

ROLE OF CHARRED WOOD, HEAT-SHOCK, AND LIGHT IN GERMINATION OF POSTFIRE PHRYGANA SPECIES FROM THE EASTERN MEDITERRANEAN BASIN

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ABSTRACT

Seeds of 22 species collected from recently burned phrygana were tested for their response to fire-type cues of charred wood and heat-shock. All *Cistus* species were stimulated by brief heat-shock, as shown in previous studies; however, none responded to charred wood. Only one of the 22 species was stimulated by charred wood, and only in dark-inhibited seeds, and this response did not occur in the light. The lack of charred-wood-induced germination is in contrast to the substantial proportion of species with this germination response reported for mediterranean-type vegetation in California, the Cape region of South Africa, and Western Australia. Phrygana has many species with heat-shock-stimulated germination, primarily in the Fabaceae and Cistaceae. This germination cue is widespread in these two families, thus, the presence of heat-shock-stimulated germination is a result of homologous, rather than convergent, adaptations in mediterranean-climate ecosystems. Germination response to light was not randomly distributed with respect to fire-type response. Heat-shock-stimulated species were almost uniformly light neutral, in contrast to more opportunistic colonizing species with non-refractory seeds, in which half of the species responded positively or negatively to light.

INTRODUCTION

Convergent evolution theory predicts that, given similar environments, organisms will be selected for similar structural and functional attributes, and in this respect mediterranean-climate ecosystems have many convergent characteristics (Cody and Mooney, 1978; Cowling and Campbell, 1980; Shmida and Whittaker, 1984; cf., Barbour and Minnich, 1990). While climate is often considered to be a primary selective force in the evolution of convergence, fire has been a potent selective force in mediterranean-climate vegetation (Kruger, 1983; Trabaud, 1994). In all five regions there is evidence that species have evolved to exploit fires for population expansion, some even being “fire-dependent” for completion of their life cycle (e.g., Gill, 1981; Keeley, 1986; Ne’eman et al., 1993a; Troumbis, 1993). Seedling recruitment is cued to postfire sites by various mechanisms. For example, geophytes are widely known

to have fire-stimulated flowering (Le Maitre and Brown, 1992), pines and cypress in the Northern Hemisphere, and many flowering plants in the Southern Hemisphere, have serotinous cones or fruits that open only after fire (Lamont et al., 1991), and a great many species have dormant seed banks stimulated by fire (Parker and Kelly, 1989; Keeley, 1995).

Fire-stimulated germination results from seed characteristics that range from ones that are relatively unspecialized to fire, to highly specialized responses. For example, burned sites have highly altered light and thermal regimes that stimulate germination, although similar cues may occur under a variety of conditions. Heat-shock is somewhat specialized to fire, although

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most heat-stimulated species respond to a temperature range (70–120 °C) that could be encountered on mineral soil within gaps in mature vegetation. Such heat-shock-stimulated germination is widespread in the Fabaceae, Rhamnaceae, Cistaceae, Convolvulaceae, Malvaceae, and Sterculiaceae, and is common in the Mediterranean Basin (Corral et al., 1990; Thanos et al., 1992; Gonzalez-Rabanal and Casal, 1993, 1995), California (Keeley, 1991), Australia (Auld and O'Connell, 1991; Bell et al., 1993), South Africa (Jeffery et al., 1988; Musil and De Witt, 1991), Chile (Munoz and Fuentes, 1989), and other ecosystems (Rolston, 1978). Seedling recruitment of such species is often, but not always, associated with burns.

A cue more specialized to fire are chemicals generated by phytomass combustion, from either smoke or charred wood, being first reported for California chaparral (Wicklow, 1977) and later for South African fynbos (De Lange and Boucher, 1990) and Australian scrub communities (Dixon et al., 1995). There is evidence that charred-wood- and smoke-stimulated germination represents a response to the same chemicals (Brown, 1993b; Keeley and Fotheringham, 1998b). It has been proposed that nitrate is the active component of charred-wood-stimulated germination (Thanos and Rundel, 1995), which would suggest a relatively unspecialized germination mechanism, as a great many weeds are nitrate-stimulated (Pons, 1989). However, there is substantial evidence that nitrate is not the cue (Baldwin et al., 1994; Keeley and Fotheringham, 1998a) and trace gases generated from smoke or oxidation of charred wood are involved (Keeley and Fotheringham, 1997).

Charred-wood-stimulated germination has not been reported for any species from the Mediterranean Basin and the primary purpose of this study was to test species from postfire phrygana communities for evidence of charred-wood-stimulated germination. Germination response to heat-shock and light were also investigated.

SPECIES AND METHODS

Seeds were collected from 1 to 3-yr-old burned sites in phrygana communities in southern Greece, including the islands of Naxos, Sandrini, and Crete, and stored at room temperature for 3 months prior to testing. Seeds were pre-treated by exposing them to dry heat in an oven for 5 min at 100 °C or 110 °C. Pine branches were charred, but not ashed, with a torch, and ground with a Wiley mill to pass a 2-mm sieve. Previous studies with chaparral species have demonstrated that the charred-wood response is not a function of the type of wood (Keeley and Pizzorno, 1986). Germination was done in 6-cm Petri dishes on filter paper with 2 ml distilled

water (n = 3 dishes of 30 seeds). Powdered charred wood (0.5 g) was added to each Petri dish and an additional 1 ml of water added. All seeds were given a one month cold (4–5 °C) stratification prior to incubation under 12 hr photoperiod (~50 $\mu\text{mol m}^{-2} \text{s}^{-1}$, Sylvania 20 W Cool White fluorescent bulbs) at 18 °C light/12 °C dark. All treatments were replicated (n = 3) in the dark and germination recorded under dim green light.

Germination was compared with a 2-way fixed effects ANOVA on arc-sine-transformed data. Interaction between light response and fire-response was analyzed by testing the null hypothesis of random association using χ^2 calculated from a contingency table.

RESULTS

For approximately half of the species collected; germination under any treatment was relatively weak; therefore, data presented here are only for the 22 species with >25% germination. Figure 1 illustrates the range of responses observed with respect to fire-type cues and light and these are summarized by species (Table 1). The majority of species had non-refractory seeds with high control germination (e.g., *Allium* and *Crucianella*, Fig. 1), but significantly reduced germination with one or the other heat treatments. Not surprisingly, all *Cistus* species were heat-shock stimulated. Only one species, *Lavendula stoechas*, exhibited a positive response to charred wood, and only in the dark, although this response was not significantly ($p > 0.05$) greater than the response to one of the heat-shock treatments (Fig. 1).

Response to light varied from light-neutral (e.g., *Cistus* spp., Fig. 1), to light-stimulated (e.g., *Misopates oronitum*, Fig. 1), to light-inhibited (e.g., *Allium* sp., Fig. 1). The ANOVA indicated a highly significant ($p < 0.01$) interaction between fire-type treatment and light treatment in all except the light neutral species. Species that showed no response to either heat, charred wood, or light (i.e., "0 0 Neutral" response, Table 1) exhibited very high control germination (50–100%) in both the light and the dark.

Response to light was further analyzed with a contingency table, and the χ^2 value showed that light response was not randomly distributed with respect to fire-type response. Relative to species with fire-type cues, non-refractory seeds had a significantly higher proportion of light-sensitive species (Table 2).

DISCUSSION

The relative lack of charred-wood-stimulated germination in this phrygana community stands in marked contrast to other mediterranean-climate ecosystems. A pre-

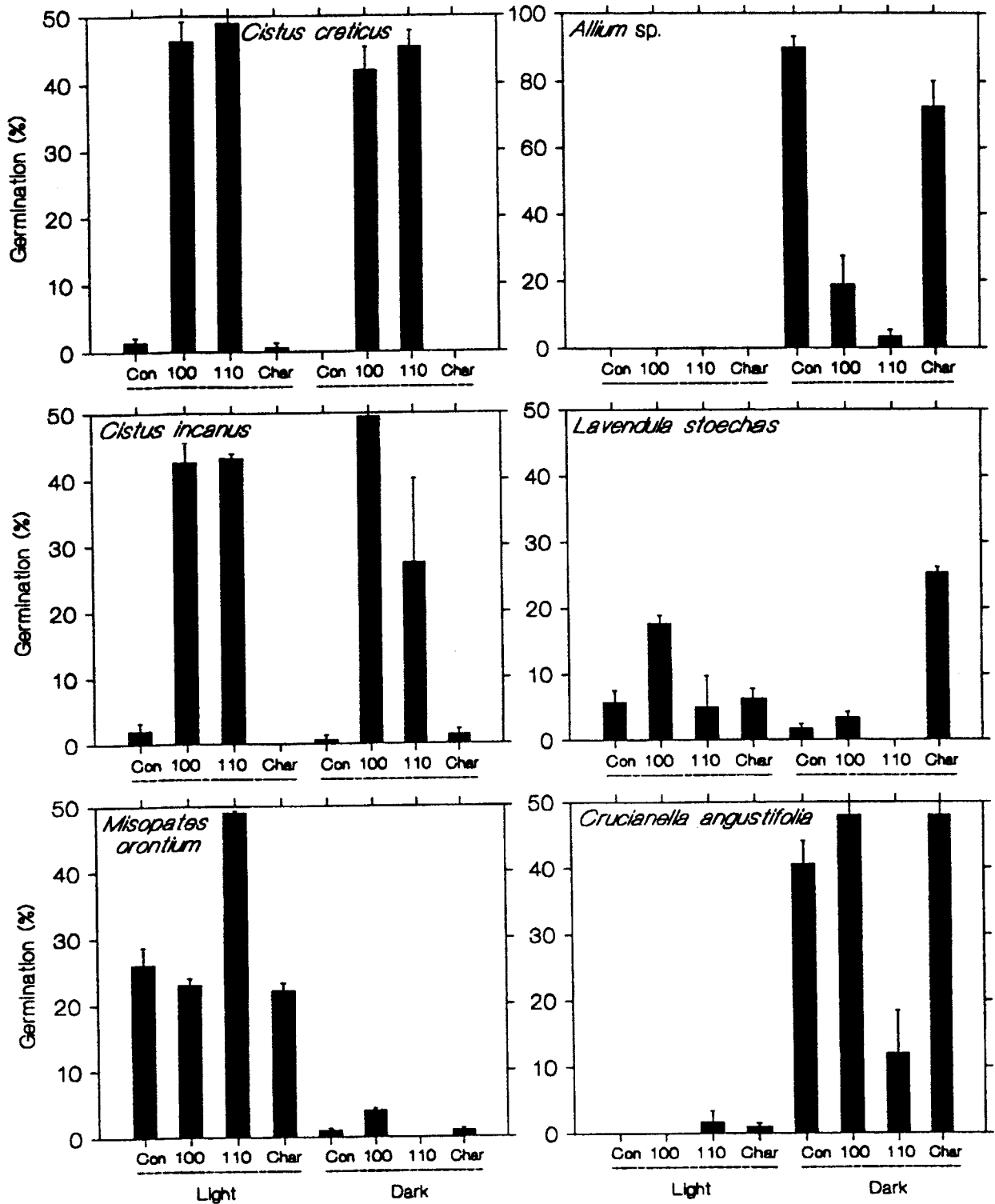


Fig. 1. Germination of selected eastern Mediterranean Basin species under control conditions, heat shock of 100 or 110 °C for 5 min, or incubated in ground charred wood in either the light or dark (vertical bars = 1 S.E.).

Table 1

Species with greater than 25% germination, and their response to heat-shock, charred wood, and light. Species were analyzed separately with a 2-way ANOVA; "+" indicates statistically significant ($p < 0.05$) increase over controls (some species were inhibited (killed?) by heat and these negative responses are not indicated), "inhibited" indicates a significant increase in germination in the dark over light, and "stimulated" indicates a significant increase in the light

Species	Family	Maximum control germination $X \pm S.E.$	Heat-stimulated	Charred-wood-stimulated	Light-response
<i>Allium</i> L. sp.	Alliaceae	90 \pm 3	0	0	Inhibited
<i>Althaea hirsuta</i> L.	Malvaceae	85 \pm 4	0	0	Neutral
<i>Ammoides pusilla</i> (Brot.)Breistr.	Apiaceae	95 \pm 7	0	0	Inhibited
<i>Asphodelus aestivus</i> Brot.	Asphodeliaceae	57 \pm 4	0	0	Neutral
<i>Atractylis cancellata</i> L.	Asteraceae	51 \pm 6	0	0	Neutral
<i>Cistus creticus</i> L.	Cistaceae	1 \pm 1	+	0	Neutral
<i>C. incanus</i> L.	"	2 \pm 2	+	0	Neutral
<i>C. monspeliensis</i> L.	"	2 \pm 1	+	0	Neutral
<i>C. salvifolius</i> L.	"	1 \pm 1	+	0	Neutral
<i>Crucianella angustifolia</i> L.	Rubiaceae	47 \pm 3	0	0	Inhibited
<i>Daucus carota</i> L.	Apiaceae	96 \pm 3	0	0	Stimulated
<i>Hedypnois cretica</i> (L.)C.Cours.	Asteraceae	76 \pm 8	0	0	Neutral
<i>Hypericum empetrifolium</i> Willd.	Hypericaceae	59 \pm 6	0	0	Neutral
<i>Lavendula stoechas</i> L.	Lamiaceae	7 \pm 4	+	+	Neutral
<i>Misopates orontium</i> (L.) Raf.	Scrophulariaceae	28 \pm 5	+	0	Stimulated
<i>Papaver</i> L. sp.	Papaveraceae	57 \pm 8	0	0	Stimulated
<i>Phlomis lanata</i> Willd.	Lamiaceae	11 \pm 6	+	0	Neutral
<i>Plantago psyllium</i> L.	Plantaginaceae	53 \pm 5	0	0	Inhibited
<i>Sarcopoterium spinosum</i> L.	Rosaceae	27 \pm 4	+	0	Neutral
<i>Teucrium</i> L. sp.	Lamiaceae	62 \pm 6	0	0	Neutral
[unidentified]	Apiaceae	87 \pm 3	0	0	Inhibited
[unidentified]	Asteraceae	77 \pm 5	0	0	Neutral

Table 2

Contingency table for germination response to light vs. fire-type cue for 22 postfire species from phrygana ($\chi^2 = 4.68$, $p < 0.01$). Based on data from Table 1

	Light-neutral	Light-stimulated	Light-inhibited
Non-refractory	7	2	5
Heat-stimulated	7	1	0
Charred-wood-stimulated	1	0	0

cise comparison of germination response across regions is complicated by the lack of randomly selected samples of the flora for any mediterranean-climate region. Assuming, however, that the data published to date are somewhat representative of the pattern for different floras, we can make some estimates about the frequency of charred-wood vs. heat-shock-stimulated germination. In California chaparral (Keeley, 1991; Keeley and Fotheringham, 1998b), 33% of the flora is stimulated by charred wood/smoke and 26% by heat-shock, which is similar to estimates for Western Australia (Bell et al.,

1993; Dixon et al., 1995) of 30% and 32% germination for charred-wood/smoke and heat-shock, respectively. South African fynbos, perhaps due to the small sample size ($n = 57$), had only 14% heat-shock-stimulated but 43% charred-wood/smoke-stimulated germination (Keeley and Bond, 1997). In contrast, the present study found no species strictly stimulated by charred wood and only one out of 22 species exhibited any response to such treatment. Other studies in the Mediterranean Basin have also reported failure of wood ash to stimulate germination (Ne'eman et al., 1993b; Gonzalez-Rabanal and Casal, 1995). However, there is an important distinction between charred wood and ash. The former is typically acidic ($pH < 5.5$) whereas the latter is highly alkaline ($pH > 11$) (Keeley and Fotheringham, 1998a), and in the chaparral flora charred wood (and smoke) induce germination whereas ash has no effect (Keeley, 1991).

While recognizing the need for more study, we cautiously conclude that Mediterranean Basin flora is relatively depauperate in charred-wood/smoke-stimulated germination, and this represents a lack of convergence

with other mediterranean-climate ecosystems. In common with other regions, however, is the importance of heat-shock-triggered germination (Table 1) and this has been well documented for many species of Cistaceae and Fabaceae (Trabaud and Oustric, 1989; Corral et al., 1990; Thanos et al., 1992; Gonzalez-Rabanal and Casal, 1993, 1995). Convergent evolution of heat-shock-triggered germination seems unlikely, and a more parsimonious hypothesis would be parallel evolution. This is based on the strong phylogenetic relationship of heat-shock species between regions; Fabaceae and Cistaceae are two of the more commonly encountered heat-shock-stimulated taxa in California as well as in the Mediterranean Basin (Keeley, 1991). Worldwide, these families are noted for being "hard-seeded" (Quinlivan, 1971; Thanos et al., 1992), a characteristic associated with heat-shock-stimulated germination.

If the studies presented here are representative, then an important difference between the Mediterranean Basin and other mediterranean-climate regions is the preponderance of species with weakly dormant seed banks capable of germinating without fire-type cues. In the present study, > 60% of the species had non-refractory seeds (Table 1), which is in contrast to < 45% for California (Keeley, 1991), Australia (Bell et al., 1993; Dixon et al., 1995), and South Africa (Keeley and Bond, 1997). While more study is required, it is interesting to speculate on the possibility that the long history of intensive land use in the Mediterranean (Shmida, 1981; Naveh, 1982) may have selected for more opportunistic type colonizing species. Such weedy species often utilize other cues for timing germination to disturbance, such as photo and thermal regimes.

The interaction between light-response and fire-response is worth comment (Table 2). In general, species that are induced to germinate by fire were neutral to lighting conditions, and this conclusion was also reached by Thanos et al. (1992), but cf. Roy and Sonie (1992). In contrast, a significantly higher proportion of non-refractory seeds have germination significantly affected by light—mostly inhibited by light—a response noted for other non-fire-type species (Thanos et al., 1991). Thus, seeds of these species are likely to remain dormant unless buried to a sufficient depth. In this study there were relatively few species stimulated by light, which is a cue proposed by Roy and Arianoutsou-Faraggitaki (1985) for cuing germination to gaps in the vegetation. The interaction between charred-wood-stimulated germination and dark observed for *Lavendula stoechas* is interesting because a very similar pattern was observed for several summer-deciduous elements in California chaparral (Keeley, 1987).

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