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Forum

Pregermination heat shock and seedling growth of fire-following Fabaceae from four Mediterranean-climate regions

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Abstract – The role of heat-shock in stimulating the germination of soil-stored seeds from fire-following plant species is well known. However, the effects of high pre-germination temperatures on subsequent seedling growth are less well understood. In this study, we examined the effect of pre-germination heat shock at five temperatures (60°, 75°, 90°, 105° and 120°C, each applied for 5 min) on the seedling growth of four, fire-following Fabaceae species from four Mediterranean-type ecosystems; *Hippocrepis multisiliquosa* (Israel), *Gastrolobium villosum* (Western Australia), *Cyclopia pubescens* (South Africa) and *Lupinus succulentus* (California). Following heat treatment and subsequent germination, seedlings were grown in controlled conditions before being harvested at either 10, 20- or 40 d old. A significant increase in mean dry weight biomass was found at 10 days for *Hippocrepis* seedlings germinated from seeds pre-heated to 90°C. However, subsequent comparison of mean dry weight biomass for seedlings of this species at 20 and 40 d old showed no significant response to heat shock pre-treatment. Similarly, an initial increase in growth of *Gastrolobium* seedlings germinated from seeds heated to 90° and 105°C disappeared as the plants matured. Seedling growth of *Lupinus* and *Cyclopia* was unaffected by the pre-germination heat treatment of their seeds. Since seedling competition is influenced by the size and growth rates of neighbouring plants, any changes in seedling growth rates as a consequence of the temperature environment experienced by their seeds, may therefore influence patterns of post-fire plant community recovery. © 2001 Éditions scientifiques et médicales Elsevier SAS

fire / hardseededness / heat shock proteins / seeds / thermal scarification

1. INTRODUCTION

Fire is a common occurrence in Mediterranean-climate regions throughout the world. Relative differences in ecosystem productivity and composition before and after fires are well known and many of the factors affecting plant community response to fire well established [28]. These factors include the effects of fire on seed release from the canopy [8, 24], germination from soil stored seed banks [3, 4], resprouting from rootstocks [5, 28], soil nutrient availability [7, 9] and changes in herbivore populations [12, 28]. By far the most widely studied phenomenon however is the role of heat shock on germination. Germination of seeds from a wide-range of fire-following plant species in Mediterranean Europe [16], South Africa [5], California [18] and Australia [11], has been shown to respond positively to temperatures often well in excess of 100°C.

The differential tolerance of seeds to high temperatures can exert a considerable influence on plant community development following fire [20, 27]. Fires, both at ground level and within the canopy, are often highly variable in intensity [28]. The effects of spatial heterogeneity within a burn site, and in particular variable fuel loads caused by the distribution of pre-existing vegetation, may significantly affect pat

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terns of post-fire seedling recruitment [22, 23, 25]. However, it is only recently that the interaction between heat shock to soil-stored seeds and seedling competition in the aftermath of a burn has been highlighted [14]. Seedling growth and competitive interactions between neighbouring seedlings may be of critical importance in determining plant community composition in the post-fire plant community [22].

In order to understand more fully how post-fire patterns of seedling distribution are controlled, it is vital that the effects of pre-germination temperature on germination and plant growth are established. While the effects of heat shock on seed germination are relatively well understood [2], we know virtually nothing about its effects on subsequent plant growth. Hanley and Fenner [10] showed how seedling survivorship and growth for the semi-serotinous pine species (*Pinus brutia* and *P. halepensis*) and the fire ephemerals (*Anthyllis vulneraria* and *Hippocrepis unisiliquosa*), was reduced by pre-germination temperatures in excess of 90°C. However, survivorship and growth for the two hardseeded species examined (*Cistus creticus* and *C. salvifolius*), were reduced only by temperatures in excess of 110°C. Hanley and Fenner [10] suggest that the ability of *Cistus* spp. seeds to withstand relatively high soil temperatures and produce seedlings able to maintain good growth rates following a burn, effectively releases them from competition with fire-following annuals. In areas where low fuel loads have resulted in lower fire intensities and temperatures, *Cistus* spp. seedlings are often competitively excluded by post-fire ephemeral species [22].

As far as we are aware, Hanley and Fenner's [10] work on Mediterranean plant species is unique in examining the effect of pre-germination heat shock on seedling survivorship and growth. Certainly there are no accounts in the literature documenting this phenomenon in other Mediterranean-climate regions. In this paper we attempt to determine how the seedling growth of four Fabaceae species from four different Mediterranean climate ecosystems is affected by heat shock.

2. MATERIALS AND METHODS

2.1. Experimental species and germination response to heat-shock treatment

Seeds of each of the experimental species were collected when fully matured from parent plants growing in natural plant communities before storage at room temperature (c. 20°C) until use. Each of the species is common, or locally abundant within its native range, and is known to respond positively to the passage of fire (*table I*). However in order to check each species' germination response to heat shock, we dry heated two hundred seeds of each species in an oven for 5 min at 60°, 75°, 90°, 105° or 120°C. To avoid problems associated with pseudoreplication of heat-shock treatments we heated seeds in single replicate batches [21]. The seeds were then set to germinate in 9 cm diameter Petri dishes containing two layers of Whatman No. 1 filter paper soaked with 15 mL of distilled water. Each species/treatment group (including the unheated control) consisted of five Petri dishes, each replicate dish containing 40 seeds. The dishes were covered with lids and randomly arranged in a dark incubator set at 15°C. Dishes were examined daily for 28 days and any seeds that had germinated were counted and removed. Germination was deemed to have occurred when the radicle was visible. Total

Table I. Characteristics of four, fire-following Fabaceae species used to determine the effect of heat-shock pre-treatments on seed germination rate and seedling growth.

Species and authority	Life history and growth form	Habitat and abundance	Fire response syndrome	Province	Year seeds collected	Location
Cyclopia pubescens (Eckl. and Zeyh.)	Erect perennial shrub	Locally abundant in post-fire fynbos [5]	Obligate Seeder	South Africa	1999	Grahamstown
Gastrolobium villosum (Benth.)	Prostrate/spreading perennial shrub	Locally abundant in post-fire jarrah forest [3]	Resprouter	Western Australia	1996	Jarrahdale
Hippocrepis multisiliquosa (L.)	Prostrate/erect annual	Common in post-fire pine forest [16]	Obligate Seeder	Eastern Mediterranean	1994	Amirim
Lupinus succulentus (Douglas ex Koch.)	Erect annual	Locally abundant in post-fire chaparral and coastal sage scrub [17]	Obligate Seeder	California	1996	El Toro

percentage germination (TPG) for each treatment was recorded at the end of the experiment. The effect of heat shock treatment on TPG was determined by one-way ANOVA following arc-sine transformation. Differences in TPG between individual treatments for each species were located by using LSD tests.

2.2. Seedling growth and survival

Two hundred seeds of each species were dry heated in an oven for 5 min at 60°, 75°, 90°, 105° and 120°C. Following heat treatment these seeds, together with seeds stored at ambient temperature, were scarified mechanically by rubbing each seed with sandpaper. Mechanical scarification ensured that a high proportion of seeds, particularly within the unheated control groups, germinated. The seeds were then set to germinate in similar conditions to those described above. Dishes were examined daily and any seeds that had germinated were counted and removed.

Newly germinated seedlings were transplanted into 50 mm diameter pots containing vermiculite. Seedlings were taken in roughly equal numbers from each of the respective temperature treatment replicates (approx. 9 per Petri dish) so that there were 45 seedlings of each species from each of the temperature treatment groups. The exact planting date was recorded for each seedling and a harvest date assigned to it. The newly potted seedlings were arranged randomly in a plant growth room set at a constant 22°C with a 12 h day/12 h night light regime. The seedlings were watered daily with tap water and once a week using a 20%, Hoagland's nutrient solution. The aim was to harvest fifteen seedlings from each species/temperature treatment group at 10, 20 or 40 d old. However, a small number of seedlings died during the course of the experiment.

Upon harvest seedlings were removed from the pots and any vermiculite carefully washed from the roots, before being dried for 48 h in an oven set at 80°C. The dry weight biomass of each plant was determined and the mean total plant dry weight biomass for each species/temperature treatment group at the three harvest dates calculated. The effect of pre-germination heat shock on seedling growth was determined for individual species by one-way ANOVA and differences between treatment means located using LSD tests. Seedling survival (i.e. the number of seedlings still alive at harvest from the 15 replicates for each species/treatment group) was examined using the *G*-test.

3. RESULTS

3.1. Germination of unscarified seeds

The effect of heat shock on TPG for the four experimental species is shown in *table II*. Germination within unheated controls and the 120°C treatment groups was very low for each species. However, heat shock significantly increased germination for three of the species over unheated controls, although the effects varied considerably from species to species. *Cyclopia* germination was greatest at 60°C before declining again to zero germination at 120°C. For *Gastrolobium* and *Lupinus*, maximum germination occurred at the higher temperatures of 90°C and 105°C respectively, although germination in each of the 75°, 90° and 105°C treatment groups was significantly higher than the control group for both species. Germination in *Hippocrepis* was very low across all treatments and although maximum germination occurred at 90°C, at only 5% it was not significantly higher than germination within the unheated controls.

3.2. Seedling growth and survival

As with the unscarified seeds, germination of scarified seeds heated to 120°C was low or non-existent.

Table II. Mean total percentage germination (± SE) of four fire-following plant species 28 d after heat shock treatment.

Species	Control	60°C	75°C	90°C	105°C	120°C	One way ANOVA
Cyclopia pubescens	3.5^{a} (1.7)	55.2^{d} (3.4)	39.6^{c} (2.0)	30.5^{c} (2.9)	14.5^{b} (1.8)	0	$F_{5,24} = 51.08$, $P < 0.001$
Gastrolobium villosum	2.2^{ab} (1.0)	4.6^{bc} (2.2)	7.1^{c} (0.5)	8.0^{c} (1.9)	6.5^{c} (1.3)	1.2^{a} (1.2)	$F_{5,24} = 5.231$, $P = 0.002$
Hippocrepis multisiliquosa	1.0 (1.0)	1.0 (1.0)	2.0 (1.2)	5.0 (1.6)	3.0 (2.0)	2.0 (1.2)	$F_{5,24} = 1.62$, $P = 0.193$
Lupinus succulentus	12.0^{b} (1.3)	15.1^{bc} (2.6)	17.5^{bc} (1.0)	19.5^{c} (2.1)	20.5^{c} (2.3)	1.5^{a} (0.9)	$F_{5,24} = 18.41$, $P < 0.001$

Results of LSD tests showing significant ($P < 0.05$) differences between treatment means following one-way ANOVA on arc-sine transformed data, are represented by different letters.

Therefore, this treatment group was not included in subsequent statistical analyses. Growth of two of the four experimental species was significantly affected by pre-germination heat treatment (*figure 1*). However for both *Hippocrepis* and *Gastrolobium*, these effects were only apparent in the youngest seedlings. Due to slow growth rate, the first harvest of *Gastrolobium* seedlings was done at 20 d old. At this age, mean dry weight biomass of seedlings from the 90°C and 105°C treatment groups was significant greater than that of the unheated control group (ANOVA $F_{4,57} = 3.180$, $P = 0.020$). It is interesting to note that these significant increases in seedling growth occurred at temperatures that also induced a significant increase in germination.

Pre-germination heat shock also resulted in increased seedling growth of *H. mulitisiliquosa* plants ($F_{4,68} = 5.372$, $P < 0.001$). Although this response was limited to 10 d old plants within the 90°C treatment group, it did match the slight increase in germination found at this temperature. Subsequent harvests of seedlings of both *Gastrolobium* and *Hippocrepis* failed to show any significant pre-germination heat shock effects on seedling growth. Furthermore, neither *Lupinus* nor *Cyclopia* showed any evidence for a significant interaction between pre-germination heat shock and seedling growth. Seedling survival of all four species was unaffected by heat shock treatment.

In tandem with mechanical scarification, heat treatment may affect the hardness of the seed coat. Such an effect could potentially influence the speed of seed emergence, and with it the size and developmental stage of emerging seedlings. In order to determine whether emergence time varied significantly between control and heat treatments, we compared the germination rates of scarified *Gastrolobium* and *Hippocrepis* seeds from the different treatments (data not shown) using the weighted percentage germination (WPG) rate approach described in Hanley and Lamont [11]. This method gives maximum weighting to seeds that germinate first, so detecting any differences in time-to-emergence in our experimental species. Although germination rates for both *Gastrolobium* and *Hippocrepis* differed greatly between control and heat treat

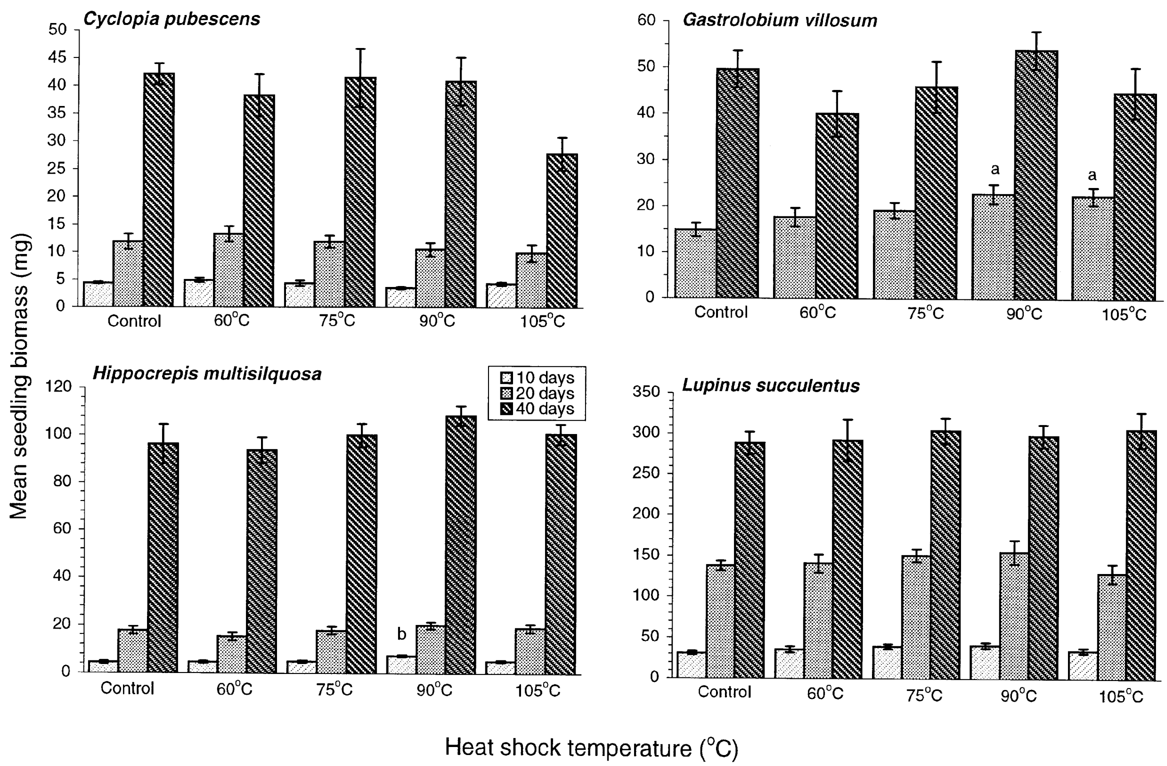

Figure 1. The effects of pre-germination heat shock on mean (± SE) dry weight biomass of seedlings of four fire-following plant species harvested at 10, 20- or 40-d old. Results of LSD tests showing significant ($P < 0.05$) differences between treatment groups following one-way ANOVA, are given as '***a***': heat shock treatment greater than untreated control, or '***b***': treatment greater than all other heat shock treatments and control.

ments, it was only at the highest temperatures that rates were significantly affected. For example, WPG was reduced for *Gastrolobium* seeds heated to 120°C (LSD $P < 0.05$) and for *Hippocrepis* seeds heated to 105°C and 120°C (LSD $P < 0.05$). For all other treatments however, including the 90°C and 105°C treatments for *Gastrolobium*, and the 90°C treatment for *Hippocrepis*, there was no significant effect of heat treatment on scarified seed germination rate.

4. DISCUSSION

This results presented here provide the first reported experimental evidence that pre-germination heat shock exerts a stimulatory effect on subsequent seedling growth. While mechanical scarification appeared to mimic closely the effects of heat shock on seed germination, a result supporting the suggestion that the stimulatory effect of heat shock is due to physical disruption of the seed coat [2, 26], we failed to find any evidence that these two factors combined were facilitating faster emergence from the seed, and consequently seedling size at harvest. Although the precise physical or ecophysiological mechanism by which increased seedling growth at particular pre-germination temperatures arises is unclear (a fact incidentally also true of the interaction between pre-germination chilling and plant performance [6]), one possibility could be the release of heat shock proteins (hsps) during heat stress. Hsps have been recorded in soybean seedlings exposed to 50°C and their release is believed to provide protection against high temperature [1]. Whether hsps are synthesised within seeds of fire-following species during fire, and whether their release affects seedling growth, is unknown. At this stage however, hsps may provide one possible future avenue of research to explain the phenomenon described here.

It is clear however that the regeneration of each of our experimental species is positively related to fire (*table I*), a response highlighted by the fact that germination of three of the four species was increased significantly following heat shock. In the case of *Gastrolobium*, there was also a close relationship between the temperature (90°C) at which maximum germination and maximum seedling growth at 20 d occurred. Although the increased growth response was only apparent in the youngest seedlings (as it was for *H. multisiliquosa*), even short-lived differences in plant size can have significant implications for competitive interactions between neighbouring plants. Initially uniform seedling populations develop a skewed distribution in favour of larger plants because larger and faster-growing seedlings are more competitive than their smaller neighbours [15, 29]. Competition is one of the key mortality factors operating at the seedling stage [14, 19, 22] and any advantage a seedling gains in terms of its size may be crucial to its survival in the plant community [13]. Fire-following species, whose growth has been stimulated as a consequence of heat shock to their seeds, may hold a competitive advantage over neighbours not exhibiting the same response.

Only one species, *H. multisiliquosa*, failed to exhibit a germination response to heat shock, although neither unheated controls nor heat-treated seeds, germinated well without mechanical scarification. Other fire-related factors, such as nitrate release or changing light intensity in addition to or combination with heat shock, may act as cues to germination for this species. However, since 10 d old *H. multisiliquosa* seedlings exhibited increased growth in the 90°C treatment, and no seedlings suffered reduced growth following pre-germination heat shock, it does appear that at the very least, the embryo is extremely resistant to the passage of fire.

This resistance was also apparent for *Lupinus* and *Cylopia*, where heat shock temperatures exceeding 100°C failed to reduce seedling growth of either species. These findings are similar to those reported for two Mediterranean *Cistus* species [10]. Although seedling growth for these species is not stimulated by pre-germination heat shock, their increased germination ability is likely to provide them with a significant competitive advantage in the post-fire environment [28]. Some species however, have been shown to exhibit reduced growth rates following heat shock. In addition to their work on *Cistus creticus* and *C. salvifolius*, Hanley and Fenner [10] showed that pre-germination temperatures exceeding 90°C for 10 min, significantly reduced growth of 56 d old *Anthyllis vulneraria* (Fabaceae) and *Pinus halepensis* seedlings.

Differences between seedling growth responses to pre-germination heat shock may simply be due to species-specific effects, something common in germination studies [2, 3, 11, 28]. For *P. halepensis* in particular, it is not surprising that a species whose seeds are protected from fire in cones held high in the canopy should demonstrate this kind of negative response. However, some differences may also be ascribed to experimental design, in particular the duration of temperature treatment applied. For instance, Hanley and Fenner [10] showed that application of pre-germination heat shock for long durations (up to 20 min), significantly reduced seedling growth of *H. unisiliquosa* plants in comparison with that of seedlings whose seeds were heated to the same temperatures, but for a shorter (5 min) period. It is also worth noting that Hanley and Fenner [10] based their conclusions on data collected from a single harvest of 56 d old plants. Given that the positive growth effects found in the present study for *Hippocrepis* and *Gastrolobium* disappeared by the time seedlings had reached 40 d old, had any of Hanley and Fenner's [10] species shown a positive response, they may well have failed to detect it.

Although only two of our four experimental species displayed a positive growth response to pre-germination heat shock, the fact that they were from two geographically distinct regions suggests that the phenomenon could exist in other fire-following species in these, and possibly other, areas. However, it is impossible to say from this study how this response varies within a suite of coexisting species. More work is required to examine how widespread this phenomenon is and to elucidate the physical or physiological mechanism by which it arises. Having done this, ecologists may then examine how heat shock-induced changes in seedling growth affect the competitive interactions between neighbouring seedlings, and ultimately influence post-fire plant community composition.

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