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Research Note

Smoke Solutions and Temperature Influence the Germination and Seedling Growth of South African Mesic Grassland Species

Habteab M. Ghebrehiwot,[1] Manoj G. Kulkarni,[2] Kevin P. Kirkman,[3] and Johannes Van Staden[4]

Authors are [1]Doctoral student, [2]Postdoctoral fellow, [3]Professor of Grassland Science, and [4]Professor and Director, Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa.

Abstract

Fire, natural or of anthropologic origin, is a recurrent phenomenon in South African mesic grassland. The species composition of these grasslands is sensitive to fire, particularly fire frequency. However, the mechanisms involved in influencing species composition are not fully understood. Currently there is a general suggestion that plant-derived smoke and smoke-isolated biologically active butenolide compound provide an important germination cue for a range of Poaceae species. Studies also show that these smoke solutions play a role in vegetative growth of many plants. We examined if this fire-response syndrome is related to the effect of plant-derived smoke-water (1:500 v/v) and smoke-derived butenolide compound (10^{-8} M) on seed germination and seedling growth of six major constituent species of the grassland. In addition, the interaction of the smoke solutions with temperature was examined by incubating seeds at a range of temperatures. Treating seeds with smoke-water and butenolide, the germination rate and final germination percentage were greater in three of the six species. *Themeda triandra* Forssk. and *Tristachya leucothrix* Trin. ex Nees showed the greatest response, with final germination increased from 43% to 67% and 35% to 63%, respectively. With increasing temperature ($> 30°$C), *Aristida junciformis* Trin. & Rupr., *Hyparrhenia hirta* (L.) Staph, and *Panicum maximum* Jacq. responded positively to the test solutions. In nearly all the species tested, smoke-water–treated seeds produced significantly longer shoots or roots. However, the degree of response varied from species to species and across different temperatures. Findings from this study suggest that plant-derived smoke and its interaction with temperature may significantly influence the germination and seedling growth of the South African mesic grassland species, which can further alter the grassland composition.

Resumen

El fuego, tanto natural como antropogénico es un fenómeno recurrente en los pastizales mesofíticos de Sudáfrica. La composición florística de dichos pastizales es sensible al fuego y particularmente a la frecuencia con la que el mismo ocurre. A pesar de ello, la comprensión de los mecanismos que influyen la composición florística es limitada. Actualmente, se cree que el humo generado por las plantas y que la butenolida biológicamente activa extraída de dicho humo, promueven la germinación de una gama de especies de las Poaceae. Experimentos anteriores también demuestran que dichas soluciones de humo tienen un rol en el crecimiento vegetativo de muchas plantas. Examinamos la relación entre este síndrome de respuesta al fuego y el efecto de humo-agua (1:500 v/v) derivado de las plantas y butenolida (10^{-8} M) derivada del humo, sobre la germinación y el crecimiento de plántulas de seis especies mayoritarias del pastizal. Además, se examinó la interacción entre las soluciones de humo y la temperatura incubando semillas en un rango de temperaturas. El tratamiento con humo-agua y butenolida incrementó la tasa de germinación y el porcentaje final de germinación de tres de las seis especies. *Themeda trianda* Forssk y *Tristachya leucothrix* Trin. ex Nees mostraron la mayor respuesta con incrementos en el porcentaje final de germinación de 43% a 67% y 35% a 63%, respectivamente. Con temperaturas crecientes ($> 30°$C) *Aristida junciformis* Trin. & Rupr., *Hyparrhenia hirta* (L.) Staph, y *Panicum maximum* Jacq. registraron respuestas positivas a las soluciones utilizadas. En casi todas las especies ensayadas las semillas tratadas con la solución humo-agua produjeron tallos y raíces significativamente más largos. No obstante, el grado de respuesta varió entre especies y a lo largo del rango de temperaturas ensayadas. Los resultados de este experimento sugieren que el humo generado por las plantas y su interacción con la temperatura podrían tener una influencia significativa sobre la germinación y el crecimiento de plántulas de especies de los pastizales mesofíticos de Sudáfrica, hecho que podría alterar aun más la composición florística del pastizal.

Key Words: butenolide, germination, grassland species, seedling vigor, smoke-water, temperature

INTRODUCTION

Fire, natural or of anthropologic origin, is a recurrent phenomenon in the mesic grasslands of South Africa (Scott 1971; Tainton 1999). Fire consumes nearly all grass cover, and post-fire grasslands are marked by a flush of vigorous vegetative regeneration from perennial tufts and new seedling

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Correspondence: Johannes Van Staden, Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa. Email: rcpgd@ukzn.ac.za

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emergence (Everson and Tainton 1984; Tainton 1999). Long-term and ongoing (1950–2008) studies of grassland fire suggest that frequent fire causes a major shift in species composition of the Tall grassland favoring the survivorship of relatively short to medium height native grasses such as *Themeda triandra* Forssk., *Tristachya leucothrix* Trin. ex Nees, and *Heteropogon contortus* (L.) Roem. & Schult. Infrequent burning or complete absence of fire often results in the replacement of these short species by relatively taller species such as *Aristida junciformis* Trin. & Rupr., *Eragrostis curvula* Nees, *Cymbopogon validus* Stapf ex Burtt Davy, and *Alloteropsis semialata* (R.Br.) Hitchc. (Everson and Tainton 1984; Fynn et al. 2005). Little is known about the germination and regeneration ecology of these species, and knowledge of grass species responses to fire and fire-related factors is generally lacking. Despite the number of studies on grassland fire, the South African native grass species are poorly represented in smoke stimulation research, and no information exists on the response of the species to plant-derived smoke and their interaction with temperature (Zacharias et al. 1988; Baxter et al. 1994). This was mainly because of the generally held view that in the South African mesic grasslands most grass reproduction takes place via vegetative reproduction, and the contribution from seed germination is minimal (Danckwerts 1984; Everson 1994).

Plant-derived smoke is widely studied for its role in seed germination and seedling growth of many plant species (Adkins and Peters 2001; Sparg et al. 2006; Van Staden et al. 2006; Kulkarni et al. 2007). In the South African fynbos (Brown 1993), Californian chaparral (Keeley and Fotheringham 1998), and Australian native grasslands (Dixon et al. 1995; Read and Bellairs 1999; Clarke and French 2005), plant-derived smoke has been shown to influence the recruitment of grass species differently. Germination enhancement has been shown in several species from different families (Adkins and Peters 2001), and a biochemically active compound, butenolide (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one), isolated from smoke has been shown to enhance seed germination and seedling vigor of diverse plant species (Van Staden et al. 2006; Kulkarni et al. 2007). Several studies also reported that the germinative role of smoke solutions is temperature dependant (Keeley et al. 1985; Gonzalez-Rabanal and Casal 1995).

The main objective of this study was to understand how and which species would positively or negatively respond to smoke and whether these species require a specific temperature to promote germination in the presence of smoke. This study was therefore conducted to determine the effects of plant-derived smoke-water and a smoke-isolated butenolide compound, at different temperatures, on germination and seedling growth of species from South African mesic grasslands.

MATERIALS AND METHODS

Species, Seed Source, and Storage

Six perennial and naturally occurring C_4 grassland species were selected for this study from typical mesic grasslands of South Africa. These are among the most commonly occurring grass species in the mesic grasslands (Acocks 1988). These species were also selected on the basis of their contrasting response to fire frequency (Everson and Tainton 1984; Fynn 2004). Mature, naked, and husked seeds were collected in 2008 from the wild around Pietermaritzburg (lat 30°24′S, long 29°40′E; 755 m above sea level). The area has a warm subtropical climate. Mean maximum summer temperature is 26.4°C in February, and mean minimum winter temperature is 8.8°C in July with occasional frost. Mean annual rainfall is 885 mm. All the seeds were stored at 5°C until they were used for germination and seedling growth experiments.

Study Species

A. junciformis Trin. & Rupr. produces a prolific amount of seed allowing it to rapidly colonize overgrazed areas. Its expansion increases as fire frequency decreases and through selective grazing (Venter 1968; Van Zyl 1998). *E. curvula*'s abundance increases, and it replaces areas of *T. triandra* in the absence of burning. It produces very high numbers of tiny seeds per head (on an average 12 000 seeds per plant) and colonizes areas of high fertility (Kirkman and Morris 2003). *Hyparrhenia hirta* (L.) has a low seed production and seed viability because of variations in seed setting between plants (Robinson and Potts 1950). It is a tall robust species commonly found on well-drained soils and often dominates old lands. It is a strong competitor in infertile habitats (Fynn et al. 2005). *Panicum maximum* Jacq. flourishes on fertile soil and is well adapted to a wide variety of environmental conditions, and fire does not cause long-term damage (Harty et al. 1983). *T. triandra* Forssk. is a fire-loving species, and it is replaced by later-successional species in the absence of regular burning (Everson and Tainton 1984; O'Connor 1997; Fynn et al. 2005). *T. leucothrix* Trin. ex Nees increases in regularly burnt but underutilized grasslands.

Smoke-Water and Butenolide Solutions

A number of studies have described the procedure of preparing smoke-water extract using different plant material (De Lange and Boucher 1990; Brown 1993; Jäger et al. 1996; Boucher and Meets 2004; Van Staden et al. 2004). In this study dry *T. triandra* leaf material (5 kg) was burnt in a 20-L metal drum, and smoke generated was passed through a glass column containing 500 mL of tap water for 45 min (Baxter et al. 1994). This smoke-extract was filtered through Whatman No. 1 filter paper and was used as stock solution. The test solution was prepared by diluting 1 mL of smoke-extract with 500 mL of distilled water (1:500 v/v). A pure butenolide (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one) used in this experiment was isolated from plant-derived smoke-water as described by Van Staden et al. (2004). The concentration of the compound tested was 10^{-8} M.

Seed Germination and Seedling Growth Experiments

Seeds were placed in 90-mm Petri dishes on two layers of Whatman No. 1 filter paper. There were four replicates of 25 seeds each. The germination was recorded daily on the emergence of the radicle (0.5 mm) from the seed coat.

Before the germination experiment, viability of the seeds was determined using 1% aqueous solution of tetrazolium salt (2, 3, 5-triphenyl tetrazolium chloride). Three replications of 25 filled seeds were soaked in distilled water for 18 h; subsequently the lemma and palea were removed and the caryopsis was bisected longitudinally with a razor blade to expose the main structures of the embryo, and half of the caryopsis was immersed in the tetrazolium solution. After 3 h of dark incubation at 25°C, caryopses with completely red stained embryos were scored as viable (Grabe 1970).

To determine the effect of different constant temperatures, seeds were incubated at 15°C, 20°C, 25°C, 30°C, and 35°C (16:8 h light:dark) with smoke solutions or distilled water (control). Filter papers were moistened with distilled water or smoke solutions when required. Germinated seeds at different temperatures were retained for 21 days in the same Petri dishes for seedling development. For seedling growth studies, the seeds were also subjected to alternating temperatures (30/15°C) under 16:8 h light:dark. Response variables considered were germination rate (GR), final germination percentage (FGP), and subsequent seedling growth (shoot and root length). Germination rate for each species was calculated by modified Timson index (Easton and Kleindorfer 2009): GR (% d^{-1}) $= \sum[(G^1/t)+(G^2/t)+\cdots+(G^t/t)]$, where G is the percentage of seed germination at one-day intervals, and t is the total number of days of the germination period. A greater value of G represent faster germination rate.

Statistical Analysis

Percentage germination data were arcsine transformed before the analysis. Germination and seedling growth data were subjected to one-way analysis of variance (ANOVA), and Tukey's post-hoc test was used for pair-wise comparison at a 5% level of significance ($P < 0.005$). General ANOVA was conducted for main effects and their interactions. Statistical packages used were GENSTAT® (GENSTAT 2008) and MINITAB® (MINITAB 2003).

RESULTS

Seed viability, as determined by tetrazolium analysis, varied among the six species of grasses tested, ranging from 30% to 92%. In comparison to the control, smoke-water treatment significantly increased GR and FGP of *A. junciformis* seeds at 35°C (Fig. 1). At this temperature shoot and root length was also significantly greater than the control (Fig. 2). The seeds of *E. curvula*, after treating with smoke-water at 30°C, showed a significant increase in FGP compared to the control (Fig. 1). A butenolide treatment significantly increased shoot length at 25°C, 30°C, and 30/15°C when compared with the control (Fig. 2). Smoke-water-incubated seeds of *E. curvula* showed a significant increase in shoot and root length of seedling only at 30/15°C over the control. Smoke-water-treated seeds of *H. hirta* showed significantly higher GR and FGP at 30°C and 35°C and at all other temperatures had a significantly greater shoot and root length than the control with the exception of 15°C (Fig. 2). Although smoke-water and butenolide treatments increased GR and FGP of *P. maximum* seeds at all tested temperatures, these results were significantly different to control only at 30°C and 35°C (Fig. 1). These treatments exhibited significantly greater shoot and root length at 35°C (Fig. 2). The seeds of *T. triandra* when treated with smoke-water at 15°C, 25°C, 30°C, and 35°C showed significantly higher GR and FGP compared to the respective controls (Fig. 1). Smoke-water also significantly increased shoot and root length of *T. triandra* seedling in comparison to the control at 25°C, 30°C, and 35°C (Fig. 2). With the exception of 15°C and 20°C, at all other tested temperatures smoke-water-treated seeds of *T. leucothrix* showed significantly higher GR and FGP in comparison to the control (Fig. 1). The shoot and root length were significantly increased by smoke-water treatment when compared with the control at 25°C, 30°C, and 35°C (Fig. 2). The overall result of this study shows that there was a significantly positive interaction between species, temperature, and treatment for GR and FGP (Table 1).

DISCUSSION

The findings from this experiment support the hypothesis that plant-derived smoke- and fire-mediated changes in temperature may significantly influence the germination and seedling growth of the common species of the Tall grassland, which is likely to alter the grassland composition. Smoke treatments at higher temperatures generally enhanced GR, FGP, and seedling growth of *H. hirta*, *T. triandra,* and *T. leucothrix* (Figs. 1 and 2). Smoke solutions tested at different constant temperatures did not show significant effect on GR and FGP of *A. junciformis* and *E. curvula*, with some exceptions at higher temperatures. This finding agrees with that of Zacharias et al. (1988), where percentage germination for *A. junciformis* remained unaffected in response to fire, although it is unknown whether *A. junicformis* failed to respond to the heat or smoke component of fire. Adkins et al. (2003) reported that smoke failed to alter the natural germination of *Aristida ramose*. According to these authors, *A. ramose* responded to smoke-water only at higher temperatures. In this study, *Aristida junciformis* also responded to high temperature of 35°C in the presence of smoke-water. Other species of *Aristida*, such as *Aristida vegans,* failed to respond to smoke-water treatments (Clarke and French 2005). Germination rate in *E. curvula* was not affected by smoke-water or butenolide treatments, but there was an improvement in FGP and shoot and root length at higher temperatures. Clarke and French (2005) found that germination percentage in *E. curvula* was increased by heat treatment but only in the presence of smoke. The other species of *Eragrostis* such as *Eragrostis tef* (Ghebrehiwot et al. 2008), *Eragrostis leptostachya,* and *Eragrostis benthamii* (Clarke and French 2005) are responsive to smoke.

Interestingly, all the growth parameters of *T. triandra* studied were significantly enhanced by smoke-water and butenolide treatment at nearly all the temperatures tested. This species is suggested to have evolved under fire regimes (Everson and Tainton 1984; O'Connor 1997). In the absence of fire such species are not capable of maintaining their population size, and they are unable to grow vigorously (Everson and Tainton 1984; Morgan and Lunt 1999). Many authors have associated such a reaction of *T. triandra* to post-fire nutrient enrichment, greater access to light and water, competitive release, and lowered productivity (O'Connor 1997; Morgan and Lunt 1999; Fynn 2004; Fynn et al. 2005). Our results clearly show that in addition to the other factors, plant-derived smoke may enhance the germination and seedling growth of *T. triandra*. Studies have showed that smoke-water was effective in

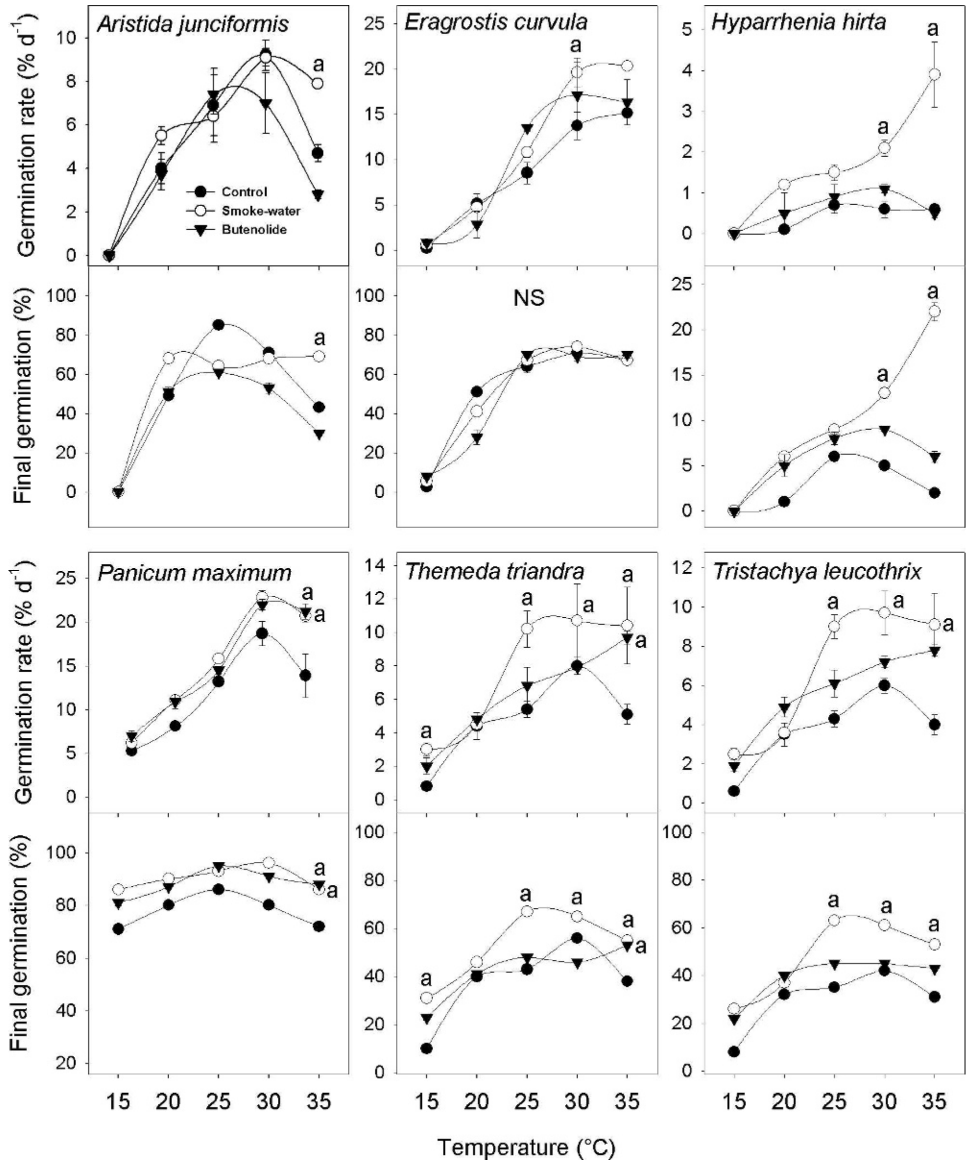

Figure 1. Effect of smoke solutions (smoke-water 1:500 v/v and butenolide 10^{-8} M) on seed germination rate and final germination percentage of six mesic grassland species at constant temperatures under 16:8 h light:dark conditions. Symbol (± SE) with letter "a" is significantly different from control according to Tukey's test ($P < 0.001$). NS = nonsignificant.

breaking the dormancy and stimulating the germination and seedling vigor of *T. triandra* (Groves et al. 1982; Baxter et al. 1994; Read and Bellairs 1999). Such fire-dependent species also responded positively to aerosol smoke treatment (Read and Bellairs 1999). *T. leucothrix*, an obligate seed-reproducing species, response to smoke-water and butenolide treatment was not that different from that of *T. triandra*. In nearly all the variables considered, *T. leucothrix* positively responded to the smoke solutions.

At higher temperatures, GR and FGP of *H. hirta* (a species with low viable seeds) were significantly enhanced when treated with smoke-water and butenolide. Smoke-water showed a pronounced effect on shoot and root growth of *H. hirta* seedlings. This indicates that *H. hirta* can establish quicker than other less-smoke-responsive species. Smoke solution treatment of *P. maximum* seeds showed an increasing trend of GR and FGP with only significant effects at higher temperatures. The result of this experiment to an extent is similar to the findings of Adkins et al. (2000), in which smoke treatment enhanced the germination rate but there was no significant change in FGP.

In this study butenolide did not show consistent effects, which may be attributed to the level of concentration used. The seeds of all grass species tested were treated with only one level of butenolide concentration, 10^{-8} M. It may show consistent results similar to smoke-water if tested with different concentrations. The effectiveness of the smoke solutions used in this experiment and the interactive effects with temperature and light is however questionable. In general, our results showed that there was a high variability in both germination and seedling growth responses of grasses. Species such as *T. triandra*, *T. leucothrix*, and *H. hirta* can be considered as more responsive to smoke than the rest of the species investigated. In conclusion, under conditions of high fire

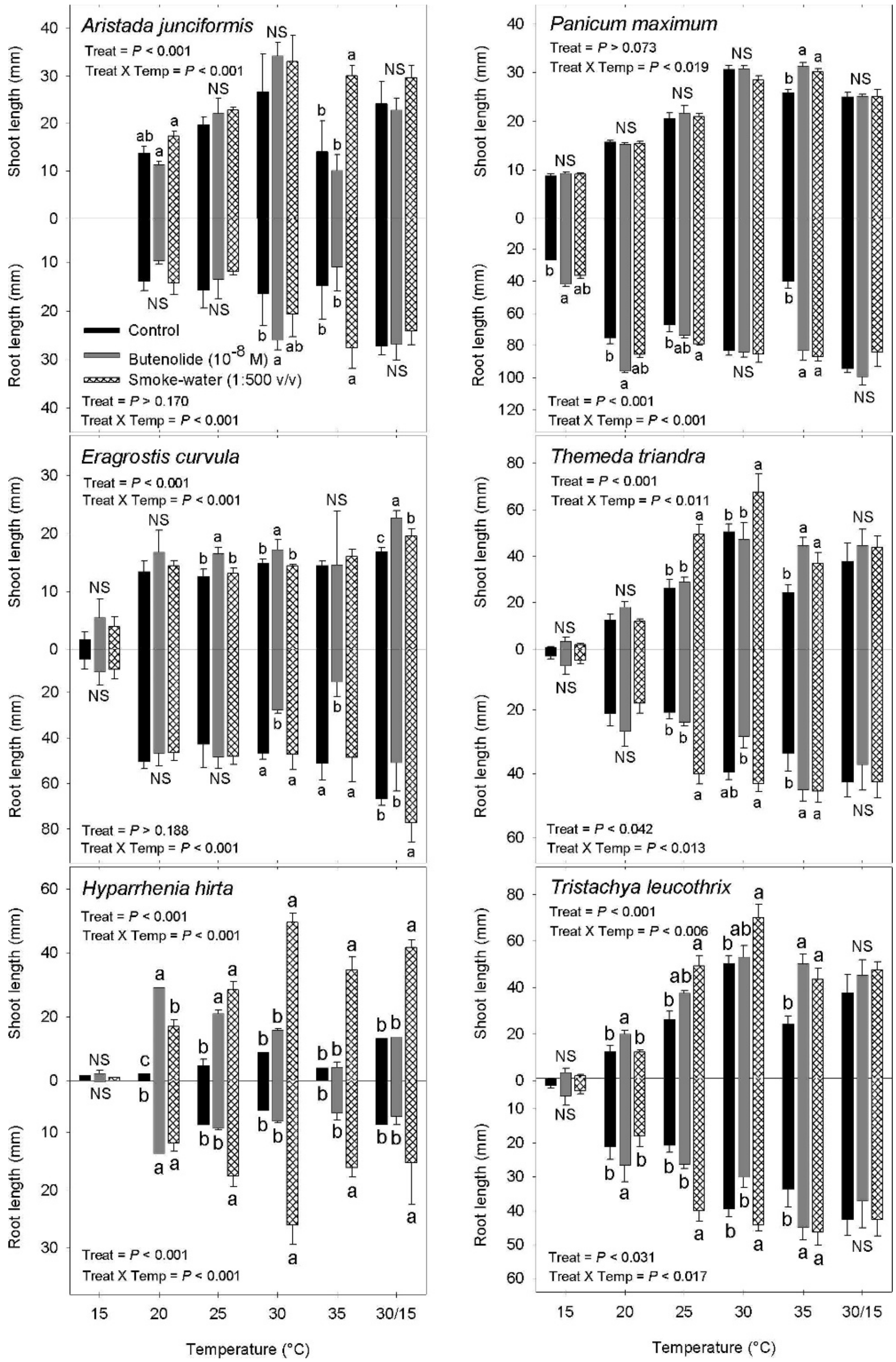

Figure 2. Effect of smoke-water (1:500 v/v) and butenolide (10^{-8} M) on shoot and root length (mm) of six mesic grassland species at different temperatures under 16:8 h light:dark conditions. Bar ($\pm$ SE) with different letter(s) is significantly different according to Tukey's test ($P < 0.001$). NS = nonsignificant; Treat $\times$ Temp = Interaction between treatment and temperature.

frequency and consequent exposure to smoke, species such as *T. triandra*, *T. leucothrix,* and *H. hirta* should show a competitive advantage over those less or non-smoke-responsive species such as *A. junciformis*, *E. curvula,* and *P. maximum*. In the long run, changes in the frequency of fire and the subsequent exposure to smoke, it is likely to influence the germination and seedling growth of grass species differently, which can affect the community composition. Recent advances in smoke research have shown that smoke can induce genes to elicit a faster germination rate and greater seedling vigor (Soós et al. 2009a, 2009b, respectively). This has opened new avenues to study genomics of smoke-responsive grasses.

Table 1. General analysis of variance with main effects and their interactions for six mesic grassland species. Treatments at constant temperatures were control, smoke-water (1:500 v/v), and butenolide (10^{-8} M).

Source	Df[1]	Sum of squares	Mean squares	*F*-value	*P*-level[2]
Germination rate (% d^{-1})					
Species (S)	5	6 073.2	1 214.6	459.0	< 0.001
Temperature (T)	4	4 142.9	1 035.7	391.4	< 0.001
Treatment (TR)	2	332.1	166.0	62.7	< 0.001
S × T	20	1 643.5	82.1	31.0	< 0.001
S × TR	10	103.2	10.3	3.9	< 0.001
T × TR	8	155.1	19.3	7.3	< 0.001
S × T × TR	40	227.4	5.6	2.1	< 0.001
Residual	270	714.4	2.6		
Total	359	13 392.1			
Final germination (%)					
S	5	30.5689	6.1137	419.2	< 0.001
T	4	8.4896	2.1224	145.5	< 0.001
TR	2	1.0308	0.5154	35.3	< 0.001
S × T	20	3.9652	0.1982	13.6	< 0.001
S × TR	10	1.2429	0.1242	8.5	< 0.001
T × TR	8	0.1476	0.0184	1.2	0.262
S × T × TR	40	1.1692	0.0292	2.0	< 0.001
Residual	270	3.9374	0.0145		
Total	359	50.5517			

[1]Degrees of freedom.
[2]Probability level.

MANAGEMENT IMPLICATIONS

It is the view of several scientists that smoke biotechnology can be a useful tool for grassland regeneration and restoration of degraded lands. Smoke stimulation can also be used as strategy for depleting the soil seed bank in weed control programs (Adkins and Peters 2001; Kępczyński et al. 2006; Daws et al. 2007, 2008). Thus, an understanding of the seed germination ecology of grasses in response to fire (smoke) can assist in predicting their potential distribution and developing effective management strategies. Findings from this experiment imply that smoke, because of its selective stimulation characteristics, may be a useful tool in grassland management. For instance, *T. triandra* and *T. leucothrix*, both obligate seed-reproducing species and among the most economically important native grass species in South Africa, are prone to extinction in areas left unburnt. On the other hand, less desirable and weedy species such as *A. junciformis* can increase in less frequently burnt situations. Our results demonstrate that part of this species turnover may originate from species responses to fire (smoke) and the interactive effect of smoke with temperature on germination and seedling growth of the species. Thus, regular burning of the mesic grassland could therefore be a useful device to restore degraded grasslands, where spontaneous colonization of smoke-responsive grass species is very limited. Furthermore, knowledge of the seed smoke interaction would assist in weed and invasive species management programs. However, large-scale and well-organized screening of the germination characteristics of the mesic grassland flora and detailed field studies on this subject are needed to quantify the net effect of smoke on grassland dynamics.

LITERATURE CITED

Acocks, J. P. H. 1988. Veld types of South Africa. Memoirs of the Botanical Survey of South Africa, No 57. Pretoria, South Africa: Botanical Research Institute. 146 p.

Adkins, S. W., P. J. Davidson, L. Matthew, S. C. Navie, D. A. Wills, I. N. Taylor, and S. M. Bellairs. 2000. Smoke and germination of arable and rangeland weeds. *In:* B. M. Bradford and K. J. Vázquez-Ramos [eds.]. Seed biology: advances and applications. Wallingford, United Kingdom: CABI Publishing. p. 347–359.

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

Adkins, S. W., N. C. B. Peters, M. F. Paterson, and S. Navie. 2003. Germination stimulation of weed species by smoke. *In:* G. Nicolas, K. J. Bradford, D. Come, and H. M. Pritchard [eds.]. The biology of seeds: recent research advances. Wallingford, United Kingdom: CABI Publishing. p. 413–420.

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

Boucher, C., and M. Meets. 2004. Determination of the relative activity of aqueous plant-derived smoke solutions used in seed germination. *South African Journal of Botany* 70:313–318.

Brown, N. A. C. 1993. Promotion of germination of fynbos seeds by plant-derived smoke. *New Phytologist* 123:575–583.

Clarke, S., and K. French. 2005. Germination response to heat and smoke of 22 Poaceae species from grassy woodlands. *Australian Journal of Botany* 53:445–454.

Danckwerts, J. E. 1984. Towards improved livestock production of sweet grassveld [thesis]. Pietermaritzburg, South Africa: University of Natal. 319 p.

Daws, M. I., J. Davies, H. W. Pritchard, N. A. C. Brown, and J. Van Staden. 2007. Butenolide from plant-derived smoke enhances germination and seedling growth of arable weed species. *Plant Growth Regulation* 51:73–82.

Daws, M. I., H. W. Pritchard, and J. Van Staden. 2008. Butenolide from plant-derived smoke functions as a strigolactone analogue: evidence from parasitic weed seed germination. *South African Journal of Botany* 74:116–120.

De Lange, J. H., and C. Boucher. 1990. Autecological studies on *Audouinia capitata* (Bruniaceae). I. Plant derived smoke as a germination cue. *South African Journal of Botany* 54:700–703.

Dixon, K. W., S. Roche, and J. S. Pate. 1995. The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. *Oecologia* 101:185–192.

Easton, L. C., and S. Kleindorfer. 2009. Effects of salinity levels and seed mass on germination in Australian species of *Frankenia* L. (Frankeniaceae). *Environmental and Experimental Botany* 65:345–352.

Everson, C. S., and N. M. Tainton. 1984. The effect of thirty years of burning on the highland sourveld of Natal. *Journal of the Grassland Society of Southern Africa* 1:15–20.

Everson, T. M. 1994. Seedling establishment of *Themeda triandra* Forssk. in the Montane grasslands of Natal [thesis]. Pietermaritzburg, South Africa: University of Natal. 176 p.

Fynn, R. W. S. 2004. Determinants of community composition and diversity in KwaZulu-Natal mesic grasslands: evidence from long-term field experiment and pot and plot competition experiment [thesis]. Pietermaritzburg, South Africa: University of KwaZulu-Natal. 235 p.

Fynn, R. W. S., C. D. Morris, and T. J. Edwards. 2005. Effect of burning and mowing on grass and forb composition in a long-term grassland experiment. *Applied Vegetation Science* 8:5–12.

GENSTAT [COMPUTER PROGRAM]. 2008. GENSTAT® version 11.1. Hemel Hempstead, United Kingdom: VSN International.

GHEBREHIWOT, H. M., M. G. KULKARNI, K. P. KIRKMAN, AND J. VAN STADEN. 2008. Smoke-water and a smoke-isolated butenolide improve germination and seedling vigor of *Eragrostis tef* (Zucc.) Trotter under high temperature and low osmotic potential. *Journal of Agronomy and Crop Science* 194:270–277.

GONZALEZ-RABANAL, F., AND M. CASAL. 1995. Effect of high temperatures and ash on germination of ten species from gorse shrubland. *Vegetatio* 116:123–131.

GRABE, D. F. 1970. Tetrazolium testing handbook for agricultural seeds. Contribution 29. *Association of Official Seed Analysts* 29:1–62.

GROVES, R. H., M. W. HAGON, AND P. S. RAMAKRISHNAN. 1982. Dormancy and germination of seed of eight populations of *Themeda australis. Australian Journal of Botany* 30:373–386.

HARTY, R. L., J. M. HOPKINSON, B. H. ENGLISH, AND J. ALDER. 1983. Germination, dormancy and longevity in stored seeds of *Panicum maximum*. *Seed Science & Technology* 11:341–351.

JÄGER, A. K., M. E. LIGHT, AND J. VAN STADEN. 1996. Effects of source of plant material and temperature on the production of smoke extracts that promote germination of light sensitive lettuce seeds. *Environmental and Experimental Botany* 36:421–429.

KEELEY, J. E., AND C. J. FOTHERINGHAM. 1998. Smoke-induced seed germination in California Chaparral. *Ecology* 79:2320–2336.

KEELEY, J. E., B. A. MORTON, A. PEDROSA, AND P. TROTTER. 1985. Role of allelopathy, heat, and charred wood in the germination of chaparral herbs and suffrutescents. *Journal of Ecology* 73:445–458.

KĘPCZYŃSKI, J., B. BIAŁECKA, M. E. LIGHT, AND J. VAN STADEN. 2006. Regulation of *Avena fatua* seed germination by smoke solutions, gibberellins A_3 and ethylene. *Plant Growth Regulation* 49:9–16.

KIRKMAN, K. P., AND C. D. MORRIS. 2003. Ecology and dynamics of *Eragrostis curvula* and *Eragrostis plana* with a view of controlling their spread in natural grasslands. Proceedings of the VII International Rangelands Congress; 26 July–1 August 2003; Durban, South Africa. 138 p.

KULKARNI, M. G., G. D. ASCOUGH, AND J. VAN STADEN. 2007. Effects of foliar applications of smoke-water and a smoke isolated butenolide on seedling growth of okra and tomato. *HortScience* 42:179–182.

MINITAB [COMPUTER PROGRAM]. 2003. MINITAB® version 12.1. State College, PA, USA: Minitab Inc.

MORGAN, J. W., AND I. D. LUNT. 1999. Effects of time-since-fire on the tussock dynamics of a dominant grass (*Themeda triandra*) in a temperate Australian grassland. *Biological Conservation* 88:379–386.

O'CONNOR, T. G. 1997. Microsite influence on seed longevity and seedling emergence of a bunch-grass (*Themeda triandra*) in a semi-arid savanna. *African Journal of Range and Forage Science* 14:7–11.

READ, T. R., AND S. M. BELLAIRS. 1999. Smoke affects the germination of native grasses of New South Wales. *Australian Journal of Botany* 47:563–576.

ROBINSON, B. P., AND R. C. POTTS. 1950. Seed setting and germination in *Hyparrhenia hirta* (L.) Stapf (South African Bluestem). *Agronomy Journal* 42:109–110.

SCOTT, D. J. 1971. Veld burning in Natal. *Proceedings of the Tall Timbers Fire Ecology Conference* 11:33–51.

SOÓS, V., A. JUHÁSZ, M. E. LIGHT, J. VAN STADEN, AND E. BALÁZ. 2009a. Smoke-water-induced changes of expression pattern in Grand Rapids lettuce achenes. *Seed Science Research* 19:37–49.

SOÓS, V., E. SEBESTYÉN, A. JUHÁSZ, J. PINTÉR, M. E. LIGHT, J. VAN STADEN, AND E. BALÁZ. 2009b. Stress-related genes define essential steps in the response of maize seedlings to smoke-water. *Functional and Integrative Genomics* 9:231–242.

SPARG, S. G., M. G. KULKARNI, AND J. VAN STADEN. 2006. Aerosol smoke and smoke-water stimulation of seedling vigor of a commercial maize cultivar. *Crop Science* 46:1336–1340.

TAINTON, N. M. 1999. Veld burning in different vegetation types. *In:* N. M. Tainton [ED.]. Veld management in South Africa. Pietermaritzburg, South Africa: University of Natal Press. p. 228–243.

VAN STADEN, J., A. K. JÄGER, M. E. LIGHT, AND B. V. BURGER. 2004. Isolation of the major germination cue from plant derived smoke. *South African Journal of Botany* 70:654–659.

VAN STADEN, J., S. G. SPARG, M. G. KULKARNI, AND M. E. LIGHT. 2006. Post-germination effects of the smoke-derived compound 3-methyl-*2H*-furo[2,3-*c*]pyran-2-one, and its potential as a preconditioning agent. *Field Crops Research* 98:98–105.

VAN ZYL, D. D. 1998. Aspects of the invasion of the southern Tall grassveld by *Aristida junciformis* sub species *junciformis* Train. ET Rupr [thesis]. Pietermaritzburg, South Africa: University of Natal. 150 p.

VENTER, A. D. 1968. The problems of *Aristida junciformis* encroachment into the veld of Natal. *Proceedings of the Grassland Society of South Africa* 3:163–165.

ZACHARIAS, P. K. J., N. M. TAINTON, AND C. OBERHOLSTER. 1988. The effect of fire on germination in five common veld grasses. *Journal of the Grassland Society of Southern Africa* 5:229–230.