-derived smoke. Although smoke was shown to be an important environmental stimulus for breaking the dormancy of native grasses, the response varied considerably between different genera and between species of the same genus. Of the 20 species examined, for only five species had smoke affected the germination rate. These findings are contradictory to other studies that showed improved germination when seeds were treated with smoke water. The reduction in germination reported here may be due to octanoic acid found in smoke water. According to Stewart and Berrie (1979), germination may be inhibited by the presence of the acid, as it has the ability to damage the cell membrane of the seed. However, the validity of this in the context of *N. glauca* needs to be determined by further study.

Effect of radiant heat on seedlings

Prescribed burning has been used as one weed control strategy in the arid zone of Australia (Leigh and Noble 1981) and elsewhere (Lonsdale and Miller 1993; Gleadow and Narayan 2007). Our study shows that although the temperatures to which the seedlings were exposed effectively killed the aboveground plants, parts of the seedlings were able to resprout days after their exposure. This is somewhat in contrast to the findings by Miller (1988), who reported that young plants are vulnerable to fire. The ability of adult plants to resprout was already reported by Florentine and Westbrooke (2005), who observed that 'dead' shrubs of *N. glauca* were able to resprout either from the branches or from close to the root collar immediately after a sporadic rainfall event. Previous studies have emphasised the need to place these invasive species under stress with herbicides before the application of fire to increase mortality (Miller *et al.* 1981; Miller 1988). Although none of the heat-treated seedlings were killed in our study, it is misleading to suggest that fire is not a suitable control method for this species. We recommend further examination of the effect of different durations of radiant heat on *N. glauca* seedlings' survival.

Conclusion

This study shows that the germination of *N. glauca* seeds is affected by several factors. Although *N. glauca* seeds are able to germinate under a range of temperature regimes, the highest germination values were observed when the seeds were exposed to alternating temperatures (30/20°C) and 12/12 h light/dark regimes. The osmotic potential study shows that this species is highly sensitive to soil moisture for its germination, confining it to areas along waterways, creek lines, and other high soil moisture areas such as earth tanks, and roadsides in arid and semi-arid landscapes.

Management implications

Although its ability to germinate under a wide range of environmental conditions increases its invasive potential, the viability of *N. glauca* seed declines substantially within the period of a year. In areas where native vegetation has been decimated, as often found around water points, the emergence or persistence of the weed may result in stock losses. The study highlights the need to prevent the development of a soil seed bank. Our study also showed that fire alone may not be sufficient

to kill individual plants, implying that larger infestations would require an integrated management approach. The removal by hand of individual plants should therefore be considered before such plants can flower for the first time.

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References

- Baskin, C. C., and Baskin, J. M. (1998). 'Seeds. Ecology, Biogeography, and Evolution of Dormancy and Germination.' (Academic Press: San Diego, CA.)
- Benvenuti, S., Macchia, M., and Miele, S. (2001). Quantitative analysis of emergence of seedlings from buried weed seeds with increasing soil depth. *Weed Science* **49**, 528–535. doi:10.1614/0043-1745(2001)049[0528:QAOEOS]2.0.CO;2
- Bewley, J. D., and Black, M. (1994). 'Seeds: Physiology of Development and Germination.' (Plenum: London.)
- BOM (2005). 'Australian Climate Zones – All Climate Classes.' (Bureau of Meteorology: Melbourne, Vic.) Available at: www.bom.gov.au/climate/averages/#climatemaps (accessed 5 April 2016).
- BOM (2013). 'Australian Climate Zones – All Climate Classes.' (Bureau of Meteorology: Melbourne, Vic.) Available at: www.bom.gov.au/climate/averages/#climatemaps (accessed 4 May 2016.)
- Boyland, D. E., Carolin, R. C., George, A. S., Jessop, J. P., and Maconochie, J. R. (Eds) (1985). 'Flora of Central Australia.' (Reed Books: Sydney.)
- Chachalis, D., and Reddy, K. N. (2000). Factors affecting *Campsis radicans* seed germination and seedling emergence. *Weed Science* **48**, 212–216. doi:10.1614/0043-1745(2000)048[0212:FACRSJ]2.0.CO;2
- Chauhan, B. S. (2013). Seed germination ecology of feather lovegrass [*Eragrostis tenella* (L.) Beauv. Ex Roemer & J.A. Schultes]. *PLoS One* **8**, e79398. doi:10.1371/journal.pone.0079398
- Chauhan, B. S., and Johnson, D. E. (2008). Seed germination and seedling emergence of giant sensitive plant (*Mimosa invisa*). *Weed Science* **56**, 244–248. doi:10.1614/WS-07-120.1
- Cunningham, G. M., Mulham, W. E., Milthorpe, P. L., and Leigh, J. H. (1981). 'Plants of Western New South Wales.' (NSW Government Printing Office: Sydney.)
- Ferrari, F. N., and Parera, C. A. (2015). Germination of six native perennial grasses that can be used as potential soil cover crops in driP-irrigated vineyards in semiarid environs of Argentina. *Journal of Arid Environments* **113**, 1–5. doi:10.1016/j.jaridenv.2014.09.002
- Florentine, S. K., and Westbrooke, M. E. (2005). Invasion of the noxious weed *Nicotiana glauca* R. Graham after an episodic flooding event in the arid zone of Australia. *Journal of Arid Environments* **60**, 531–545. doi:10.1016/j.jaridenv.2004.07.015
- Florentine, S. K., Westbrooke, M. E., Gosney, K., and Ambrose, G. (2006). The arid land invasive weed *Nicotiana glauca* R. Graham (Solanaceae): population and soil seed dynamics, seed germination patterns and seedling response to flood and drought. *Journal of Arid Environments* **66**, 218–230. doi:10.1016/j.jaridenv.2005.10.017
- Gavel, T., Treweeke, R., and Saunders, D. (Eds) (2010). 'Managing Invasive Native Scrub to Rehabilitate Native Pastures and Open Woodlands.' (Central West Catchment Management Authority: Wellington, NSW.)
- Geerts, S., and Pauw, A. (2009). African sunbirds hover to pollinate an invasive, hummingbird-pollinated plant. *Oikos* **118**, 573–579. doi:10.1111/j.1600-0706.2008.17167.x
- Gleadow, R. M., and Narayan, I. (2007). Temperature thresholds for germination and survival of *Pittosporum undulatum*: implications for management by fire. *Acta Oecologica* **31**, 151–157. doi:10.1016/j.actao.2006.07.003
- Global Invasive Species Database (GISD) (2016). Species profile *Nicotiana glauca*. Available at: www.iucngisd.org/gisd/species.php?sc=1453 (accessed 14 March 2016).
- Goodspeed, T. H. (1954). The genus *Nicotiana*. *Chronica Botanica* **16**, 1–536.
- Hassan, H., El-Hameed, T. Z. A., and Nasr, E. (2014). Ecological and phytochemical studies on *Nicotiana glauca* from Egypt. *Egyptian Journal of Experimental Biology (Botany)* **10**, 87–95.
- Hernández, H. M. (1981). Sobre la biología reproductiva de *Nicotiana glauca* Graham: una maleza de distribución cosmopolita. (On the reproductive ecology of *Nicotiana glauca*, a cosmopolitan weed.). *Boletín de la Sociedad Botánica de México* **41**, 47–73.
- Kim, S., and Park, C. (2008). Gibberellic acid-mediated salt signaling in seed germination. *Plant Signaling & Behavior* **3**, 877–879. doi:10.4161/psb.3.10.6247
- Köppen, W. (1900). Versuch einer Klassifikation der Klimate, Vorzugsweise nach ihren Beziehungen zur Pflanzenwelt. *Geographische Zeitschrift* **6**, 593–611.
- Leigh, J. H., and Noble, J. C. (1981). The role of fire in the management of rangelands in Australia. In: 'Fire and the Australian Biota'. (Eds A. M. Gill, R. H. Groves and I. R. Noble.) pp. 471–495. (Australian Academy of Science: Canberra.)
- Lonsdale, W. M., and Miller, I. L. (1993). Fire as a management tool for a tropical woody weed: *Mimosa pigra* in Northern Australia. *Journal of Environmental Management* **39**, 77–87. doi:10.1006/jema.1993.1055
- Luna, B., Moreno, J. M., Cruz, A., and Fernandez-Gonzalez, F. (2007). Heat-shock and seed germination of a group of Mediterranean plant species growing in a burned area: an approach based on plant function types. *Environmental and Experimental Botany* **60**, 324–333. doi:10.1016/j.envexpbot.2006.12.014
- Mahmood, A. H., Florentine, S. K., Chauhan, B. S., McLaren, D. A., Palmer, G. C., and Wright, W. (2016). Influence of various environmental factors on seed germination and seedling emergence of a noxious environmental weed: green galenia (*Galenia pubescens*). *Weed Science*, In press. doi:10.1614/WS-D-15-00184.1
- Milberg, P., Andersson, L., and Thompson, K. (2000). Large-seeded species are less dependent on light for germination than small-seeded ones. *Seed Science Research* **10**, 99–104. doi:10.1017/S0960258500001118
- Miller, I. L. (1988). Aspects of the biology and control of *Mimosa pigra* L. PhD Thesis, University of Sydney, NSW, Australia.
- Miller, I. L., Nemestothy, L., and Pickering, S. E. (1981). '*Mimosa pigra* in the Northern Territory.' Technical Bulletin No. 51. (Department of Primary Production Division of Agriculture and Stock: Darwin, NT.)
- Mizrachi, N., Levy, S., and Goren, Z. (2000). Fatal poisoning from *Nicotiana glauca* leaves: identification of anabasine by gas chromatography/mass spectrometry. *Journal of Forensic Sciences* **45**, 14761J. doi:10.1520/JFS14761J
- Moore, R. P. (1985). 'Handbook on Tetrazolium Testing.' (International Seed Testing Association: Zurich, Switzerland.)
- Moore, G. M., Rowan, K. S., and Blake, T. J. (1977). Effects of heat on the physiology of seedlings of *Eucalyptus obliqua*. *Australian Journal of Plant Physiology* **4**, 283–288. doi:10.1071/PP9770283
- Moreno, J. M., Cruz, A., Fernández, F., Luna, B., Pérez, B., Quintana, J. R., and Zuazua, E. (2004). Ecología del monte mediterráneo en relación con el fuego: el jaral-brezal de Quintos de Mora (Toledo). In: 'Avances en el Estudio de la Gestión del Monte Mediterráneo'. (Eds R. Vallejo and J. A. Alloza.) pp. 17–45. (Fundación Centro Estudios Ambientales del Mediterráneo: Spain.)

- Panter, K. E., Weinzweig, J., Gardner, D. R., Stegelmeier, B. L., and James, L. F. (2000). Comparison of cleft palate induction by *Nicotiana glauca* in goats and sheep. *Teratology* **61**, 203–210. doi:10.1002/(SICI)1096-9926(200003)61:3<203::AID-TERA8>3.0.CO;2-I
- Read, T. R., and Bellairs, S. M. (1999). Smoke affects the germination of native grasses of New South Wales. *Australian Journal of Botany* **47**, 563–576. doi:10.1071/BT97124
- Schueller, S. K. (2004). Self-pollination in island and mainland populations of the introduced hummingbird-pollinated plant, *Nicotiana glauca* (Solanaceae). *American Journal of Botany* **91**, 672–681. doi:10.3732/ajb.91.5.672
- Sims, D. N., James, R., and Christensen, T. (1999). Another death due to ingestion of *Nicotiana glauca*. *Journal of Forensic Sciences* **44**, 14484J. doi:10.1520/JFS14484J
- Stewart, R. R. C., and Berrie, A. M. M. (1979). Effect of temperature on the short-chain fatty acid-induced inhibition of lettuce seed germination. *Plant Physiology* **63**, 61–62. doi:10.1104/pp.63.1.61
- Tadmor-Melamed, H., Markman, S., Arieli, A., Distl, M., Wink, M., and Izhaki, I. (2004). Limited ability of Palestine Sunbirds *Nectarinia osea* to cope with pyridine alkaloids in nectar of tree tobacco *Nicotiana glauca*. *Functional Ecology* **18**, 844–850. doi:10.1111/j.0269-8463.2004.00929.x
- Trabaud, L. (1979). Etude du comportement du feu dans la Garrigue de Chêne kermès à partir des températures et des vitesses de propagation. *Annals of Forest Science* **36**, 13–38. doi:10.1051/forest/19790102
- Woolley, J. T., and Stoller, E. (1978). Light penetration and light-induced seed germination in soil. *Plant Physiology* **61**, 597–600. doi:10.1104/pp.61.4.597
- Wu, G., and Du, G. (2007). Germination is related to seed mass in grasses (Poaceae) of the eastern Qinghai-Tibetan Plateau, China. *Nordic Journal of Botany* **25**, 361–365. doi:10.1111/j.0107-055X.2007.00179.x