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Perspectives on the contributions by South African researchers in igniting global research on smoke-stimulated seed germination

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ABSTRACT

There is the age-old saying, "where there is smoke, there is fire", and while the important effects of fire as an ecological driver has been well known, and the effect of heat on seed germination previously well studied, it is amazing that it took until around 1990 for the "penny to drop" as far as the physiological effects of smoke on plant communities is concerned. Here, we describe some of the historical events that took place in South Africa in terms of discoveries of smoke-stimulated seed germination that ultimately led to the recognition of a new group of plant growth regulators. These findings underscored and sparked many research studies in other countries which have contributed greatly to elucidating the role of smoke as a germination cue, and its importance in ecological systems and potential use in seed technology.

1. Introduction

Plant-derived smoke, and several bioactive compounds (stimulatory and inhibitory) from plant-derived smoke are now known to play an intriguing role in regulating germination of many species. There is also evidence that some of these compounds may affect plant growth and development. The discovery of the role of smoke as a germination cue has led to many international studies relating to the ecology, physiology and chemistry of plant-derived smoke. This article gives the personal accounts of three South African scientists who were instrumental in 'igniting' research on smoke-stimulated seed germination in South Africa during the early 1990s, and which soon led to great interest across the world. While not widely recognized, nor understood, many indigenous peoples were utilizing "smoke" in Africa, Australia, as well as in the Americas for uses including various ethnomedicinal uses, for food preservation, or as a pesticide (Pennacchio et al., 2010). As far as seed germination is concerned, it is interesting that as far back as 1941, Parija et al. (1941) reported that smoke could decrease the after-ripening period of rice (Pandey et al., 2013), and Wicklow (1977) showed that extracts from charred wood were able to stimulate the germination of *Emmenanthe penduliflora*. This was confirmed by other researchers, with sterling work by Keeley and others on species from California chaparral (Keeley and Pausas, 2018).

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2. Recognition that plant-derived smoke stimulates seed germination

While employed at Kirstenbosch National Botanical Gardens and trying to get acquainted with the flora of the Cape Floristic Region in the late 1980s, Dr. H De Lange decided to follow up the suggestions of Mr. D Clark on the conservation status of *Audouinia capitata* (L.) Brongn. (Fig. 1) in the Cape of Good Hope Nature Reserve. This research was supported by the then Chief Director of the National Botanical Institute at Kirstenbosch, Prof JN Eloff.

A comprehensive project was outlined to investigate the role of fire in the regeneration of this threatened species. Workers on fire suggested a plethora of factors to be considered. Amazingly, smoke was never mentioned as a possible cue. Many obstacles were raised to experimentation with fire in this reserve (a "botanical shrine") in an area where no burns had occurred for 11 years. Experimentation eventually took place in 1989, and much attention was given to fire and heat as combined factors. During experimentation, the smoke made data recording difficult and persisted for hours after the flames were gone. Dr. De Lange, coming from the Citrus and Subtropical Research Institute at Nelspruit, had no preconceived ideas about fires and concluded that the effect on plant regeneration was largely due to the smoke generated by fires.

A further observation, which pointed to the improbable effect of temperature as the major factor on the soil seed bank, came from temperature readings. The average soil temperature at a depth of 10 mm was only 57.3 °C in the burned area soon after the fire, and at the

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Fig. 1. *Audouinia capitata* at Sirkelsvlei in the Cape of Good Hope Nature Reserve. (Photo by JH De Lange)

experimental plots outside the area it was 54.4 °C when the heat sensitive labels employed were removed late afternoon after the burn; a mere 3 °C difference.

These observations pinpointed to smoke as the possible "stimulus" in the investigation on post-fire regeneration. Several studies were done to test this assumption. The first trial was undertaken in an *A. capitata* population at Olifantsbos in the Cape of Good Hope Nature Reserve, a population that had escaped fire for approximately 20 years. Smoke was generated in a 130-L drum on 22 March 1989. Using bellows, the smoke was blown for 30 min into each of the plastic tents erected on nine 0.5 × 0.5 m areas containing seeds from adjoining *A. capitata* plants. A relatively long pipe was used which allowed the smoke to cool before entering the tents, as shown in Fig. 2 (De Lange and Boucher, 1990).

These sites were visited regularly for several months with no seedlings to be seen and the "smoke idea" was eventually considered to be a wild-goose chase. However, in October, seven months following the smoke-tent treatments, field observations were made in another population several kilometers away from the original Olifantsbos test site. Although a negative result in the latter population had already been accepted, it was nevertheless decided to make a detour during the return hike to visit this population once more. Plants of all species in this 20-year post-fire area were moribund with no seedlings to be seen. At the nine treated plots, however, there were small green islands of recently emerged seedlings amidst the brown, barren surroundings (Fig. 3). It was an emotional moment and was cherished for quite a while on that late afternoon of a beautiful day in the South African spring of 1989.

Following a study of the embryology in this species, it was understood why there was such a long delay for the seedlings to emerge (De Lange et al., 1993). At 15 weeks after cross-pollination, the heart-shaped embryos enter dormancy, as was seen with all seeds in the indehiscent fruits

Fig. 2. Apparatus used to pump smoke from smoldering plant material in a 130-L drum into plastic tents positioned over *A. capitata* seed banks in the Olifantsbos population on 22 March 1989 (De Lange and Boucher, 1990).

Fig. 3. Emergence of *A. capitata* seedlings in one of the nine 0.5 × 0.5 m areas given a smoke-tent treatment seven months earlier. Each of the small recently-emerged seedlings is indicated by means of a small label (De Lange et al., 1993).

recovered from the seed bank. Following application of a germination stimulus (i.e. gibberellic acid or smoke treatment), renewed growth of the rudimentary embryo is initiated and this results in a much-delayed germination and emergence of seedlings under field conditions, as much as seven months after the stimulus.

The soil surface around the *A. capitata* populations at Cape Point is generally very white (Fig. 1). Thus, the brown discoloration of these whitish sandy soils following smoke-tent treatments implicated the deposition of chemical residues. A similar conclusion was made each time after any lengthy periods of exposure to the clouds of smoke. When getting rid of smelly clothes and having a shower/bath after such a spell and observing the distinctly brown colored water running down the body, the general extent of the residual of smoke particles was realized. Gradually, one also becomes aware of the displeasure of others in close proximity, before bathing.

Consequently, the chemical nature of the smoke stimulus was investigated in a small-scale trial. A smoke extract was prepared and fractionated using chloroform (De Lange and Boucher, 1990). The treatments are shown in Table 1. Seed-containing fruits (indehiscent) were soaked for 24 h in distilled water (control), in a crude smoke extract or in two fractions of the crude smoke extract. A total of 28 fruits were planted in 100-mL sand-filled plastic cups in each of the treatments. In an additional treatment the same number of fruits were planted and given a smoke-tent treatment. After 16 weeks, the germination (emergence) was scored. Under favorable laboratory conditions *A. capitata* seeds germinate much earlier than under field conditions. The stimulatory effect of smoke on seed germination was once more demonstrated and it was realized that the active principle(s) could be extracted with organic solvents.

The highlight of this botanical adventure was when the nine patches of recently-emerged seedlings, at the sites given a smoke-tent treatment seven months earlier, were found in the Olifantsbos population. The planned data collection during the burn in the Sirkelsvlei population failed utterly due to smoke, tears and blurred vision. Initially, this was a great disappointment and embarrassment but ultimately proved to

Table 1
Effect of different treatments on the germination of *Audouinia capitata*. (From De Lange and Boucher, 1990)

Treatment	Germination %
Control (distilled water)	3.6
Smoke-tent	14.3
Crude smoke extract	17.9
Chloroform fraction	21.4
Aqueous phase after chloroform extraction	39.3

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