Effects of Plant-derived Smoke and Heat Treatment on Seed Germination of an Australian Native Plant, *Goodenia scaevolina* F. Muell.

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Summary

A water soluble part of smoke derived from leaves of an endemic Australian tree, Forest oak (*Allocasuarina torulosa*) is effective in stimulating seeds of a native Australian plant, *Goodenia scaevolina* F. Muell. to germinate. The smoke solution was made by burning 25 g of dried senescent or fresh leaves and dissolving the smoke in a liter of distilled water. The highest seed germination rate occurred on a 1/50 dilution of a saturated smoke solution on Murashige & Skoog (MS) medium. The components of smoke and heat are two factors considered to be involved in the smoke effects. The heat treatment of seeds at 80 °C for 1 to 10 min, combined with a smoke solution, was significantly effective on seed germination. However, the effect of heat treatment did not last long as germination rates became even lower than that of the untreated control seeds 8 weeks after the treatment. The smoke solution showed a similar effect to treatment with 1000 ppm gibberellic acid (GA₃) on seed germination at the start. The germination rate in 100 days on smoke medium was equivalent to those between 100 and 10 ppm GA₃. The germination rate on 1/50 smoke medium increased in the 8 months after the smoke solution was made, indicating that some changes might have occurred in the smoke solutions during their storage.

Key Words: gibberellic acid, *Goodenia scaevolina* F. Muell., heat, seed germination, smoke.

Introduction

Seeds of native plants are often difficult to germinate because of their dormancy. Seed dormancy is an important characteristic governing the dynamics of plant populations. Fire-stimulated seed germination has been reported for fynbos species of *Asteraceae*, *Ericaceae* and *Restionaceae* (Brown et al., 1993). The absence of seedlings from unburnt vegetation in scrub-heath was observed in southwestern Australia (Meney et al., 1994). In the northern part of Western Australia, *Goodenia scaevolina* F. Muell. is widely spread in the woodland. It is an erect subshrub with mauve corollas. The family *Goodeniaceae* consists of 5 genera, *Dampiera*, *Scaevola*, *Velleia*, *Lechenaultia* and *Goodenia*. They are characterized by having a cup shaped indusium around the top of the stigma (Wheeler et al., 1991) in which pollen is collected before flowering (Fig. 1). The species is self-incompatible; it has a high potential as ornamental plants because of its colors and shapes.

Native plants are usually extremely difficult to germinate by conventional techniques. Elliot and Jones (1992) found *Goodenias* to be difficult and time-consuming to germinate, requiring 1 to 6 months. Fire has long been

observed as a vehicle for seedling germination in fire prone plant communities, such as fynbos in South Africa (Le Maitre and Midgeley, 1992), chaparrals in North America (Keeley et al., 1985) and in a range of habitats in Australia (Gill, 1981; Bell et al., 1993). The phenomenon that fire stimulates seed germination was discovered, including the effects of dry heat (Auld and O'Connell, 1991; Bell and Bellairs, 1992), ethylene and ammonia (Van de Venter and Esterhuizen, 1988). The effect of plant-derived smoke was also studied for South African and Australian plant species (De Lange

Fig. 1. Flower of *G. scaevolina* F. Muell. The arrow indicates the indusium. Bar = 1 cm.

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and Boucher, 1990; Brown, 1993; Brown et al., 1993; Dixon et al., 1995). Exposure of dormant seeds to cold gaseous smoke derived from burned native vegetation had a positive influence on germination in native western Australian plants (Dixon et al., 1995).

However, there are no reports on the effects of water soluble part of smoke on seed germination, and the employment of smoke into tissue culture system. Understanding seed germination is a basic requirement for efficient breeding to realize various genotypes through cross hybridization. The ecological system of vegetation induced by fire would be applicable to gain many synchronously germinated plants (Baldwin et al., 1994). The objectives of this study were to improve seed germination by investigating the effect of smoke solution and heat, and to introduce the mechanisms of smoke-stimulated germination to the tissue culture system for efficient plant breeding and cultivation using native plants.

Materials and Methods

Plant material

In this study, seeds of *Goodenia scaevolina* F. Muell., purchased in 1994 from Nindethana Seed Service Pty. Ltd. (Albany, Australia), but they were collected from plants growing in the company holding in 1987. They were sealed and stored in the dark at room temperature. The weight of a thousand seeds was 0.87 g, and the seeds was 7-year-old at the time of this study.

Preparing smoke solution

Allocasuarina torulosa, an Australian wild tree called Forest oak, which was growing in the garden of Gosford Horticultural Research and Advisory Station (Gosford, Australia), was used (Fig. 2A, B). Using a beekeeper's smoker (Fig. 2C, D), 25 g of dried senescent leaves were burned while holding the spout of the smoker into a mouth of a 2-liter glass bottle containing 1 liter distilled

Fig. 2. Preparation of smoke solution and seed germination on the smoke medium. (A) *A. torulosa*. (B) Intact leaves (top) and separated leaves into individual units (bottom) of *A. torulosa*. Bar = 1 cm. (C) Green leaves (left), brown leaves (middle) of *A. torulosa* and a beekeeper's smoker (right). (D) Making smoke solution using beekeepers' smoker (right) by burning leaves inside (the lid is open) and glass bottles containing distilled water (left; before introducing smoke, right; after shaking with smoke). (E) Smoke solutions of Smoke brown (left) and Smoke green (right). (F) Seed germination on media with no smoke, 1/100, 1/50 and 1/10 smoke dilution (from left to right).

water. The smoke, derived from the leaves, was pushed by the smoker's bellows and put into the head space above the distilled water in the bottle (Fig. 2D). To dissolve the smoke into the water, the bottle was shaken by hand for 1 min (about 150 times) until the smoke in the bottle disappeared. Then more smoke was put into the head space of the bottle and shaken in the same way. This procedure was repeated until the smoke introduced into the jar would not dissolve or disappear. The solution was then considered saturated by the smoke. To be sure of saturation, this procedure was repeated for an hour (48 times) until smoke was no longer generated. Finally, the color of the water changed to dark brown (Smoke brown). Dried green leaves from the same materials were also used to make another smoke solution (Smoke green, Fig. 2E, right) in the same manner, for comparison with Smoke brown (Fig. 2E left). The smoke solutions were filtered through No. 1 Whatman filter paper to eliminate solid bodies and stored at room temperature.

Concentration of smoke solution

The smoke solution (Smoke brown) was added into Murashige & Skoog (MS) medium, containing $30 \text{ g} \cdot \text{liter}^{-1}$ sucrose and $8 \text{ g} \cdot \text{liter}^{-1}$ agar (pH 5.9 before autoclaving). The concentrations of the smoke solution in MS medium were 1/2, 1/10, 1/100, 1/1000 and 1/10000 on a volume / volume basis. The medium without smoke solution was used as the control. Seeds of *G. scaevolina* were surface-sterilized by 70% ethanol for 30 sec, followed by 1% sodium hypochlorite for 30 min. After the treatment, they were rinsed three times with sterile distilled water. Fifty seeds were inoculated to individual 250-ml plastic jars containing 40 ml of the six different concentration media. Each treatment was replicated five times. The jars were incubated at 25°C under 2000 lx and a 16-hr photoperiod. The germinating seeds were counted every day for 50 days after the inoculation.

Three concentrations of smoke solution diluted with MS medium, 1/10, 1/50 and 1/100, were also tested for determining the optimum concentration for seed germination based on the results of a previous experiment.

Density of seeds

Effects of seed density on germination rates were examined. The densities were 5, 10, 20 and 50 seeds per jar. The concentration of smoke solution was 1/50 of MS medium (1/50 smoke medium), which was the same as the heat treatment experiment. The conditions of incubation and the number of repetition were the same as above.

Heat treatment

Dried seeds of *G. scaevolina* were wrapped in aluminum foil and treated at 80°C in an electric oven for 0, 1, 2, 5, 10, 20, 40 and 80 min. Immediately after the heat

treatment, the seeds were surface-sterilized and inoculated on Smoke brown medium and incubated as above. The concentration of smoke solution to MS medium was adjusted to 1/50. Germinating seeds were counted daily for 30 days.

The effect of duration after the heat treatment at 80°C for 10 min was also examined by storing the seeds in the dark for 0 day, 1 day, 1 week, 2 weeks, 4 weeks and 8 weeks after the heat treatment prior to inoculation. No heat treatment was set as the control plot. The medium used for this experiment was MS medium with and without 1/50 of smoke solution (Smoke brown). Fifty seeds were inoculated per jar and maintained as described above, and their germination rate were determined daily for 30 days.

Effect of Gibberellic acid

The effects of 1/50 Smoke brown and green solution were compared with 10, 100 and 1000 ppm gibberellic acid (GA_3) using 50 seeds per jar as above; an equal number of untreated seeds placed on the basal MS medium was used as the control. The germinating seeds were counted daily for 100 days.

Results

Effect of smoke on seed germination

The germination rate of the control seeds on MS medium was less than 1%, whereas that of 1/100 smoke medium was 18.0 % (Fig. 3) which is significantly higher than those of 0 (control), 1/10000, 1/1000 and 1/2 at the 5 % level ($\text{LSD}_{0.05}$). The cumulative germination rates reached maximum in about 30 days. Of the 1/100, 1/50, and 1/10 strength smoke media, the 1/50 had the highest cumulative germination rate (Fig. 2F, 4).

Effect of seed density

There were no significant differences in cumulative germination rates in 30 days among the 5, 10, 20 and 50 seeds per jar (Fig. 5).

Fig. 3. Effect of concentration of smoke solution on cumulative seed germination rates in *G. scaevolina* F. Muell. $\text{LSD}_{0.05}$: Least significant difference at the 5% level.

Fig. 4. Effect of the concentrations of smoke solution, 1/10, 1/50 and 1/100, on cumulative seed germination rates in *G. scaevolina* F. Muell. $LSD_{0.05}$: Least significant difference at the 5% level.

Fig. 5. Effect of seed density on cumulative seed germination rates in *G. scaevolina* F. Muell.

Fig. 6. Effect of heat treatment on cumulative seed germination rates in *G. scaevolina* F. Muell. $LSD_{0.05}$: Least significant difference at the 5% level.

Effect of heat treatment on seed germination

The heat treatment was significantly effective on seed germination according to the analysis of variance (ANOVA), especially when inoculated on a smoke medium (Table 1). Heat treatments of 1, 2, 5, and 10 min yielded significantly ($LSD_{0.05} = 4.6\%$) higher germination rates than those treated for 20, 40, 80 min

Table 1. ANOVA for seed germination rate in heat treatment and smoke media.

Source	Degree of freedom	Sum of square	Mean of square	F - value
Heat duration	7	732.77	104.68	117.246**
Smoke media	1	0.01	0.01	0.001
Error	7	42.49	6.07	

** significant at 1% level.

Table 2. Effect of duration after heat treatment on cumulative germination rates for 30 days.

Duration after heat treatment	Germination rate (%)	
	MS medium	Smoke medium
no treatment	0.0	15.0ab ^z
0 day	0.0	16.5ab
1 day	0.0	14.3ab
1 week	0.0	12.7ab
2 weeks	0.0	24.2bc
4 weeks	0.0	14.2ab
8 weeks	0.0	1.8a

^z Different letters indicate significant difference at 5% level, by LSD test.

and no heat treatment (Fig. 6). There was no significant difference between Smoke brown and Smoke green.

Heat treatment alone was not significantly effective because no heat-treated seeds without a smoke solution germinated on the normal MS medium (Table 2). However, with smoke medium, seed germination rate became highest in 2 weeks after the heat treatment; a significant difference at the 5% level occurred between those of 2 and 8 weeks incubation; the effectiveness of heat treatment had a tendency to decrease after 2 weeks.

Comparison between smoke solution and gibberellic acid treatments

At the higher concentration of GA_3 , more seeds germinated initially than did Smoke brown or green treatment. Subsequently, the smoke treatment promoted a significant increase in seed germination in consistent with a previous experiment. The germination rate at 100 ppm GA_3 was slower than that on the smoke medium at the onset of the trial. But the germination rate of the latter became equally high as that of 1000 ppm GA_3 . However, the final cumulative germination rates of the smoke media reached a plateau between 10 and 100 ppm GA_3 (Fig. 7).

Discussion

G. scaevolina seeds germinated poorly on the normal MS medium (Fig. 3), though MS medium provides all

Fig. 7. Comparison of smoke solution and gibberellic acid on cumulative seed germination rate in *G. scaevolina* F. Muell.

the essential mineral elements for growth and a range of vitamins and amino acids. None of these components overcame the enforced eco- and/or endodormancy. The role of the mucilage that emerged from epidermal cells of the seeds when they are soaked (Fig. 8) is unknown. Todd-Bockarie et al. (1993) reported that the disruption of only the outer seed coat surface (scarification) was needed to overcome the dormancy in *Cassia sieberiana*. Priming seeds in polyethylene glycol solution also affects germination *Apium graveolens* L. (Drew and Dearman, 1993).

The smoke medium at 1/50 dilution stimulated seed germination the most; at higher concentrations the germination rates were reduced. Similar effects were found with the use of wood ash (Neeman et al., 1993). Smoke solutions derived from brown and green leaves were equally stimulative, indicating that leaf maturity is not a factor.

Our results agree with those of Brown et al. (1993) who found that direct plant-derived smoke treatment improved the seed germination rate in *Erica* species. However, our experiment also involved heat as well as smoke. Higher germination rates were achieved by heating seeds at 80 °C for various durations. Similarly, Musil and De Witt (1991) and Auld and O'Connell (1991) tested seeds of the fynbos families at 80–120 °C for up to 15 min and 80–100 °C for 1 to 120 min, respectively.

In this study, heating seeds of *G. scaevolina* promoted their germination, provided the smoke medium was used in conjunction (Table 1 and Fig. 6). Heat treatments at 80 °C for 1–10 min improved seed germination rates significantly higher than for 20, 40 and 80 min or heating alone, which indicates that some constituent(s) in smoke was essential for breaking dormancy. However, post-heat treatment storage for 8 weeks reduced germination rates below that of no treatment, indicating that seeds may revert back to the dormant stage. This idea needs to be tested by repeating the smoke treatment as the results might explain why seeds occasionally do

Fig. 8. Seeds of *G. scaevolina* F. Muell. (A) Dry seeds (top) and wet seeds (bottom). Bar = 1 mm. (B) Scanning electron micrographs of the mucilaginous structure on the seed coat. Bar = 100 μ m. (C) Magnified part of the boxed area in (B). Bar = 10 μ m.

not germinate even after a wildfire.

Baldwin et al. (1994) identified 71 compounds in wood smoke, but their combinations did not stimulate germination to the same degrees as did smoke. Because GA_3 is effective as smoke (Table 3), the latter may function as a trigger to induce GA_3 synthesis.

Ethylene, which is the active ingredient in smoke (De Lange and Boucher, 1990), has been also reported to be effective for seed germination comparable to the effect of fire (Van de Venter and Esterhuizen, 1988). Fire-stimulated seed germination has been attributed to ethylene and ammonia in smoke in fynbos species (Brown, 1993). In this study, the water soluble part of smoke was

Table 3. Summary for the results of seed germination rate treated by smoke and heat.

Experiment	Germination rate in 30 days (%)	Condition	Referring figure
Concentration of smoke	15.0	1/100 of smoke	Fig. 1
	13.0	1/10 of smoke	
	0.4	No smoke	
Optimum concentration of smoke	15.9	1/50 of smoke	Fig. 2
	12.7	1/100 of smoke	
	10.1	1/10 of smoke	
	0.7	No smoke	
Heat treatment	39.8	1/50 of smoke with 2 min of heat	Fig. 4
	23.4	1/50 of smoke with no heat	
Comparison of smoke with GA ₃	64.8	1000 ppm GA ₃	Fig. 5
	32.8	100 ppm GA ₃	
	6.4	10 ppm GA ₃	
	27.6	1/50 of smoke	
	2.4	No smoke	

examined in which ethylene is insoluble so that ethylene is not involved in this case.

In summary, our results (Table 3) reveal that the germination rate on the 1/50 of smoke medium increased for 8 months from 15.9% in the second experiment (Fig. 4), 23.4 % in the forth (Fig. 6) and 27.6 % in the last (Fig. 7). The effect of smoke can be maintained by autoclaving the solutions at 121 °C, but their effectiveness may diminish because some changes in the smoke solution might occur with time. Furthermore, the germination rate of these 7-year-old seeds on the normal medium without smoke solution also increased from 0.4% in the first experiment (Fig. 3) to 0.7% in the second and 2.4% in the last (Fig. 7) with time. Thus, this aspect of post-harvest seed storage and the mechanism of breaking seed dormancy need to be investigated for further research. The introduction of smoke solution into *in vitro* germination may be useful for plant breeding and cultivation in getting many genotypes from the native materials and their progenies which are difficult to germinate.

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オーストラリア野生植物 *Goodenia scaevolina* F. Muell. の種子発芽に及ぼす植物由来の煙と熱処理の効果

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摘 要

オーストラリアの野生植物 *Goodenia scaevolina* F. Muell. の種子発芽が、オーストラリア固有の樹木、フォレストオーク (*Allocasuarina torulosa*) の葉から生じた煙の水溶液によって促進されることが明らかとなった。煙水溶液は、この樹種の落葉または新鮮葉 25 g を燃焼させて発生した煙を蒸留水 1 リットルに飽和させて作製した。煙水溶液の濃度を 1/50 に希釈して添加した MS 培地で、発芽率は最も高い値を示した。煙の効果には、煙に含まれる成分と熱の 2 つの要因が考えられる。煙水溶液とともに、種子に 80 °C で 1~10 分間の熱処理

をすることにより、発芽率は有意に増加した。しかし、熱処理の効果は長期間は持続せず、処理後 8 週間を経た種子を用いた場合には、むしろ無処理よりも発芽率は低くなった。煙水溶液の発芽促進効果は、発芽初期にはジベレリン酸 1000 ppm に類似し、100 日後には、発芽率はジベレリン酸 10 ppm と 100 ppm との中間の値を示した。1/50 の濃度の煙培地での発芽率は、煙水溶液作製後の 8 か月間で日数が増すにしたがって高くなったことから、この間に煙水溶液に何らかの変化が生じたことが考えられた。