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Impact of Gaseous Smoke Treatment on Germination and Seedling Emergence of the Cape Flats Sand Fynbos Species

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ABSTRACT

The Cape Flats Sand Fynbos (CFSF) is critically endangered vegetation because of many factors which affect them negatively such as urbanisation, agricultural production and invasive alien species. To help inform us better on the protection and conservation of this critically endangered group of plants, an understanding of smoke derived pregermination cues on their seeds could be an invaluable contribution towards their restoration on the landscape. We conducted a greenhouse experiment to investigate the impact of smoke on pre-germination of seeds directly or indirectly through soil by mimicking field conditions after burn. Three different smoke treatments were evaluated based on their effects on germination of many CFSF plant species especially critically endangered groups. Our results showed that plant-derived smoke enhanced seed germination of a couple of native Fynbos plant species, and that, treatment success was species-specific, with pre-smoked seeds, soil pre-germination treatment and the two combined all capable of significantly boosting germination. We therefore recommend that Fynbos seeds be pre-smoked before sowing, especially in the absence of a prescribed burn.

Keywords: Dormancy, Enhanced germination, Plant-derived smoke, Restoration, Smoke-stimulated, Pre-smoke

INTRODUCTION

South Africa is home to the endemic Fynbos biome, a major component of the Cape Floristic Region (CFR) biodiversity hotspot [1]. The Fynbos is a distinctive vegetation type occurring in a small belt of the Western Cape, mainly along the coastal and mountainous areas with a Mediterranean-type climate [2]. The Fynbos biome is composed of two vegetation groups namely: Fynbos and Renosterveld, however this study focuses on the Fynbos group only. The Fynbos group comprises three main plant groups, i.e., proteoids, restioids and ericoids [2]. The Fynbos also has defining vegetation types such as grassy fynbos, mountain fynbos, limestone fynbos and sand plain fynbos. Plants here (in the Fynbos biome) are categorised into sclerophyllous (hard, tough and leathery leaves) and microphyllous (small leaved).

Among the distinctive set of eleven vegetation types found in the city of Cape Town, the Cape Flats Sand Fynbos (CFSF) is the critically endangered type of fynbos [3]. The CFSF has only 14% of its original extent remaining, although in a degraded state owing to infestations by Australian acacias (*Acacia cyclops, Acacia saligna*) [2]. With *Acacia saligna* being the most problematic of the two species hence often used as a measure of invasion in South Africa [2]. This is even so for post fire seedling establishment especially in Fynbos.

Several factors can hinder post-fire establishment of seedlings [4]. These include unfavourable soil conditions [5,6], competition from established plants and herbivores [7]. Other sources of competition for seedling establishment

are invasive alien species [8], dormancy [9] and climatic conditions [10]. Other related factors which affect post fire seedling establishment are, light and favourable temperature conditions [11] (i.e., increased light, moisture and temperature) which removes litter and taller plants. Other factors may also include increased resource availability and reduced competition and herbivores [12-14]. Sometimes seed germination can also be hindered by dormancy of the seeds with several germination cues needed to break this dormancy.

Seeds of different plant species have developed a range of dormancy strategies which allows for their dispersal before germination. Dormancy thus becomes a vital survival mechanism which favours propagation of seeds to establish plant populations. As a result, special conditions are normally required to break this dormancy (e.g. cold stratification, light, etc.) to enable seed to germinate. Baskin and Baskin, 2004 [9] classified seed dormancy into five categories namely: Physiological, morph physiological, physical and combinational dormancy. Physiological dormancy requires chemical changes to break. Physical dormancy requires the seed coat to be broken for water to enter (i.e., heat, desiccation). Our focus for this study was on smoke that is believed to break physiological dormancy [15].

Fynbos species are adapted to fire and their seeds respond to physical cues indirectly associated with fire (i.e., altered light, temperature, moisture) and/or germination cues directly associated with fire (i.e., smoke, heat pulse, chemicals in ashes/charred wood) [16,17]. Direct fire-related germination cues include heat shock [18], smoke and/or smoke-derived products [19,20] and combinations of these cues [21]. Plant-derived smoke is known to improve seed germination [20-24] owing to chemicals in smoke-derived extracts (i.e., butenolides, ethylene, and ammonia) that stimulates germination [19,20].

According to Ooi [15] smoke had a positive impact on seeds that remained morphologically dormant, after physiological dormancy had been broken during burial or stratification. He further stated that, seed germination increased significantly after pre-treatment with smoke. On the other hand, species with deep physiological dormancy are unlikely to respond to smoke if their dormancy is not overcome already via seasonal high temperatures. This show that under certain circumstances seeds needs pre-heat treatment before smoke treatment can be effective. Many of the Ericaceae and Rutaceae are physiologically dormant, while many Asteraceae are known to have a large non-dormant fraction [9].

De Lange and Boucher [25] reported the first plant-derived smoke-enhanced germination in the fynbos shrub *Audouinia capitata*. Later, Brown [16] found that the germination response in smoke-treated seeds improved for several families and genera of the fynbos. After that, Dixon and Roche [26] also reported that there was positive response to smoke pre-germination treatment for 45 out of 94 native Western Australian plant species. Furthermore, there was a positive germination response of smoke on seeds in Californian chaparral [23,24]. For restoration purposes, smoke is either applied to seeds in gaseous or aqueous form in either in situ or under laboratory or greenhouse condition [25]. For germination to be stimulated, seeds may need to be exposed directly to fresh smoke chemicals. To use scarce seed resources efficiently in active restoration, it is important to know whether it is beneficial to pre-treat seeds with smoke even when sowing into a post-fire environment.

In this study, we investigated the importance of pre-treating seeds and their substrate soils with gaseous smoke to germination and seedling emergence under greenhouse conditions. It measures the effectiveness of three different smoke treatments on germination success in a selection of typical CFSF plant species. Specifically, we compared germination of untreated seeds placed on smoke-treated soils (i.e., post-fire sowing) versus smoke-treated seeds placed in untreated soils and a combination of both under greenhouse condition. We also investigated the effectiveness of smoke pre-germination treatments in either soil or seeds by mimicking field conditions after a burn under greenhouse conditions with the aim of giving recommendations for restoration.

MATERIALS AND METHODS

Site description

The study site was the Blaauwberg Nature Reserve (BBNR) located about 25 km north of the city centre of Cape Town (CCT) (33°45'29.79"S 18°27'58.13"E°), on the West Coast, between Big Bay, Bloubergstrand and Melkbosstrand in the Western Cape, South Africa. The Blaauwberg Nature Reserve falls within the West Coast Biosphere. The Cape

Flats sand fynbos is endemic alongside other numerous vegetation types in the fynbos biome in the City of Cape Town. The BBNR conserves a unique combination of three vegetation types, of which CFSF dominates. These are, the Cape Flats Dune Strandveld (endangered), Swartland Shale Renosterveld (critically endangered), and Cape Flats Sand Fynbos (also critically endangered) [2], as well as the transitional zones between them. Within these vegetation types in the reserve are 559 plant species of which 47 are listed in the Red List of threatened plant species [2,3,27]. This research focused on the Cape Flats Sand Fynbos, which has less than 14% of the original extent remaining. Invasive alien vegetation, mostly *Acacia saligna* is one of the most problematic species that is degrading most of the remaining habitat in this area. Some of the plant species commonly found in the Cape Flats Sand Fynbos include *Ursinia anthemoides, Staavia radiata, Watsonia meriana, Leucadendron salignum, Serruria decipiens, Thamnochortus punctatus, Willdenowia incurvata, Phylica cephalantha, Passerina corymbosa and Sparaxis viollosa.*

Species collection, cleaning and storage

The main fynbos plant guilds selected for this study were, Restioid (wiry reed-like graminoids in the family Restionaceae); Ericoid (fine-leaved shrubs in the families of Asteraceae, Ericaceae, Rhamnaceae, Rutaceae, Thymelaeaceae); Proteoid (larger shrubs of the family Proteaceae); annual forbs and geophyte (mainly winter ephemeral species in the families' Amaryllidaceae, Colchicaceae, Haemodoraceae and Iridaceae) (Table 1). Seeds were collected in Blaauwberg Nature Reserve and in natural vegetation remnants along the N7 road including the Friend's patch (a previously invaded site in BBNR that has recovered to a functional fynbos community [28].

Family	Species and naming authority	Common name Growth form		Germination cues	Survival mode
Asteraceae	Chrysocoma ciliata L.	Bitterbos	Perennial woody shrublet	Smoke	Reseeder
Asteraceae	Metalasia densa (Lam.) P.O. Karis	Blombos	Ericoid Shrub	icoid Shrub Light/smoke/heat [6,21]	
Asteraceae	Senecio elegans L.	Wild cineraria	Annual herb	Warm and cold stratification [6]	Reseeder
Asteraceae	Seriphium incanum (Thunb.) Pers.	Slangbos	Shrub	Unknown [6]	Reseeder
Asteraceae	<i>Ursinia anthemoides</i> (L.) Poir. Subsp. Anthemoides	Marigold	Annual herb	Warm and cold stratification [6]	Reseede
Ericaceae	Erica mammosa Salisb.	Nine-pin heath	Ericoid Shrub	Dry heat/low temperatures [6]	Resprout
Ericaceae	Erica plumosa Thunb	Wolheide	Ericoid Shrub	Heat/smoke [22]	Reseede
Haemodoraceae	Wachendorfia multiflora (Klatt) J.C. Manning and Goldblatt	Dwarf mirrorface	Geophyte	Smoke [21]	Resprout
Iridaceae	Babiana villosula	Hairy	Geophyte	Variable [7,21]	Resprout
Iridaceae	Watsonia meriana (L.) Mill. Var. meriana	Suurkanol	Geophyte	Variable [7,21]	Resprout
Proteaceae	Leucadendron salignum P.J. Bergius	Knopbos	Proteoid shrub	Smoke/scarification/warm and cold stratification [16]	Resprout
Proteaceae	Protea repens (L.) L.	Common sugarbush	Proteoid shrub	Smoke	Serotino reseede
Proteaceae	Protea scolymocephala (L.) Reichard	Thistle sugarbush	Proteoid shrub	Smoke/hot water/scarification	Serotino
Proteaceae	Serruria fasciflora Salisb. Ex Knight	Common pin spearhead	Proteoid shrub	Cold/fluctuating temperatures [16]	Reseede
Restionaceae	Thamnochortus punctatus Pillans	Dotty dangle reed	Restioid	Smoke and heat [16]	Reseede
Rhamnaceae	Phylica cephalantha Sond.	Sandveld hardleaf	Ericoid Shrub	Smoke [6]	Resprout
Rutaceae	Agathosma imbricata (L.) Willd	Boegoe	Ericoid Shrub	Scarification	Resprout
Rutaceae	Diosma oppositifolia L.	Bitter buchu	Ericoid Shrub	Heat/scarification/fluctuating temperatures [7]	Resprout
Thymelaeaceae	Passerina corymbosa Eckl. ex C.H. Wright	Common gonna	Ericoid Shrub	Alternating temperature [6,21]	Reseede

Table 1: Names and seed characteristics of target species of Cape Flats Sand Fynbos used in the experiments designed to give recommendations for improved germination in large-scale restoration projects. Superscript numbers used to refer to the reference source for germination cues

Seeds of 17 species were collected by hand, when they were ripe and ready for use. This followed previous collection method by Newton et al. [29]. Targeted species were collected based on their phenological (flowering and seeding

time) information for the restoration experiments as per Millennium Seed Bank Partnership (MSBP)'s standards of harvesting [30]. Seeds were collected using various methods, including bagging plants, hand picking, stripping and collecting stems for ripening of seeds at room temperature. Large populations of seeds per each species were collected for the experiments [30,31].

Mature seeds were placed in paper bags and stored under dry and cold conditions of 15% relative humidity and 15°C temperature in the Millennium Seed Bank project cold dry storage room at Kirstenbosch Botanical Gardens seed room [32].

Seed debris were cleaned by sieving using Seed Hand Test Sieves (Round Brass of varying sizes) before being aspirated using Zigzag aspirator (Selecta Industries Netherlands) to remove chaff [6]. Clean seeds were then weighed and packed in sealed paper bags and labelled with a batch number and species name. In this study, the term 'seed' refers to plant unit of dispersal/diaspora.

Germination study

Germination study was conducted in a greenhouse with a clear corrugated PVC roof at the Forestry Department at Stellenbosch University. The surrounding shade nets in the greenhouse and a fan ensured the daily temperatures fluctuated between $8-25^{\circ}$ C, while the roof protected the soil from rain. Study species were chosen based on growth form, regeneration mode and availability of seeds (Table 2). The different smoke-treatments were applied to 160 seeds of each species (4 replicates × 40 seeds each) [33]. Each tray was divided between two species (each species' seed was allocated 150 mm space), a small-seeded and a large-seeded species, to accommodate all species within the available space. The four pre-germination treatments tested were [1] both untreated seeds and soil (control treatment) [2] Untreated soil with pre-smoked seeds (smoke-treated seeds) [3] Pre-smoked soil and untreated seeds (smoke-treated soil) [4] Pre-smoked seeds and soil (smoke-treated seeds and soil combination).

Seed trays $(300 \times 270 \times 100 \text{ mm})$ were lined with a layer of non-woven fabric to retain sand. Dry river-washed sand was used as the first layer of soil (30 mm deep) and the tray was then filled with soil from the BBNR site (70 mm deep). Soil was collected from the mole rat heaps, which are free of Acacia leaf litter at BBNR in uncleared, invaded fynbos. This was done to exclude *Acacia saligna* seeds from collected soil. However, soil collected from the field was not sieved, thus unwanted seedlings were weeded. Dry seeds (placed in replicates of 4×40 per tray and spread evenly) soil, a combination of soil and seeds were placed in trays lined with non-woven fabric to retain seeds and/or soil, then placed in a steel-framed plastic tent. Fynbos biomass was burnt (mixed fresh and dry fynbos plant material) (total worth 25 kg in weight) in a large metal drum. Smoke was then pumped into the tent through a black plastic pipe (1 m long) [21,22,25] using a petrol leaf blower. The use of long pipe allowed the smoke to cool before entering the tent. For single treatments, the seeds were placed in trays ($300 \times 270 \times 100$ mm) then smoke-treated before sown in soil whereas soil samples in trays ($300 \times 270 \times 100$ mm) were smoke-treated before seeds were sown. The combination of dry soil and seeds were pre-smoked in a $10 \times 10 \times 6$ m steel-framed tent, which was sealed with plastic.

Species name	Control	Smoke-treated seeds only	Smoke-treated soil only	Smoke-treated seeds & soil (combination)	F	р
			Normally distributed data responses			
Agathosma imbricata	-	+	+	+	21.25	0.00
Chrysocoma ciliata	-	+	+	+	8.19	0.00
Leucadendron salignum	-	+	+	+	12.88	0.00
Metalasia densa	-	+	+	+	9.45	0.00
Passerina corymbosa	-	+	+	+	6.48	0.01
Protea repens	-	+	+	+	24.84	0.00
Ursinia anthemoides	_	+	+	+	5.55	0.01
Wachendorfia multiflora	_	+	+	+	7.71	0.00
Watsonia meriana	-	+	+	+	20.69	0.00
Erica mammosa	-	+	-	+	4.26	0.03
Erica plumosa	-	+	-	+	55.85	0.00
Serruria fasciflora	-	-	+	+	4.63	0.02
Senecio elegans	-	-	-	-	0.66	0.60
Thamnochortus punctatus	-	-	+	-	5.72	0.01
		Not Norma	lly distributed data responses	5		
					H (3, N=16)	Р
Diosma oppositifolia	-	-	-	+	10.332	0.02
Phylica cephalantha	-	-	-	-	11.587	0.01
Protea scolymocephala	-	-	-	-	5.62	0.13

Table 2: Slope responses from the cumulative frequency curves of these species

Trays were removed after 60 min, watered and then transferred to benches in the greenhouse. Large seeds were buried in rows 2 cm deep into the soil. Small seeds were mixed in with dry sand of which a thin layer was spread evenly over the soil surface. Single treatment trays were moistened to field capacity before planting any seeds; this was done by adding 200 ml of water to each tray. The trays were kept moist using an automated irrigation system that was operational every three days for 3 min, providing sufficient water (approximately 5 mm) to keep the soil moisture above wilting capacity. Trays were rotated weekly to account for minor variations in light intensity, temperature and amount of water in the greenhouse. No fertilisers were added. Seedlings (defined as such when true leaves were observed) were counted weekly, identified and marked with toothpicks. The experiment ran for over 24 weeks.

Statistical analysis

All statistical analyses were performed using the Statistica software version 13.2 [32] with an Alpha (α)-level of 0.05. The results for each of the species and all data pulled together were tested for normality using Shapiro Wilk's test followed by normal or Kruskal-Wallis one-way ANOVA tests to measure the differences in seedling emergence between the various treatments. Normal or Kruskal-Wallis ANOVAs were used because results for our species data composed of both normal and non-normal distribution and, we measured only the differences between treatment since the experiment was conducted under a near homogenous environmental condition (greenhouse) (W=0.84 for species counts, W=0.91 for total individual counts, P<0.00).

'Seedling emergence' defined as the overall percentage of seeds surviving germination following a pre-germination stimulus was used to determine the response of the different species to the different pre-germination treatments. Microsoft Excel was used to generate cumulative frequency curves (pre-germination treatments vs. time, number of days) per species. In this study, the term 'emergence rate' refers to how quickly seedling emergence occurs over a given period and is compared using cumulative germination curves. To show seedling emergence in relation to different smoke pre-germination treatments, we generated cumulative curve plots (Supplementary Table).

RESULTS

Seedling emergence in response to smoke treatments

A Kruskal-Wallis H (3, N=416) test showed that there was a statistically significant difference between the emergence of treated materials (smoke treated seeds and soils and their combination) and untreated materials (none smoked seeds and soils) in terms of counted individual seedlings or all species pulled together (χ^2 =13.38 p=0.004; Figure 1 and Supplementary Table). At the species level however, these results varied. Seeds of different species responded differently to the smoke treatments (Table 2). About nine species (*A. imbricata, C. ciliata, L. salignum, M. densa, P. corymbosa, P. repens, Ursinia anthemoides, W. multiflora* and *Watsonia meriana* responded positively to all the types of pre-smoke-treatment (i.e., seeds only, soil only and a combination of both).

In addition, there were positive responses for *Serruria fasciflora* to smoke-treated soils only and a combination of smoke-treated seeds and smoked treated soils. However, this species responded negatively to smoke treated seeds only. *Thamnochortus punctatus* responded negatively to a single treatment of smoked seeds only and to a combination of smoke treated soils and seeds but positively to smoked soil single treatment. There was a positive response of seedling emergence of *E. plumosa* and *E. mammosa* when their seeds were pre-treated with smoke only as well a combination of smoke treated seeds and soils but not smoke treated soils only. There was a positive response of seedling emergence of *D. oppositifolia* when both seeds and soils were treated with smoke. However, negative responses were recorded for this species when its soil substrates were pre-treated with smoke only as well as pre-treated seeds only. There was no response of *S. elegans*, *P. cephalantha* and *P. scolymocephala* to any pre-treatment in terms of seedling emergence.

In addition, the slope responses from the cumulative frequency curves of these species followed the same pattern as results in Table 2. These curves indicated that smoke-treated seeds responded more quickly than seeds in smoke-treated soil in the following species: *Erica plumosa, Erica mammosa* although the slope of germination was very similar in smoked seeds and smoked soil. The main difference was that the final germination was greater in smoke-treated seeds compared with smoke-treated soil), *Protea repens* and *Passerina corymbosa* (Figure 1).

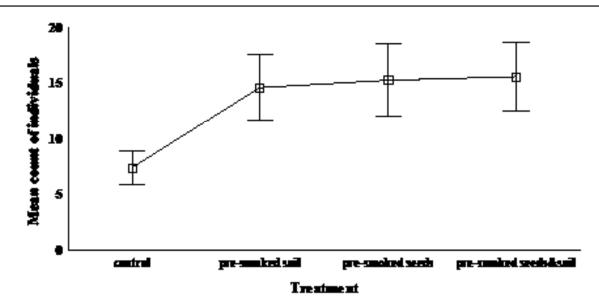


Figure 1: A graph of mean (\pm SE) counts of seeds of CFSF (all species together) in response to different pre-germination treatments of smoke and untreated seeds

A combination of smoke-treated seeds and soil initiated a faster germination rate response than in smoke-treated seeds alone for *Chrysocoma ciliata*, *Diosma oppositifolia*, *Senecio elegans*, *Phylica cephalantha*, *Thamnochortus punctatus* and *Ursinia anthemoides*.

A faster germination rate response was recorded in the smoke-treated soil than in the combination of seeds and soil treatment in *Metalasia densa*, *Serruria fasciflora*, *Protea scolymocephala*, *Agathosma imbricata*, *Leucadendron salignum*, and *Wachendorfia multiflora*. Germination rates in *Watsonia meriana*, however, did not differ among smoke pre-germination treatments (Figure 2).

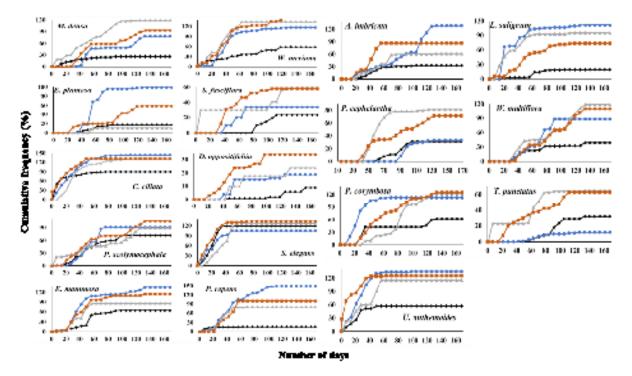


Figure 2: Graphs showing the percent germination of species of Fynbos plants after 161 days of observation under controlled conditions in the green house. Black lines with rhombus points=control (untreated seeds), blue lines with circular points=pre-smoke treated seeds only, grey lines with triangular points=pre-smoke treated soils only and brown lines with square points=a combination of pre-smoke treated soils and seeds in a greenhouse experiment

DISCUSSION

Rate of seedling emergence

As recorded in earlier studies [16,17,21], smoke treatment enhances germination of many fynbos plant species. This was further substantiated in this study where all species but two showed high germination rate after smoke-treatment. Smoke-treated seeds showed improved germination through enhanced vigour and growth when exposed to aerosol smoke. This result also agrees with another study in Norway where smoke was found to be a more effective germination cue across all functional groups compared to ashes in northern heathland ecosystems in Lygra, Lindas [34]. Smoke-stimulated germination is common in a wide range of plants from both fire-prone [16] and fire-resistant environments [35] which enables species that lack heat-stimulated germination are transferred to seeds in gaseous or aqueous form and this may occur directly from smoke or be secondarily transferred from soil particles to seeds [17]. According to Keeley and Fotheringham [24] smoke is more effective in combination of soil and smoke-treated seeds in the California chaparral community in *Caulanthus heterophyllus* (Brassicaceae), *Emmenanthe penduliflora* (Hydrophyllaceae) and *Silene multinervia* (Caryophyllaceae). This result agrees with our results for this study. This finding also agrees with studies conducted on western Australian plants species that responded positively to smoke and or extracts [22,26].

This study also showed that although smoke is an important germination cue for most CFSF species and guilds, the different species investigated require different pre-germination treatments cues to maximise their germinability. This is even more important considering results of an earlier study where 221 Fynbos plant species from more than 10 families recorded only 54% seed germination improvement after smoke treatment [16,21]. Perhaps this percentage could have increased had they considered treating the soils of where these seeds were sown with smoke. This is a clear difference between these previous studies and this study.

In general, species response to the different pre-germination treatments showed some patterns associated with growth forms and regeneration mode. Species with the same growth form or regeneration mode varied in their response to the different smoke-treatments suggesting that responses to smoke-treatments were species-specific in agreement with findings from [26]. Germination in both large-seeded and small-seeded species was enhanced significantly by smoke pre-germination treatments. Likewise, Dixon and Roche [26] found that both large- and small-seeded plant species responded equally positively to different smoke pre-germination treatments in Western Australian.

Recommendations for restoration and research needs

Seeds should be pre-smoked in a tent and, if restoration efforts include controlled burns, smoke treatment should be applied to seeds. This is because, in many cases, sowing may be between one month and five months after fire and smoke chemicals in the soil may have dissipated over time, especially with interim rainfall. It is therefore important for seeds to be sown in the field before the winter rainfall (from May to August) to allow species sufficient time to establish in moist soil conditions. Smoke effects may be seasonally operative [11], with the stimulatory effects declining in late autumn [25], thus timing of the seed smoke pre-germination treatment could be important [36]. The persistence of combusted plant-derived smoke impregnated in the soil deteriorates with time, thus affecting the smoke's germination activity or stimulatory effects on seeds [35-38]. Based on this study, the high germination rates of *W. meriana, C. ciliata, E. mammosa, P. scolymocephala, S. elegans, P. corymbosa* and *U. anthemoides* in control treatment is not an option. This is to ensure a structurally diverse and fully functional fynbos; hence, the underrepresented guilds should be targeted for re-introduction.

We conclude that, plant-derived gaseous smoke pre-germination treatments enhance seed germination in numerous CFSF species. The success of the type of gaseous smoke pre-germination treatment applied, however, was species-specific. Seeds sown in one year may germinate in the following year; however, results of this study are of considerable importance as using smoke pre-germination treatments to improve germination may be critical to successful restoration programmes that depend on seed sowing and prescribed burns. This experiment might have underestimated final seedling emergence in the field. Other factors could have influenced seed germination treatments used in this study is species-specific. Nonetheless, smoke-treated seed will be a quite efficient approach and if cost or practicality are not limiting, then a combination of smoke-treated seeds and soil can be more effective in restoration programs. Here is a figure summarising findings from this research (Figure 3).

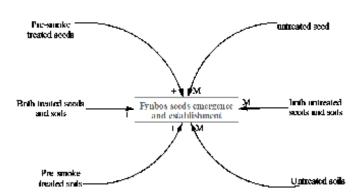


Figure 3: A Summary of findings of pre-smoke treated CFSF seeds in a greenhouse experiment. Positive enhancement=+, Mixed response (including + and no response)=M. Here the positive enhancement sign at the end of pre-smoke treated seeds, soils and both means, these pre-treatments enhance seed emergence and establishment. Also, the mixed response at the end of arrows of untreated seeds, soils and both means, the pre-treatments have mixed responses when applied to seeds before sowing

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