Short Communication

Autecological studies on *Audouinia capitata* (Bruniaceae). I. Plant-derived smoke as a seed germination cue

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Accepted 10 September 1990

Seeds of *Audouinia capitata*, a threatened fynbos species, are known to germinate under natural conditions only after fires. Experimental results are presented which demonstrate that seed germination is initiated by chemical factor(s) found in smoke, derived from burning fynbos plant material.

Dit is bekend dat sade van *Audouinia capitata*, 'n bedreigde fynbos spesie, onder natuurlike toestande slegs na vure ontkiem. Eksperimentele resultate word aangebied waarin daar gedemonstreer word dat ontkieming van saad deur chemiese faktor(e) aanwesig in rook afkomstig van brandende fynbos plantmateriaal, geïnisieer word.

Keywords: *Audouinia capitata*, fire, fynbos, germination, smoke

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Audouinia capitata (L.f.) Brongn. (Bruniaceae) is a threatened monotypic fynbos plant, categorized by Hall & Veldhuis (1985) as 'vulnerable'. It was decided to study the autecology of *A. capitata* because: (i) young plants are rarely found in nature; (ii) the plant has a decided horticultural potential; (iii) to our knowledge nobody has succeeded in cultivating the plant; (iv) the Bruniaceae is a family containing many threatened species and is endemic to the south-western, southern and south-eastern Cape with the single exception of an outlier (*Raspalia trigyna* which occurs in southern Natal); (v) it could serve as a model to investigate the processes of rarification and extinction of plants in the western Cape.

Fire regimes play a major role in pyric successions in fynbos and other heathlands (Gill & Groves 1981; Kruger 1984; Kruger & Bigalke 1984; Cowling *et al.* 1987; Kruger 1987). Fire-stimulated germination of seed has been reported for a wide variety of fynbos species (Levyns 1935; Martin 1966; Boucher 1981; Moll & Gubb 1981; Bond *et al.* 1984; Brits 1986).

Various factors have been suggested to explain the fire-mediated germination response by heathland species such as alterations in the levels of: allelopathic chemicals, competition, microbial populations, scarification, soil nutrients, and of temperature regimes (Renbuss *et al.* 1973; Boucher 1981; Warcup 1981; Brits 1986; Cowling *et al.* 1987; van de Venter & Esterhuizen 1988). Enhanced germination in a number of Californian chaparral species following exposure to charred wood (Keeley 1986) and extracts of charred wood (Keeley & Pizzorno 1986) have been reported previously. Extracts of post-fire ash, however, was found not to exert any significant influence on the germination of *Leucospermum cordifolium* seeds (Pool 1985).

Seeds of *Audouinia capitata* are known to be difficult to germinate under ordinary nursery conditions while seedling recruitment in nature has only been observed after veld fires. Observations during an experimental burn in February 1989, which will be reported on more fully elsewhere (de Lange 1991), indicated that scorching of soil-stored, indehiscent fruits and increased sub-soil temperatures during fire were unlikely to have induced the germination of *Audouinia* seed.

A preliminary investigation into the induction of *Audouinia capitata* seed germination by chemicals contained in smoke is reported on in this communication.

An initial investigatory trial was undertaken in a population of 90 plants at Olifantsbos in the Cape of Good Hope Nature Reserve. This population had escaped fire for approximately 20 years.

Smoke was generated in a 130-l drum using a mixture of dry and fresh plant material gathered in the surrounding vegetation on the 22 March 1989. The smoke was blown, using bellows, through a pipe into plastic tents erected on nine 0.5 × 0.5-m areas containing seed from different adjoining living mature plants for 30 min each. The system allowed the smoke to cool before it entered the tents (Figure 1). Hereafter this treatment will be referred to as the 'smoke-tent treatment'.

No seedlings were found to germinate in surrounding untreated areas whereas a total of 128 *A. capitata* seedlings were recorded in the nine treated areas by the end of October 1989. The most responsive plot is shown in Figure 2.

The promising results obtained in the above experiment, and the distinctly discoloured soil surface after the application of smoke, indicated a possible chemical basis for the smoke-mediated enhancement of seed germination. A further experiment was designed to test this hypothesis using smoke extracts prepared as follows: smoke, generated in an 18-l drum, was forced to bubble through distilled water using compressed air (Figure 3). A 500-ml aliquot of the resultant

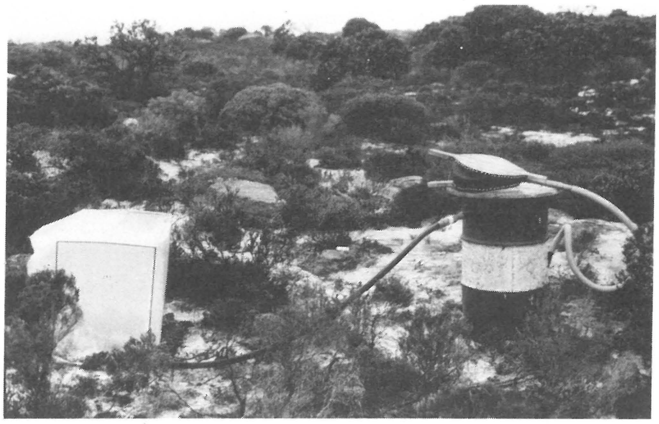

Figure 1 Apparatus used to pump smoke from burning fynbos plant material in a drum into plastic tents placed onto natural *A. capitata* seed banks in the unburned Olifantsbos population.

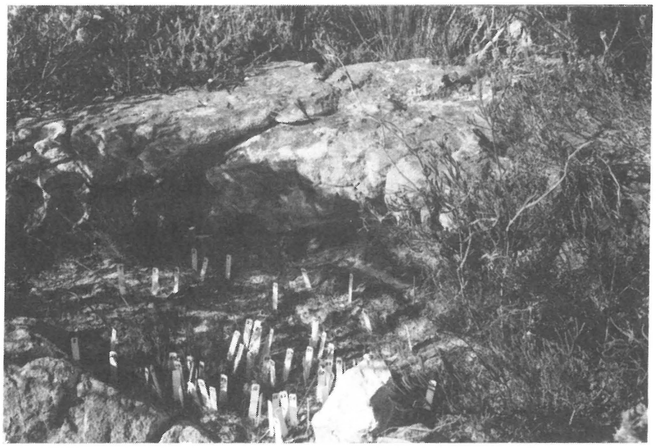

Figure 2 Prolific emergence of *A. capitata* seedlings in the Olifantsbos population, seven months after applying a smoke-tent treatment. Each seedling is indicated by means of a small plastic label.

brown water solution at pH 3.3 was extracted three times with chloroform, using 500-ml chloroform each time. The combined chloroform extracts were evaporated to dryness and redissolved in 500 ml distilled water.

Twenty-eight seed-containing fruits were soaked for 24 h in each of the different extract fractions (crude extract, chloroform fraction and the aqueous phase left after chloroform extraction). The same number of fruits were soaked for 24 h in distilled water, in 50 mg l^{-1} gibberellic acid and in 2.1 $\times$ 10^{-4} ml l^{-1} ethrel. A further 28 fruits were planted in 100-ml soil-filled plastic cups and were given the smoke-tent treatment. As seed-set percentages in *A. capitata* are extremely low, a large number of the indehiscent fruits had to be dissected partially to ensure that the same number of seeds were used in each treatment. The partial exposure of seed in fruits to determine whether seed was present, led to many seeds of this threatened plant to be discarded because of damage. The fruits were dusted with thiram fungicide and planted in sand in transparent plastic cups with the fruits placed against the cup walls to facilitate early recording of germination and detection of microbial contamination. The fruits were kept in the dark in an alternating temperature growth chamber (16 h at 10°C, 8 h at 25°C). Germination was scored for a 16-week period.

Table 1 Germination percentages of *A. capitata* seeds scored over a 16-week period following 24-h soaking in the listed solutions or the smoke-tent treatment (n = 28)

Treatment	% Germination
Control (distilled water)	3.6
Ethrel (2.1 $\times$ 10^{-4} ml l^{-1})	3.6
Gibberellic acid (50 mg l^{-1})	14.3
Smoke-tent	14.3
Smoke extract — *Fraction A	17.9
Smoke extract — *Fraction B	21.4
Smoke extract — *Fraction C	39.3

*A: Crude extract, B: chloroform extract, C: aqueous phase (see text for details)

Comparison of results presented in Table 1 shows that the germination of seed in the 'smoke-tent' and smoke extract treatments is stimulated to levels equal to or higher than that in the gibberellic acid treatment. These results confirm findings in the Olifantbos experiment in which application of the 'smoke-tent' treatment resulted in the emergence of large numbers of seedlings. The results also indicate that the active principle(s) can be extracted chemically.

Ethrel was found to be ineffective in stimulating the germination of *Audouinia* seed during an earlier trial where isolated seeds had been soaked for 24 h in this chemical at 2.1 $\times$ 10^{-4} and 2.1 $\times$ 10^{-5} ml l^{-1} concentrations. This was confirmed during the present experiment.

It can be concluded that ethylene is probably not the active ingredient in the smoke. This conclusion is supported by the positive response in the 'aqueous phase' treatment in which a negligible ethylene concentration would have been expected (Prof. B.V. Burger, Univ. Stellenbosch, pers. comm.). The use of synthetic ethylene gas in these experiments, however, would have provided a more decisive conclusion.

A third experiment was performed to investigate the role of season in the smoke-mediated regulation of seed germination. A total of 646 intact fruits, representing approximately 100 seeds, were planted in each of eight small (200 $\times$ 200 $\times$ 100

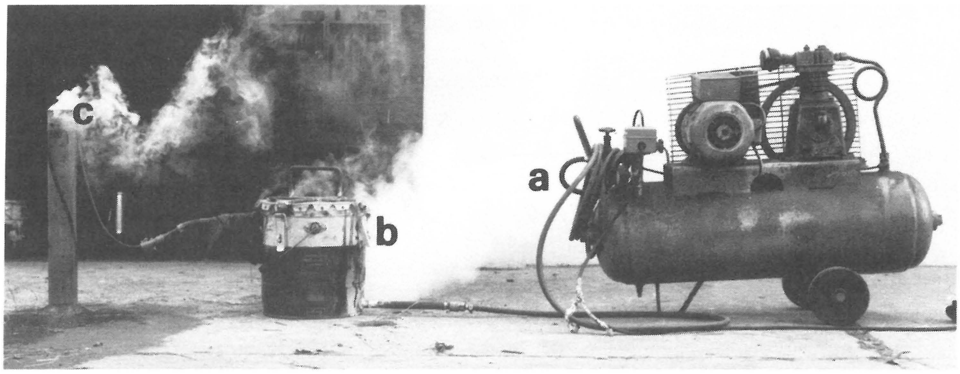

Figure 3 Apparatus used to extract water-soluble chemicals in smoke. (a) Air compressor, (b) smouldering plant material in 18-l drum, (c) smoke bubbling through distilled water.

Table 2 Results of germinating *A. capitata* seeds planted in glass cages and buried *in sito* in November 1989; seeds were subsequently subjected to different treatments. Germination counts as at 23 June

Treatment	Number of days since treatment	Number of seeds germinated
Untreated	–	0
Smoke-tent, 7 February	136	29
Smoke extract, 19 March	96	20

mm) glass cages, in such a way that germination and pre-emergence growth of seedlings could be monitored. A fine plastic gauze was glued to the bottom of each container to ensure direct soil contact and unimpeded vertical movement of soil moisture. Each cage was placed in an identically-sized hole in the ground in the Klaasjagers Plateau (Cape of Good Hope Nature Reserve) population of *A. capitata* during early November 1989.

One cage was left untreated; another was treated with a crude extract of smoke on 19 March 1990 while smoke-tent treatments were applied to the other cages on 23 November 1989, 5 January 1990, 7 February 1990, 17 March 1990, 12 May 1990 and 16 June 1990. The complete results, with their ecological implications, will be reported separately.

Results of only the control, smoke extract and 7 February smoke-tent treatments are presented. Preliminary results (Table 2) again indicate higher germination levels in the smoke-tent treatments as well as in the treatment where the crude smoke extract had been applied onto the soil surface in comparison to the control treatment.

An intriguing question at this stage is whether other fynbos species would respond in a similar fashion to smoke or smoke extracts. An affirmative answer could have several implications of scientific and commercial significance. Several other species are currently being investigated to test for the wider applicability of the results. Seasonal responses to smoke treatment which could be a useful means of establishing the optimal timing of controlled burns, are also being investigated.

This method could be used to test for the presence of seed in localities where plants have become extinct. It could also be used as a 'cleaner' method to reintroduce plants into the wild, thereby reducing the chance of introducing contaminated soil.

Identification of the responsible chemical substance(s) and elucidation of the biochemical mechanisms in the triggering of seed germination are further important eco-physiological facets which are under investigation.

The preliminary results presented here are extracted from a more detailed study being undertaken by the first author for a Ph.D. degree at the University of Stellenbosch under the second author and Prof. J.J.A. van der Walt.

Acknowledgements

The whole-hearted cooperation of the staff of the Western Cape Regional Services Council at Klaasjagersberg and Noordhoek; building of the smoking apparatus by Messrs C.E. Trautman and W. Newman of Kirstenbosch National Botanic Garden and photographic assistance by Mrs J. Loedolff, are gratefully appreciated.

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