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SEED GERMINATION OF SIERRA NEVADA POSTFIRE CHAPARRAL SPECIES

JON E. KEELEY[1,2], THOMAS W. MCGINNIS[1], AND KIM A. BOLLENS[1]
[1] U.S. Geological Survey, Western Ecological Research Center, Sequoia National Park, Three Rivers, CA 93271, USA
jon_keeley@usgs.gov
[2] Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA 90095 USA

ABSTRACT

The California chaparral community has a rich flora of species with different mechanisms for cuing germination to postfire conditions. Here we report further germination experiments that elucidate the response of several widespread shrub species whose germination response was not clear and include other species from the Sierra Nevada, which have not previously been included in germination studies. The shrubs *Adenostoma fasciculatum* and *Eriodictyon crassifolium* and the postfire annual *Mentzelia dispersa* exhibited highly significant germination in response to smoke treatments, with some enhanced germination in response to heating as well. The shrubs *Fremontodendron californicum* and *Malacothamnus fremontii* were stimulated only by heat-shock treatments. Seeds buried in the soil for one year exhibited substantially higher germination for controls and most treatments. In the case of two postfire annuals, *Mimulus bolanderi and M. gracilipes,* germination of fresh seed was significantly greater with smoke or heating but after soil storage, over two-thirds of the control seeds germinated and treatment effects were not significant. These two annuals are generally restricted to postfire conditions and it is suggested that control germination of soil-stored seed may be a light-response (which was not tested here) as previously reported for another chaparral species in that genus.

Key Words: chaparral, fire, germination, heat-shock, seed dormancy, smoke.

Wildfires are a natural feature of California landscapes and many plant communities are composed of species that are well adapted to such periodic disturbances. The most widespread vegetation type in the state is chaparral and its postfire response has been well studied (Keeley 2000). Regeneration is from residual species present prior to the fire and colonization plays a relatively minor role in the postfire recovery. Resprouts from stems or roots and seedling recruitment from seed banks are the primary means of regeneration. A substantial number of species of both herbaceous and woody life forms produce dormant seeds that accumulate in soil seed banks (Parker and Kelly 1989).

Fire-triggered germination is the result of either heat-shock or chemical products of combustion, and species appear to utilize one or the other of these modes. Heat-shock stimulated germination is widespread in the Fabaceae, Rhamnaceae, Convolvulaceae, Malvaceae, Cistaceae, and Sterculiaceae, and is found in many ecosystems (Keeley and Fotheringham 2000). While an exhaustive study of germination characteristics for these taxa is lacking, those that use this strategy are described as "hard seeded," with a prominent waxy cuticle and a dense palisade layer of sclerids that enforces dormancy by forming a water-impermeable barrier. Brief heat-shock above 75°C is generally sufficient to induce imbibition by loosening cells in selected parts of the seed coat. This is sufficient to overcome dormancy in many species, although in some, heat-shock must be coupled with light and/or cold stratification (Keeley 1987).

For a substantial number of species with fire-triggered germination, heat-shock has no effect on germination, rather germination is induced by chemicals from combustion products (Keeley 1991). Charred wood was first shown to stimulate germination in the postfire annual *Emmenanthe penduliflora* (Wicklow 1977) and later reported for many other chaparral species (Keeley 1991). Smoke also stimulates germination of this species and can trigger germination directly and indirectly by binding to soil particles, followed either aqueous or atmospheric transfer to seeds (Keeley and Fotheringham 1997). Some chaparral species have complex germination behavior that couples smoke cues with other environmental cues such as cold stratification. One of the more interesting characteristics is that reported for *Dicentra chrysantha, Dendromecon rigida and Trichostema lanatum,* where seed dormancy could only be broken by a combination of long term soil burial followed by smoke (Keeley and Fotheringham 1998). Smoke also has been reported from species indigenous to mediterranean-climate shrublands in Australia (Roche et al. 1997) and South Africa (Brown 1993). One interesting observation made by researchers in the latter country is that commercial food flavoring known as "liquid smoke" is equally effective in triggering

germination of many smoke stimulated species (Jager et al. 1996).

The bulk of the studies in California chaparral have focused on southern California species and very little attention has been directed at other parts of the state. In addition, the vast majority of work has been directed at annual species and less attention has been given to the germination behavior of the dominant shrub species. For example, the nearly ubiquitous *Adenostoma fasciculatum* (chamise) has never been tested for its response to smoke and prior studies have reported seemingly conflicting responses. For example, Stone and Juhren (1953) reported that heat alone would stimulate germination of *A. fasciculatum,* however, others have reported that heat does not stimulate germination but charred wood does (Parker 1987; Keeley 1987). Similar conflicting reports are noted for other woody species such as *Fremontodendron californicum* (Keeley 1991).

The purpose of the present study was to examine germination behavior of woody and herbaceous species from recently burned chaparral in the Sierra Nevada foothills. Postfire response of chaparral in this region has received relatively minimal study (Stocking 1966; Parsons 1976; Rundel et al. 1987; Rice 1993) and very little on natural fire-type cues for germination. Stocking (1966) reported that several Sierra Nevada fire following species such as the shrub *Malochothamnus fremontii,* a central California endemic, were stimulated to germinate by seed coat scarification. However, both smoke-stimulated and heat-stimulated species respond to this artificial treatment (Keeley and Fotheringham 1998), so it is unknown what the natural germination cue is for this species. Similarly, Rogers (1949) reported that a Sierra Nevada manzanita, *Arctostaphylos viscida,* is stimulated to germinate by scarification but could not identify the fire cue. Our study focuses on eight species that recruit seedlings in first year burned sites in the Sierra Nevada. We chose species that are either endemic to the region and not previously studied or more widespread species for which we could not ascribe any clear germination response. We investigated the effect of a range of heat-shock treatments and liquid smoke treatments on fresh seeds and seeds buried outside in soil for approximately one year.

Methods

Species and Experimental Design

Eight species were selected for study, five shrubs and three annuals. Seeds from the annuals were collected from recently burned sites in Fresno County and seeds of the shrubs were from mature chaparral in Fresno and Tulare counties. Nomenclature is according to Hickman (1993). Seeds were cleaned of fruit material and other debris and stored dry in paper bags at room temperature for approximately 6 months, or in nylon mesh bags in soil outdoors in Three Rivers, Tulare County, California (520 m elevation) for approximately one year.

Understanding germination response to fire cues such as smoke or heat-shock is complicated by the fact that the same factors that stimulate germination at particular levels are lethal at higher levels. Levels stimulatory to some species may be lethal to other species and levels stimulatory to some species may be insufficient to trigger germination in other species (Keeley and Fotheringham 1998). As a consequence, correctly interpreting negative responses requires a response curve that includes a range of levels from low to high for both smoke and heat.

For our smoke treatment we used a commercial liquid smoke (Wright's Concentrated Hickory Seasoning, B&G Foods, Inc.) diluted with distilled water. This product comes in a highly concentrated form and preliminary trials suggested the appropriate range to use was dilutions of 1:100, 1:500 and 1:1000. Heat-shock treatments included 80°C for 1 hr, and 5 min at 100°C, 110°C, 130°C, 140°C and 150°C. The three highest heating treatments were not done on soil-stored seeds due to limited seed availability. Thus, there were a total of 9 treatments and a control. Each treatment was replicated three times. Replicates comprised 30 seeds each for all species except for those with much larger seeds and smaller seed collections; n = 15 for *Arctostaphylos viscida* and *Malachothamnus fremontii* and n = 10 for *Fremontodendron californicum.*

Germination Experiments

Seeds to be heat-treated were placed in 70 × 15 mm aluminum dishes and treated in a forced convection oven. For the brief heat treatments, there was substantial spatial variation in temperature in different parts of the oven, although this was not a complication for the 1 hour treatment. Trials using thermocouples indicated that between the front and the back of the oven temperatures might have varied as much as ± 5°C at low temperatures and as much as ± 10°C at the highest temperature. In addition there was temporal variation due to the drop in temperature when dishes at room temperature were placed inside. To reduce this variation, multiple batches of replicates were treated separately. Nonetheless, temperatures reported here are the average temperature in the middle of the oven and only approximate the temperature any given replicate was exposed to. They are not meant to clearly define temperature optima but rather evaluate whether or not there is a germination response to heat-shock *per se.*

Germination was conducted in 60 × 15 mm sterilized polystyrene petri dishes with two pieces of 55 mm Whatman No. 1 filter paper and germination was initiated with the addition of 1.5–2.0 ml of distilled water, depending on the size of the seeds, for controls and heat-treated treatments or with a similar quantity of liquid smoke dilutions. Petri dishes

TABLE 1. ANALYSIS OF VARIANCE FOR GERMINATION EXPERIMENTS WITH FRESH LAB-STORED AND ONE YEAR SOIL-STORED SEEDS.

	Fresh lab stored seed			One year soil-stored seed		
Species	df	*F*-statistic	*P*-value	df	*F*-statistic	*P*-value
Adenostoma fasciculatum						
Treatment	9	9.748	<0.001	6	6.749	<0.01
Error	20			14		
Arctostaphylos viscida						
Treatment	9	1.976	>0.05	6	1.129	>0.05
Error	20			14		
Eriodictyon crassifolium						
Treatment	9	7.648	<0.001	6	19.550	<0.001
Error	20			14		
Fremontodendron californicum						
Treatment	9	13.094	<0.001	6	20.878	<0.001
Error	20			14		
Malacothamnus fremontii						
Treatment	9	14.870	<0.001	6	63.834	<0.001
Error	20			14		
Mentzelia dispersa						
Treatment	9	14.178	<0.001	6	62.236	<0.001
Error	20			14		
Mimulus bolanderi						
Treatment	9	2.881	<0.05	6	9.749	<0.001
Error	20			14		
Mimulus gracilipes						
Treatment	9	8.568	<0.001	6	5.644	<0.01
Error	20			14		

were placed in a single layer on plastic trays and enclosed in heavy plastic zip-lock bags to reduce evaporation and transfer of gases between treatments. Trays were stored for 1 month under variable light conditions at ~4°C, followed by incubation at alternating 12 h in the light at 18°C and 12 h in the dark at 12°C. Seeds were examined weekly for at least 6 weeks and germinated seeds recorded and removed. Germination was determined as the emergence of the epicotyl, and for the smaller seeds, was done under a 7× dissecting scope, once a week for one month.

A subsample of seeds was cut and if seeds were hollow or shrunken they were considered inviable. This was considered an upper estimate of viability as no further tests were performed. Final germination was expressed as a percentage of apparently viable seeds. Percentage germination was arcsin transformed prior to analysis. Treatments were analyzed with one-way ANOVA. Pairwise comparisons were made with the Bonferroni post hoc test. For a few cases in which treatments indicated increased germination, but it was not statistically significant, a power analysis was performed to determine the necessary sample size required to obtain a significant difference ($P < 0.05$) from controls. For these analyses the average observed standard deviation for controls and treatments was used. All analysis and graphics were run with SYSTAT 10.2 (www.systat.com).

RESULTS

Freshly collected seeds exhibited significant treatment effects with the exception of *Arctostaphylos viscida* (Table 1). Although most species exhibited some increased germination in response to both smoke and heat-shock, only one or the other was significantly different than controls (Fig. 1). Two shrubs, *Adenostoma fasciculatum* and *Eriodictyon crassifolium,* and two herbs, *Mentzelia dispersa* and *Mimulus bolanderi,* exhibited significantly greater germination for one or more of the smoke treatments over controls, but not for any of the heating treatments. Two shrubs, *Fremontodendron californicum* and *Malacothamnus fremontii,* and one herb, *Mimulus gracilipes,* had significantly greater germination for one or more of the heating treatments, but not for any of the smoke treatments. *Arctostaphylos viscida* germination did increase with smoke treatment but with the sample size of $n = 3$ it was not significant. Power analysis indicated that with the variance observed in these data

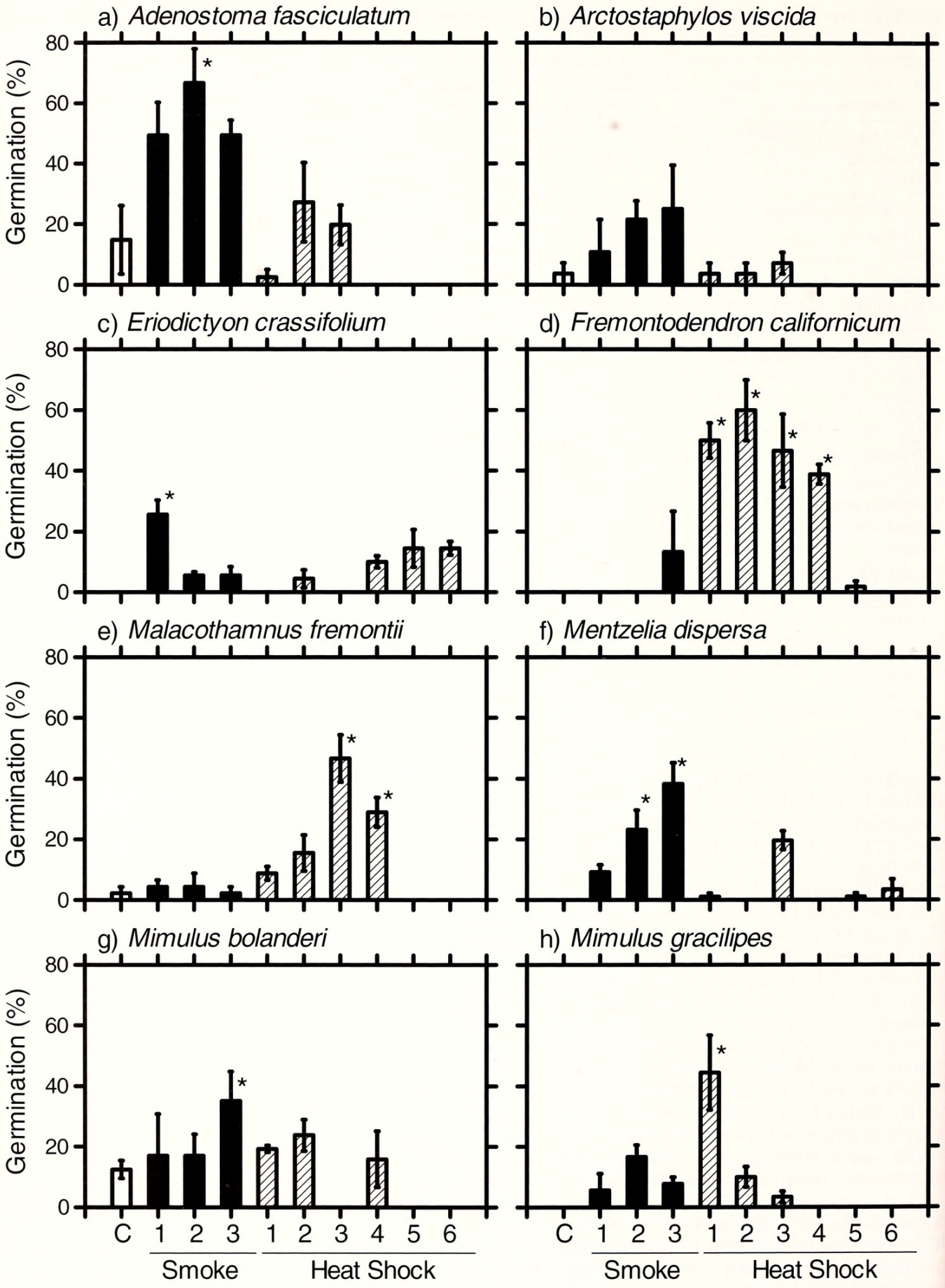

FIG. 1. Germination of freshly collected lab-stored seed of Sierra Nevada chaparral species in response liquid smoke (closed bars) at dilutions of 1 = 1:100, 2 = 1:500, 3 = 1:1000 and heat-shock (hashed bars) of 1 = 80°C for 1 hr, 2 = 100°C for 5 min, 3 = 110°C for 5 min, 4 = 130°C for 5 min, 5 = 140°C for 5 min, and 6 = 150°C for 5 min. Treatments significantly less than controls (open bars) at $P < 0.05$ are indicated with a *.

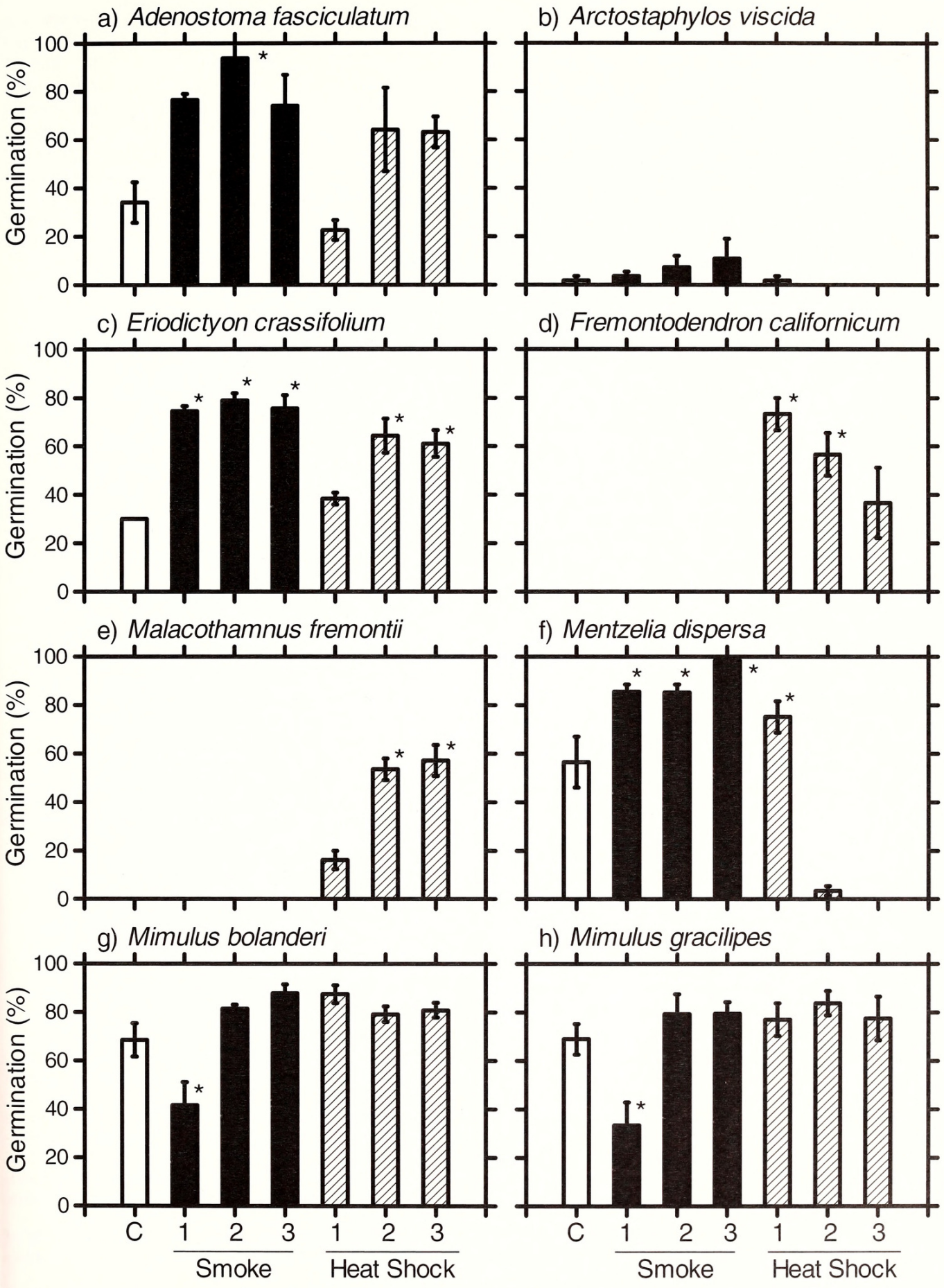

FIG. 2. Germination of seeds stored in outdoors in soil for approximately 1 year; in response to liquid smoke (closed bars) at dilutions of 1 = 1:100, 2 = 1:500, 3 = 1:1000 and heat-shock (hashed bars) of 1 = 80°C for 1 hr, 2 = 100°C for 5 min, and 3 = 110°C for 5 min (higher heating treatments not done on these seeds). Treatments significantly less than controls (open bars) at $P < 0.05$ are indicated with a *.

that it would require a sample size of n = 68 to detect a difference at the $P < 0.05$ level.

Germination of soil-stored seeds was greater for all species, with the exception of *Arctostaphylos viscida* (Fig. 2). In most cases, control germination was significantly greater than in freshly collected seeds. Responses to treatments were generally unchanged with a few differences. In *Adenostoma fasciculatum,* smoke was the only treatment that resulted in significantly greater germination than controls. Heating treatments did not increase germination significantly and power analysis indicated a sample size of n = 40 would be required to demonstrate a significant effect at the $P < 0.05$ level. In the case of *Eriodictyon crassifolium* and *Mentzelia dispersa,* germination was significantly increased by both smoke and heating, whereas with freshly collected seed it was only significant with smoke. For both *Mimulus* species control germination was over 60% and the only significant effect was reduced germination for the highest smoke concentration. However, both smoke and heat-shock did increase germination and power analysis indicated that with a sample size of n = 40 this would be significant at the $P < 0.05$ level.

Discussion

For the chaparral species studied here seedling recruitment is largely restricted to the first postfire growing season. Colonization plays a minor role and the vast majority of recruitment arises from dormant soil-stored seed banks (Keeley 2000). Although seed banks have not been investigated for most of these species, we can infer their existence because they do not have propagules designed for long distance dispersal, and the time between fires and the first growing season is outside the season of seed dispersal. Our germination experiments indicate fire is a potential trigger for germination of the dormant seed bank and that these species in Sierra Nevada chaparral respond to either heat-shock or smoke.

This study clarifies the germination response of the widespread *Adenostoma fasciculatum,* which had not previously been clearly elucidated; heat-triggered germination (Stone and Juhren 1953) and other reports indicated chemical products of combustion in charred wood stimulated germination (Parker 1987; Keeley 1987). Here we found smoke to significantly increase germination, and in all likelihood the stimulatory chemicals are the same as in charred wood (Keeley and Fotheringham 2000). Heat may also increase germination but power analysis indicated that we would need much larger sample sizes to demonstrate a significant effect, thus heat-shock is perhaps a minor factor in the fire-stimulated germination response or it responds to a specific temperature range and duration not tested here.

The Sierrean *Arctostaphylos viscida* germination is triggered by smoke and is similar to other species in the genus, which also have been reported to respond to combustion products such as charred wood (Keeley 1991). The very low germination is also quite similar to that reported for other species in the genus and suggests that either our crude estimate of viability was way off or there are other factors that are necessary to trigger germination of the entire seed bank.

For heat-stimulated species, significantly greater germination was observed when heated for 5 min between 100–130°C. For all species, heat-shock treatments of 5 min duration 140°C and above were apparently lethal.

These studies also provide further examples of changes in germination behavior following long term soil storage. Previous studies have shown that for several chaparral species a combination of soil burial followed by smoke treatment could overcome dormancy in deeply dormant seed banks, although burial alone could not (Keeley and Fotheringham 1998). The present study shows that soil storage generally increased germination of controls and treatments for most species. One interesting effect was the greatly increased control germination of *Mimulus* species following soil burial and the increased sensitivity to concentrated smoke solutions (Fig. 2). The fact that two-thirds of the control seeds germinated after soil burial seems to be at odds with the natural history of these two postfire annual species that are largely restricted to recently burned sites. One clue to what might explain this apparent discrepancy is the report of light-dependent dark-imposed dormancy for the chaparral species *Mimulus aurantiacus,* which can be overcome by smoke. In the light, this species germinates readily without smoke, but in the dark its germination is triggered by smoke.

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