# The influence of temperature and water stress on germination of a serotinous member of the Proteaceae, *Leucadendron xanthoconus*.

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**Abstract**

Seeds of Leucadendron xanthoconus (O.Knutze)K.Schum were germinated at the temperature regimes of 20/10 °C, 15/10 °C and 10/10 °C (10hr light/14hr dark). At each temperature regime, seeds were subject to a range of moisture stresses by imbibition and incubation in Polyethylene glycol solutions at a range of water potentials (0, -0.5, -1.25, -2.5, -5, -10, -15 and -20 bars). The realized percentage germination was significantly affected by both temperature and water potential. No germination occurred at -10 to -20 bars. At intermediate water potentials (-0.5 to -2.5 bars) germination was greatest for the 15/10 °C treatment while at 0 and -5 bars there was no difference in germination between the two warmer regimes. All incubations at 10 °C showed low germination percentages. Relating these data to soil surface temperatures and moisture contents measured at a recently burnt site, it was found that moisture conditions favouring germination occurred from autumn to spring. However, the occurrence of an optimal temperature of 15/10 °C was restricted to the winter months. Thus temperature seems to have a stronger influence than moisture in regulating germination.

## Introduction

An important survival trait in the fire-prone Cape fynbos is serotiny (canopy-stored seed). Serotinous species of the Proteaceae that dominate mesic shrublands in the Cape mountains (*Aulax*, *Leucadendron* and *Protea*) retain their seeds in cones for a number of years, to be released in large quantities after fire (Bond et al 1984). Since most serotinous species survive fire only as seeds, the post-fire environment into which these seeds are released is important for the re-establishment of the species. Post-fire environmental conditions are determined by the season of burn (Le Maitre 1987).

It has been shown that seedling establishment is most successful following autumn burns and often less favourable and/or highly variable after summer fires. Spring and winter were found to be unfavourable seasons for seedling recruitment of serotinous species. Seeds released from the cones after a spring fire fail to germinate until the rainy season and thus lie dormant for many months (van Wilgen & Viviers 1985), increasing the probability of seed loss to predation and decay (Bond 1984). A winter burn might provide suitable post-fire conditions for germination but it reduces the time available for seedling establishment before the summer drought. Successive winter and spring fires could rapidly cause local extinction in serotinous species. Thus, present management of the fynbos is partly determined by the response of serotinous Proteaceae to fires of different rotations and seasonalities (Bond et al 1984).

By noting the post-fire season at which maximum seedling recruitment occurs, the above mentioned workers have deduced the overall climatic factors affecting germination but have not considered the micro-climatic conditions of the immediate seed environment. Manders & Cunliffe (1987) criticize these studies in which established plants were simply counted. They believe that since germination and establishment are generally observed together, a greater understanding of this phase of the life cycle could be reached by detailed studies of germination. Seed germination should occur at a time which will favour survival of the seedling (Mayer & Poljakoff-Mayer 1975). Therefore the physical determinants of seed germination could contribute to a better understanding of the seasonal constraints of post-fire recruitment and result in a more confident prediction of post-fire succession of serotinous species.

Two of the major microclimatic conditions altered by fire are soil surface temperature and moisture content (Kruger 1987). This study aimed at determining the optimal temperature range for the germination of seeds of Leucadendron xanthoconus (O.Knutze) K.Schum, a non-sprouting shrub which is dominant in many fynbos communities of the south western Cape (Williams 1972). The effect of moisture stress on germination was also investigated since this species appears to be restricted to mesic habitats (Williams 1972) (400-1000mm $yr^{-1}$), a feature which Midgley (1987) regards as typical of serotinous species.

The data obtained in this study under controlled environmental conditions could lead to an understanding of the cues involved in seed germination. It is generally accepted that a suitable combination of rainfall and temperature act as the the major determinants of seed germination (Bradstock & Myerscough 1981, Bond 1984). According to Rebelo (1986) the prevention of germination in the unfavourable dry summer can theoretically be regulated by means of a low temperature requirement. Van Wilgen & Viviers (1985) suggest that a low temperature plus moisture cue controls germination while Kruger (1972) & Brits (1983) (both cited by van Wilgen & Viviers 1985) found that in some cases moisture alone can trigger germination.

The results of these studies would indicate the optimum temperature and moisture conditions for the germination of L.xanthoconus and thus the season of burn which would optimize post-fire recruitment of this species. It may also help understand the poor recruitment following winter burns when moisture appears to be adequate and temperatures are low.

**Study Site**

Cones of L.xanthoconus were collected randomly from 15 year-old female plants (about 2m high) in mesic Mountain Fynbos at Highlands Forest Reserve (34° 16´S, 19° 06´E), about 15km south east of Grabouw. L.xanthoconus is the dominant shrub at the site. The understorey was dominated by Chondropetalum hookerianum (Masters)Pill., Ceratocaryum sp. and Erica cristata Duffer.

The general climate of the site is cool-temperate, humid and windy. The mean annual rainfall is 928mm and occurs predominantly (65%) in the winter season (May-October). The soils (quartzitic and shale-derived colluvium overlying weathered shale) are typically acid, nutrient-poor and seasonally waterlogged (Davis 1988).

## Materials and Methods

### Seed treatment

The cones collected were all >1 year old and thus contained mature seeds. The release of seeds was induced by placing the cones in a drying oven at room temperature (25°C) for about 10 days. Seeds were hand-sorted, selecting a uniformly sized and coloured batch to be used for the germination trials.

Polyethylene glycol (chemically pure P.E.G. 6000 supplied by Unilab, molecular weight 6000-7500) was used as the osmotica made up to provide different solute potentials since it has the greatest effect on reducing the seed water potential without being absorbed and is not toxic to germination (McDough 1976). The concentrations of P.E.G. made up were 0, 4.6, 9.2, 13.8, 20, 28.5, 36.5 and 42 g/100ml distilled water which gave water potentials of 0, -0.5, -1.25, -5, -10, -15 and -20 bars respectively. These concentrations, corresponding to the desired water potentials, were obtained from Michel's (1983) relationships of water potential for P.E.G. solutions at 25°C as a function of concentration.

Seeds were imbibed in solutions at each water potential for 24 hours under partial vacuum (to ensure a uniform potential within all the seeds at each solution potential). The imbibed seeds were then transferred onto a single sheet of Whatman no.1 filter paper (9cm diameter) which was soaked in the appropriate imbibition solution, sprayed with 0.1M benlate (osmotically inactive

fungicide) and placed in a petri dish (8cm diameter). For each temperature regime, 10 seeds per petri dish, replicated 5 times for each imbibition concentration (=50 seeds per concentration, 400 seeds per temperature) were set up for the incubation trial. Each batch of 50 seeds was placed in a plastic bag (to prevent dehydration) and incubated in the Electrocool temperature controlled incubators which have a temperature and light setting. The availability of three incubators limited the study to three different temperature regimes: 20/10 °C, 15/10 °C and a constant 10 °C. Where temperatures were alternated, the higher and lower temperature was maintained for 10 and 14 hours respectively. For each temperature regime, the light also alternated concurrently: 10 hours light, 14 hours dark.

Germination

The petri dishes were checked regularly, each time recording the number of germinated seeds. A seed was considered to have germinated when the radicle had emerged.

An initial trial run of germination rates at 0 bars water potential over the temperature regimes indicated that an incubation period of approximately one month was sufficient to allow maximum germination. The second germination study included seed imbibitions and incubations at water potentials of 0, -5, -10, -15 and -20 bars within the temperature regime 20/10 °C, 15/10 °C and 10/10 °C. When no germination was observed other than at 0 bars, additional treatments of -0.5, -1.25 and -2.5 bars were included in the study. After the one month incubation

period, the seeds imbibed between -5 and -20 bars were hydrated with distilled water to observe the ability of the seeds to germinate after a period of moisture stress.

Statistical Analyses

A Two-way ANOVA tested the effect of temperature and water potential on arcsine transformed germination percentages. A multiple range analysis indicated the temperature / water potential ranges at which significant differences occurred.

## Results

There was no germination of seeds imbibed in -10, -15 and -20 bars P.E.G. solutions. The progress of germination at each of the water potentials 0, -0.5, -1.25, -2.5 and -5 bars for seeds incubated at the three temperature regimes are presented in Figures 1 to 5.

Germination was usually first observed after 14 days and then often occurred in many seeds simultaneously. However, in the 10/10 °C incubations, germination was delayed to about 20-24 days except at higher water stress (-5 bars, Figure 5) where germination only occurred after 28 days. Where water was not limiting (0 bars, Figure 1), there was no difference in the percentage germination at 15/10 and 20/10 °C. Both showed similarly high germination (80-90%). While a slight reduction in water potential from 0 to -0.5 bars had very little effect on germination at 15/10 °C, it resulted in a 25% drop in germination at 20/10 °C. Thus at the lower water potentials (-0.5 to -2.5 bars, Figures 2 to 4) a difference in germination between 15/10 and 20/10 °C became apparent, with 15/10 °C always resulting in higher germination. However, 15/10 and 20/10 °C incubations showed similar germination results again at -5 bars (Figure 5). All incubations at 10/10 °C showed large relative reductions of germination rates as well as of the maximum germination percentage realized at the end of the incubation period. The maximum percentage germination reached under any temperature regime decreased as the water potential of the seeds decreased.

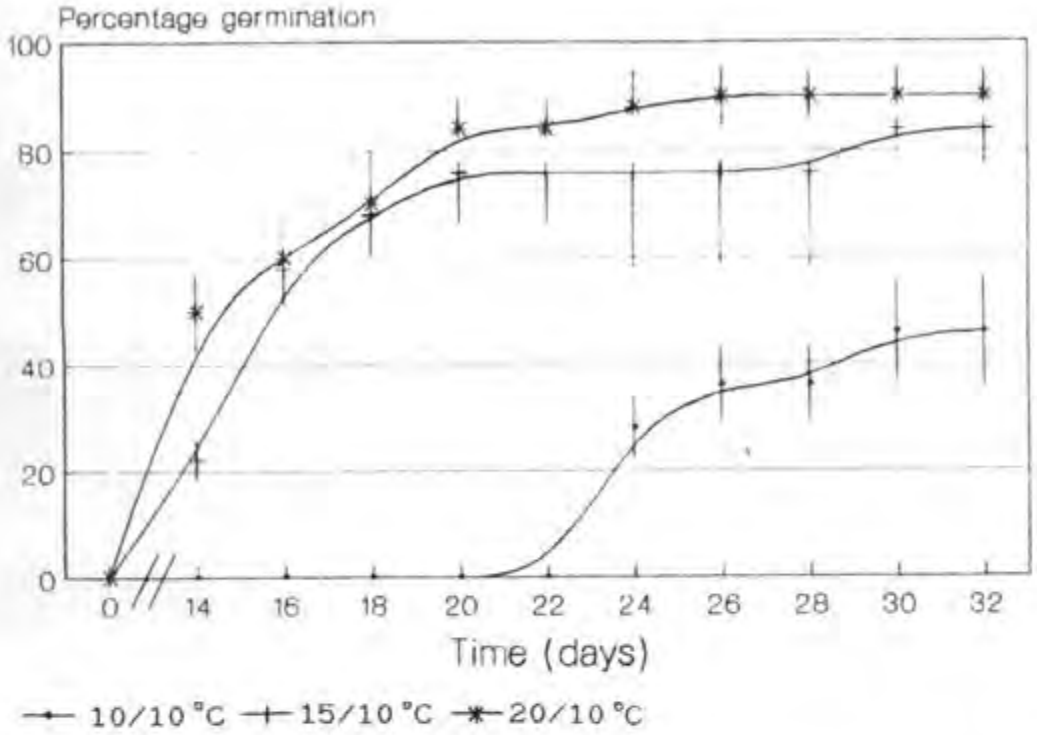


**Figure 1** Percentage germination (means ± 1 S.E.M.) of L.xanthoconus seeds at the 3 temperature regimes at a water potential of 0 bars. Vertical bars represent S.E.M.

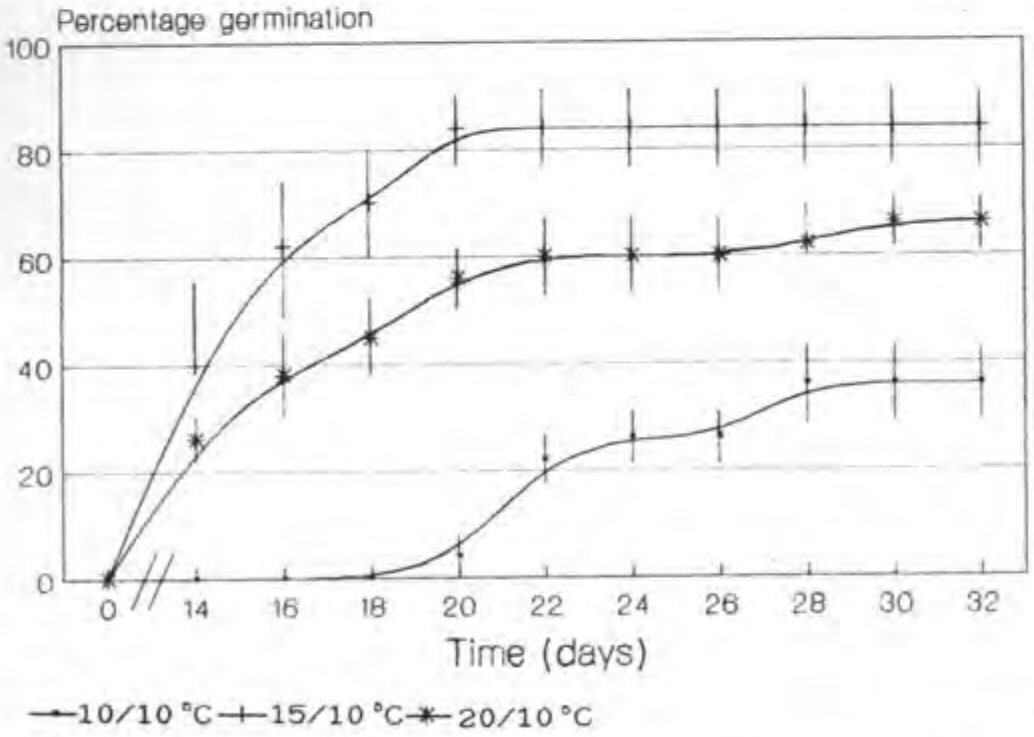


**Figure 2** Percentage germination (means ± 1 S.E.M.) of L.xanthoconus seeds at the 3 temperature regimes at a water potential of -0.5 bars. Vertical bars represent S.E.M.

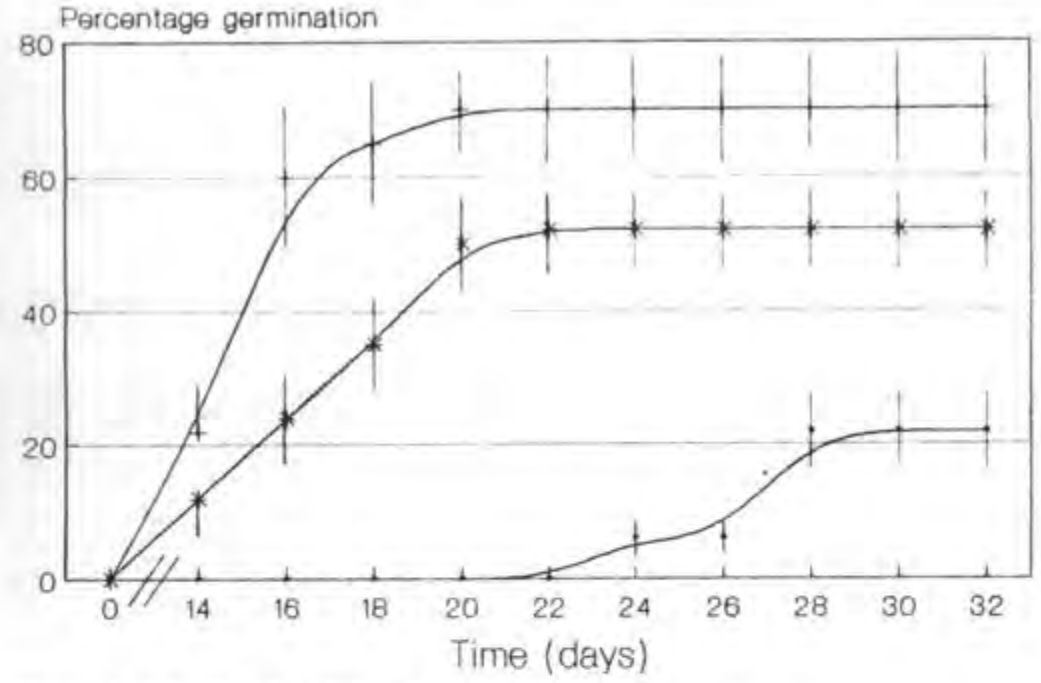


**Figure 3** Percentage germination (means ± 1 S.E.M.) of L.xanthoconus seeds at the 3 temperature regimes at a water potential of -1.25 bars. Vertical bars represent S.E.M.

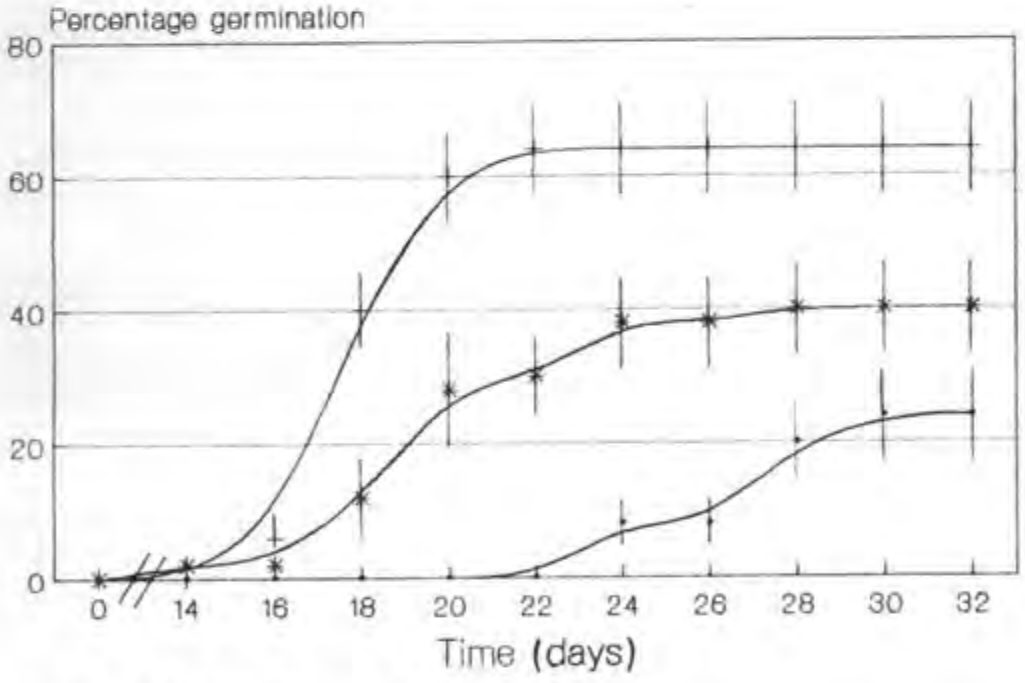


**Figure 4** Percentage germination (means ± 1 S.E.M.) of L.xanthoconus seeds at the 3 temperature regimes at a water potential of -2.5 bars. Vertical bars represent S.E.M.

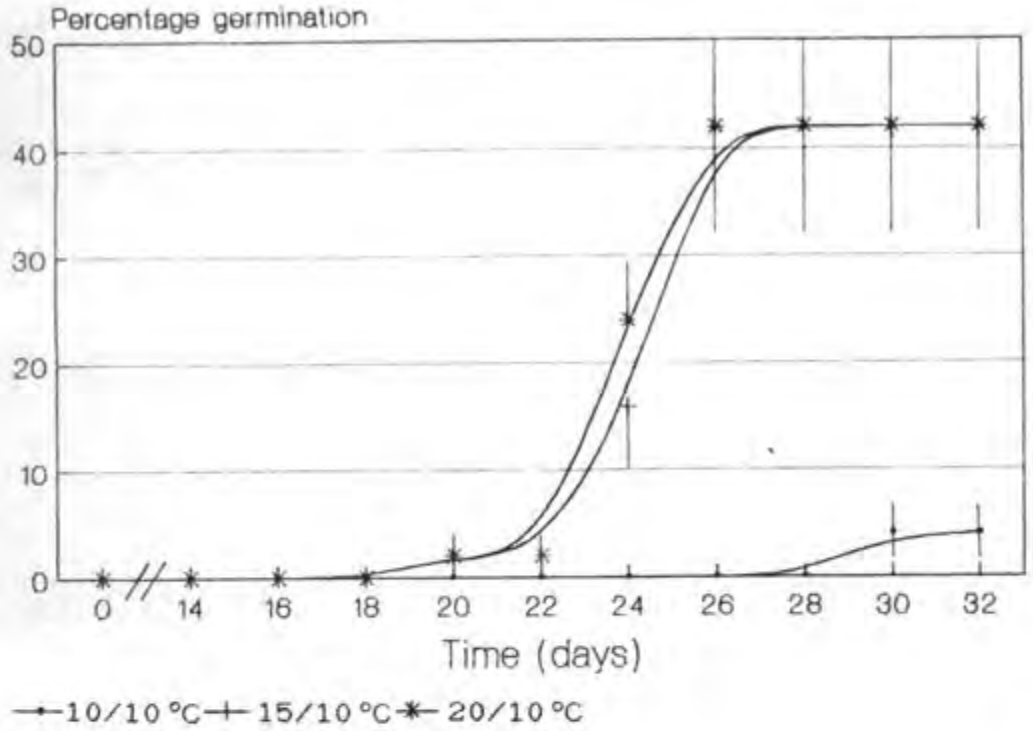


**Figure 5** Percentage germination (means ± 1 S.E.M.) of L.xanthoconus seeds at the 3 temperature regimes at a water potential of -5.0 bars. Vertical bars represent S.E.M.

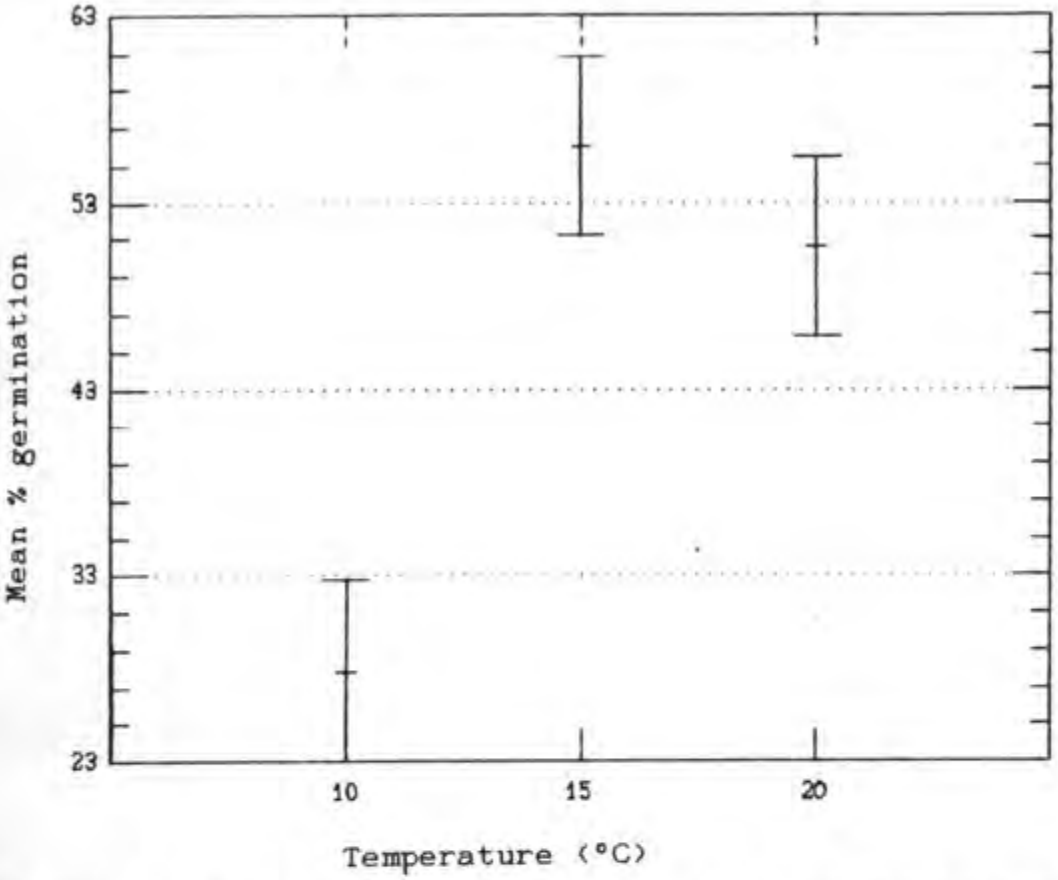


**Figure 6** 95% Confidence intervals for mean germination at the temperature range.

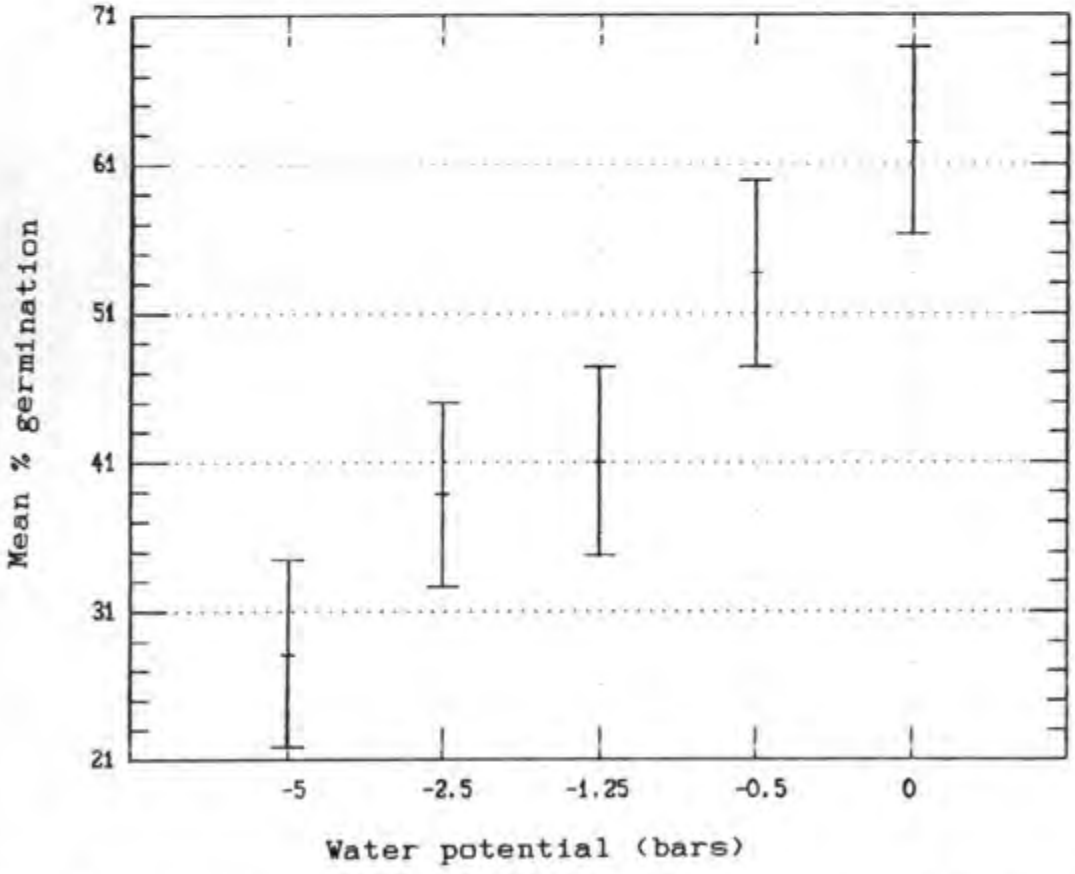


**Figure 7** 95% Confidence intervals for mean germination at the range of water potentials.

This was particularly evident at 20/10°C, where the decreasing water potential resulted in the greatest reductions in germination (Figures 1 to 5).

Two-way ANOVA showed that both temperature and water potential had a significant effect (temperature , F=38.3, P<0.001; water potential F=18.4, P<0.001) on percentage germination realized at the end of the 32 day period. However, the effects of these two factors were indepedent of one another i.e. the interaction of temperature and water potential was not significant (F=1.1; P=0.35). A multiple range analysis relating germination to temperature indicated that germination at 10/10 °C was significantly lower (P<0.001) than at 15/10 and 20/10 °C; there was no significant difference between germination at 15/10 and 20/10°C (Figure 6). Germination showed a linear response to water potential; germination at -5 to -1.25 bars was significantly lower (P<0.001) than at -0.5 and 0 bars (Figure 7).

The seeds at -5 to -20 bars showed germination after being hydrated (Table 1). A response to temperature and water potential was still evident. Excluding contaminated samples, generally an increase in temperature and water potential favoured germination.

It was noted that subsequent to radicle emergence in seeds at 0 and -0.5 bars (irrespective of temperature), growth continued until a seedling with two leaves and a small rooting system developed. A decreasing water potential appeared to inhibit seedling development. At -5 bars a small radicle emerged which usually withered within a few days.

**Table 1** Mean germination percentages (± 1 S.E.M.) after hydration

| Time after hydration (days) | Temperature (°C) | Water potentials (bars) | | | |
|---|---|---|---|---|---|
| | | -5 | -10 | -15 | -20 |
| 0 | 10 | 4 +2.4 | 0 | 0 | 0 |
| | 15 | 20 +9.0 | 0 | 0 | 0 |
| | 20 | 22 +8.0 | 0 | 0 | 0 |
| 7 | 10 | 10 +0 | 8 +3.7 | 0 | 0 |
| | 15 | 16 +6.7(d) | 38 +7.3 | 2 +2 | 0 |
| | 20 | (d) | 58 +2 | 12 +4.9 | 0 |
| 11 | 10 | 12 +2 | 16 +6.7 | 0 | 0 |
| | 15 | 12 +5.8 | 40 +5.4 | 15 +6.5 | 0 |
| | 20 | (d) | 15 +6.5(d) | 28 +3.7 | 2 +2 |
| 17 | 10 | 12 +2 | 16 +6.7 | 0 | 0 |
| | 15 | 12 +5.8(d) | 42 +8.6 | 20 +9 | 0 |
| | 20 | (d) | 15 +6.5(d) | 35 +8.7 | 6 +4 |

(d) indicates decay of seeds as a result of fungal contamination.

## Discussion

The absence of seedlings and young plants in mature stands of L.xanthoconus indicate that this species is incapable of establishment in the inter-fire period (Kruger 1987). Therefore, only seeds released after a fire and the death of the parent plants will germinate and establish. Since these serotinous seeds germinate at or very near the soil surface (Brits 1986a), they will be exposed to the extreme environmental conditions of the post-fire soil surface (Kruger 1987). Environmental data including total monthly precipitation, soil moisture and water potential and monthly soil surface temperatures were obtained from Davis (1988) and G.Davis (unpublished data). These data were collected from a site cleared by means of a controlled burn in February 1985, situated adjacent to the 15 year old study site.

Most rain at the site falls between May and August, resulting in soil surface (30mm depth) water potentials being at or close to 0 bars. However, soil water potentials from April to November seem to fall within the range at which seed germination was observed (0 to -5 bars). Kruger's (1987) determinations of surface soil moisture contents for a burnt site in L.xanthoconus-dominated vegetation at Jakkalsrivier showed very similar trends. Thus if moisture availability was the cue for post-fire germination and a late winter/spring (August to October) fire occurred, germination might still occur but the seedlings are unlikely to survive the summer drought. In this study it was observed that L.xanthoconus seedlings are sensitive to water stress -

at -5 bars, radicles that emerged soon withered thus inhibiting further seedling development. This would seriously reduce post-fire recruitment of the species. However, the tendency of serotinous species to occur in moist habitats (Midgley 1987) suggests that they are suited to the situation of soil moisture levels being favourable over the greater portion of the year. Thus another factor must be operating to ensure that seeds only germinate during the most suitable period.

The effect of temperature on germination in this incubation study (Figure 6) might suggest that in its natural habitat, germination of L. xanthoconus seeds is controlled by the requirement for an optimal temperature regime (15/10 °C). Soil surface temperatures monitored at noon at the Highlands burnt site during summer ranged from 35 to 60 °C (G. Davis, unpublished data). Thus not only is soil moisture limiting during this season, but the extremely high soil temperatures would prevent germination. Brits (1986) suggests that dormancy in serotinous species is imposed physiologically in the embryo by a low temperature requirement only, which in nature synchronizes germination with the first winter season following dispersal. The daily maximum winter soil surface temperature range at Highlands burnt site was 15 to 20 °C (G. Davis, unpublished data). Relating this to the results of this study (Figure 1), maximum germination will occur during the winter months when moisture availability is high, resulting in high soil and seed water potentials. Even though germination at 15/10 and 20/10 °C is not significantly different (Figure 6), the

tendency of higher germination at 15/10 than at 20/10 °C when water availability is only slightly limiting (Figures 2 to 4) may be of importance in terminating germination at the end of the moist cool winter period. During the spring months, water availability is reduced but is still sufficient to allow germination. However, G. Davis (unpublished data) recorded noontime soil surface temperatures >30 °C during spring. Thus the lowered optimal germination temperature at intermediate water potentials (apparent from September to November) inhibits germination before the onset of the higher temperatures of spring and summer. This again suggests the controlling influence of temperature over germination, inducing a summer drought-avoiding dormancy (van Wilgen & Viviers 1985).

Thus it seems that while moisture is necessary for successful germination in the post-fire environment, temperature acts as the cue that regulates germination. Disagreement exists amongst earlier workers as to whether temperature, moisture or a combination of these factors is the primary cue controlling germination (van Wilgen & Viviers 1985). Incubation trials such as this study might indicate the relative significance of the factors affecting germination. However, to understand the mechanisms involved in controlling germination, it seems necessary to investigate the physiological processes of seed germination and dormancy.

When the moisture level of the seed environment is high, uptake of water (imbibition) occurs until a constant potential is achieved. This hydration activates metabolic processes and

prepares the seed for the visible germination stage. Since active metabolism involves enzyme activity (Bewley & Black 1978) and enzymes function within an optimum temperature range (Curtis 1983), the regulation of germination by temperature may be by enzyme control. Thus even though imbibition of L.xanthoconus seeds may hydrate the enzymes and their substrates during a large portion of the year (excluding only the dry summer months), enzyme activity and subsequent germination is inhibited until the favourable temperatures during the winter months.

Thus far, the ecology and physiology of germination has been considered for the serotinous L.xanthoconus seeds released into the post-fire environment. However, perhaps this information can be used to understand the prevention of establishment in the interfire period. Kruger (1987) compared microclimatic factors of burnt and unburnt sites at Jakkalsrivier. The most obvious difference between these sites is vegetation canopy cover which in turn modifies the respective microclimates.

Soil moisture contents in summer are the same on the burnt as on the unburnt site. During the wetter season there are markedly higher surface soil moisture contents on the burnt site, due mainly to the lower foliage surface area from which evaporative losses occur (Kruger 1987). However, the soil moisture contents of the unburnt sites still maintain water potentials at which germination was shown to occur in this study. Kruger's (1987) temperature data showed higher soil surface temperatures during the day on the burnt site and cooler temperatures at night.

Considering the germination responses to temperature of this study, it is apparent that the temperature regime at the unburnt site would still allow germination during the winter period, while maintaining dormancy during summer. Since diurnal temperature variation is different at the burnt and unburnt site, this may be an influential factor affecting germination. The smaller daily variation at the unburnt site might inhibit germination. Brits (1986b) showed that hard-seeded Proteaceae species require a specific diurnal variation in temperature to attain maximum germination, and this was achieved only at a daily low and high temperature corresponding to the daily minimum and maximum temperatures of burnt fynbos during winter. Perhaps a similar requirement exists for serotinous seeds.

Therefore it seems that the only microclimatic factor that could possibly limit seedling germination and establishment in the interfire situation is temperature with the actual daily minima and maxima being important. Under the generally moist climatic regime at Highlands and Jakkalsrivier, it is unlikely that soil moisture content would influence establishment in the mature vegetation.

The hydration of L.xanthoconus seeds after subjection to a range of moisture stress indicated that these seeds are tolerant of drought conditions. The germination recovery rates showed a response relative to the water potential of the initial stress treatments. This was as a result of rehydration with similar amounts of distilled water apparently insufficient to overcome

the initial stress treatments. It is believed that seeds are not only tolerant of moisture stress but may even benefit from a cycle of dehydration and hydration before germination. This is known as seed "hardening or priming" (Hegarty 1977) and may explain the observed "profuse" germination of L.xanthoconus seeds one year after a spring burn (Kruger 1972, cited by van Wilgen & Viviers 1985) during which time the seeds would have experienced a period of drought followed by hydration (winter rains).

In conclusion, it seems that this study substantiates the hypothesis that the optimum temperature and moist regime for seed germination (low diurnally fluctuating temperature of 15/10°C and high soil moisture content, 0 to -0.5 bars) coincides with the post-fire microclimatic conditions provided the fire occurs during the optimal fire season (autumn) followed by the cool wet conditions of the winter months.

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