

Two Novel Uses For *Arabidopsis thaliana* (L.) Heynh Seeds.

Marcello Pennacchio, Lara Jefferson and Kayri Havens
Institute for Plant Conservation, Chicago Botanic Garden.



Phytotoxic Bioassays

Introduction

We evaluated the potential use of *Arabidopsis thaliana* (L.) Heynh (Brassicaceae) seed for use as part of a set of standard target species (STS) in phytotoxic bioassays. In our study, we tested seven potent allelochemicals and compared their effects between the seeds of Columbia (Col-3) ecotype of *A. thaliana* with those of the more commonly used lettuce seeds.

Materials and Methods

Seven known and commercially available allelochemicals were used as test solutions in this study. The allelochemicals tested were tannic acid (0.1, 1.0 and 10.0 mM), juglone (1, 10 and 100 μ M), cinnamic acid (0.01, 0.1 and 1.0 mM), caffeic acid (0.001, 0.01 and 0.1 M), coumarin (0.01, 0.10 and 1.00 μ M), salicylic acid (0.01, 0.1 and 1 mM) and 1,8-cineole (0.65, 6.5 and 65 mM). The control was distilled water. There were four replicates for each trial. A total of 25 lettuce and 50 *A. thaliana* seeds were used in each replicate. These were sealed in 90 mm Petri dishes and kept in an incubator. We recorded Final Germination % (FG%), Rate of Germination (RG) and Mean Period to Final Germination (MPFG). Prior to data analyses with one-way ANOVAs, FG% was arcsine transformed while RG and MPFG were log transformed.

Results

The Columbia (Col-3) ecotype of *A. thaliana* exhibited greater sensitivity to the seven potent allelochemicals than did lettuce seeds, which, in some cases, did not respond at all to those substances.

Table 1. The effects of coumarin on FG%, RG and MPFG of *A. thaliana* (Col-3 ecotype) and lettuce seeds.

Conc.	FG%		RG (d)		MPFG (d)	
	At	Ls	At	Ls	At	Ls
Control	100.0 ± 0.0 a	100.0 ± 0.0 a	27.8 ± 4.4 a	24.1 ± 0.4 a	2.0 ± 0.2 a	1.1 ± 0.1 a
0.01	59.5 ± 2.1 b	78.0 ± 15.7 ab	9.1 ± 0.8 b	4.6 ± 1.0 c	3.7 ± 0.3 b	4.7 ± 0.3 b
0.1	37.5 ± 5.7 c	70.0 ± 10.9 ab	5.9 ± 0.9 b	10.8 ± 2.1 ab	3.5 ± 0.1 b	2.0 ± 0.1 c
1	0.0 ± 0.0 d	54.0 ± 9.6 b	0.0 ± 0.0 c	31.1 ± 1.8 b	0.0 ± 0.0 c	1.7 ± 0.1 c
P-Value	< 0.001	0.015	< 0.001	< 0.001	< 0.001	< 0.001

Different letters indicate significant differences between extract concentrations, using Tukey's Compromise. FG% = final germination percentage; RG = rate of germination (d); MPFG = mean period to final germination (d); At = *Arabidopsis thaliana*; Ls = *Lactuca sativa*. See Methods for calculation of RG and MPFG.

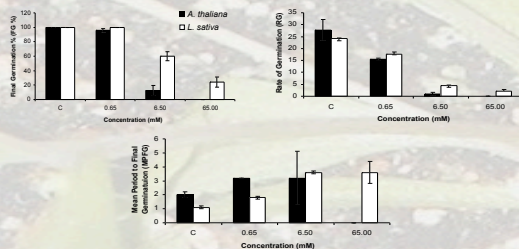


Fig. 1. The effect of 1,8-cineole on FG% (top left), RG (top right) and MPFG (bottom) of *A. thaliana* seeds.

Smoke and Germination

Introduction

The ability for plant-derived smoke to inhibit seed germination is poorly understood. To better understand this phenomenon, we tested the effects of eight different aerosol smoke treatments on the Columbia (Col-3) ecotype of nondormant *A. thaliana* seeds.

Materials and Methods

A total of eight smoke treatments were tested with durations of 0, 1, 2, 4, 8, 16, 32 and 64 min periods of aerosol smoke, which was produced by burning hay in a burner built in our laboratory. We conducted four separate seed germination smoke trials (Trials 1-4) with *A. thaliana* seed. 1) seeds were treated and germinated in 55 mm Petri dishes and kept in an incubator set at 10o/20oC and with a 12-hour light/12-hour dark cycle. 2) *A. thaliana* seed directly sown onto a germination soil mixture. These seeds were kept in a greenhouse where they were watered frequently with a fine mist of overhead water. 3) This trial was similar to the second. Untreated *A. thaliana* seeds were directly sown onto the germination soil, but only after the soil had already been treated with the eight smoke treatments. 4) Pre-imbibed *A. thaliana* seeds were treated with smoke and then rinsed with running water for 2 min. Data analyses were the same for Phytotoxic Bioassays.

Results

Prolonged exposures to smoke inhibited germination in *A. thaliana* seeds unless they were rinsed with water. Smoke treatments greater than 4 minutes were enough to inhibit germination. Seeds that were immediately rinsed following smoke treatment were not inhibited. Sowing seeds on soil previously treated with smoke were also inhibited following long exposures to smoke. Smoke treatments that ranged from 2-8 minutes significantly increased RG.

Table 2. The effects of aerosol smoke on FG%, RG and MPFG of *A. thaliana* (Col-3 ecotype).

Time	Seeds in Petri Dishes (Trial 1)			Seeds rinsed after smoke (Trial 4)		
	FG%	RG	MPFG	FG%	RG	MPFG
0	100.0 ± 0.0 a	12.5 ± 0.0 a	2.0 ± 0.0 a	99.2 ± 0.8 a	9.6 ± 0.4 a	2.18 ± 0.05 a
1	98.5 ± 1.5 a	6.1 ± 0.2 b	4.6 ± 0.2 b	98.7 ± 0.9 a	7.9 ± 0.5 a	2.22 ± 0.09 a
2	20.5 ± 6.7 b	1.3 ± 0.4 c	4.3 ± 0.5 b	97.3 ± 0.9 a	8.1 ± 0.8 a	2.14 ± 0.06 a
4	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 c	97.4 ± 2.6 a	8.0 ± 1.5 a	2.59 ± 0.20 a
8	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 c	97.3 ± 1.9 a	9.4 ± 0.9 a	2.21 ± 0.06 a
16	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 c	97.8 ± 1.3 a	10.2 ± 2.4 a	2.21 ± 0.14 a
32	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 c	97.2 ± 1.7 a	10.3 ± 1.4 a	2.22 ± 0.09 a
64	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 c	98.9 ± 1.0 a	8.1 ± 1.4 a	2.23 ± 0.02 a
P-Value	< 0.001	< 0.001	< 0.001	0.884	0.689	0.118

Different letters indicate significant differences between extract concentrations, using Tukey's Compromise. FG% = final germination percentage; RG = rate of germination (d); MPFG = mean period to final germination (d); At = *Arabidopsis thaliana*; Ls = *Lactuca sativa*. See Methods for calculation of RG and MPFG.

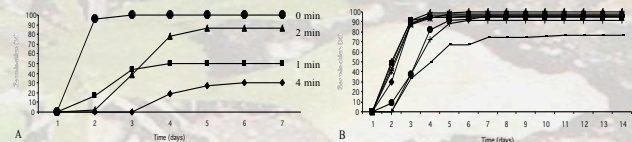


Fig. 2. Cumulative germination of *A. thaliana* seeds following smoke treatments (Trials 1(A) and 2 (B)).

Overall Discussion

Arabidopsis thaliana appears to be more sensitive to phytochemicals than lettuce seeds and is therefore useful for inclusion as part of a set of standard target species in phytotoxic bioassays. This may help identify less potent allelochemicals. *A. thaliana* seeds are also responsive to both the promotive (RG only) and inhibitory effects of smoke. This species is used extensively in laboratories throughout the world for a variety of plant studies. More than 10 000 references to this species alone exist in the literature. In addition, its entire genome has been mapped. This represents an incredible resource that has already helped researchers understand plant growth and development, interactions between plants and pathogens, plant pattern formation, plant physiology and plant biochemistry. A number of useful mutants are available that are resistant to phytotoxins. This may therefore serve as another attractive model for helping to identify the mechanisms of resistance to certain toxic substances. Proteomic studies will probably also play a useful role. Studies of this type have already started unraveling some of the mysteries of seed germination in *A. thaliana*.

Contact details: Marcello Pennacchio (mpennacchio@chicagobotanic.org)
Lara V. Jefferson (ljefferson@chicagobotanic.org)
Kayri Havens (khavens@chicagobotanic.org)
1000 Lake Cook Road, Glencoe, IL, 60002, USA.

Pennacchio, M., Jefferson, L.V. and Havens, K. (2005). *Arabidopsis thaliana*: a new test species for phytotoxic bioassays. *Journal of Chemical Ecology* (in press).