South African Journal of Botany 90 (2014) 87–92

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Smoke-water enhances *in vitro* pollen germination and tube elongation of three species of Amaryllidaceae

H.B. Papenfus, A. Kumari, M.G. Kulkarni, J.F. Finnie, J. Van Staden *

Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville, 3209, South Africa

ARTICLE INFO

Article history:
Received 7 October 2013
Received in revised form 16 October 2013
Accepted 22 October 2013
Available online 18 November 2013

Edited by MI Daws

Keywords:
Karrikinolide
Pollen germination
Pollen tube
Smoke-water
3,4,5-Trimethylfuran-2(5*H*)-one

ABSTRACT

Smoke-water prepared from burning plant material and smoke-derived compounds significantly promote seed germination and enhance growth of many plant species. Since large amounts of smoke are generated and released into the air during wildfires, it is possible that angiosperm pollen germination and pollen tube elongation may be affected by plant-derived smoke even when the plants are some distance from the fire. We assessed the effect of smoke on pollen germination and pollen tube elongation for three species of Amaryllidaceae that occur naturally in areas prone to winter fire in South Africa. *In vitro* pollen germination and pollen tube growth of *Clivia gardenii*, *Cyrtanthus mackenii* and *Scadoxus multiflorus* were assessed by preparing hanging drop slides with different concentrations of smoke-water, karrikinolide and 3,4,5-trimethylfuran-2(5*H*)-one combined with Brewbaker and Kwack's medium and a sucrose and boric acid medium. These slides were incubated for 2 h at 25 °C. Pollen germination and pollen tube lengths were recorded by capturing images with a compound microscope aided by a digital camera. Low concentrations of smoke-water (1:1000 and 1:2000 v:v) significantly increased pollen germination and pollen tube length in the three species, when applied alone or in combination with either Brewbaker and Kwack's medium or sucrose and boric acid medium. Low concentrations of smoke-water significantly increased *in vitro* pollen germination and pollen tube growth even when no additional calcium was added. Consequently, smoke from grassland fires may have favourable implications for the reproductive process of flowering plants.

1. Introduction

Fire has a great influence on vegetation ecology. Shrub species from fire-prone ecosystems show diverse responses to fire with post-fire regeneration evident in shrublands around the world (Keeley and Zedler, 1978; Kruger, 1977). Smoke generated from burning plant material is now widely recognized as an important germination and seedling growth cue for many plant species (Downes et al., 2013; Kulkarni et al., 2007; Light and Van Staden, 2004). In fire-prone areas, fire-stimulated flowering is also a common phenomenon, especially in herbaceous plants (Gill and Groves, 1981; Rundel, 1981). For example, flowering of *Cyrtanthus ventricosus* (Amaryllidaceae), a fynbos geophyte commonly known as the 'fire-lily', is associated with fire (Le Maitre and Brown, 1992; Olivier and Werner, 1980). Fire also plays a role in mass flowering of *Watsonia borbonica* (Iridaceae) which leads to abundant fruit set (Le Maitre, 1984) and seedling recruitment (Kruger, 1978; Kruger and Bigalke, 1984; Le Maitre, 1984). Greater fruiting has also been observed in the grasstree *Xanthorrhoea preissii*, in summer-burnt populations compared to autumn- and spring-burnt populations (Lamont et al., 2000). Despite numerous reports of fire-stimulated flowering, no study has been carried out on the effect of smoke on angiosperm pollen.

Smoke-water (SW), prepared by bubbling plant-derived smoke through distilled water, has been used extensively as a "smoke equivalent" in many studies (Adkins and Peters, 2001; Baxter et al., 1994; Doherty and Cohn, 2000; Drewes et al., 1995; Kulkarni et al., 2011). Some of the active principles in smoke have been identified and the activity is still being studied. Karrikinolide (KAR_1), the first biologically active compound isolated from smoke, promotes seed germination in a wide variety of species (Chiwocha et al., 2009; Light et al., 2009). These reviews indicate that smoke-water and the smoke-derived compounds act like plant growth regulators in stimulating seed germination. 3,4,5-Trimethylfuran-2(5*H*)-one (trimethylbutenolide (TMB)) is also present in plant-derived smoke and inhibits the promotory activity of KAR_1 (Light et al., 2010).

In vitro pollen growth studies are largely aimed at determining the growth requirements of pollen from different plant species (Abdelgadir et al., 2012; Bolat and Pirlak, 1999; Brewbaker and Kwack, 1963; Franklin-Tong, 1999; Khatun and Flowers, 1995; Kumari et al., 2009; Lyra et al., 2011; Sato et al., 1998; Tuinstra and Wedel, 2000; Wang et al., 2004). Various plant growth regulators such as auxins, brassinosteroids, cytokinins and gibberellic acid have been found to induce pollen germination and pollen tube growth in several species (Bamzai and Randhawa, 1967; Hewitt et al., 1985; Singh et al.,

* Corresponding author. Tel.: +27 33 260 5130.
E-mail address: rcpgd@ukzn.ac.za (J. Van Staden).

0254-6299/$ – see front matter © 2013 SAAB. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.sajb.2013.10.007

2002; Smith, 1942; Voyiatzis and Paraskevopoulou-Paroussi, 2000). We predict that given the plant growth regulator-like germination inducing effects of smoke, smoke may also have an effect on pollen.

Fire is a common phenomenon in most regions of South Africa with the majority of the flora being exposed to smoke from naturally occurring wildfires. Fire plays an important role as a germination stimulant and assures the reproductive success of many plant species. It is possible that smoke from these fires may affect the reproductive success of plants at the pollen level. In the present study, the effect of smoke-water and two smoke-derived compounds was tested on pollen germination and pollen tube growth of three Amaryllidaceae species, all of which are generally found in fire-prone areas.

2. Materials and methods

2.1. Flower and pollen collection

The three Amaryllidaceae species used in this study were *Clivia gardenii* Hook., *Cyrtanthus mackenii* Hook.f. subsp. *mackenii* and *Scadoxus multiflorus* (Martyn) Raf. subsp. *multiflorus*. These species produce hermaphrodite flowers in an umbel arrangement. Flowers were collected from the University of KwaZulu-Natal's botanical garden (Pietermaritzburg, South Africa; S 29° 37.50′, E 30° 24.23′). Following anthesis, anthers were collected from flowers between 07:00 and 09:00 in the morning.

2.2. Pollen viability

Three pollen staining methods were used to differentiate between viable and dead pollen. For all three staining methods, a small quantity of pollen grains was transferred to a drop of dye, mixed thoroughly to a homogenous pollen suspension using a pin and covered with a cover slip. Pollen grains were observed with an Olympus AX70 fluorescence microscope (Camera Nikon DS-Ri1, Tokyo, Japan). The number of viable pollen grains was recorded in each field of view out of the total number of pollen grains. Pollen grains were observed in four different fields of view (replicated four times). The dyes used were: (1) Aqueous 2,3,5-triphenyl tetrazolium chloride (TTC, Merck) [pollen grains that turned red under fluorescence were considered viable (Abdelgadir et al., 2012; Hauser and Morrison, 1964; Khatun and Flowers, 1995; Stanley and Linskens, 1974)]. (2) Aniline blue-lactophenol (ANB, Merck) staining solution consisting of 5 mL phenol, 20 mL lactic acid, 40 mL glycerol, and 20 mL distilled water (Kearns and Inouye, 1993) [pollen grains were considered viable if they fluoresced blue (Kearns and Inouye, 1993; Khatun and Flowers, 1995; Wang et al., 2004)]. (3) Fluorescein diacetate (FDA) (Sigma-Aldrich) dissolved in acetone (2 mg mL^{-1}) and used in combination with 10^{-6} M sucrose solution was used for the fluorochromatic reaction (FCR) [pollen grains that fluoresced brightly were taken as viable (Heslop-Harrison and Heslop-Harrison, 1970; Heslop-Harrison et al., 1984; Jain and Shivanna, 1988; Kearns and Inouye, 1993; Khatun and Flowers, 1995; Shivanna and Heslop-Harrison, 1981; Wang et al., 2004)]. The microscope slides for all three staining methods were kept in humidity chambers (>90% RH) and placed in the dark for 1 h at 25 °C.

2.3. In vitro pollen germination and pollen tube elongation

All test solutions were evaluated for activity individually and in combination with either Brewbaker and Kwack's (BWK) medium and sucrose or boric acid (SB) medium. The BWK medium was prepared by making a 10% sucrose solution to which 100 mg L^{-1} boric acid, 300 mg L^{-1} calcium nitrate, 100 mg L^{-1} potassium nitrate and 200 mg L^{-1} magnesium sulphate were added (Brewbaker and Kwack, 1963; Shivanna and Rangaswamy, 1992). The SB medium consisted of a 10% sucrose solution with 100 mg L^{-1} boric acid (Kumari et al., 2009; Linskens, 1967; Shivanna and Rangaswamy, 1992). Smoke-water (SW) was prepared by burning 5 kg dry *Themeda triandra* (Poaceae) leaf material in a 20 L metal drum and bubbling the smoke through 500 mL distilled water for 45 min. The SW solutions, 1:500 (v:v), 1:1000 (v:v) and 1:2000 (v:v), were prepared by diluting 1 part SW in 500, 1000 and 2000 parts distilled water. Karrikinolide and 3,4,5-trimethylfuran-2(5*H*)-one [trimethylbutenolide (TMB)] were isolated from SW as described in Van Staden et. al. (2004), and Light et al. (2010), respectively. The different liquid media used were as follows: (1) distilled water; (2) BWK; (3) SB; (4) gibberellic acid, GA_3 (10^{-4} M); (5) GA_3 (10^{-5} M); (6) SW (1:500); (7) SW (1:1000); (8) SW (1:2000); (9) BWK + SW (1:500); (10) BWK + SW (1:1000); (11) BWK + SW (1:2000); (12) SB + SW (1:500); (13) SB + SW (1:1000); (14) SB + SW (1:2000); (15) karrikinolide, KAR_1 (10^{-6} M); (16) KAR_1 (10^{-7} M); (17) KAR_1 (10^{-8} M); (18) BWK + KAR_1 (10^{-6} M); (19) SB + KAR_1 (10^{-6} M); (20) TMB (10^{-3} M); (21) BWK + TMB (10^{-3} M) and (22) SB + TMB (10^{-3} M). Since the 10^{-6} M concentration of KAR_1 gave the best results in the absence of the sucrose media, this concentration was used in combination with the two sucrose media. Similarly, the 10^{-3} M TMB solution gave the best results, and no other concentrations were therefore included. All chemicals that are not otherwise specified were obtained from Sigma-Aldrich.

Six hanging drop slides were prepared for each test solution (replicated four times). A thin film of petroleum jelly was applied to the rim of the cavity of each cavity slide to prevent evaporation of the test solutions. A consistent amount of pollen grains were transferred from the anthers to each hanging drop slide and mixed into a homogenous pollen suspension using a pin. Preparation of the slides was scheduled in a manner that allowed for the images to be captured exactly 2 h following incubation at 25 °C. Images were taken using a compound microscope (Olympus AX70; Camera Nikon DS-Ri1, Tokyo, Japan). Pollen grains were considered germinated when an intact tube had emerged from them. The percentage of germinated pollen grains was calculated by counting the number of germinated pollen grains out of the total number of pollen grains per field of view. Pollen tube lengths were determined by analysing the images captured using the on-board NIS elements BR4.00.016 software. Mean pollen tube length was calculated as the average length of 20 pollen tubes per replicate (replicated three times).

2.4. Statistical analysis

Statistical analysis of the data was conducted using a one-way analysis of variance (ANOVA) (GenStat®, Edition 14). Significant differences were determined using the Duncan's multiple range test ($P < 0.05$).

3. Results

3.1. Pollen viability

The three staining methods, TTC, ANB and FCR successfully stained and indicated viable pollen grains. Viability ranged between 92.2%–94.2%, 76.9%–90.1% and 75.0%–91.0% for *C. gardenii*, *C. mackenii* and *S. multiflorus*, respectively (Fig. 1). No significant differences were found between the three staining methods.

3.2. In vitro pollen germination

In vitro pollen germination was initiated within the first 30 min with BWK and SB media. Optimum pollen germination was achieved with BWK medium. In the absence of the media (BWK medium and SB medium), low concentrations of SW (1:1000 and 1:2000) significantly increased germination in all three species (Table 1). Gibberellic acid (10^{-4} and 10^{-5} M) also produced significantly higher germination compared to the water control (Table 1). In the presence of both media, low concentrations of SW (1:1000 and 1:2000) significantly increased germination in *C. gardenii* and *S. multiflorus* (Fig. 2A and C). Karrikinolide (10^{-6} M) showed significantly greater pollen germination

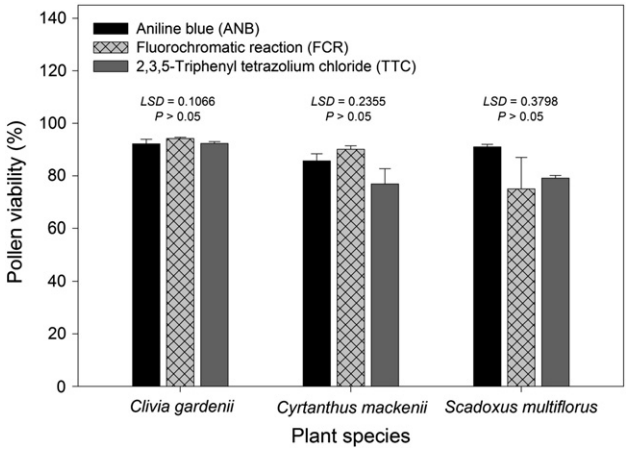

Fig. 1. Pollen viability of *Clivia gardenii*, *Cyrtanthus mackenii* and *Scadoxus multiflorus* as determined by three fluorescent staining methods ($n = 16$).

in all three species compared to the controls in the absence of media (Table 1) and produced significantly higher germination in *C. gardenii* when incubated with both media (Fig. 2A). Trimethylbutenolide showed significantly higher pollen germination percentages for *S. multiflorus* and *C. gardenii* compared to their respective controls in the absence of media (Table 1) but had no significant effect on germination of any species when combined with BWK or SB medium (Fig. 2).

3.3. In vitro pollen tube elongation

In the absence of media, low concentrations of SW (1:1000 and 1:2000) significantly increased pollen tube length compared to the other treatments in all three species (Table 1). Gibberellic acid (10^{-4} and 10^{-5} M) produced significantly longer pollen tubes compared to the water control in *C. mackenii* (Table 1). Smoke-water (1:1000 and 1:2000) used in combination with BWK and SB produced significantly longer pollen tubes compared to their respective controls in all three species (Fig. 3). In one instance, KAR_1 (10^{-6} M) significantly increased *C. gardenii* pollen tube length compared to the other treatments in the absence of media (Table 1) and produced significantly longer pollen tubes in *S. multiflorus* in the presence of both media (Fig. 3E and F). Trimethylbutenolide produced significantly longer pollen tubes in *S. multiflorus* compared to the control in the absence of media (Table 1). This compound also produced significantly longer pollen tubes in *C. mackenii* and *S. multiflorus* when combined with both media (Fig. 3C, D, E and F).

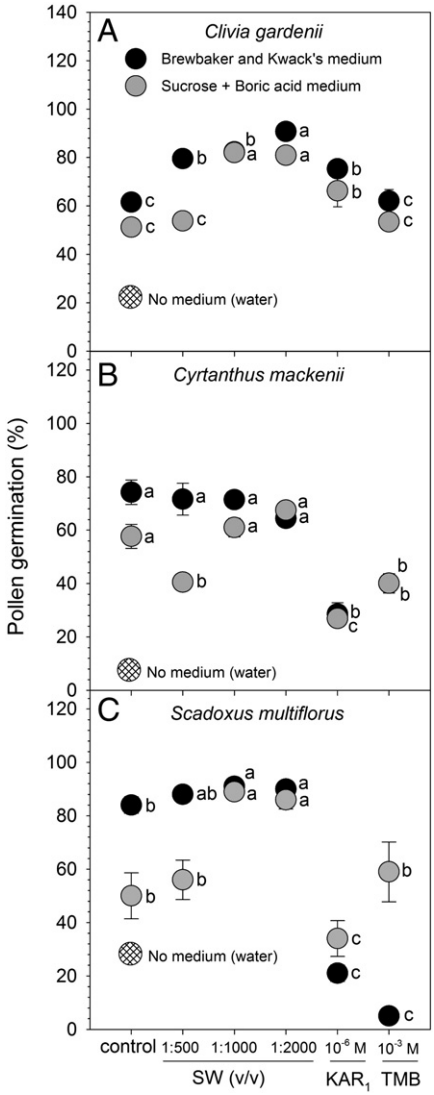

Fig. 2. The effect of different concentrations of smoke-water (SW), karrikinolide (KAR_1) and trimethylbutenolide (TMB) on pollen germination of three Amaryllidaceae species applied in combination with two sucrose media. Symbols (±SE) of each species and medium with a different letter are significantly different according to Duncan's multiple range test ($P < 0.05$) ($n = 24$).

Table 1
The effect of gibberellic acid (GA_3), smoke-water (SW), karrikinolide (KAR_1) and trimethylbutenolide (TMB) on pollen germination ($n = 24$) and pollen tube length ($n = 60$) of three Amaryllidaceae species in the absence of media.

Treatments	Species					
	Clivia gardenii		*Cyrtanthus mackenii*		*Scadoxus multiflorus*	
	Germination (%)	Tube length (μm)	Germination (%)	Tube length (μm)	Germination (%)	Tube length (μm)
Water (control)	24.1 ± 2.5 fg	29.5 ± 1.6 e	7.5 ± 2.2 d	49.5 ± 4.8 c	26.0 ± 6.4 e	90.7 ± 13.6 de
GA_3 (10^{-4} M)	59.9 ± 5.1 bc	45.7 ± 4.2 de	30.1 ± 2.0 b	101.3 ± 8.9 b	82.8 ± 1.2 ab	58.0 ± 4.6 e
GA_3 (10^{-5} M)	57.4 ± 3.5 c	55.0 ± 2.5 d	25.8 ± 1.5 b	105.3 ± 8.9 b	75.2 ± 2.2 ab	60.7 ± 5.0 e
SW (1:500 v:v)	59.8 ± 2.1 bc	42.1 ± 2.3 de	21.1 ± 1.2 bc	78.5 ± 9.6 bc	36.1 ± 3.6 de	213.5 ± 7.1 a
SW (1:1000 v:v)	70.3 ± 1.8 ab	170.5 ± 9.5 b	40.6 ± 3.7 a	166.3 ± 16.7 a	84.2 ± 2.5 a	188.7 ± 35.9 ab
SW (1:2000 v:v)	71.6 ± 4.7 a	111.6 ± 4.5 c	40.6 ± 6.9 a	172.8 ± 17.2 a	78.8 ± 1.3 ab	222.2 ± 7.6 a
KAR_1 (10^{-6} M)	50.6 ± 3.1 cd	241.6 ± 10.8 a	25.6 ± 2.5 b	67.2 ± 7.8 c	69.3 ± 3.0 cd	128.7 ± 8.5 cd
KAR_1 (10^{-7} M)	32.7 ± 4.9 ef	43.7 ± 6.6 de	10.5 ± 3.6 cd	46.4 ± 6.1 c	66.4 ± 6.1 abc	141.8 ± 5.2 c
KAR_1 (10^{-8} M)	17.3 ± 1.6 g	29.9 ± 3.1 e	10.9 ± 1.2 cd	62.2 ± 8.3 c	67.2 ± 1.7 abc	75.5 ± 6.3 e
TMB (10^{-3} M)	44.4 ± 3.5 de	28.4 ± 2.1 e	8.4 ± 1.5 d	56.2 ± 5.3 c	72.8 ± 1.4 abc	153 ± 21.4 bc

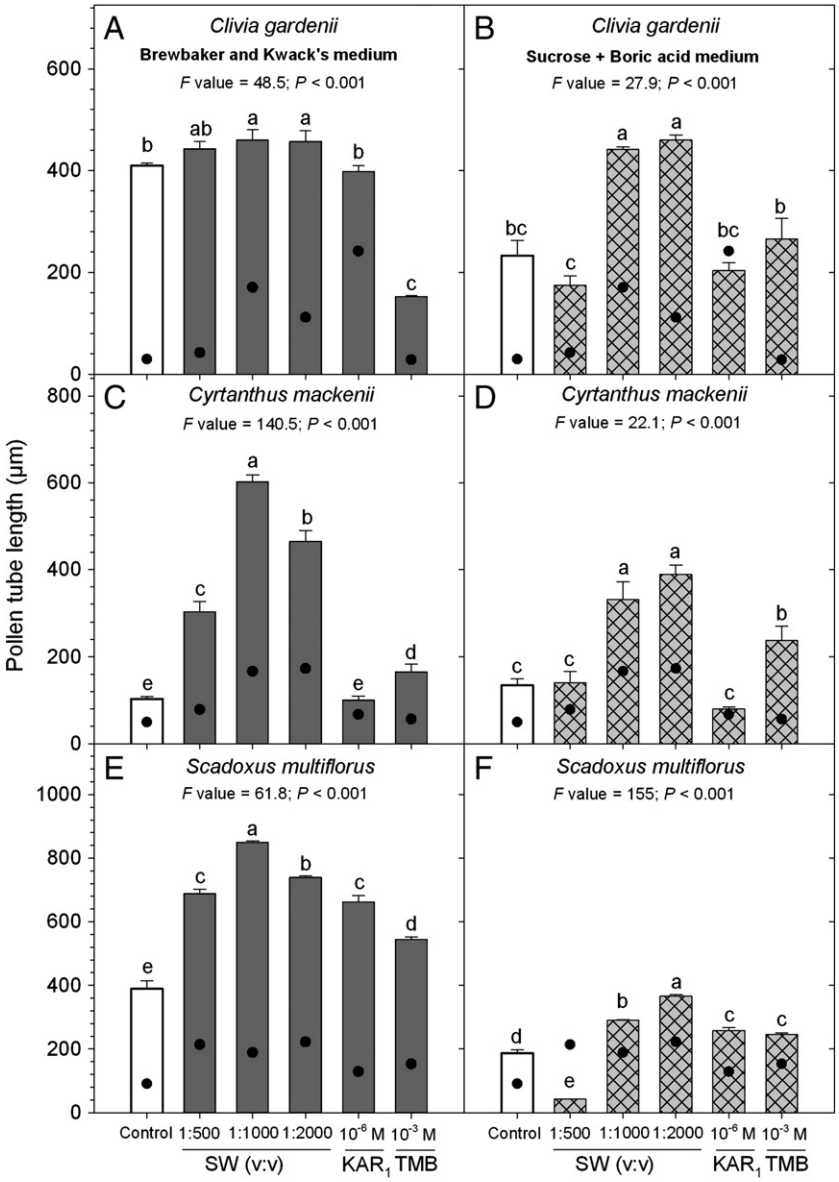

Fig. 3. The effect of different concentrations of smoke-water (SW), karrikinolide (KAR_1) and trimethylbutenolide (TMB) on pollen tube length of three Amaryllidaceae species. Bars (±SE) of each species and medium with different letters are significantly different according to Duncan's multiple range test ($P < 0.05$). Dark symbols on the bars indicate mean pollen tube length without media ($n = 60$).

4. Discussion

Many studies have reported on the growth requirements and viability of pollen of certain groups of plant species (Abdelgadir et al., 2012; Bolat and Pirlak, 1999; Brewbaker and Kwack, 1963; Franklin-Tong, 1999; Khatun and Flowers, 1995; Kumari et al., 2009; Lyra et al., 2011; Sato et al., 1998; Tuinstra and Wedel, 2000; Wang et al., 2004). Addicott (1943) showed that certain vitamins and growth substances have the potential to increase pollen germination and elongation under *in vitro* conditions. The addition of growth regulators such as auxins, brassinosteroids, cytokinins and gibberellic acid stimulate pollen germination and pollen tube growth in several species (Bamzai and Randhawa, 1967; Hewitt et al., 1985; Singh et al., 2002; Smith, 1942; Voyiatzis and Paraskevopoulou-Paroussi, 2000). These studies indicated that pollen growth can be manipulated in *in vitro* systems and that plant growth regulators play a role in the normal germination and growth of pollen.

This is the first report on the use of SW and smoke-derived compounds to promote pollen germination and pollen tube growth. In the absence of sucrose containing media, low concentrations of SW (1:1000 and 1:2000) significantly increased pollen germination and pollen tube elongation in all three species, respectively (Table 1). These results were significantly different to the GA_3 treatments. This is an important result since these solutions contained no added sucrose, calcium (Ca) and boron which are known to be prerequisites for successful *in vitro* pollen germination and pollen tube elongation (Brewbaker and Kwack, 1963). This indicates that SW has the ability to stimulate pollen germination and pollen tube growth even when the precise medium requirements are not met. If SW stimulates pollen growth in a wide variety of species then it could be a useful tool in breeding studies.

Maximal pollen germination and pollen tube lengths were found when the pollen grains were treated with low concentrations of SW in the presence of BWK medium. Although the BWK medium supplied

the necessary sucrose and Ca, SW (1:1000) still resulted in significantly higher pollen germination percentages (Fig. 2) and longer pollen tubes in all three studied species (Fig. 3). This result indicates that there might be other compounds present in SW that stimulate pollen growth. This idea is supported by the SB + SW (1:2000) results (Fig. 3), which showed that pollen tube length was doubled compared to the respective SB control in all three tested species. Considering that the SB medium contained no added Ca, these results indicate that SW has the ability to alleviate the Ca requirement for germination and tube growth of *in vitro* germinated pollen.

The seed germination stimulating effect documented for KAR_1 (Chiwocha et al., 2009; Light et al., 2009) and the inhibitory effects of TMB (Light et al., 2010) were not analogues to the effects when applied to *in vitro* germinated pollen. Thus, compared with a promotory effect of KAR_1 on seed germination, this compound reduced pollen germination for *C. mackenii* and *S. multiflorus* and only stimulated germination in *C. gardenii* (Fig. 2). Similarly, TMB reduced pollen germination in *C. mackenii* and *S. multiflorus* compared to the respective controls but had no effect on *C. gardenii* pollen (Fig. 2). Nonetheless the inhibitory activity of this compound on pollen germination was much less than the effect it has on seed germination (Light et al., 2010). Although KAR_1 and TMB showed some promotory activity in terms of pollen tube growth (Fig. 3), these treatments were not as consistent as the SW treatments. Since there is no cell division during pollen tube elongation, SW could function in stimulating the mobilisation of the sucrose rich reserves in the pollen grains. The inconsistent activities of KAR_1 and TMB on pollen germination could be a consequence of their ability to stimulate cell division rather than reserve mobilisation.

Flowers produced by the Amaryllidaceae family have great potential for hybridization and commercialization (Niederwieser et al., 2002). However, the main constraints are irregular flowering and difficulty in manipulating flowering. Since low concentrations of SW (1:1000 and 1:2000) consistently showed significantly higher pollen germination percentages and longer pollen tubes in this study, such treatments have potential to increase the reproductive success of species in the Amaryllidaceae. It is, however, also important to determine whether SW has a similar effect on species from other plant families. The findings of this study are crucial for investigating post-fire flowering of smoke-responsive and non-responsive plant species.

Acknowledgements

We thank the National Research Foundation (NRF), South Africa and the University of KwaZulu-Natal (UKZN), South Africa for financial support. The Microscopy and Microanalysis Unit of UKZN, Pietermaritzburg, is thanked for the use of the microscopy facilities.

References

Abdelgadir, H.A., Johnson, S.D., Van Staden, J., 2012. Pollen viability, pollen germination and pollen tube growth in the biofuel seed crop *Jatropha curcas* (Euphorbiaceae). South African Journal of Botany 79, 132–139.

Addicott, F.T., 1943. Pollen germination and pollen tube growth, as influenced by pure growth substances. Plant Physiology 18, 270.

Adkins, S., Peters, N., 2001. Smoke derived from burnt vegetation stimulates germination of arable weeds. Seed Science Research 11, 213–222.

Bamzai, R., Randhawa, G., 1967. Effects of certain growth substances and boric acid on germination, tube growth and storage of grape pollen (*vitis* ssp.). Vitis 6, 269–277.

Baxter, B., Van Staden, J., Granger, J., Brown, N., 1994. Plant-derived smoke and smoke extracts stimulate seed germination of the fire-climax grass *Themeda triandra*. Environmental and Experimental Botany 34, 217–223.

Bolat, I., Pirlak, L., 1999. An investigation on pollen viability, germination and tube growth in some stone fruits. Turkish Journal of Agriculture and Forestry 23, 383–388.

Brewbaker, J.L., Kwack, B.H., 1963. The essential role of calcium ion in pollen germination and pollen tube growth. American Journal of Botany 50, 859–865.

Chiwocha, S.D., Dixon, K.W., Flematti, G.R., Ghisalberti, E.L., Merritt, D.J., Nelson, D.C., Riseborough, J.-A.M., Smith, S.M., Stevens, J.C., 2009. Karrikins: a new family of plant growth regulators in smoke. Plant Science 177, 252–256.

Doherty, L.C., Cohn, M.A., 2000. Seed dormancy in red rice (*Oryza sativa*). XI. Commercial liquid smoke elicits germination. Seed Science Research 10, 415–421.

Downes, K.S., Light, M.E., Pošta, M., Kohout, L., van Staden, J., 2013. Comparison of germination responses of *Anigozanthos flavidus* (Haemodoraceae), *Gyrostemon racemiger* and *Gyrostemon ramulosus* (Gyrostemonaceae) to smoke-water and the smoke-derived compounds karrikinolide (KAR_1) and glyceronitrile. Annals of Botany 111, 489–497.

Drewes, F., Smith, M., Van Staden, J., 1995. The effect of a plant-derived smoke extract on the germination of light-sensitive lettuce seed. Plant Growth Regulation 16, 205–209.

Franklin-Tong, V.E., 1999. Signaling and the modulation of pollen tube growth. Plant Cell 11, 727–738.

Gill, A.M., Groves, R., 1981. Fire Regimes in Heathlands and Their Plant-Ecological Effects. In: Specht, R.L. (Ed.), Heathlands and related shrublands: analytical studies (Ecosystems of the world). Elsevier, Amsterdam, pp. 61–84.

Hauser, E.J.P., Morrison, J.H., 1964. Cytochemical reduction of nitroblue–tetrazolium as an index of pollen viability. American Journal of Botany 51, 748–753.

Heslop-Harrison, J., Heslop-Harrison, Y., 1970. Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. Biotechnic & Histochemistry 45, 115–120.

Heslop-Harrison, J., Heslop-Harrison, Y., Shivanna, K.R., 1984. The evaluation of pollen quality, and a further appraisal of the fluorochromatic (FCR) test procedure. Theoretical and Applied Genetics 67, 367–375.

Hewitt, F., Hough, T., O'Neill, P., Sasse, J., Williams, E., Rowan, K., 1985. Effect of brassinolide and other growth regulators on the germination and growth of pollen tubes of *Prunus avium* using a multiple hanging-drop assay. Functional Plant Biology 12, 201–211.

Jain, A., Shivanna, K.R., 1988. Storage of pollen grains in organic solvents: effects of organic solvents on leaching of phospholipids and its relation to pollen viability. Annals of Botany 61, 325–330.

Kearns, C.A., Inouye, D.W., 1993. Techniques for Pollination Biologists. University Press of Colorado, Colorado, Niwot.

Keeley, J.E., Zedler, P.H., 1978. Reproduction of chaparral shrubs after fire: a comparison of sprouting and seeding strategies. American Midland Naturalist 99, 142–161.

Khatun, S., Flowers, T.J., 1995. The estimation of pollen viability in rice. Journal of Experimental Botany 46, 151–154.

Kruger, F.J., 1977. Ecology of Cape fynbos in relation to fire. Symposium of Environmental Consequences of Fire and Management in Mediterranean Ecosystems, United States Department of Agriculture and Forest Service General Technical Report WO, pp. 391–396.

Kruger, F.J., 1978. Some Aspects of the Demography of *Watsonia pyramidata* (andr.) Stapf in relation of Fire. Joint SAAB and GSSA congress, Bloemfontein.

Kruger, F.J., Bigalke, R.C., 1984. Fire in Fynbos. In: Booysen, P.V., Tainton, N.M. (Eds.), Ecological Effects of Fire in South African Ecosystems. Springer Berlin Heidelberg, pp. 67–114.

Kulkarni, M.G., Ascough, G.D., Van Staden, J., 2007. Effects of foliar application of smoke-water and a smoke-isolated butenolide on seedling growth of okra and tomato. Hortscience 42, 179–182.

Kulkarni, M.G., Light, M.E., Van Staden, J., 2011. Plant-derived smoke: old technology with possibilities for economic applications in agriculture and horticulture. South African Journal of Botany 77, 972–979.

Kumari, A., Komal, R., Rajesh, R., Pandey, A.K., 2009. *In Vitro* pollen germination, pollen tube growth and pollen viability in *Trichosanthes dioica* Roxb. (Cucurbitaceae). The International Journal of Plant Reproductive Biology 1, 147–151.

Lamont, B.B., Swanborough, P.W., Ward, D., 2000. Plant size and season of burn affect flowering and fruiting of the grasstree *Xanthorrhoea preissii*. Austral Ecology 25, 268–287.

Le Maitre, D., 1984. A short note on seed predation in *Watsonia pyramidata* (Andr.) Stapf in relation to season of burn. South African Journal of Botany 50, 407–415.

Le Maitre, D.C., Brown, P.J., 1992. Life Cycles and Fire-Stimulated Flowering in Geophytes. In: Van Wilgen, B.W., Richardson, D.M., Kruger, F.J., Hensbergen, H.J. (Eds.), Fire in South African Mountain Fynbos. Springer Berlin Heidelberg, pp. 145–160.

Light, M.E., Burger, B.V., Staerk, D., Kohout, L., Van Staden, J., 2010. Butenolides from plant-derived smoke: natural plant-growth regulators with antagonistic actions on seed germination. Journal of Natural Products 73, 267–269.

Light, M.E., Daws, M.I., Van Staden, J., 2009. Smoke-derived butenolide: towards understanding its biological effects. South African Journal of Botany 75, 1–7.

Light, M.E., Van Staden, J., 2004. The potential of smoke in seed technology. South African Journal of Botany 70, 97–101.

Linskens, H.F., 1967. Pollen. In: Ruhland, W. (Ed.), Handbuch der Pflanzenphysiologie, Berlin and New York, pp. 368–406.

Lyra, D., Sampaio, L., Pereira, D., Silva, A., Amaral, C., 2011. Pollen viability and germination in *Jatropha ribifolia* and *Jatropha mollissima* (Euphorbiaceae): species with potential for biofuel production. African Journal of Biotechnology 10, 368–374.

Niederwieser, J.G., Terblanche, M., Spreeth, M.H., 2002. Potential of South African members of the Amaryllidaceae for new crop development. Acta Horticulturae 570, 359–365.

Olivier, W., Werner, W., 1980. The genus *Cyrtanthus* Ait. Veld and Flora 66, 78–81.

Rundel, P.W., 1981. Fire as an Ecological Factor. In: Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H. (Eds.), Physiological Plant Ecology I. Springer, Berlin Heidelberg, pp. 501–538.

Sato, S., Katoh, N., Iwai, S., Hagimori, M., 1998. Establishment of reliable methods of *in vitro* pollen germination and pollen preservation of *Brassica rapa* (syn. *B. campestris*). Euphytica 103, 29–33.

Shivanna, K.R., Heslop-Harrison, J., 1981. Membrane state and pollen viability. Annals of Botany 47, 759–770.

Shivanna, K.R., Rangaswamy, N.S., 1992. Pollen Biology: A Laboratory Manual. Springer-Verlag, Berlin.

Singh, D.P., Jermakow, A.M., Swain, S.M., 2002. Gibberellins are required for seed development and pollen tube growth in *Arabidopsis*. Plant Cell 14, 3133–3147.

Smith, P.F., 1942. Studies of the growth of pollen with respect to temperature, auxins, colchicine and vitamin B1. American Journal of Botany 29, 56–66.

Stanley, R.G., Linskens, H.F., 1974. Pollen: Biology, Biochemistry and Management. Springer, Berlin, Heidelberg and New York.
Tuinstra, M.R., Wedel, J., 2000. Estimation of pollen viability in grain sorghum contribution no. 99-212-J from the Kansas Agric. Exp. Stn. Crop Science 40, 968–970.
Van Staden, J., Jäger, A.K., Light, M.E., Burger, B.V., 2004. Isolation of the major germination cue from plant-derived smoke. South African Journal of Botany 70, 654–659.
Voyiatzis, D., Paraskevopoulou-Paroussi, G., 2000. The effect of photoperiod and gibberellic acid on strawberry pollen germination and stamen growth. IV International Strawberry Symposium, 567, pp. 257–260.
Wang, Z.-Y., Ge, Y., Scott, M., Spangenberg, G., 2004. Viability and longevity of pollen from transgenic and nontransgenic tall fescue (*Festuca arundinacea*) (Poaceae) plants. American Journal of Botany 91, 523–530.