The effect of smoke treatment on the germination on four species of Mesembryanthemum: some preliminary observations

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Abstract

The effect of plant derived smoke on the germination, extent of eosine dye penetration and amount of abscisic acid (ABA) in the seeds of two Karoo non-fire-prone species: *Ruschia caroli* and *Drosanthemum speciosum*, and two fynbos fire-prone species: *R. macowanii* and *D. stokoei*, of Mesembryanthemum were investigated. The seed coat characteristics of each species were also investigated.

Pierce *et al.* (1995) found that smoke promotes seed germination in the Karoo species *R. caroli* (scarified) and *D. speciosum*, whereas smoke has no effect on the germination of *R. macowanii* and *D. stokoei* (fynboss species). Results presented in this investigation support those of Pierce *et al.* (1995). The extent of dye penetration in smoke treated seeds of *D. speciosum* was significantly greater then that in untreated seeds of this species. This was due to the breakdown of some barrier, apparently in the membrane between the endosperm and the seed coat, by smoke. Seed ABA levels were reduced by smoke treatment in all four species, however this reduction was more pronounced in the smoke responsive species. Seeds coat studies revealed that the least responsive species was the only one to be covered by a waxy cuticle.

Seed dormancy was a feature of the species in which seed was improved by germination, whereas the seeds of the unresponsive species, *R. macowanii* and *D. stokoei* were non-dormant. Hence, smoke appears to act as a dormancy release cue, having no, or little effect, on the seed germination of non-dormant species. Results from this study indicate that the mechanism of smoke triggered dormancy release involves the chemical alteration of the membrane bounding the endosperm and the reduction in ABA concentration in the species investigated.

Introduction

Dormancy

Seed dormancy is a common feature of the diverse flora of the world. Dormant seeds, even though viable, fail to germinate, despite the prevail of favourable chemical and physical environments for germination. This is as a result of internal dormancy mechanisms which have to be overcome by or removed before germination can take place (Bewley and Black (1994). Those authors identify two categories of dormancy: coat imposed or coat enhanced dormancy; and embryo dormancy. Both types of dormancy may exist simultaneously or successively within a species.

As the name implies, in coat imposed dormancy it is the seed coat[1] that causes dormancy. The isolated embryo is able to germinated in the absence of seed coat tissue. The effects of coat imposed dormancy include: the interference of water uptake, interference with gas exchange, mechanical restraint, prevention of the exit of inhibitors from the embryo and the supply of inhibitors to the embryos (Bewley and Black, 1994).

Embryo dormancy is a feature in which the embryo is itself dormant. Two factors have been identified as important in maintaining this type of dormancy: cotyledons and germination inhibitors. Where cotyledons impose dormancy, removal of these organs results in dormancy release embryos remain dormant. With respect to germination inhibitors: one chemical inhibitor that is thought to play a major role in dormancy is abscisic acid (ABA). Various examples in which ABA is involved in maintaining embryonic dormancy have been demonstrated, see Bewley and Black (1994).

Smoke as a germination cue

Seeds may evolve certain dormancy release strategies that are suited to their environment. Characteristic environmental features, such as rain in deserts, may be used as cues for breaking dormancy. In numerous habitats, the occurrence of periodic fires is a natural phenomenon. Fire often plays a major role in the succession and structure of plant

[1] A loose term referring to the tissue enclosing the embryo which may include the endosperm, pericarp or extrafloral organs.

communities in these habitats (de Lange and Broucher, 1990). A number of fire related dormancy breaking cues may therefore have evolved. Several such cues have been suggested and include: changes in the levels of allelochemicals, microbial populations, scarification competition nutrients and temperature regimes and more, recently described, smoke (de lange and Broucher, 1990).

Sweeny (1956, cited in Keeley *et al.*, 1985) found that seeds usually requiring scarification, germinated without scarification when treated by burning wood shavings on the surface of the soil. He assumed that the improved germination was due to the increased temperature resulting from the burning. However, attempts to duplicate this effect using heat failed. Later, Jones and Scheilder (1980, cited in Keeley *et al.*, 1985) demonstrated that the germination of *Emmenanthe penduliflora* is stimulated by charred wood. At this stage it was unknown what component of charred wood stimulated the germination in this species. It was, however, determined that germination could also be induced with an aqueous extract of charred wood (Keeley *et al.*, 1985). Similarly, Keeley *et al.* (1985) found that the germination of several post fire dominant Californian chaparral species is stimulated by charred wood and aqueous extracts of charred wood.

Van der Venter and Esterhuizen (1988) considered the possibility that gasses released during a burn may also constitute germination cues. They tested this for two species of Ericaceae: *Erica sessiliflora* and *E. hebecalyx*. Heat pretreatment, as well as exposure to both dry and imbibing seeds to ethyl and ammonia, stimulated germination of *E. hebecalyx* seeds but no but had no effect in *Erica sessiliflora* (van der Venter and Esterhuizen, 1988).

The first conclusive report indicating that smoke itself stimulated germination came from a study conducted by de Lange and Broucher (1990) on *Audouinia capitata* (Bruniaceae). This species is characteristically difficult to germinate. However, when seeds were treated *in situ* with cool smoke derived from burning a mixture of fresh and dry plant material, or with crude smoke extract, germination improved significantly. In a study in which twenty eight fynbos species were screened for stimulated germination responses to *ex situ* smoke and smoke extract treatments, twelve showed a statistically significant improvement in seed germination (Brown, 1993). These included fynbos species belonging to the families:

Asteraceae, Restionaceae, Ericaceae and Proteaceae.

The promotive effect of smoke on seed germination in species from Western Australia, also a fire prone system, has recently been reported. Dixon *et al.* (1995) demonstrated that in of 45 out of 94 native Western Australian species, that are difficult to geminate, germination was improved with smoke treatment or aqueous extracts of smoke. Furthermore, under controlled conditions, those authors found that smoke-treated seeds from some species showed earlier germination than control seeds. In other species, smoke treated seeds continued to germinate for several weeks after controls had achieved full germination. The species that responded positively to smoke treatment include members of the Rutaceae, Dilleniaceae, Proteaceae, Myrtaceae, Cypressaceae and Thymelaeaceae (Dixon *et al.*, 1995).

Smoke/smoke extract promoted (hence forth collectively refereed to as smoke treatment) seed germination is emerging as a widespread phenomena, applying to species that do not even occur fire-prone habitats and have not have evolutionary histories to cause the evolution of such a response. For example van Staden *et al.* (in press, cited in van Staden *et al.*, 1995) have even found that plant derived smoke extract stimulates the germination of light sensitive Grand Rapids lettuce seeds in the dark. The evolutionary significance of smoke-stimulated germination should therefore be debated.

Pierce *et al.* (1995) hypothesised that smoke treatment would promote germination in fire prone species, but not in non-fire-prone species. The hypothesis was tested by comparing the effect of smoke treatment on 16 fynbos-fire prone species, and 6 Karoo non-fire-prone species of Mesembyanthemaceae. Those authors found that only half the fynbos fire-prone species responded to smoke treatment. More surprisingly, all but one of the karoo non-fire-prone species showed a significant positive responses to smoke treatment. The authors suggested that: "the chemical component of smoke which promotes germination may be acting on a general germination inhibitor". Furthermore they make the point that the ecological significance of reported germination responses to smoke may be obscure. This highlights two questions that need to be answered 1) what component(s) of smoke are involved in stimulating seed germination; and, 2) what are the mechanisms involved in smoke-promoted seed germination. The answers to these questions, when and if they emerge,

need to be evaluated in terms of their evolutionary significance and universality.

Components

Elucidating what component(s) of smoke cause dormancy release or stimulate germination is of great commercial importance to the horticultural industry, and many of the recent investigations on the effect of smoke on seed germination have concentrated on determining the component(s) of smoke that are responsible for the observed trend of improved germination. Although some smoke components that promote germination have been isolated, no single component has yet been isolated that can entirely claim responsibility for the broad trends observed. Furthermore, the various active components of smoke have different effects on different species (Fortheringham, pers comm.).

For example: Sutcliffe and Whitehead (1995) found that ethylene, an important component of plant-derived smoke, stimulated seed germination in *Cyclopodia intermedia* and *C. subternata*. Additionally those authors determined that treatment with short-chain saturated fatty acids, eg. octanoic acid, (also a component of smoke) resulted in increased sensitivity to ethylene, and consequently, improved germination. Sutcliffe and Whitehead (1995) concluded that these compounds are, at least in part, responsible for smoke-promoted germination. Baxter *et al.* (1994) however demonstrated that ethryl and ethylene were unable to stimulate germination in the grass *Themeda triandra* at a wide range of concentration tested. Those authors also demonstrated that germination of *T. triandra* seeds is stimulated by plant derived smoke and aqueous smoke extract. Baxter *et al.* (1994) concluded that ethylene is not the active component of smoke that promotes seed germination. So some species respond to ethylene, as a component of smoke whereas others do not. The ethylene in smoke can therefore not be considered as the universal (or primary) active component of smoke. Baxter *et al.* (1994) suggested "the bioactive component of plant-derived smoke may originate from a commonly occurring source, possibly being the thermal breakdown product of hemicellulose or cellulose". More specifically the compound is suggested to be an oligosaccharide-type molecule, a product of the thermal breakdown of xylan or other hemicelluloses having glucauronic acid side chains (Baxter *et al.*, 1994).

The effectiveness of smoke derived the burnt material of twenty seven different species of plant in stimulating germination of *Themeda triandra* seed was tested by Baxter *et al.* (1995). Smoke from eighteen of the twenty seven species screened significantly improved germination. Those authors concluded that the effectiveness of germination-stimulating smoke varies, depending on the source. Investigations have also found that the active component can be produced be dry heating plant material. For example, the germination-stimulating components of *Passerina vulgaris* smoke can also be obtained by dry-heating leaf material of this species at temperatures as low as 80°C. Baxter *et al.* (1995) thus suggest that the relationship between temperature and the level of activity of germination-stimulating compounds in plant derived smoke may be important.

Nitrate ion is the most common soil chemical known to promote germination in laboratory conditions (Thanos and Rundel, 1995). Thanos and Rundel (1995) demonstrated that the addition of optimal concentrations (c. 10mM) of nitrate promoted seed germination in the fire annuals *Emmenanthe penduliflora* and *Phacelia grandiflora* and to a lesser extent, the fire adapted shrub *Sulvia mellifera*. Ammonium ions were also effective in *P. grandiflora* and *S. mellifera*. Those authors found that in all three species tested, the effect of nitrogenous substances on seed germination was nearly identical to that produced by an extract of charred wood (and probably smoke). Californian chaparral is deficient in nitrogen but concentrations of available nitrogen are found in comparatively high concentrations after burning. The nitrate and ammonium concentrations required to induce germination are very close to the increased values encountered after a fire in otherwise nitrogen-poor chaparral soil. Therefore the post-fire germination flush observed in chaparral may be induced by the increased levels of available nitrogen produced after fires (Thanos and Rundel, 1995). Stock and Lewis (1986) also found that total nitrogen and exchangeable $NH^{4+}N$ concentration at the soil surface increased significantly after fires in coastal fynbos, creating a favourable environment for germination.

Mechanisms involved in smoke-stimulated germination

The type of studies conducted on smoke-stimulated seed germination are often geared towards screening numerous species for positive germination responses to smoke treatment. Very few studies have investigated the mechanisms of dormancy release caused by smoke treatments.

Understanding what processes smoke induces in seeds resulting in dormancy release may help to understand what components of smoke are relevant in stimulating germination. Keeley (1991, cited in Bell *et al.*, 1993) suggested that the mode of action of the active compound in charred wood (and probably smoke) could be related to 1) triggering a biochemical change in the embryo, 2) inactivating an inhibitor, or 3) chemically breaking the seed coat or the membranes beneath.

Baxter *et al.* (1994) found that the smoke-promoted seed germination increases relative to the degree of imbibition. Following this they suggested that "smoke may act on an enzyme system or on phytohormone metabolism". Whitehead and Bossé, (1991, cited in Sutcliffe and Whitehead, 1995) and Whitehead and Vasiljevic (1993, cited in Sutcliffe and Whitehead, 1995) found that "the increase in ethylene sensitivity caused by short-chain saturated fatty acids is related to its ability to increase ethylene binding by altering certain membrane properties". This mechanism is implicated in smoke-promoted seed germination (Sutcliffe and Whitehead, 1995).

Aims

The broad aim of this study was to investigate the effect of smoke on some basic physiological responses of seeds in order to gain a deeper understanding of the phenomenon of smoke-stimulated germination. This was done on seeds occurring in the fire-prone (fynbos) and non-fire prone (Karoo) regions. Thus, more specifically:

1) The effect of smoke on seed germination of the fynbos Mesembryanthemum species *Ruschia macowanii* and *Drosanthemum stokoei* and the karoo Mesembyanthemum species *Ruschia caroli* and *Drosanthemum speciosum* was tested.

2) Patterns of water penetration in smoke treated and control (unsmoked) seeds of these species were investigated.

3) Seed coat attributes of all four species were investigated using SEM, to determine weather there were any distinctive features that correlated with the above parameters.

4) The effect of smoke on ABA levels before and after smoke treatment were determined.

By comparing the results from the above investigations, it was hoped to determine noticeable differences in the seed physiology of a) smoke-responsive and unresponsive species, and b)

non-responsive species occurring in fire-prone habitats to responsive species occurring in non-fire-prone habitats. The later comparison was anticipated to clarify the evolutionary discrepancy found by Pierce *et al.* (1995) that non-fire prone species screened are responsive to smoke whereas fire prone species screened often were not.

Materials and methods

Species

In order to make comparisons such as those outlined above, it is necessary to compare species that are closely related. The family Mesembryanthemum is a useful group for such purposes as it has linages that are shared between fire-prone habitats (fynbos) and non-fire-prone habitats (Pierce *et al.*, 1995). Two such genera that each have represented species in fire-prone and non-fire-prone habitats are *Ruschia* and *Drosanthemum*. Thus two species, each representing the two different habitats, from each of these genera were chosen for this study. These species are: *Ruschia macowanii* (fynbos species) and *Ruschia caroli* (Karoo species); and *Drosanthemum stokoei* (fynbos species) and *Drosanthemum speciosum* (Karoo species). A previous study conducted by Pierce, *et al.* (1995) found that the germination of both *R. macowanii* (Fynbos) and *D. stokoei* (Fynbos) is unresponsive to smoke treatment whereas *R. caroli* (Karoo) and *D. speciosum* (Karoo) showed improved germination in response to smoke treatment. Seeds of *R. macowanii*, *D. stokoei* and *D. speciosum* were obtained from the seeds bank at Kirstenbosch Gardens. Mature, current year's seed of *R. caroli* were collected from plants growing in the Worcester Botanical Gardens.

Germination experiments

Germination experiments consisted of two smoke treatments *viz.* a) seeds were treated with smoke and b) seeds were soaked in water for twenty four hours, then treated with smoke; and one control (non-smoke) treatment. In the case of *R. caroli* the smoke and control treatments, but not the water+smoke treatment, were repeated on scarified seeds. Seeds were scarified by exposure to 50% KOH for 15 minutes.

For both the treatments and the control, eight replicates of twelve seeds each were used for each species. Seeds were placed on three layers of Schleicher and Schüll filter paper in Petri dishes.

Smoke treatment was conducted as follows: Petri dishes containing the seeds were placed into a sealed cardboard box. Smoke was generated by using a bee keepers smoker. The plant material used to generate smoke was a mixture of fresh and dried *Passerina vulgaris* as the smoke of this species is known to be effective in promoting seed germination (Baxter *et al.*, 1995). Smoke was activly pumped into the box for five minutes through a pipe which fitted into a small inlet on the box. Seeds were left in the box for twenty four hours and then removed.

In all treatments the filter paper was moistened with equal amounts of 0.5 % benlate fungicide solution and the Petri dishes covered with lids. The Petri dishes from the three different treatments were kept separate until they had been placed into three different clear plastic bags which were securely sealed. This was done to avoid contamination of the control seeds from volatile gases from the smoke treated seeds. Seeds were germinated in a controlled environment chamber (photon flux density $49m^{-2}s^{-1}$) at $10^0C/20^0c$ for 14/10 hr in light dark. Pilot trials by Pierce *et al.* (1995) indicated that these are suitable conditions. Seeds were checked for germination on days 3, 7, 13, 20, 30, 38, 48, and 58. Germination was scored as radical emergence.

Statgraphics 6.O (STSC Inc.) was used to perform one way ANOVAs, on arc sine transformed germination proportion data for each of the species. This was done to determine if significant differences in germination response occurred among the three treatments within species. A multivariate ANOVA was conducted incorporating the data of all four species to determine if there were significant differences in germination response among the different species. In both all tests results were significant if $p < 0.05$ ie. at the 95% confidence level.

Dye penetration

Fotheringham found a correlation between the extent of dye penetration and the level of dormancy in smoke treated seeds, thus the same procedure was applied in this study. The extent of dye penetration through the seed tissues was investigated for all treatments except water+smoke and scarified *R. caroli* seeds. Care was taken to select only undamaged for the experiment. Half the seeds from each species were smoke treated as described above. The remaining half served as a control. Seeds from each treatment and each species were placed

in separate vials. Seeds were then left to stand in 1% w/v Eosine dye for 6, 11, 16 and 21 days respectively. At each interval 15 seeds were removed for sectioning with a freezing microtome. Prior to sectioning, seeds were rinsed with distilled water for about 30 seconds and then rinsed in 70% alcohol for about 10 seconds. This was done to remove excess surface dye. Sections (approximately 10 Ym thick) were placed onto glass slides, where they adhered upon drying. Sections were not set in any media as this would have caused the dye to run, obscuring true patterns of penetration.

Three categories of dye penetration were used in scoring individuals: 1) no dye penetration through the seed coat (class 1); 2) dye penetration through the seed coat but not into the endosperm (class 2); and 3) dye penetration completely through the endosperm and into the embryo (class 3). Data was recorded as proportion of seeds in each category. In Statistica for Windows (version 4.3) a Kruskal-Wallis test for non-parametric data was used to test whether there is a significant difference in pattern of dye penetration between control and smoke treated seeds for each species at the 95% confidence level.

Seed coat studies

The nature of the seed coat of all four species was investigated using scanning electron microscope (SEM) imagery. This was done to detect any obvious differences in seed coat that may correlate with smoke-stimulated germination. Six seeds from each species were glued to Cambridge stubs with colloidal graphite, then coated with gold palladium. The seeds were viewed in a Cambridge 200 SEM. In all four species whole seed, seed sculpture and hilum region were photographed.

Hormone studies

ABA levels of smoke treated and non-smoke treated (but **not** water+smoke treated) seeds were determined for all four species. ABA levels were not determined for scarified control and smoke treated seeds of *R. caroli*.

Seeds of all these species are very small (< 1mm) therefore extractions were done on whole seed material. Due to the limited amount of material available only two samples of 0.2g per treatment per species were used. ABA was extracted from finely ground material by an

overnight incubation in 2ml of 70% methanol, containing 20mg.l^{-1} butylhydroxy toluene and 50mg.l^{-1} ascorbic acid. The particulate matter was removed by centrifugation at 10000 ppm for ten minutes. The supernatant was filtered using a 0.45μm PFTE filter and then passed though a C^{18} Sep-pak column. The resulting filtrate was reduced to dryness in a Savent vacuum concentrator. The samples were then redissolved in methanol and triplicate alliquats dispensed for radioimmunoassay (RIA). Presence of interfering substances was tested for as described in Farrant *et al.* (1993). Calculations were done using an online computer and securia data reduction RIA package (Packard Instrument Company, 1986 publication No. 169-3016). The ABA analysis was done by the department of horticulture at the University of Stellenbosch. Result are expressed as nannograms of ABA/gram of seed material.

Results

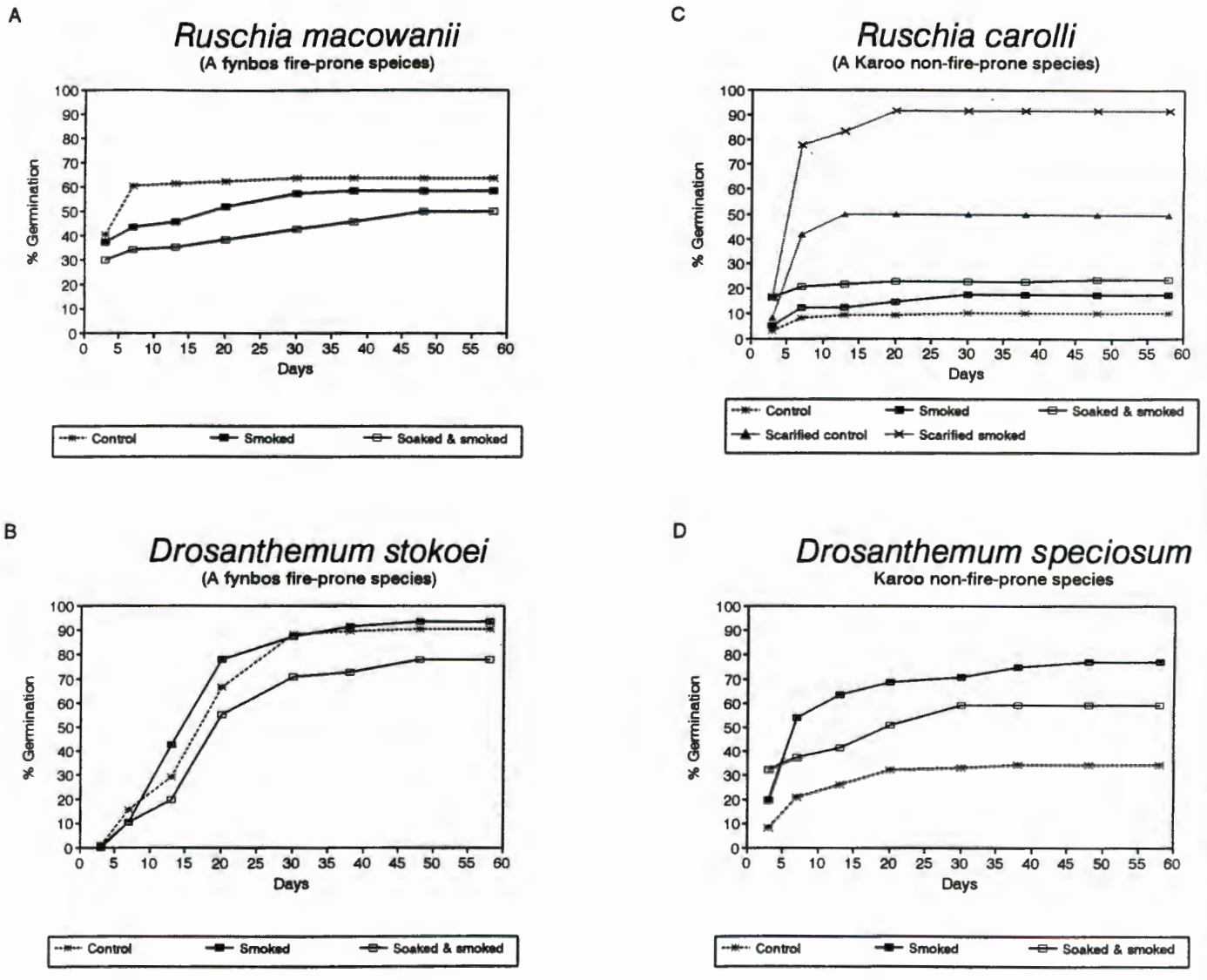
Germination experiments

Results from the ANOVAs conducted on germination data for each species are presented in Table 1. The time course of germination for the four species are shown in Figures 1a-d.

Table 1. Probabilities of accepting the null hypothesis (no effect or interactions among the treatments on germination). Probabilities generated by one way ANOVAs conducted arc sine transformed germination data for each species. When asterisk is shown, the null hypothesis is rejected ($p < 0.05$).

Species	P values	Contrast* (S-SW; S-C; SW-C)
R. macowanii (fynbos)	0.0677	
D. stokoei (fynbos)	0.0099 *	SW-C; S-SW
R. caroli (Karoo)	0.0256 *	SW-C
R. caroli (scarified)	0.0000 *	C-S
D. speciosum (Karoo)	0.000 *	SW-C; S-SW; S-C

* Contrast shows which groups were significantly different in their germination response.



Figures 1a-d. The effect of: smoke treatment; soaking seeds for twenty-four hours prior to smoke treatment; and control treatment on the percentage germination of four species of Mesembryanthemum. A, *R. macowanii*. B, *D. stoekoei*. C, *R. caroli*. D, *D. speciosum*.

Smoke had no effect on seed germination of *R. macowanii* and *D. stokoei* (Figs. 1a&b, Table 1). In both these species soaking seeds prior to smoke treatment reduced germination to below control levels. This reduction was significant in *D. stokoei* (Table 1). Smoke had no effect on the germination of *R. caroli* seeds, but water+smoke significantly improved germination of this species (Fig. 1c, Table 1). The germination of scarified *R. caroli* seeds improved significantly with smoke treatment (Fig.1c, Table 1). The effect of water+smoke on scarified seeds is unknown as this was not tested. In *D. speciosum* there was a significant difference among all the three treatments (Fig. 1d, Table 1). Smoke improves germination in this species, as does water+smoke, but not to the same extent. Results from the water+smoke treatment for *R.macowanii*, *D. stokoei* and *D. speciosum* indicate that soaking seeds in water before smoke treatment inhibits the effectiveness of smoke in promoting germination or germination itself in these species.

Table 2. Probabilities of accepting the null hypothesis (no effect or interactions among the treatments on seed germination). Probabilities generated by a multivariate ANOVA conducted on the combined arc sine transformed germination data of all species. When asterisks is shown, the null hypothesis is rejected ($p < 0.05$).

Source of variation	**P-values**
Species	0.0000 *
Treatments	0.0001 *
Interaction of species + treatment	0.0000 *

Results from the Multivariate ANOVA suggest that there is a significant difference in germination among species and among treatments. There is also a significant interaction between species and treatment, which is expected from results of individual ANOVAs. All species were found to be significantly different in their germination responses except for *R. macowanii* and *D. stokoei*.

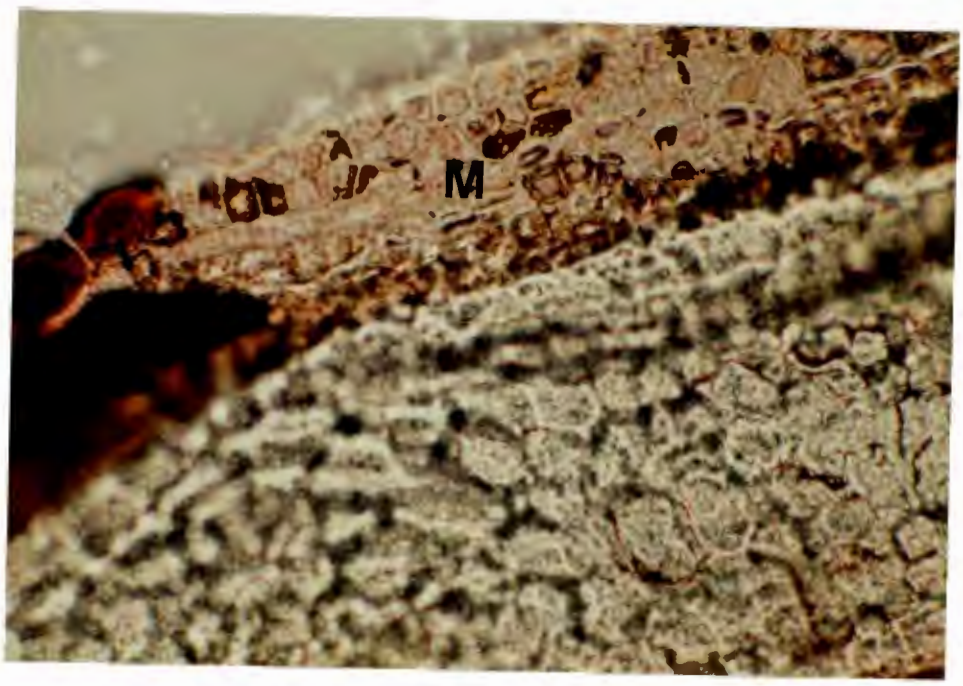
Dye penetration

Plates 1a-c are sections through seeds showing the degree of dye penetration. Figures 2a-d & 2a-d illustrate the patterns of dye penetration, over time, of control and smoke treatments for the four species investigated.

a

Plate 1a-c. Sections through seeds showing the tree classes of dye penetration: 1a no penetration. A, No penetration through seed (class 1). B, Penetration through the seed coat but not into endosperm (class 2), where M= the membrane below the seed coat into which dye has penetrated. B, Complete penetration (class 3). The magnification of Plates a&c = 10 x 1.25 x 12.8. The magnification of Plate c = 40 x 1.2 x 12.8.

b



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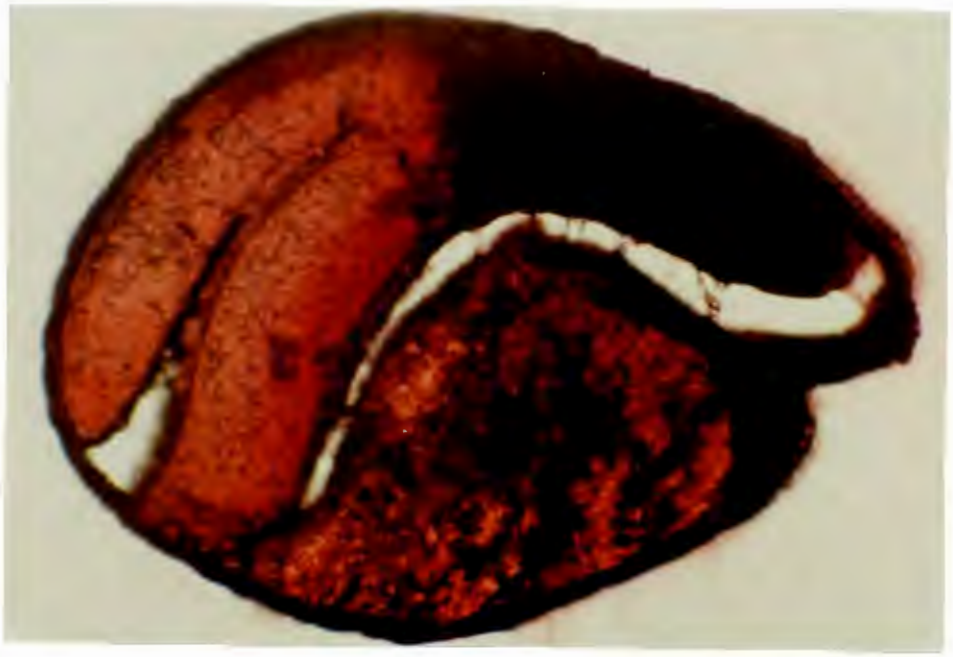
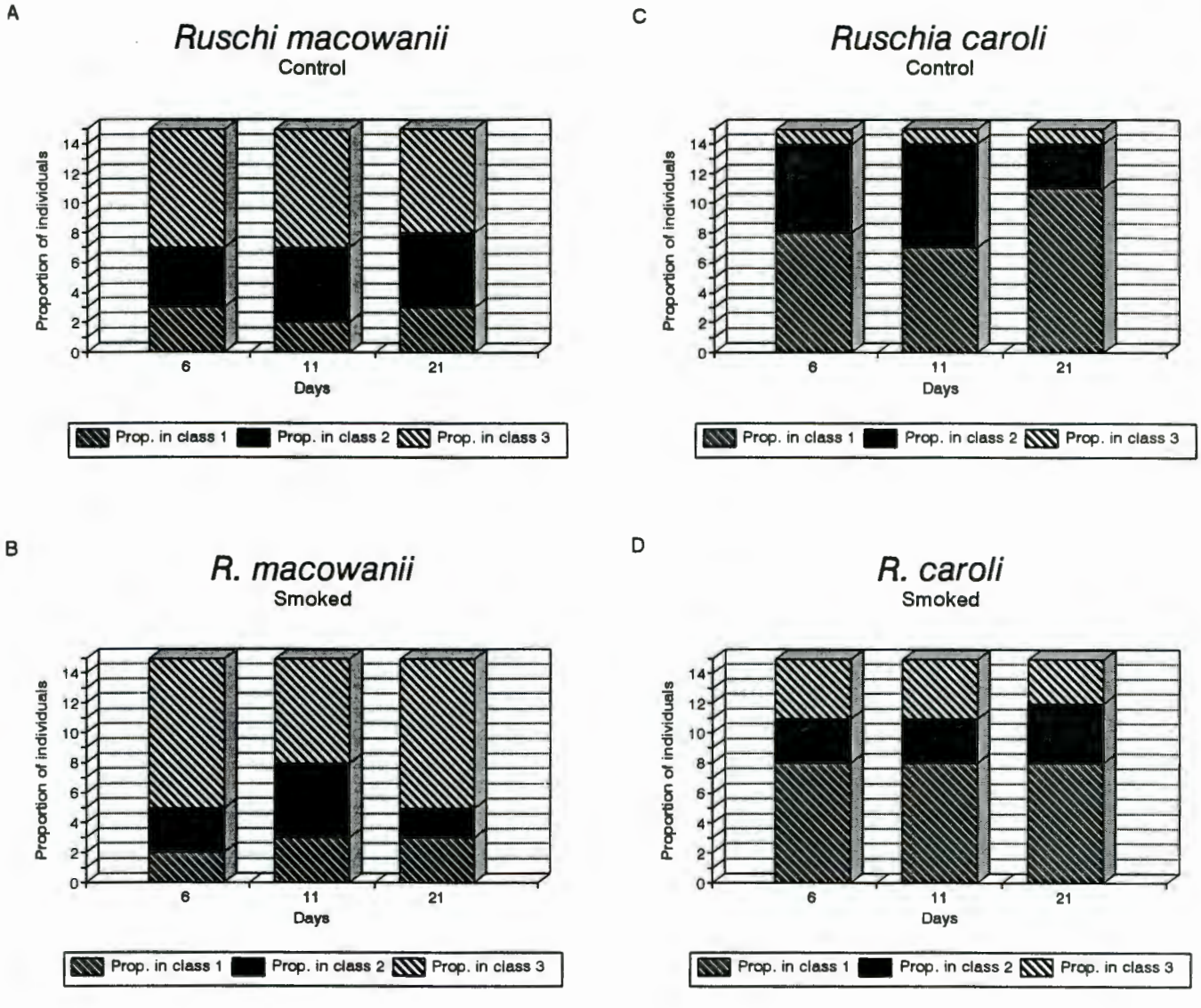
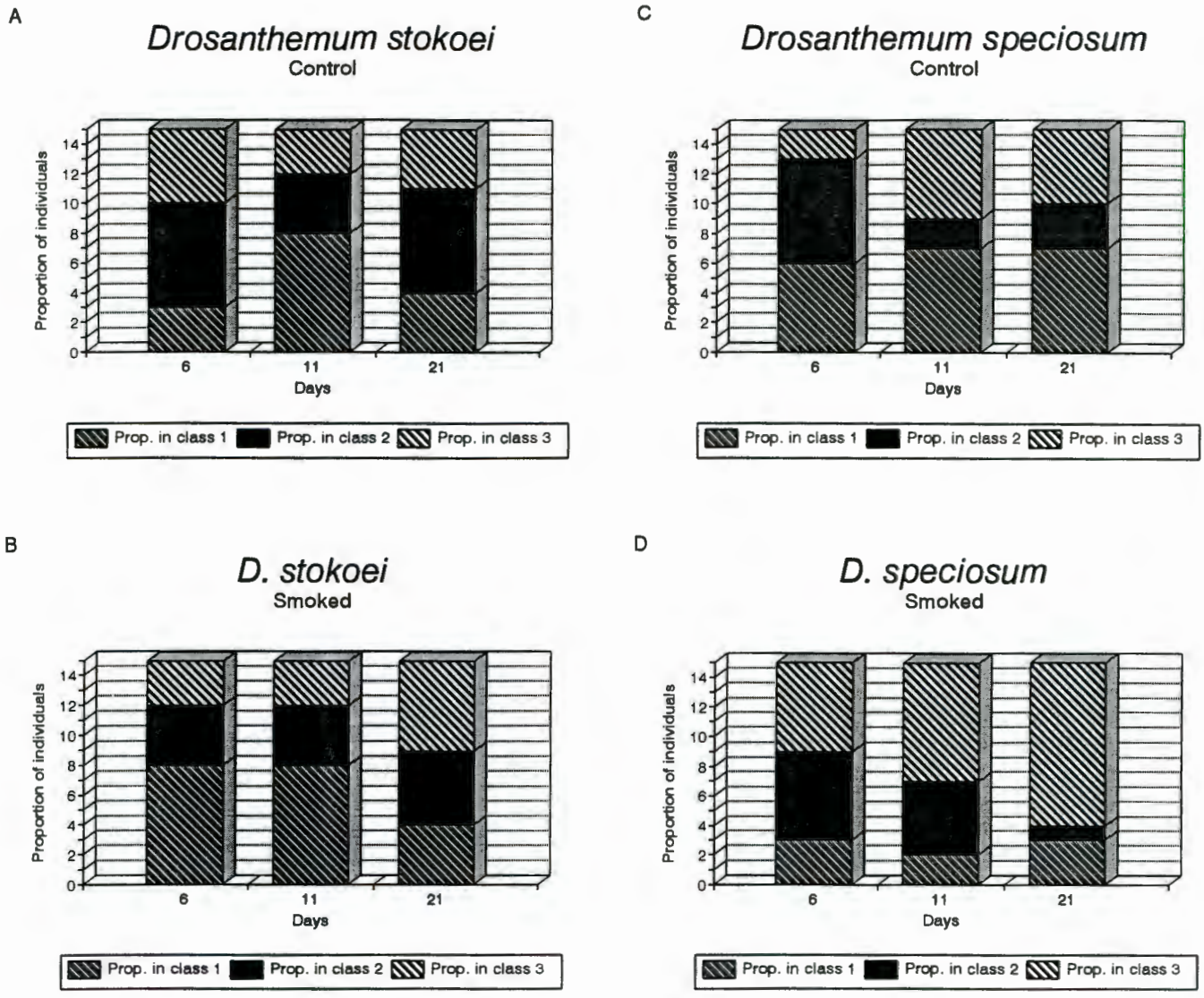




Figures 2a-d. Proportion of seeds in each class of dye penetration in control and smoke treated seeds of each species. Class 1 = the proportion of seeds in which no dye penetration had taken place. Class 2 = the proportion of seeds in which there was dye penetration through seed coat but not into the endosperm. Class 3 =the proportion of seeds in which complete dye penetration occurred. A, *R. macowanii* (control). B, *R. macowanii* (smoke treated). C, *R. caroli* (control). D, *R. caroli* (smoke treated).



Figures 3a-d. Proportion of seeds in each class of dye penetration in control and smoke treated seeds of each species. Class 1 = the proportion of seeds in which no dye penetration had taken place. Class 2 = the proportion of seeds in which there was dye penetration through seed coat but not into the endosperm. Class 3 =the proportion of seeds in which complete dye penetration occurred. A, *D. stokoei* (control). B, *D. stokoei* (smoke treated). C, *D. speciosum* (control). D, *D. speciosum* (smoke treated).

The Kruskal-Wallis test found no significant differences in dye penetration between smoke-treated and control seeds of *R. macowanii*, *D. stokoei* and *R. caroli*. In *R. macowanii* dye penetrated through to the embryo (class 3) in the majority of seeds for both control and smoke treatments (Fig. 2a&b). The pattern of dye penetration for this species correlates with result from the germination experiments. The proportion of seeds in which dye penetrated through to the embryo in *D. stokoei* is less that what is expected from the germination results (Fig. 3a&b). The reason for this discrepancy is unknown.

Dye penetration patterns in *R. caroli* (Fig. 2c&d) also correlated with germination data. Although there was some penetration through to the embryo, it generally stopped at the seed coat. The proportion of seeds in which complete penetration occurred was higher for smoke treated seeds that for controls, but this was not significant. Preliminary data on dye penetration in scarified *R. caroli* seeds (not presented here) indicates that there is a significant difference in dye penetration between scarified smoke treated and scarified control seeds. Here too, a greater proportion of smoke treated seeds were completely penetrated compared to control seeds.

Kruskal-Wallis tests showed that the only significant difference in dye penetration occurred between smoked and control seeds of *D. speciosum* on day 21 ($p=0.0426$) (Fig. 3c&d). The proportion of seeds in which no penetration had taken place was much greater in control versus smoke treated seeds. The same is true for the proportion of seeds in which penetration was through the seed coat but not into the endosperm. Thus, dye penetrated more readily into the embryo in seeds which were smoke treated compared to the control.

Seed coat studies

Images obtained from SEM are shown in Plates 3a-5d. All the seeds have a similar shape (Plates 3a-d). *R. caroli* is the largest, followed by *R. macowanii*, *D. stokoei* and *D. speciosum* is the smallest. All species except *R. macowanii* had sculptured seeds coats (Plates 4a-d). *D. stokoei* was the most elaborately sculptured species, followed by *D. speciosum* and *R. caroli*. The coat of *R. macowanii* (Plates 3b&4b) was smooth and waxy.

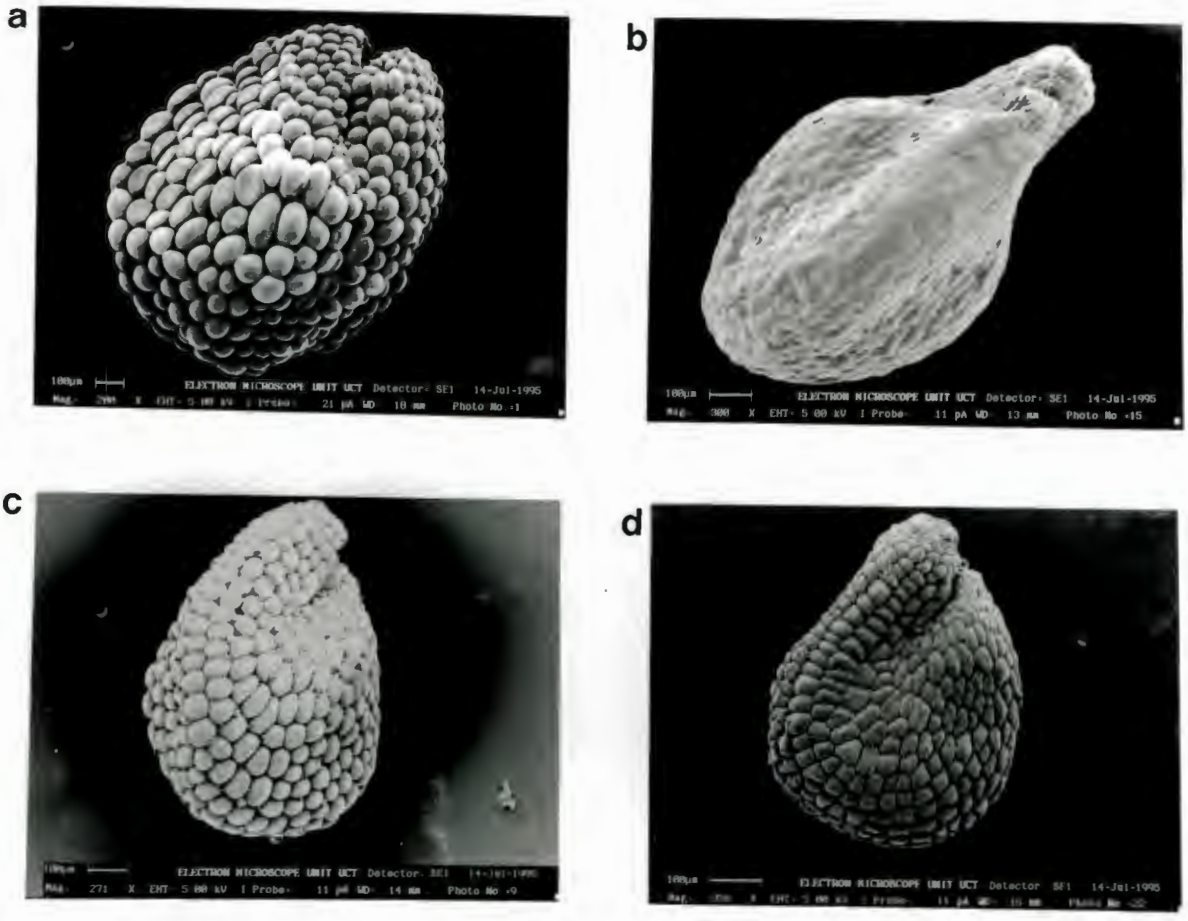
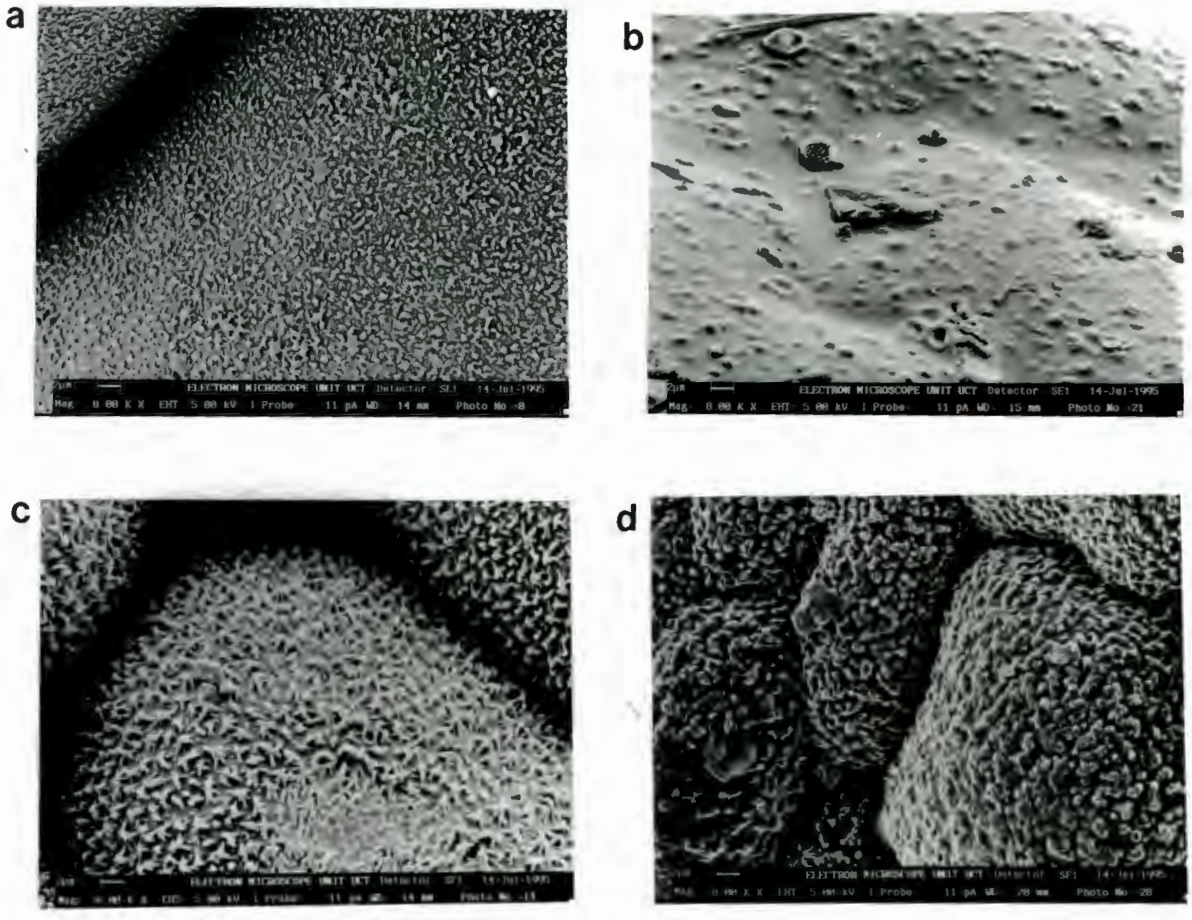
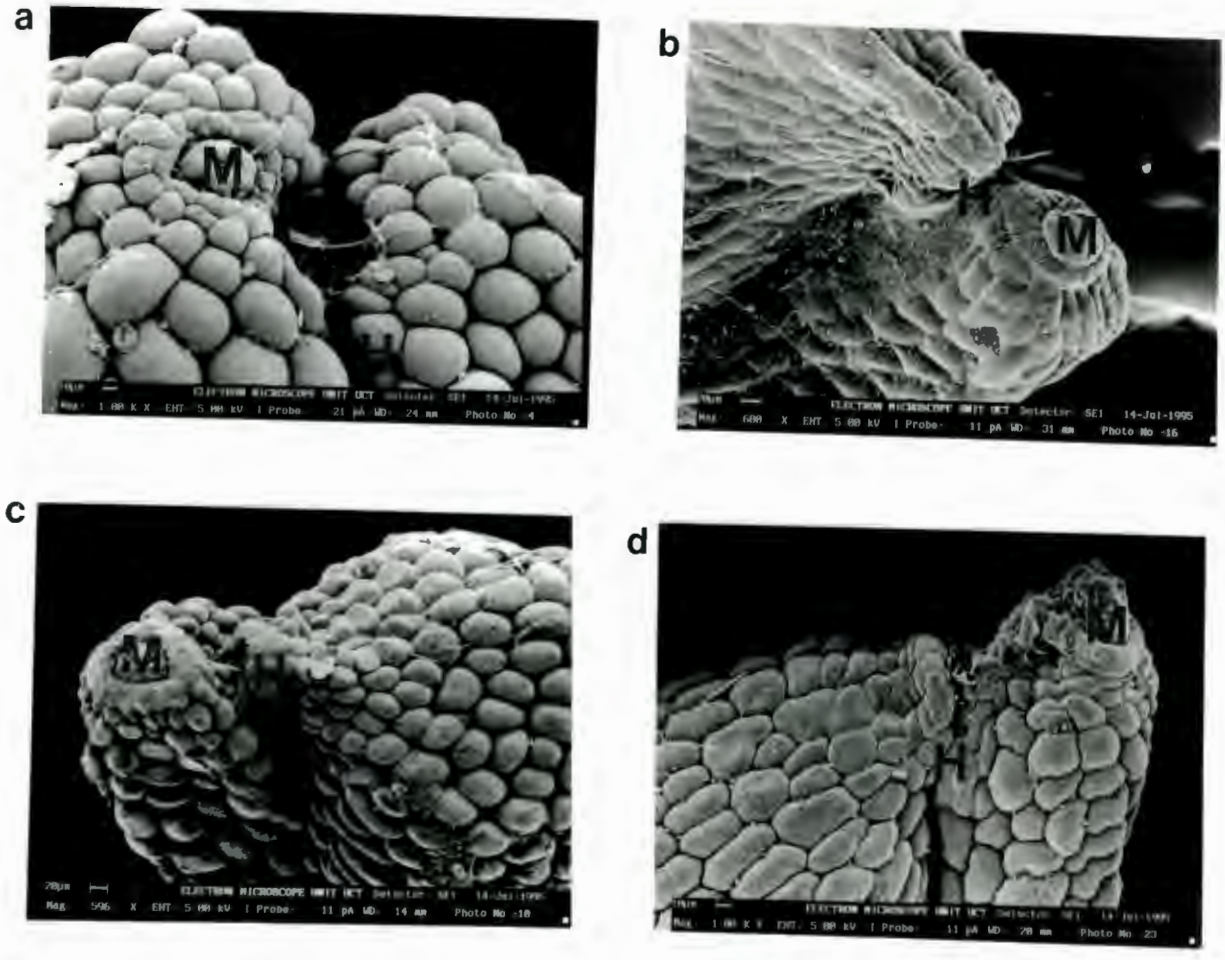


Plate 3a-d. SEM images of the whole seeds. A, *R. caroli* (scarified seeds responsive to smoke). B, *R. macowanii* (unresponsive to smoke). C, *D. stokoei* (smoke-responsive). D, *D. speciosum* (unresponsive to smoke).



Plates 4a-d SEM images of seed coat sculpture. A, *R. caroli*. B, *R. macowanii*. C, *D. stokoei*. D, *D. speciosum*.



Plates 5a-d. SEM images of the hilar-micropyle regon. A, *R. caroli*. B, *R. macowanii*. C, *D. stokoei* . D, *D. speciosum* . H = hilum, M = structure thought to be the mycropyle.

The sculptured surfaces and relatively loosely packed seed coat cells of *D. stokoei*, *D. speciosum* and *R. caroli* may be conducive to efficient gas exchange and subsequent water uptake. The waxy cuticle and apparently tightly packed seeds of *R. macowanii* may inhibit such processes.

In all species, a structure, assumed to be the micropyle, lay adjacent to the hilum, which was deeply incised (Plates 5a-d). It appeared that this structure may be a site of gas exchange and water penetration. Sectioning for dye penetration confirmed this (see Plate 6). Photos showed that on all the four species, fungal spores are present on the seed coat cells.

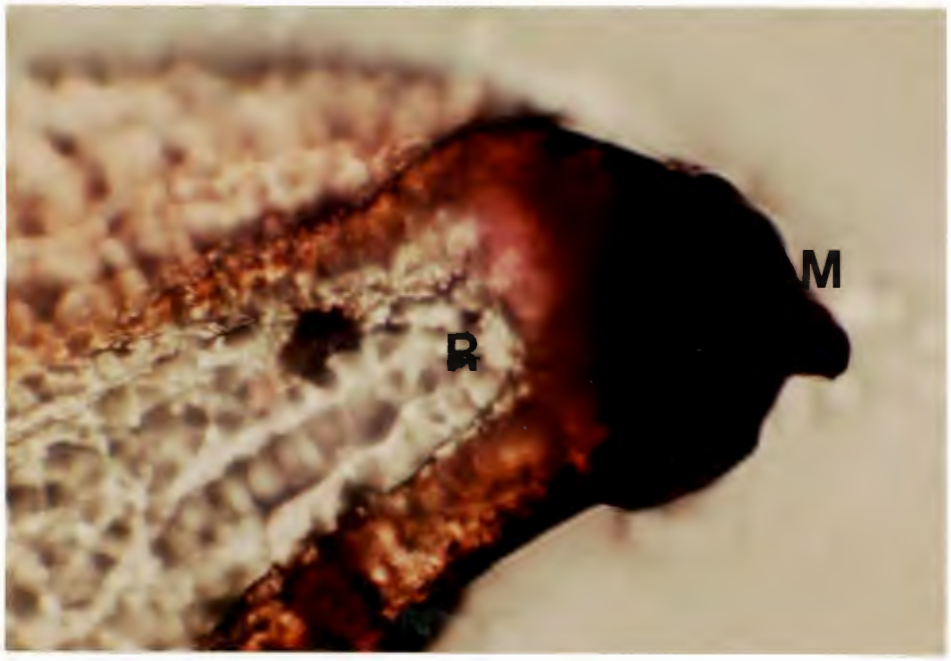


Plate 6. Dye penetration though the micropyle in *D. speciosum*. Magnification = 1.25 x 40 x 12.8. M = microphyle, R= root tip.

Hormone studies

Table 3. The mean levels of ABA (ng/gram) from 12 replicates, in control and smoke treated seeds, and the percentage in change of ABA between smoke and control treatments, for each species.

Species	Control: ABA in ng/gram	Smoke treated: ABA in ng/gram	Percentage difference
R. macowanii	42.78	35.68	16.60%
R. caroli (unscarified)	18.87	13.45	28.72%
D. stokoei	72.91	48.21	33.88%
D. speciosum	53.49	31.24	41.60%

From the results presented in Table 3 we can deduce, firstly, that the levels of ABA in the seeds of the *Drosanthemum* species are higher than those of the *Ruschia* species. However, the ABA levels of the dormant seeds in each genus are higher than those occuring in the non-dormant members of each genus. Secondly, ABA levels are always reduced by smoke in these four species. Thirdly, the percentage change of ABA between smoke and control treatments was greater for the Karoo species (both smoke-responsive) *R. caroli* and *D. speciosum*, than for the fynbos species (both unresponsive to smoke). Due to the fact that assays were of whole seed material, we were unable to ascertain the position of the ABA, ie whether is occurred in the embryo or the seed coat.

Discussion

Germination trials

Ruschia macowanii and *Drosanthemum stokoei* although both occurring in the Fynbos fire-prone habitat, do not exhibit smoke-promoted seed germination. However, germination of the Karoo non-fire prone species, *Ruschia caroli* (scarified) and *Drosanthemum speciosum* is promoted by smoke treatment. These results support those obtained by Pierce *et al.* (1995). Differences in the germination responses to smoke among species may be attributed to differences in sensitivity to the active component of smoke (Sutcliffe and Whitehead, 1995), or, possibly, differences in dormancy strategies.

From our experiments we deduced that the fynbos species tested, (*R. macowanii* and *D. stokoei*) germinate easily and appear to lack dormancy mechanisms. Conversely, the Karoo species, (*R. caroli* and *D. speciosum*) do possess dormancy mechanisms. To improve the germination of *R. caroli*, seeds need to be scarified, hence germination is constrained by coat imposed dormancy. Smoke as well as scarification further promotes dormancy, indicating that this species may exhibit embryo dormancy too, which is released by smoke. *D. speciosum* seeds appear to exhibit embryo imposed dormancy, which is released by smoke. Thus we can conclude that for the species tested here, smoke-promoted germination is a feature of seeds that have internal dormancy mechanisms, and is not a feature of species that lack such mechanisms. Results from previous reports (see Dixon *et al.*, 1995, and references there in) offer further support that dormant seeds respond positively to smoke. However, the are no apparent comparisons between the effect of smoke on dormant versus non-dormant seeds.

The germination percentages of seeds that were smoke treated reported in this study were higher than those reported by Pierce *et al.* (1995) for these species. This could, in part be attributed to the fact that Pierce *et al.* (1995) used aqueous extracts of smoke in their smoke treatment. The results from this study indicate that the presence of moisture in seeds prior to smoke treatment inhibits the effect of smoke to some extent in all species except *R. caroli*. Similar, but less pronounce inhibition may occur when aqueous extract of smoke is used in place of dry smoke. The mechanism of inhibition may have something to do with the effect of water on the sites or biochemical pathways that respond to smoke.

Sutcliffe and Whitehead (1995) found that imbibition of seeds with smoke-saturated water for one hour stimulated germination in *Cyclopodia intermedia* but longer treatment resulted in drastic inhibition of seeds germination in both *C. intermendia* and *C. subternata*. Those authors suggested that inhibition of germination by the continued presence of these short-chain saturated fatty acids such as octanoic acid, "could be the result of their incorporation into cell membranes in such a way that the integrity of the membranes is affected". From the results in the study presented here, and those indicated in Sutcliffe and Whitehead (1995), it seems likely that the presence of water during, or prior, to smoke treatment may play some role in the inhibition mechanism prosed by Sutcliffe and Whitehead (1995), or others not yet described.

The fact that the Karoo species, *R. caroli* and *D. stokoei* come from a non-fire prone environment and yet show improved germination after smoke treatment indicates that smoke cued germination is not an evolved feature. This offers support to the argument of Baxter *et al.* (1995) that the wide range of positive responses of seed germination to plant derived smoke indicates the active component(s) of smoke may originate from components that occur commonly in plants (Baxter *et al.*, 1995).

Dye penetration

Dye penetration studies corroborate the above suggestion: dormant seeds are responsive to smoke whereas non-dormant seeds are not. The non-dormant seeds, *R. macowanii* and *D.stokoei* show no significant change in dye penetration patterns between smoke and control treatments. Personal observations in the mechanisms of water uptake indicate that the water uptake in *R. macowanii* appears to occur through mechanical breakages in the seed coat and entrance though the hilum. In *D. stokoei* water uptake also occurs freely though the hilum, and possibly though the micropyle.

In the dormant species, *R. caroli* and *D. speciosum* water penetration is inhibited in at the site of the hilum and at the membrane below the seed coat that binds the endosperm. Dye penetration data reported in this study on *D. speciosum*, and preliminary results for scarified *R. caroli* seeds suggest that treating the seeds with smoke over comes both these barriers, thereby releasing dormancy.

From dye penetration and germination experiments, Fotheringham (pers. comm.) suggested that some type of membrane or barrier between the seed coat and the endosperm that prevents water uptake and gaseous exchange. She further suggests that this barrier is chemically deteriorated by a component of smoke. As previously mentioned Sutcliffe and Whitehead (1995) suggest that the action of short-chain saturated fatty acids, a component of plant derived smoke, help improve germination by increasing ethylene sensitivity. Those authors suggest that this occurs by the change in membrane properties by the action of short-chain fatty acids. Thomas, (pers comm.), also suggested that smoke may effect the properties of the membranes. His theory is that smoke stimulates ion pumps within the membranes. The suggestions of Thomas (pers. comm.) and Sutcliffe and Whitehead (1995) of Sutcliffe support the observations in this report and that of Fotheringham (pers comm.), that the barrier that

smoke alters, resulting in dormancy release, is the membrane surrounding the endosperm.

Seed coat characteristics

R. macowanii is unique in having a waxy cuticle. This is the only species that showed a negative germination response to smoke. The negative effect of smoke on this species could either be the result of the smoke causing the cuticle to become even less hydroscopic, though some chemical interaction. Alternatively, the presence of smoke residue on the waxy cuticle, may cause the seed to become less permeable to water and gas exchange. As mentioned earlier, this is a non dormant species, and despite the waxy cuticle dye penetration studies showed that water uptake through the hilum is not hindered.

The dormant species *R. caroli* and *D. speciosum* have no apparent block to water, although *R. caroli* needs to be scarified. This suggests some form of embryo imposed dormancy is present in both species.

The presence of structures, assumed to be micropyles, in all four species that correspond to the root cap is noteworthy. From observations made in the dye penetration experiment, dye penetration appeared to take place through this structure, particularly in smoke-responsive, smoke treated seeds. These structures may therefore be important sites of gas exchange and water penetration. The may therefore be sites at which smoke released dormancy may operate.

One observation that was made during the germination experiments was the development of fungal infections. Only seeds that had been smoke treated, or soaked and smoke treated, became infected with fungus. Control seeds of all species however did not become noticeably infected with fungus. This may suggest that the germination of fungal spores may be stimulated in some way by smoke. These fungal communities could play a role in breaking seed dormancy through mechanically degrading the seeds coat. In the environment of the petri dish, the fugal infections became detrimental to seed germination, but if the development of these fungal communities is more controlled in natural environments, it may confer a germination advantage.

Some work has provided evidence for the role of fungi in dormancy release. " Natural scarification occurs in the environment whereby microorganisms erode or breakdown the seed coat prior to germination" (Morpeth *et al.*, 1995). Techniques that enhance microbial growth have been used that result in higher germination percentages in *Rosa corymbifera* 'Laxa'. The addition of a compost activator increased the microbial activity, and improved seed germination. Hence the breakdown of seed coats by smoke enhanced microbial activity may be an important smoke associated mechanism in releasing dormancy in seeds that have coat imposed dormancy. In smoke-responsive seeds which exhibit embryo imposed dormancy, the components of smoke will be acting directly on the seed, specifically membranes and germination inhibitors.

ABA Analysis

ABA is thought to play a major role in seed dormancy in many species and could be considered a general germination inhibitor (Bewley and Black, 1994). Our results show that smoke does reduce the level of ABA in seeds in both smoke-responsive (dormant) species and unresponsive (non-dormant) species. If the percentage change was similar in dormant versus non dormant seeds, it may indicate that a reduction in ABA levels caused by smoke treatment does not confer an improved germination response. However, the percentage change in ABA was greater in the responsive species than in the unresponsive species. Thus, smoke induced reductions in the level of ABA in smoke responsive dormant seeds very well play a role in releasing dormancy. However, this is, as yet, speculative. The reduction in ABA levels by smoke may be the primary process involved in releasing dormancy in smoke-responsive species. However, the fact that ABA levels are also reduced in non-responsive species indicates that this may not be the case. Smoke reduced ABA levels are more likely to be a step in a more complex chain of events, uncommon to unresponsive (non-dormant) species, that ultimately results in dormancy release. The possibility that the smoke-responsive species (*R. caroli* (scarified) and *D. speciosum*) may differ from the unresponsive species in their sensitivity to changes in ABA levels, should also be considered (Bewley and Black, 1994).

Due to the fact that the ABA extractions were done on material from intact seeds, the position in the seeds in which ABA occurs in each species is unknown. It is possible that

ABA occurs in different positions (embryo or seed coat) in the smoke-responsive dormant species to that in the unresponsive, non-dormant species (Bewley and Black, 1994). If such differences occur, they may, in part, explain why changes in the level ABA were associated with smoke-stimulated response in some species but not others. The interaction between smoke, dormancy, ABA and improved germination clearly requires further rigorous testing.

Future research

The effect of smoke treatments on scarified *R. caroli* sees was tested only for its effect on the germination. Further experiments need to be conducted for *R. caroli* on: the effect of water+smoke on germination of scarified seeds; the patterns of dye penetration of control and smoke treated scarified seeds; and the levels of ABA in smoke and control treated scarified seeds. The effect of smoke+smoke water on ABA levels, and dye penetration needs to be investigated in all the species.

Interactions between smoke and fungal spores, and the subsequent effect of fungal activity on seed germination is also of interest.

Final remarks

For the four species of Mesembryanthemum investigated, smoke-promoted germination is a characteristic of dormant seeds but not non-dormant ones. Whether this is a general trend is unknown. The germination of non-dormant seeds in some species may be enhanced by smoke, therefore, in studies conducted in this field, the distinction between smoke-stimulated germination, and smoke-induced dormancy release should be made. Further studies undertaken on the effect of smoke on seed germination should determine, or incorporate information, on the type of dormancy and germination mechanisms characteristic of the species intended for investigated

There are different physiological responses following, smoke treatment, between species whose seeds are smoke-responsive to those which are unresponsive. These include: changes in the patterns of water penetration possibly related to changes in membrane properties, and greater reductions in the level of ABA in responsive species compared to unresponsive species. However, these may be only a few effects, or mechanisms, out of several involved

in smoke-induced dormancy release.

The major difference between the fire-prone fynbos species that were unresponsive to smoke, and the non-fire-prone Karoo species was that the Karoo species were dormant whereas the fynbos species were non-dormant. The occurrence or absence of dormancy may explain the discrepancy found in the germination response of fire-prone fynbos species and non-fire prone Karoo species to smoke found by Pierce *et al.* (1995). It would be worthwhile therefore to compare the physiological responses of closely related dormant and non-dormant species from fire prone habitats to dormant and non-dormant species from non-fire-prone habitats.

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