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Karrikinolide residues in grassland soils following fire: Implications on germination activity

H.M. Ghebrehiwot [a], M.G. Kulkarni [a], G. Szalai [c], V. Soós [b], E. Balázs [a,b], J. Van Staden [a,*]

[a] Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa
[b] Department of Genomics, Agricultural Institute of the Hungarian Academy of Sciences (ARI-HAS), Brunszvik u2, 2462 Martonvásár, Hungary
[c] Department of Plant Physiology, Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences (ARI-HAS), Brunszvik u2, 2462 Martonvásár, Hungary

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ABSTRACT

Smoke plays a positive role in promoting seed germination and enhancing post-germination processes. The compound in smoke is 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (KAR_1). Recently a structurally related butenolide [3,4,5-trimethylfuran-2(5*H*)-one, (trimethylbutenolide, TMB)], which inhibits germination and reduces the effect of KAR_1, was isolated. The mechanisms of action and interaction of these karrikins are unknown. In addition, the ecological significance of fires in altering soil-smoke-chemistry and the spatial dimensions of the influence on burnt sites and neighbouring areas are undetermined. This study quantified KAR_1 and TMB residues in soils following fire and assessed the germination activity of burnt soil extracts. Soil samples from 0 to 2, 2 to 4, 4 to 6 and 6 to 8 cm depths were extracted using dichloromethane and bioassayed using *Lactuca sativa* L. achenes (seeds). At all soil depths, *L. sativa* seeds exhibited significantly greater percentage germination when treated with burnt soil extracts compared to the no-burn soil (control). The *L. sativa* seeds also showed significantly greater percentage germination when treated with soil extracts from the adjacent plots. Compared to the no-burn soil, higher concentrations of KAR_1 and TMB were detected in the surface layers of the burnt soils. Considerable concentrations of KAR_1 and TMB were also detected in no-burn soil indicating that sources other than fire may also generate karrikins. Findings of this study imply that post-fire increases in KAR_1 residues in the soil may influence soil seed bank stimulation of certain smoke-responsive plant communities in both burnt and adjacent non-burnt areas.

1. Introduction

The history of fire and smoke on earth is as old as the planet itself. Fire has affected ecosystems for millions of years and its major role in shaping global vegetation patterns is one of the oldest observations of the natural world (Bond and Van Wilgen, 1996; Lamont and He, 2012). It is also recognized that fire (burning) induces immediate changes in the physical, biological, mineralogical and chemical properties of the post-fire environment as a result of direct exposure to higher temperatures and ash deposition (Christensen and Muller, 1975; Van Staden et al., 2000). A universal response of frequently burnt grassland ecosystems is the marked transformation in species composition that follows (Bond and Keeley, 2005). Traditionally, it had been assumed that some plant species are eliminated by frequent fires and that species adapted to fire increase in abundance in the burnt grassland ecosystems (Taylor, 1973; Krefting and Ahlgren, 1974). Consequently, most previous studies on the effects of fire on regeneration (germination) of flammable ecosystems focused on the physical attributes (Christensen and Muller, 1975) providing limited information on the chemical stimuli of fire, e.g. smoke and charred wood (Wicklow, 1977).

However, since the pioneering study by De Lange and Boucher (1990), the role of smoke as a factor regulating seed germination has fascinated botanists and the subject has been extensively examined (Van Staden et al., 2000). To date, plant-derived smoke (aerosol and solutions) and smoke-isolated karrikinolide [3-methyl-2*H*-furo[2,3-*c*]pyran-2-one] (hereafter named as KAR_1) have been shown to be broadly effective stimulants that enhance seed germination of greater than 1200 species in more than 80 genera worldwide (Dixon et al., 2009).

Although, in general, smoke provides a positive stimulus for germination, a negative effect of high concentrations of smoke-water on germination has also been well demonstrated (Light et al., 2002; Daws et al., 2007). This negative effect has been attributed to some inhibitory compounds that are present in plant-derived smoke solutions. Recently, another butenolide compound i.e. 3,4,5-trimethylfuran-2(5*H*)-one (hereafter named as TMB), which inhibits germination and significantly reduces the effect of KAR_1, when applied simultaneously, was also isolated from smoke (Light et al., 2010). However, the ecological significance of grassland fires (burning) in altering the soil-smoke-chemistry (with respect to the amount of KAR_1 and TMB residues in the soil) and the spatial (vertical and horizontal) dimensions of the influence on burnt and unburnt neighbouring sites are still unknown.

Vegetation burning produces huge clouds of smoke which can drift into neighboring unburnt areas (Andreae, 1991; Preston and Baldwin,

* Corresponding author. Tel.: +27 33 2605130.
E-mail address: rcpgd@ukzn.ac.za (J. Van Staden).

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1999). In an event of rain, such clouds of smoke emissions in the air would dissolve into the moisture, essentially producing a smoke solution. Smoke may also leach into the soil either in a gaseous or liquid form influencing the soil seed bank (Read et al., 2000; Crosti et al., 2006). There is a general suggestion that rain washing and drainage to rivers from burnt catchments can be an important terrestrial process that directly influences germination of aquatic plants (Van Staden et al., 2000). Therefore, knowledge of the effect and role of fire in altering soil-smoke chemistry and the subsequent influence on the germinable soil seed bank is ecologically significant from a conservation, restoration and weed control perspective (Roche et al., 1997). This is particularly true for fire-prone grasslands and savannas in South Africa and elsewhere, where fires of anthropologic or natural origin are common occurrences.

The objectives of the present study were therefore: (1) to assess if grassland burning affects the soil-smoke content [with respect to the concentration and distribution of both butenolide compounds (KAR_1 and TMB) present in smoke]; 2) to determine if germination activity of burnt/unburnt/adjacent soil extracts is related to the amount and distribution of smoke-compound residues in soils following a burn. This study consisted of two sections: a) GC–MS analysis of burnt soil to determine the concentration and distribution of KAR_1 and b) *Lactuca sativa* L. seeds bioassays to determine the germination activity of the burnt soil extracts. *L. sativa* seeds are particularly responsive to smoke extracts and provide a rapid bioassay to evaluate the biological activity of various extracts (Drewes et al., 1995). The primary aim with the measurement of smoke related germination cues deposited in the burnt sites was merely to reveal the concentration change and spatial pattern of these germination active compounds immediately after fire.

2. Materials and methods

2.1. Site description, burning and soil sampling

The sites used for collecting burnt, unburnt and adjacent soil samples are located at Ukulinga, a research farm of the University of KwaZulu–Natal, Pietermaritzburg (29° 24′ E; 30° 24′ S). The plots are situated on top of a small plateau ranging in altitude from 838 to 847 m a.s.l. Soils at the site are classified as Westleigh forms (Soil Classification Working Group, 1991). The vegetation of the area is classified as southern Tall Grassveld and is dominated by herbaceous species, mainly *Themeda triandra* Forssk, *Heteropogon contortus* Beauv. ex Roemer and J. A. Schultes and *Tristachya leucothrix* Trin. ex Nees (Acocks, 1988). The sward height averaged 50 ± 6.33 cm (mean ± SE of 20 random samples).

On 23 March 2011, a back fire (against wind direction) was applied on the grassland site under the following weather conditions: air temperature of 25 °C, wind speed of 5.4 km h^{-1} and average relative humidity of 62%. To keep the fire within the demarcated plots, plants surrounding the plot (edge effect of 2 m) were cut short and soaked with water before burning. Burning commenced at 12:00 am and was sustained for only a few seconds. Subsequently (20 min after the cessation of burning), soil samples were collected from four depths, i.e. 0–2, 2–4, 4–6 and 6–8 cm using a 3 cm diameter auger from plots under three different conditions (see Table 1). Soil sampling was carried out with maximum care to avoid potential contamination. The auger used was finely calibrated. A hammer was used to drive the auger carefully down in to the soil to cut a piece of the required layer e.g. 0–2 cm. The soil was in a semi-moist state, and thus there was no cross contamination between and among the samples. The no-burn soil samples were collected from sites protected from fire (burning) since 1950 (>60 years) 1 week before burning the plots used for collecting burnt and adjacent soil samples. To assess the effect of burning on soil smoke chemistry and to examine the vertical and horizontal migration of the smoke-derived compounds (i.e. KAR_1 and TMB), 80 soil samples (4 treatments × 4 depths × 5 replications) were collected and tested for germination activity and used for extraction of karrikins (KAR_1 and TMB).

Table 1
Soil core samples collected before and after burning a mesic grassland site at Ukulinga Research Farm of the University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Treatment	Abbreviation	Conditions under which soil core samples were collected
No-burn	NB	Soil cores collected from no-burn plots not burnt for more than 60 years (since 1950)
Burnt	BP	Soil cores collected from burnt plots immediately after burning
Adjacent 5 m	AP-5m	Soil cores collected from unburnt but adjacent to burnt plots after burning (5 meters away)
Adjacent 10 m	AP-10m	Soil cores collected from unburnt but adjacent to burnt plots after burning (10 meters away)

2.2. Fuel load and fuel moisture

Prior to burning, available fuel loads and fuel moisture content were estimated by collecting dead and live plant material from 5 quadrats (1 × 5 m) along an established 50 m transect. In the laboratory, the samples were oven-dried at 120 °C for 72 h. Fuel load and fuel moisture content were calculated as follows: fuel moisture content (wet basis) = (total mass of wet biomass − total mass of dry biomass)/total mass of wet biomass × 100 (Anderson and Kothmann, 1982). Dry biomass weights per square meter were converted to tonnes per hectare.

2.3. Soil extraction methods

Soil samples were individually stored in clear plastic bags at −70 °C. Each soil sample was crushed using a mortar and pestle and sieved to remove stones and large organic materials. Thereafter, 20 g of each soil sample ($n = 80$) was extracted with three volumes (100 and 50 mL × 2) of distilled (purified) dichloromethane. Dichloromethane was used as an extracting solvent, because it is known that the smoke-derived karrikinolide (KAR_1) can be easily extracted with this solvent (Van Staden et al., 2004). Preliminary experiments comparing dichloromethane and water for preparing the soil extracts showed that the dichloromethane extraction was suitable for detecting comparable germination activity. This option also provides effective and rapid drying of the extracts and thus reduces possible loss of the active compounds in smoke. Dichloromethane was evaporated and the residue redissolved in 10 mL of distilled-water. This extract was then diluted to give a dilution level of 1:100 v/v and was used to initiate germination of the *L. sativa* seeds throughout the study. Smoke-water tends to have a "dual regulatory" effect on germination, since high concentrations of smoke-water inhibit germination, whereas lower concentrations have a promotive effect (Drewes et al., 1995; Soós et al., 2012). While specific data are unavailable (Light et al., 2009), this dilution level was chosen in an attempt to simulate smoke concentration levels that soil stored seeds at a moderate depth may experience, especially in situations where vegetation fires are followed by sufficient rainfall.

2.4. Seed source and bioassay

Germination activity of the soil extracts was assessed using mature achenes (seeds) of *L. sativa* L. cv. Grand Rapids (Peto Seeds, Saticoy, USA). Four replicates of 25 seeds each were placed on two sheets of Whatman No. 1 filter paper in 70 mm plastic Petri dishes moistened with 2.5 mL of the test solutions and distilled H_2O serving as control. The seeds, kept in light-proof boxes, were incubated in the dark at 25 ± 0.5 °C for 24 h, after which germination was recorded. All manipulations were conducted under a green safe light (0.5 μmol m^{-2} s^{-1}) (Drewes et al., 1995) and germination was determined as radicle