Germination activity of smoke residues in soils following a fire

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Abstract

A simple and rapid bioassay was implemented to detect the germination activity of extracts from soils in pre/post-burn conditions. Soil samples taken from burnt, unburnt and adjacent plots at depths of 0–2, 2–4, 4–6 and 6–8 cm before and after burning mesic grassland in South Africa were analysed for germination activity over an eight-week period. Soil samples were extracted using dichloromethane and bioassayed using Grand Rapids lettuce (Lactuca sativa L.) achenes (seeds). The Grand Rapids lettuce seeds exhibited greater germination percentages when treated with extracts from burnt soil compared to the other plots. The magnitude of the germination activity declined with time since the burn. The Grand Rapids lettuce seeds also exhibited significantly higher germination when treated with unburnt soil extracts compared to the control (distilled water) which indicates the existence of other factors controlling germination in unburnt soil. Germination activity in the adjacent plots decreased with time. These findings indicate that the germination activity of the smoke derived from burning plant-material diffuses into the soil and its persistence declines with time. Considering that the soil seed bank contains viable seeds, at a moderate depth, and that they are initially unaffected by the heat of the fire, then smoke residues following a fire can influence the germination and recruitment of plant species that are responsive to smoke-derived compounds and are represented in the germinable soil seed bank.

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1. Introduction

It has been recognised that fire as a disturbance factor affects both the physiochemical properties of the soil and the atmospheric chemistry (Andreae, 1991; Raison, 1979). The role of the physical (e.g. light, temperature) and chemical (e.g. nutrients) factors associated with fire in influencing plant species recruitment is also well known (Raison, 1979; Ross and Harper, 1972). Since the 1990s, however, the role of smoke and aqueous smoke solutions as a new factor regulating seed germination has fascinated plant biologists and the subject has been extensively examined (Van Staden et al., 2000). To date, plant-derived smoke has been shown to stimulate the germination of many fire-dependant and fire-independent species. In total, over 1200 species from 80 genera representing a wide diversity of species from both fire and non-fire prone environments have shown marked germination increases in response to plant-derived smoke (Brown and Botha, 2004; Dixon et al., 2009). The effects of plant-derived smoke may extend well beyond germination stimulation of certain smoke-responsive species. Smoke also stimulates somatic embryogenesis (Senaratna et al., 1999), root initiation (Taylor and Van Staden, 1996) and flowering (Keeley, 1993). It also acts in enhancing seedling vigour and accelerating plant growth (Sparg et al., 2006).

Over the last two decades, following the first report on smoke-stimulated seed germination (De Lange and Boucher, 1990), researchers have attempted to isolate and characterise the active chemical(s) in plant-derived smoke (Chiwocha et al., 2009; Light et al., 2009). The identity of a highly active germination triggering compound in both plant-derived (Van Staden et al., 2004) and cellulose-derived smoke (Flematti et al., 2004) has
been identified as 3-methyl-2H-furo[2,3-c]pyran-2-one. This smoke-derived compound (a butenolide) is a simple organic compound and has been shown to increase both the level and the rate of seed germination, widen the environmental range over which germination can occur and have a positive effect on seedling vigour (Light et al., 2009). Butenolide is formed during pyrolysis reactions and may have an effect during fires, in the immediate post-fire environment, considerable periods after the fire, or perhaps more importantly, in plant communities long distances away from the active fire site. Though research has answered several relevant questions behind the discovery of this butenolide, knowledge of the effects of fire on altering the soil–smoke chemistry and the persistence of the active germination triggering compound in the soil, in the context of its water-soluble nature, is still lacking. The spatial and temporal dimensions of fire and smoke influence on the chemistry of burnt sites and the sites surrounding the burn are also unknown.

Smoke is a highly complex mixture of particulate matter (ash and soot), droplets and gases emitted when a material undergoes combustion or pyrolysis. Vegetation fires produce huge clouds of smoke which can drift into neighbouring unburnt areas carried by wind (Andreae, 1991; Keeley and Fotheringham, 1997). 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 stimulating the soil seed bank (Crosti et al., 2006; Read et al., 2000). Hence, knowledge of the effect of fire on soil–smoke chemistry and the subsequent influence on the germinable soil seed bank is important from conservation, restoration and weed control perspectives, particularly with regard to the fire-prone grasslands of South Africa, where the use of fire for managing the health of the grassland is a common practice (Scott, 1971). Despite the wide use of fire as a management tool, its effects on soil seed bank germination and the subsequent impact on grassland composition is still unknown.

The objectives of the present study were: (1) to investigate if grassland burning affects the soil–smoke content; (2) to determine if this soil property differs along depths in the soil profile; (3) if the activity of smoke along the soil core differs with presence or absence of rain; (4) if the soil-smoke activity differs with time since the day of burn (persistence). In this study we detected the relative smoke concentration by the biological activity of the burnt or otherwise unburnt soil extracts to germinate seeds of light-sensitive lettuce (Lactuca sativa L. cv. Grand Rapids) achenes (hereafter referred to as seeds). Grand Rapids lettuce seeds are particularly responsive to smoke extracts and are effectively used as a rapid bioassay to evaluate the biological activity of various extracts (Drewes et al., 1995).

2. Materials and methods

2.1. Site description, burning and the soil samples

The sites used for collecting burnt soils were 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. The sites had not been burnt for the previous 2 years. Soils at the site were classified as Westleigh forms (Soil Classification Working Group, 1991). The vegetation of the area is classified as southern Tall Grassveld (Acocks, 1988). The sward varied between 40 and 60±6.33 cm in height (mean±SE of 20 random samples) and was dominated by herbaceous species, mainly Themeda triandra Forssk, Heteropogon contortus Beauv. ex Roemer & J. A. Schultes and Tristachya leucothrix Trin. ex Nees.

On 29 May 2008, a head fire was applied on the grassland site under the following weather conditions: air temperature 30 °C; wind speed 6 km h⁻¹ and relative humidity of 52%. 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 12.30 pm and was sustained for only a few seconds. It was deemed to be sufficient to deposit enough smoke onto the soil. Soil samples were subsequently 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). To determine if the presence or absence of rain following a fire affected the vertical distribution of smoke activity along the soil profile, approximately 8×10⁻⁷ mm³ of water (simulated rain) was sprayed over 1 m² on marked plots and soil core sampling was done 10 min after the water had completely drained. With the exception of the artificially sprayed (simulated) rain, no rainfall was recorded during the eight-week sampling period after the burn. To assess the effect of burning on soil–smoke activity and to examine the persistence of the influence through time, 96 soil samples (6 treatments X4 depths X4 replications) were collected for successive weeks, i.e. 0 (immediately after burn), 2, 4 and 8 weeks after burn. Hence, a total of 312 soil samples were collected and tested for germination activity.

2.2. Fuel load and fuel moisture

Prior to burning, available fuel loads were estimated by collecting dead and live plant material from 5 quadrats (1 × 5 m) along an established 50 m transect. Following collection,

<table>
<thead>
<tr>
<th>Treatments Abbreviated as</th>
<th>Conditions under which soil cores were collected</th>
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<tbody>
<tr>
<td>Unburnt, no water</td>
<td>UBNW</td>
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<tr>
<td>Unburnt, watered</td>
<td>UBW</td>
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<tr>
<td>Adjacent, no water</td>
<td>ANW</td>
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<tr>
<td>Adjacent, watered</td>
<td>AW</td>
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<tr>
<td>Burnt, no water</td>
<td>BNW</td>
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<tr>
<td>Burnt, watered</td>
<td>BW</td>
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Table 1

Soil core samples collected before and after burning a mesic grassland site in South Africa.
samples were oven dried at 120 °C for 72 h to obtain an estimate of available fuel load and fuel moisture content. Fuel load and fuel moisture content were calculated as: fuel moisture content (wet basis) = (total mass of wet biomass – total mass of dry biomass) / total mass of wet biomass X 100 (Anderson and Kothmann, 1982). Dry biomass weights per square metre were converted to tonnes per hectare.

2.3. Soil extraction methods

Soil samples were individually stored in clear plastic bags at 5 °C. Each soil sample was crushed using a mortar and pestle and sieved to remove stones and organic material. The soil samples were extracted with three volumes (100 mL and 50 mL X 2) of distilled (purified) dichloromethane. Dichloromethane was used as an extracting solvent, because it is known that the smoke-derived butenolide can be 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 a suitable method. The dichloromethane was evaporated and the residue redissolved in 10 mL of distilled-water. This extract was diluted giving three concentrations (1:1, 1:10 and 1:100 v/v). These extracts were then used to initiate germination of the Grand Rapids lettuce seeds.

2.4. Seed source and the lettuce seed bioassay

Mature achenes (seeds) of L. sativa L. cv. Grand Rapids (Peto Seeds, Saticoy, USA) were used in this study. Four replicates of 25 seeds each were placed on two layers of Whatman no. 1 filter paper in 65 mm plastic Petri dishes moistened with 2.2 mL of distilled water (control) or the test solutions. The seeds were kept in light-proof boxes and moistened with 2.2 mL of distilled water (control) or the test solutions. A solvent blank extract (dichloromethane extract prepared from soils of 0–5 cm depth of the BW plot) was also tested. To verify the effect of smoke on germination, the Grand Rapids lettuce seeds were also subjected to smoke–water (1:500 v/v) and butenolide (10⁻⁸ M) solutions. A solvent blank extract (dichloromethane extract prepared in the manner of the soil extracts without soil sample) was also tested.

2.5. Statistical analysis

The data obtained from the experiments were arcsine transformed and subjected to one way analysis of variance (ANOVA). Tukey’s multiple range test at a significance level of 5% was calculated. GenStat (version 11.1, Rothamsted Research Harpenden, UK) statistical package was used to analyse the data of these experiments.

3. Results

3.1. Fuel load and fuel moisture content

The fuel load of the unburnt areas was estimated to be 1.24±0.18 (SE)t ha⁻¹. The moisture content of the fuel was 19.23±0.91%. The soil moisture content of the site was 10.33±0.87%. Such a fuel load for the given moisture content was sufficient to produce hot fires and smoke and all the dead and live grass cover was consumed rapidly.

3.2. Germination activity of the soil extracts

Smoke–water and butenolide solutions resulted in a 3-fold significant increase in percentage germination (>80%) of Grand Rapids lettuce seeds compared to controls (Fig. 1). Results of the germination activity of soil extracts are given in Fig. 2. At the first time point, soil extracts of BNW (see Table 1 for the abbreviations used) and BW plots from 0 to 2 and 2 to 4 cm depths tested with different concentrations, showed significantly higher percentage germination than the soil extracts from other plots (Fig. 2a). Soil extracts of the same plots from a depth of 6–8 cm tested with higher concentrations (1:1 and 1:10), showed a significant increase in percentage germination compared to other plots (Fig. 2a). After 2 weeks, most of the concentrations of extract prepared from soils of 0–2, 2–4 and 4–6 cm depths of BNW and BW plots, exhibited a significantly greater germination than the soil extracts from other plots (Fig. 2a). At a depth of 6–8 cm, only the highest concentration (1:1) significantly increased in percentage germination compared with the other concentrations tested (Fig. 2a). In the soil samples collected after 4 weeks from 0 to 2 and 2 to 4 cm depths of BNW plots, the concentration of 1:10 of these soil extracts showed a significant increase in percentage germination compared to other plots (Fig. 2a). At a depth of 6–8 cm, only the highest concentration (1:1) significantly increased in percentage germination compared with the other concentrations tested (Fig. 2b). At a depth of 2–4 cm this increase in percentage germination was achieved at both 1:1 and 1:10 concentrations (Fig. 2c).

Fig. 1. Germination of Grand Rapids lettuce seeds imbibed in smoke–water (SW 1:500 v/v), butenolide (B 10⁻⁸ M), dichloromethane (D) and distilled-water (W) after 24 h of incubation at 25 °C in the dark. Asterisks denote a significant (P<0.05) difference from distilled-water and dichloromethane control; error bars indicate SE.
Fig. 2. The effect of various soil extracts obtained from six different burning treatments at three dilution levels on the germination of Grand Rapids lettuce seeds after 24 h in the dark at 25 °C: (a) 0 week after burn; (b) 2nd week after burn; (c) 4th week after burn; and (d) 8th week after burn. Asterisks denote a significant ($P < 0.05$) difference from the unburnt control plots; error bars indicate SE.
extracts from depths of 4–6 and 6–8 cm of BNW and BW plots with a concentration of 1:10 showed significantly higher percentage germination than the low or high concentrations. The tested soil extracts from other plots were also significantly different to 1:10 concentration of BNW and BW plots (Fig. 2c). After 8 weeks, a high concentration (1:1) of soil extracts from depths of 0–2 and 2–4 cm of BNW and BW plots showed greater percentage germination than other treatments or plots. No significant germination activity was observed in extracts from unburnt or adjacent plots. Table 2 of ANOVA shows that treatment, concentration, depth, time and their interactions were highly significant ($P<0.001$).

4. Discussion and conclusions

Studies conducted on various vegetation types worldwide suggest that post-fire increases in germination and massive seedling emergence of certain species are due to direct heat (Read et al., 2000; Trollope, 1984; Wills and Read, 2002), increases in nutrient levels (Harper, 1977; Kinloch and Friedel, 2005), better access to light (Hulbert, 1988), smoke-induced germination (Brown, 1993; Brown and Van Staden, 1997) and the combined effect of the heat shock and smoke (Gilmour et al., 1995). The tested soil extracts from other plots were also significantly different in percentage germination than the low or high concentrations. After 8 weeks, a high concentration (1:1) of soil extracts from depths of 0–2 and 2–4 cm of BNW and BW plots showed greater percentage germination than other treatments or plots. No significant germination activity was observed in extracts from unburnt or adjacent plots. Table 2 of ANOVA shows that treatment, concentration, depth, time and their interactions were highly significant ($P<0.001$).

We found that the soil–smoke activity, as detected by the ability of the various soil extracts to stimulate germination of the Grand Rapids lettuce seeds, significantly differed among treatments, dilution levels, soil depth and time since the burn. The highest variation was accounted for by time followed by the treatments (Table 2). In comparison to the water control, the Grand Rapids lettuce seeds exhibited greater germination percentages when treated with burnt soil extracts (at 0–4 cm depths) throughout the 8-week period. This enhanced germination of Grand Rapids lettuce seeds in response to burning (smoke) is in consensus with previous studies on smoke-induced germination of various species (Brown, 1993; Brown and Van Staden, 1997). These results imply that the smoke produced by fire penetrates and incorporates into the soil and would play a significant role in enhancing germination of certain smoke-responsive species stored in the soil seed bank (Read et al., 2000; Wills and Read, 2002). The stimulatory role of fire (smoke) on the germination of the soil seed bank may be related to soil depth (Pearl, 1984; Read and Bellairs, 1999; Stevens et al., 2007), dilution level (Stevens et al., 2007) and time since burning (Pearl, 1984; Preston and Baldwin, 1999). The water treatment (simulated rain) did not affect the vertical distribution of smoke activity along the core. This could probably be because of the amount of the simulated rain applied and the high water holding capacity of the Westleigh soils at the site with more clayey topsoil (Soil Classification Working Group, 1991) not allowing the smoke compounds to permeate deeper. Recently, Stevens et al. (2007) have demonstrated that the germination compound in plant-derived smoke can be leached through the soil profile, using an experimental system with a column of silica sand. Evidently, smoke activity in the burnt plots was higher in the topsoil than in the lower soil profiles. The smoke activity declined through time, maybe due to the water-soluble nature of the germination compound in smoke, only allowing it to persist in the soil for a limited period of time (Brown and Van Staden, 1997; De Lange and Boucher, 1990; Preston and Baldwin, 1999). However, a similar study conducted by Preston and Baldwin (1999) suggested that the germination compound in smoke can remain in the soil up to 7 years.

Interestingly, in 0 week after burning (Fig. 2a), germination activity in the adjacent areas was significantly higher than in the unburnt control. However, starting from the 4th week, germination activity was significantly lower in the adjacent plots compared to the unburnt control (Fig. 2), or identical to the water control (Fig. 1). Though the reason(s) for this rapid loss of germination activity is/are unknown, it could be due to the effect of allelochemicals and/or some inhibitory compounds present in plant-derived smoke. It is common in nature that allelopathic compounds released from standing vegetation to the seed environment can inhibit pre and/or post-germination growth (Keeley et al., 1985). For instance, Javaid et al. (2006) found that germination and growth of Parthenium hysterophorus L. was significantly suppressed by root and shoot extracts of allelopathic crops. An alternative interpretation is that smoke contains some inhibitory compounds that may block the action of the stimulator, preventing germination of seeds (Daws et al., 2007; Light et al., 2010). Both, the stimulator and inhibitor signals in smoke can be transported to unburnt areas carried by wind, causing opposing effects on germination. Preston and Baldwin (1999) have found evidence that the germination compound in smoke can be transported by wind and water into adjacent unburnt areas (some 40 m away from a burnt site). These authors also demonstrated the existence of negative, germination-inhibiting factors in burning plant material. Hence, wind and water transport of the smoke compounds, may not essentially stimulate germination in adjacent unburnt areas, because the litter-derived factors may not be able to override the positive effects of the smoke cue. In many cases, the negative role of smoke on germination depended on its concentration and some species-specific inhibitors in smoke (Daws et al., 2007; Light et al., 2010).

One of the most striking results obtained from this study is that unburnt soil extracts exhibited greater germination percentage compared to the water control results. This probably suggests that other organic factors (besides smoke) may be present in the soil due to the degradation of soil organic matter and such chemicals may play a role in promoting germination (Dixon et al., 1995; Keeley and Fotheringham, 1997). In other words, smoke (burning) may only play a supplementary role in promoting germination of species in the post-fire environment (Dixon et al., 1995). It is well known that ethylene promotes germination in seeds from a wide range of species, including Grand Rapids lettuce seeds (Jäger et al., 1996). Therefore, seed banks of certain species may react additively to a number of cues in the habitat.
The marked positive germination responses that are observed following a fire are the result of the sum total of effects of smoke and non-smoke factors in the habitat. From previous studies on smoke-stimulated germination, certain grass species which dominate South African mesic grasslands (e.g. *H. contortus*, *T. triandra* and *T. leucothrix*) have shown a positive germination and vigour response to smoke (Ghebrehiwot et al., 2009), though they are generally unable to survive fire (Zacharias et al., 1988). Hence, considering that the soil seed bank consists of viable seeds, at a moderate depth and that they are initially unaffected by the heat of the fire, smoke should give a competitive advantage to the smoke-responsive species over those less responsive species, and such a competitive advantage would likely impact on the composition of the grassland. However, detailed field studies on the reaction of the soil seed bank to fire and smoke is needed to gain better insight into the effect of fire on issues related to soil, smoke and germination.

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