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Research Letter

Allelopathic Effects of Plant-Derived Aerosol Smoke on Seed Germination of *Arabidopsis thaliana* (L.) Heynh.

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The role that plant-derived smoke plays in promoting seed germination is well documented, but little is known about its ability to inhibit seed germination. To better understand this phenomenon, we tested the effects of eight aerosol smoke treatments on the Columbia-3 ecotype of nondormant *Arabidopsis thaliana* (L.) Heynh. seeds. Our results revealed that aerosol smoke significantly inhibits germination when seeds were exposed to prolonged periods of aerosol smoke. Short durations of smoke treatments significantly promoted the rate of germination of *A. thaliana* seed. We briefly discuss this dual regulation of smoke and its possible impact on conservation and restoration practices. We also propose that plant-derived smoke may be another vehicle by which allelochemicals can be introduced into the environment.

1. INTRODUCTION

Much has been written in recent times about the effects of aerosol smoke and smokesolutions on stimulating seed germination. The use of smoke as a germination cue, first reported by De Lange and Boucher [1], was significant in breaking dormancy in the fynbos species, *Audouinia capitata* (L.) Brongn. (Bruniaceae). Since then, several studies have focused on the ability for smoke to promote germination in a variety of plant species in South Africa, Australia, and North America [2–6]. Pennacchio et al. [7] and Jefferson et al. [8] have recently discovered that smoke also promotes the germination of a variety of tall-grass prairie species in the midwest regions of North America. As a result, smoke is now increasingly being used as an ecological and restoration tool throughout the world in a variety of conservation practices, in land management, and for the promotion of wild and other plants [4], including indigenous medicinal plants [9]. Researchers who have screened large numbers of plants often report that the seed of some species are inhibited by smoke. This phenomenon occurs when seeds are exposed to prolonged periods or high concentrations of smoke and smoke solutions [2, 10].

We further investigate the role that this dual regulation of smoke has on seed germination. Plant-derived smoke may also be a vehicle by which natural allelochemicals can be introduced into an area and affect the germination of some plant species. Allelopathy refers to the interactions of secondary metabolites between neighboring plants, as well as interactions between plants and other organisms, such as bacteria, fungi, and algae [11]. Secondary compounds, or allelochemicals, can induce both inhibitory and promontory effects on organisms and may play important roles in shaping plant and microbial communities. These substances are usually released during the degradation of plant material. Allelopathic substances are, however, also released into the atmosphere as volatiles that eventually settle onto soil surfaces [12]. We suggest that allelochemicals may also be carried along with smoke's particulate matter and exert a significant impact on plant communities, especially when they are exposed to prolonged periods of smoke haze.

2. MATERIALS AND METHODS

Smoke treatments

Mature, nondormant seeds of *Arabidopsis thaliana* (Columbia Col-3 ecotype) were purchased from Lehle Seeds (Round Rock, Tex, USA). We previously showed that *A. thaliana* seeds are sensitive to a variety of inhibitory

phytochemicals and are therefore ideal for use as a test species for screening agents of that nature [13]. A total of eight smoke treatments were tested with durations of 0-, 1-, 2-, 4-, 8-, 16-, 32-, and 64-minute periods of aerosol smoke. We burned straw in a burner that was built in our laboratory to produce aerosol smoke.

We conducted four separate seed germination smoke trials (Trials 1–4) with *A. thaliana* seed. In the first trial, seeds were treated and germinated in 55 mm Petri dishes lined with moistened Whatman filter paper No. 1. Seeds were treated with the eightdifferent durations of aerosol smoke and were then immediately sealed in Petri dishes with Parafilm (Trial 1). These were kept in an incubator (Low Temperature, Precision Scientific Inc (Chicago, USA)) set at 10/20°C and with a 12-hour light/12-hour dark cycle. There were eight replicates of 25 seeds for each treatment. The purpose of this trial was to determine the effects of aerosol smoke on seed in which smoke was continuously acting on the seeds.

In Trial 2, *A. thaliana* seeds were directly sown above ground onto a germination soil mixture (Fafard, Agawam, Mass, USA). These were then transferred with the soil to the smoke chamber where they too received the eight smoke treatments. There were eight replicates with 25 seeds in each replicate. These seeds were kept in a greenhouse, where they were watered frequently with a fine mist of overhead water. This trial specifically aimed at exploring the effects of aerosol smoke on seeds that were frequently watered and therefore rinsed. The third trial (Trial 3) was similar to the second. Untreated *A. thaliana* seeds were directly sown onto the germination soil, but only after the soil they had already been treated with the eight smoke treatments. This aspect of the study, which was also conducted in the greenhouse with the automatic sprinkling system, focused on the effects of smoke-treated soil on the germination of untreated seeds.

In the final trial (Trial 4), we treated preimbibed *A. thaliana* seeds with the eight smoke regimes, left them for an hour, and then rinsed the seeds with running water for 2 minutes. These seeds were transferred to the germination soil and were germinated in the greenhouse with the same sprinkling system described above. There were only four replicates of 25 seeds for this aspect of this study, which aimed to research the effects of immediate rinsing of smoke-treated seeds.

3. DATA ANALYSIS

Germination counts were performed daily for up to a period of 14 days or until germination ceased. To determine whether or not any of the treatments had an affect on seed germination, final germination percentage (FG%), rates of germination (RG) and mean period to final germination (MPFG) were calculated according to other researchers [14]. Final germination percentages were arcsine transformed prior to statistical analyses [15]. Both RG and MPFG data were log transformed. Data were analyzed using the statistical package Systat (San Jose, USA) (version 10.2). A one-way ANOVA was performed on all results. Probabilities of less than 0.05 were considered statistically significant. Differences between means were determined using Tukey's compromise test.

4. RESULTS

A. thaliana seeds treated and germinated in Petri dishes (Trial 1) did not germinate if they received four or more minutes of smoke treatment (Table 1). The average FG% for those seeds that did germinate was 98.50 ± 1.50 and 20.50 ± 6.74% for 1 and 2 minutes, respectively, (Table 1). A one-way ANOVA and Tukey's compromise test revealed that germination in the 2-minute group of seeds was significantly lower than that of the 0- and 1-minute groups ($P < .001$; Table 1). The RG of seeds treated for 2 minutes (1.28 ± 0.39) was also significantly ($P = .000$) decreased compared to the 0-minute (12.47 ± 0.03) and 1-minute (6.07 ± 0.22) groups. The MPFG, in contrast, increased from 2.01 ± 0.01 at 0 minute to 4.63 ± 0.16 at 1 minute ($P = .000$) and 4.34 ± 0.51 at 2 minutes ($P = .000$; Table 1).

The FG% of seeds sown and treated in germination soil, and which were watered regularly (Trial 2), was significantly inhibited following 64 minutes of smoke time (Table 1). The inhibitory effects of smoke on FG% did not manifest themselves in seeds treated for 32 minutes or less (Table 1). The RG of seeds treated for 2–8 minutes was significantly increased ($P < .001$). The MPFG significantly increased from 2.80 ± 0.09 at 0 minute to 3.70 ± 0.01 at 16 minutes, 3.90 ± 0.08 at 32 minutes, and 4.20 ± 0.06 at 64 minutes ($P < .001$).

The results for untreated seeds sown on smoke-treated soil (Trial 3) revealed no significant differences in FG% (Table 1). There were, however, significant decreases in RG between the 0- and 64-minute smoke treatments ($P = .000$) and between the 2-minute treatment and each of the16- ($P = .009$), 32- ($P = .042$), and 64-minute ($P = .000$) treatments (Table 1). There were no significant differences observed for MPFG (Table 1). The FG%, RG, and MPFG of seeds treated with smoke and then immediately rinsed with distilled water (Trial 4) did not significantly change ($P = .844$, .689, and .118, resp.), with all seeds germinating above 97% within 3 days (Table 1).

5. DISCUSSION

It is not surprising that prolonged exposures to plant-derived aerosol smoke exert an inhibitory effect on seed germination in this and other plant species. Aerosol smoke carries with it a number of compounds and oxidatively abrasive chemicals that could potentially be introduced into the environments in which the smoke occurs. Known volatile allelochemicals, such as camphor, alpha-pinene, alpha-phelandrene and eugenol, are often released by plants in fire-prone environments [12]. At high enough doses, all these induce inhibitory allelopathic effects on seed germination in some species. Our results suggest that treating soil with long exposures to smoke, and then sowing *A. thaliana* seeds onto that soil, also significantly decreases rate of germination in its seeds.

Plant species that occur in environments that experience unusually long exposures to plant-derived smoke may similarly be affected by its allelochemicals. Several factors often combine to confine smoke hazes in a localized area for extended periods. Local weather conditions, such as inversions and prevailing winds, immediately following fires play

Table 1: Mean ± SE for FG%, RG and MPFG results for the four seed germination trials: Petri dish trial (Trial 1), seed and soil treated with smoke (Trial 2), soil only treated with smoke (Trial 3) and seeds rinsed prior to sowing (Trial 4) following the eight smoke treatments with aerosol smoke. Different letters represent significant differences in means.

Smoke time (Min)	Seeds germinated in Petri dishes (Trial 1)			Seeds and soil treated with smoke (Trial 2)		
	FG%	RG	MPFG	FG%	RG	MPFG
0	100.00 ± 0.00 a	12.47 ± 0.03 a	2.01 ± 0.01 a	95.5 ± 1.71 a	18.0 ± 0.56 b	2.8 ± 0.09 b
1	98.50 ± 1.50 a	6.07 ± 0.22 b	4.63 ± 0.16 b	94.5 ± 2.06 a	19.6 ± 0.38 ab	2.5 ± 0.02 a
2	20.50 ± 6.74 b	1.28 ± 0.39 c	4.34 ± 0.51 b	99.5 ± 0.50 a	20.4 ± 0.18 a	2.6 ± 0.04 ab
4	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 c	96.5 ± 2.06 a	19.4 ± 0.42 ab	2.6 ± 0.06 ab
8	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 c	98.0 ± 1.16 a	19.1 ± 0.23 ab	2.7 ± 0.07 ab
16	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 c	96.0 ± 2.16 a	13.8 ± 0.21 c	3.7 ± 0.01 c
32	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 c	92.0 ± 1.63 ab	12.4 ± 0.04 d	3.9 ± 0.08 cd
64	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 c	76.5 ± 2.87 b	9.9 ± 0.21 e	4.2 ± 0.06 d
P-Value	< .001	< .001	< .001	< .001	< .001	< .001
	Soil treated only with smoke (Trial 3)			Seeds rinsed after smoke (Trial 4)		
0	98.50 ± 1.05 a	11.22 ± 0.20 ab	2.43 ± 0.11 ab	99.16 ± 0.83 a	9.65 ± 0.45 a	2.18 ± 0.05 a
1	98.50 ± 1.05 a	12.02 ± 0.18 a	2.09 ± 0.04 a	98.70 ± 0.87 a	7.97 ± 0.53 a	2.22 ± 0.09 a
2	99.00 ± 1.00 a	11.50 ± 0.41 ab	2.29 ± 1.25 a	97.34 ± 0.96 a	9.10 ± 0.83 a	2.14 ± 0.06 a
4	98.00 ± 0.76 a	12.04 ± 0.00 a	2.08 ± 0.03 a	97.43 ± 2.57 a	8.07 ± 1.53 a	2.59 ± 0.20 a
8	98.50 ± 1.50 a	11.24 ± 0.25 ab	2.39 ± 0.08 ab	97.30 ± 1.87 a	9.41 ± 0.88 a	2.21 ± 0.06 a
16	98.50 ± 1.05 a	9.99 ± 0.36 b	2.79 ± 0.13 b	97.75 ± 1.30 a	10.21 ± 2.44 a	2.21 ± 0.14 a
32	99.50 ± 0.50 a	10.33 ± 0.52 b	2.75 ± 0.20 b	97.25 ± 1.66 a	10.27 ± 1.35 a	2.22 ± 0.09 a
64	91.43 ± 3.75 a	4.40 ± 0.26 c	5.42 ± 0.17 c	98.95 ± 1.05 a	8.06 ± 1.43 a	2.23 ± 0.02 a
P-Value	.118	< .001	< .001	.884	.689	.118

an important role in that process. The inhibitory effects on the seeds affected by the resultant smoke are likely to be decreased if there is sufficient rainfall immediately following a prolonged exposure to it. The results of this study suggest that inhibitory substances can be rinsed out with water. Interestingly, this study also revealed that the promoter in smoke does not appear to be affected by rinsing the seeds. Smoke treatments ranging from 2–8 minutes significantly increased rate of germination in *A. thaliana* seeds that were frequently watered in the greenhouse. The dual regulation of plant-derived smoke on *A. thaliana* seed may make this species a useful test subject when trying to determine the mechanisms of action of the inhibitory and promontory compounds in smoke.

In conclusion, competitive and antagonistic interactions such as those reported here, as well as the need for water or rainfall to leach out any inhibitor, may be important considerations for those who routinely manage land and coordinate conservation practices in post-fire environments [10]. The results of this study also suggest that smoke is another vehicle by which allelopathic substances could potentially be introduced into the environment.

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