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DOES SMOKE DERIVED FROM VICTORIAN NATIVE VEGETATION STIMULATE GERMINATION OF DORMANT SOIL-STORED SEED?

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The effectiveness of Victorian (local) plant-derived smoke in stimulating germination of soil-stored seeds was compared with that of commercial sources from Western Australia and South Africa, for soil samples from a *Eucalyptus baxteri* (Bentham) Maiden & Blakely ex. J. Black heathy-woodland in the Grampians National Park, western Victoria, using a glasshouse experiment. Smoke from all three sources enhanced seedling emergence relative to no treatment (control). Seedling densities for the Victorian and Western Australian smoke treatments were not significantly different, but were higher than those for the South African smoke. There were also significant differences in species richness and composition among smoke treatments. Mean richness was highest in the Western Australian and lowest in the South African smoke treatments. Differences in species composition were again greatest between samples treated with Victorian or Western Australian smoke and those treated with South African smoke. Smoke clearly acts as a trigger for germination in some species. However, comparisons here were complicated by different methods of smoke production. Further research is required to identify the chemical constituents of smoke which influence seed germination, and the optimum concentration(s) of smoke in relation to germination.

Key words: plant-derived smoke, seed germination, soil seed bank, *Eucalyptus* woodland.

RECENT research has revealed that plant-derived smoke can act as a trigger for breaking seed dormancy in numerous species across a range of families from fire-prone plant communities (De Lange & Boucher 1990; Brown 1993; Baxter et al. 1995; Dixon et al. 1995; Pierce et al. 1995; Enright et al. 1997; Marsden-Smedley et al. 1997; Keeley & Fotheringham 1998). In addition, smoke may enhance seed germination of some species from non-fire-prone environments (Pierce et al. 1995) including common vegetables (Drewes et al. 1995; Thomas & Van Staden 1995). Research has been undertaken to identify the active constituents of plant-derived smoke and the mechanisms by which smoke stimulates germination (Baldwin et al. 1994; Drewes et al. 1995; Thomas & Van Staden 1995; Van Staden et al. 1995a, 1995b; Jäger et al. 1996; Keeley & Fotheringham 1998). However, so far, neither the promotive constituents nor modes of action are fully understood.

This paper investigates whether plant-derived smoke obtained from a local (Victorian) source is as effective in stimulating the germination of dormant soil-stored seeds as are (now commercially available) smoke extracts derived from South African and Western Australian vegetation where smoke-stimulated germination has already been illustrated (Brown 1993; Dixon et al. 1995).

Soil samples from a heathy *Eucalyptus baxteri* (Bentham) Maiden & Blakely ex. J. Black woodland in the Grampians National Park, western Victoria, were used in a glasshouse seed bank germination experiment to describe and evaluate the effects of the three smoke types on the density, species richness and species composition of the germinants. Based on a preliminary study by Enright et al. (1997) which showed enhanced germination using leaf material from the Victorian tree, *E. baxteri*, we hypothesised that smoke derived from local (ie. Victorian) vegetation would show an equivalent stimulatory effect to smoke originating from other geographical areas and plant species combinations, reflecting the generality of smoke as an evolved cue for germination of dormant soil-stored seeds in fire-prone environments.

MATERIALS AND METHODS

In March 1997 eight replicate surface soil samples (200 × 200 mm) to 50 mm depth were collected at random from a *E. baxteri* heathy-woodland last burned 14 years ago, near Golton Gorge in the Grampians (Gariwerd) National Park, western Victoria. Air dried samples were thoroughly mixed

and split into 20 uniform subsamples to provide five $\times 1.5$ kg replicates for each treatment. Sub-samples were placed in aluminium trays ($280 \times 170 \times 40$ mm) and then treated for 24 h with either Victorian, Western Australian or South African smoke in the form of 400 mL of a concentrated aqueous solution poured on each tray. A control treatment was established by using tap water only for the fourth set of replicate samples.

The aqueous solution for the Western Australian smoke treatment was a 1:10 mixture of smoky water (Seed Starter, Australian Smoky Water, Kings Park and the Botanic Gardens, Perth, Western Australia) and tap water. The South African smoke solution was produced by soaking 10 smoke-infused filter papers (Instant Smoke Plus, Kirstenbosch National Botanical Institute, Cape Town, South Africa) in 2 litres of water. Instant Smoke Plus also contains a small amount of added gibberellic acid which is a known seed germination stimulant (Brown & Van Staden 1997). The Victorian smoke solution was derived from eight smoke-infused filter papers (Whatmans No. 1, 180 mm diameter) soaked in 2 litres of water. The latter filter papers were prepared from the foliage of *E. baxteri* and mixed understorey shrub layer vegetation (comprising a number of species primarily from the plant families Myrtaceae, Proteaceae and Epacridaceae) from the site of soil sample collection in Grampians National Park, using the method described by Enright et al. (1997). The spectral signatures of the aqueous solutions were examined using a UV spectrophotometer, and the solution concentrations adjusted to give approximately equal absorbance levels (i.e. approximately equal solution concentrations).

After the soil samples had been soaked they were spread to a depth of 2–3 cm over a mixture of sphagnum moss, peat moss and washed sand in plastic germination trays (280×340 mm). Glass-house tray positions were randomised fortnightly, and emerging seedlings recorded weekly for a period of 150 days between May and October 1997. Nomenclature for plant species follows Ross (1996).

Total seedling densities were compared between treatments using one-way analysis of variance (ANOVA) with Tukey's HSD post hoc comparison of means test to determine whether results differed between treatments. No logarithmic transformation was necessary due to approximately normally distributed total density data. The Kruskal–Wallis Test was used for differences between treatments in species richness as well as in seedling densities for selected species. Ordination by multi-dimensional scaling (MDS) (Minchin 1987) was

performed to explore differences in overall species composition between treatments. Anosim (Clarke 1993) was used to estimate the significance of difference in species composition between treatments. Both of these analyses were based on Bray–Curtis dissimilarities among species that occurred in at least two samples. As the present research concentrates on differences between smoke treatments, the control samples were excluded from these ordination analyses.

RESULTS

All smoke treatments led to a significant increase in seed germination compared with the control treatment (Table 1), and the density of emergent seedlings was also significantly different between smoke treatments (ANOVA: d.f. = 2,12; $F = 43.77$; $P < 0.001$). Seedling densities for the Victorian and Western Australian smoke treatments, with means of $12\,547 \pm 449$ and $12\,055 \pm 184$ seedlings m^{-2} respectively, were not significantly different, but were higher than those for the South African smoke treatment (8258 ± 379 seedlings m^{-2}).

Paralleling the results for seedling densities, all smoke treatments showed increased species richness relative to the control (Table 1), and again, there were also significant differences between smoke treatments (Kruskal–Wallis Test: $P < 0.01$). Total species richness (summed across replicates) was the same for Victorian and Western Australian smoke (37 species), but was slightly lower for the South African smoke treatment (33 species). Highest mean richness was recorded for Western Australian smoke with 23.8 ± 1.2 species per sample, followed by Victorian smoke with 22.4 ± 0.4 . Lowest richness was observed for South African smoke (19.0 ± 0.8 species per sample).

Strong positive germination responses to the smoke treatments were observed for 15 out of 16 species for which seedling densities were sufficient to make statistical testing possible, the only exception being *Stuartina muelleri* (Table 2). Seven of the 16 species tested showed significant differences between the smoke treatments: *Isolepis marginata* (Cyperaceae), *Ixodia achillaeoides* (Asteraceae) and *Leucopogon glacialis* (Epacridaceae) had highest germination levels in the Victorian and Western Australian smoke treatments; *Centrolepis aristata* (Centrolepidaceae), *Epacris impressa* (Epacridaceae), and *Stylidium soboliferum* (Stylidiaceae) seedling densities were highest in the Victorian smoke treatment, while *Opercularia scabrida* (Rubiaceae) showed strongest germination

response to Western Australian smoke. No species showed maximum germination response in the South African smoke treatment.

The two-dimensional MDS ordination (stress = 0.07) showed consistent differences in the locations of samples based on treatment type (Fig. 1). Samples for Victorian and Western Australian smoke showed high scores on axis 1, suggesting little variation in species composition between these two treatments, while the South African smoke samples were clearly separated, having low scores on the first axis. Sample scores on this axis

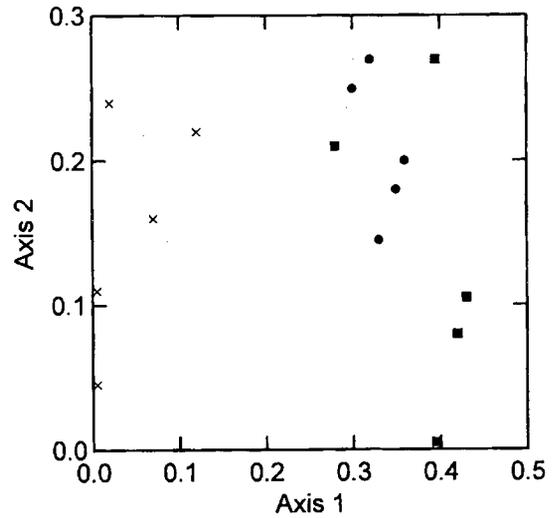


Fig 1. Two-dimensional multidimensional scaling (MDS) ordination of seedling floristic (density) data. Note: Control samples were excluded from this analysis. Treatment type: ■ = Victorian smoke; ● = Western Australian smoke; × = South African smoke.

Treatment type	Total number of seedlings	Mean density m^{-2} (\pm se)	Total number of species	Mean species richness per sample (\pm se)
Control	541	4227 \pm 184a	24	13.8 \pm 1.4a
Victorian smoke	1606	12 547 \pm 449c	37	22.4 \pm 0.4c
Western Australian smoke	1543	12 055 \pm 184c	37	23.8 \pm 1.2c
South African smoke	1057	8258 \pm 379b	33	19.0 \pm 0.8b

Table 1. Total number of seedlings, mean density (\pm se) m^{-2} , total number of species and mean species richness (\pm se) per sample for soil seed bank germination treatments. Density values (in columns) followed by the same letter are not significantly different from one another (Tukey's HSD test: $P > 0.05$).

Species	Control	Western Australian smoke	South African smoke	Victorian smoke	P
<i>Aira elegans</i>	3	6	9	11	0.617
<i>Centrolepis aristata</i>	55	91	79	136	0.028
<i>Centrolepis strigosa</i>	234	455	380	435	0.089
<i>Crassula closiana</i>	20	65	61	53	0.567
<i>Drosera glanduligera</i>	13	50	27	32	0.072
<i>Epacris impressa</i>	2	43	17	86	0.003
<i>Hydrocotyle callicarpa</i>	40	171	133	155	0.171
<i>Isolepis marginata</i>	16	131	41	124	0.009
<i>Ixodia achillaeoides</i>	24	151	60	180	0.007
<i>Laxmannia orientalis</i>	16	30	32	43	0.311
<i>Leptospermum myrsinoides</i>	3	9	5	8	0.332
<i>Leucopogon glacialis</i>	14	53	22	51	0.046
<i>Opercularia scabrada</i>	0	32	0	9	0.005
<i>Stuartina muelleri</i>	21	16	19	15	0.883
<i>Stylidium soboliferum</i>	2	28	12	52	0.006
<i>Wahlenbergia gracilentia</i>	62	154	129	145	0.338

Table 2. Total seedling number per treatment (total surface area of 0.128 m^2) for species represented by more than 20 seedlings. Significance of difference ($P < 0.05$) between smoke treatments is based on the Kruskal-Wallis Test (significance indicated by bold type), Note: Data for the control treatment were excluded from the statistical tests listed.

largely reflected variations in seedling densities, with Victorian and Western Australian smoke treatment samples showing high densities, and South African smoke treatment samples low densities, for a number of species including *I. marginata*, *I. achillaeoides*, *L. glacialis* and *E. impressa*. Some within-treatment variation is expressed on the second MDS axis, although Victorian smoke samples tend to be located with lower scores, and Western Australian samples with higher scores, reflecting differences in seedling densities for species such as *Drosera glanduligera*, *O. scabrida* (both more common in Western Australian smoke treatment), *E. impressa* and *S. soboliferum* (more common in Victorian smoke treatment).

Anosim based on a Bray–Curtis dissimilarity matrix, compared the 15 samples using 39 species. The test yielded an R statistic value of 0.71, reflecting a significant difference in the mean between- versus within-treatment rank dissimilarities (Anosim: rw = 26.27; rb = 63.69; $P < 0.001$).

DISCUSSION

While all three smoke sources led to increased germination responses when compared to the control, the Victorian and Western Australian smoke treatments were more effective in stimulating germination of dormant soil-stored seeds from Victorian heathy *Eucalyptus* woodlands than was the South African smoke. There were also significant differences in species composition between the samples treated with South African smoke and those treated with Victorian or Western Australian smoke, but little difference between species composition for the latter two treatments.

The different outcomes of the smoke treatments may be caused by differences in concentration levels of the aqueous smoke solutions and their constituent chemistries. While we attempted to standardise solution concentrations, spectrographs indicated slight differences in concentration, and in chemical make-up between the three smoke treatments. Keith (1997) has shown that smoke solutions in high concentration stimulated seed germination in *Epacris stuartii*, while low concentrations failed to promote the breaking of seed dormancy. On the other hand, light-sensitive lettuce seeds responded negatively to highly concentrated aqueous smoke extracts (Jäger et al. 1996). The density of germinants for the Victorian smoke treatment described here is also an order of magnitude higher than that described by Enright

et al. (1997) for bulk soil samples collected from the same area a few years earlier. The aqueous smoke solution used by Enright et al. (1997) was derived from a single species only (*E. baxteri*) and its concentration is unknown, so that issues of concentration and chemical composition are probably important.

Jäger et al. (1996) suggested that temperature and speed of combustion of plant material may influence the smoke-induced promotion of germination. They heated dry *Themeda triandra* leaves over a range of temperatures from 140°C to 240°C and found that the stimulatory compounds were produced at temperatures between 160°C and 200°C. Higher temperatures apparently led to loss of the active components due to their volatilisation and/or decomposition (Jäger et al. 1996). We have no information on the temperature conditions under which the smoke extracts were prepared, nor on the potential loss of active constituents of smoke relating to the different methods of smoke production and storage (ie. aqueous solution versus impregnated filter papers). However, the similar levels of germination density and species richness achieved by the Victorian (smoke-impregnated filter papers) and Western Australian (aqueous-smoke solution) treatments suggests that these factors may not have differed greatly by method.

The role of the source of plant material (ie. different chemical compositions) in influencing the germination of *T. triandra* was tested by Baxter et al. (1995) using smoke produced independently from 27 grassland species. Although the extent of stimulatory effects varied considerably among the different smoke types, germination of *T. triandra* was enhanced by the smoke derived from 26 of the 27 species (Baxter et al. 1995). Furthermore, aqueous smoke extracts prepared from the leaves of different plant species as well as extracts produced by heating agar and cellulose promoted the germination of light-sensitive lettuce seeds (Jäger et al. 1996). Baxter et al. (1995) and Jäger et al. (1996) concluded that the active compounds of plant-derived smoke appear widespread. The present results support this conclusion. Keeley & Fotheringham (1998) have reported recently that, while quantitatively important constituents of smoke including nitrate and ammonium failed to trigger germination in 25 chaparral species tested, nitrogen dioxide was effective for four of these species.

Enright et al. (1997) found that smoke could substitute for heat as a cue for germination in some Australian native plant species, but identified only one species that responded solely to smoke (*Stylidium soboliferum*). On the other hand, smoke did not stimulate germination of hard-seeded

species which needed heat to crack the seed coat (eg. many members of the Fabaceae). The identification of smoke-stimulated species has great potential value in the commercial development of the Australian flora (eg. in horticulture and floriculture) and provides a fertile ground for future research (Dixon et al. 1995). Additionally, studies focussing on the stimulatory effects of plant derived smoke from different communities (including ecosystems where fire is uncommon) will be useful in further testing the general role of smoke as a germination trigger. A standardised methodology for production of smoke extracts would facilitate comparative analyses and the aqueous solution method of Dixon et al. (1995) is recommended since it avoids the unknown (but possibly deleterious) effects of drying on some of the active constituents of smoke which is inherent in the filter paper method.

Although laboratory experiments now clearly illustrate that smoke can stimulate the germination of viable, but dormant, soil-stored seeds, there is, so far as we are aware, no field evidence for stimulation of germination beyond the fire front by wind-blown smoke. Transect studies across fire boundaries would prove interesting in testing whether the concentration and duration of smoke production was sufficient to produce a fire-induced germination response outside the burned area. The field tests used by Dixon et al. (1995) subjected soils to levels of smoke known to produce a germination response in the laboratory and do not address this question. Alternatively, it may be the heating of dead organic matter within the surface layers of the soil as the fire front passes, rather than of living, above-ground plant material, that provides the 'smoke treatment' to buried seeds, in which case little or no cross-boundary germination would be expected.

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