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Title page

Positive germination response of oriental mustard (*Sisymbrium orientale* L., Brassicaceae) to plant-derived smoke

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Positive germination response of oriental mustard (*Sisymbrium orientale* L., Brassicaceae) to plant-derived smoke

Abstract

Plant-derived smoke enhances seed germination for numerous species, but the effect of smoke can vary with germination conditions and seed dormancy state. A highly variable germination response to the active compound(s) of smoke has been published in the literature for the annual weed *Sisymbrium orientale* L. In a laboratory experiment, we tested the effect of an aqueous smoke solution (smoke-water) on the seed germination of *S. orientale* after 2 months and 13 months of dry storage (in August 2013 and 2014, respectively) at room temperature under alternating light and constant darkness. It was hypothesized that smoke-water enhances germination, but the smoke response varies with light or dark conditions. At both germination dates, smoke-water treatment consistently increased the final germination percentage under both light conditions: from 54-66% to 82-84% in the light and from 73-77% to 92-99% in the dark. Germination was higher in the dark than in the light, irrespective of smoke-water treatment. To our best knowledge, this is the first study demonstrating a positive germination response of *S. orientale* to smoke-water. These results can improve our understanding of the smoke responsiveness for this species, and can potentially increase the efficiency of weed management.

Keywords: Brassicaceae, laboratory experiment, light, smoke-water, weeds

Introduction

Plant-derived smoke or its aqueous solution (smoke-water) have been documented to enhance seed germination and/or seedling growth for numerous wild species, particularly in fire-prone Mediterranean ecosystems (Brown 1993; Dixon et al. 1995; Keeley and Fotheringham 1998; Figueroa et al. 2009; Moreira et al. 2010; Downes et al. 2014), but also in temperate regions where a number of arable weeds also responded positively (Adkins and Peters 2001; Daws et al. 2007; Stevens et al. 2007). The germination stimulating capacity is mainly attributed to karrikinolide (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, KAR_1), a butenolide-type compound identified in smoke (Flematti et al. 2009, 2015). The positive response of plants to smoke can potentially be utilized in weed control by the application of smoke or KAR_1 in croplands to enhance and synchronize the germination of weed seeds from the soil seed bank, followed by the eradication of the emerged seedlings before sowing a crop (Adkins and Peters 2001; Kulkarni et al. 2011; Kamran et al. 2014). However, there is increasing evidence that smoke responsiveness is not an absolute, static trait of a species, and the sensitivity of seeds to smoke-derived chemicals can vary with germination conditions (e.g. temperature and light), population (i.e. seed lot) and seed dormancy state (Tieu et al. 2001; Baker et al. 2005; Stevens et al. 2007; Long et al. 2011; Downes et al. 2014).

Oriental mustard (*Sisymbrium orientale* L.) is an annual arable and ruderal weed from the Brassicaceae family, which includes a number of weeds responsive to germination stimulant(s) in smoke (Daws et al. 2007; Stevens et al. 2007; Long et al. 2011; Mojzes and Kalapos 2014). This species is distributed throughout Europe (Tutin et al. 2001; Rūrāne and Rose 2015), and also in other parts of the world including Australia, the United States and East Asia. It occurs most frequently in grain and other crop fields, fallow lands, secondary pioneer grasslands and ruderal vegetation along roadsides (Virtue and Thomas 1999; Zhou et al. 2007; Pinke and Pál 2008; Abella et al. 2009). The seeds of this species are strongly dormant at maturity (< 20% germination), and require an after-ripening period to achieve a moderate to high germination (e.g. about 60% in the light after 6 months (Chauhan et al. 2006) or 80-84% after 2 months (Boutsalis and Powles 1998). Seeds show the greatest seedling emergence when placed on the soil surface (Chauhan et al. 2006), and can persist in the soil for 3-4 years (Boutsalis and Powles 1998). In previous studies, seed germination or seedling establishment of *S. orientale* decreased (Stevens et al. 2007) or was not affected (Mojzes and Kalapos 2014; Tormo et al. 2014) by smoke-water treatment. Charred wood, which most probably contains similar active compounds as smoke, also diminished the germination of 14-18-month-old seeds of this species (from 94% to 74%: Keeley and Keeley 1987). However, KAR_1 could enhance the germination of freshly collected seeds at alternating temperature in the light, or dormancy-breaking treatments could induce seeds to become responsive to KAR_1 when germinated in darkness (Stevens et al. 2007; Long et al. 2011). Furthermore, Long et al. (2011) found seasonal fluctuation in the effect of KAR_1 associated with changes in the dormancy state of seeds over a 2-year burial, but only when seeds were germinated in darkness. These somewhat contradictory results point out the needs to further explore the species' response to germination stimulants in smoke.

This study aimed at investigating the effect of smoke-water on the seed germination of *S. orientale* under alternating light and constant darkness (referred to as light and dark, respectively hereafter) in a laboratory experiment. It was hypothesized that smoke-water enhances germination, but the smoke response varies with light or dark conditions. The results of this study can improve our understanding of the smoke responsiveness of this species, and can potentially be utilized in weed management.

Materials and methods

About 6000 seeds from at least 10 individuals of one population were collected from ruderal sand vegetation along a roadside near Fót (47^{o} 38' N, 19^{o} 11' E), at the border of the Gödöllő Hills, Hungary between 29.06.2013 and 06.07.2013. Seeds were stored in a paper bag in darkness at 22 ± 1 ^{o}C and c. 40% RH until used for germination tests, which were conducted in August 2013 and 2014. The second test in August 2014 was performed to verify the results of the first one in the previous year. Seeds that appeared viable based on colour and shape were selected for the experiment.

Smoke-water was prepared by burning dry litter of lawn grass mixture of *Festuca rubra* L. and *Lolium perenne* L., and tap water was sprinkled through the smoke 8-10 times, resulting in a concentrated smoke-water solution. Based on our previous study (Mojzes and Kalapos 2014), an 1:2 v/v aqueous dilution of smoke-water prepared this way was effective in enhancing germination, thus it was used in this experiment for smoke-water treatments. In each test, five replicates of 20 seeds were placed in Petri dishes on five layers of 8-cm diameter discs of absorbent cellulose wad (Hartmann Pehazell), which were moistened with 6 ml tap water (control) or smoke-water (treatment). Germination tests were performed in a growth room at 21 ± 2 ^{o}C daily fluctuation. This was close to the temperature (i.e. the daily average of 20 ^{o}C), at which the seeds of this species were able to germinate in several other laboratory experiments under light or both light and dark conditions (Cousens et al. 1993; Chauhan et al. 2006; Long et al. 2011; Karimmojeny et al. 2014). For germination in the light, seeds received diffuse daylight (about 12-h photoperiod with a midday average PPFD of 80 $\mu mol\ m^{-2}\ s^{-1}$). For dark conditions, Petri dishes were placed in a lightproof box. Final germination percentages were recorded after no further germination was observed for 7 days. Seeds were considered to have germinated when the radicle protruded ≥ 2 mm.

For the two germination dates separately, a generalized linear model (2-way factorial ANOVA) with a binomial distribution and a logit link function was used for analyzing the effect of smoke-water treatment and light conditions as explanatory variables on final germination using binary data (germinated or not) for each seed. The statistical tests were performed in Dell Statistica (data analysis software system; Dell Inc. (2015), version 13 (available at http://software.dell.com), and differences were considered significant at $p < 0.05$.

Results and Discussion

In August 2013, 2 months after harvest, the germination of *S. orientale* seeds with water (control) was 54% in the light and 73% in the dark, and similar values were observed in August 2014, after 13 months of dry storage (66% and 77% in the light and dark, respectively; Fig. 1). At both dates and light conditions, final germination was reached within a week. In previous studies, similar or lower germination percentages (≤ 61%) were reported for *S. orientale* seeds after 1-12 months of dry after-ripening indoors (in a laboratory or greenhouse) depending on e.g. germination temperature and light, and the conditions and duration of after-ripening (Chauhan et al. 2006; Long et al. 2011; Karimmojeny et al. 2014). In addition, Karimmojeny et al. (2014) demonstrated that competition in the maternal environment and seed position on the mother plant also affected the germination of this species.

In line with our hypothesis, smoke-water treatment as main effect significantly increased germination compared to the control at both germination dates (to 82-84% in the light and 92-99% in the dark; Table 1, Fig. 1). This indicates that active compounds in smoke contributed to the dormancy alleviation of seeds or could act as a germination stimulant, depending on the definition (Finch-Savage and Footitt 2012; Thompson and Ooi 2013). To our best knowledge, this is the first study demonstrating a positive germination response to smoke-water for *S. orientale*, contrasted with previous studies that reported negative effect (Stevens et al. 2007) or no response of germination or seedling establishment (Mojzes and Kalapos 2014; Tormo et al. 2014). These results together, further support the previous findings indicating that the responsiveness to germination stimulants in smoke is not an absolute characteristic of a species, but can highly vary with environmental conditions and intrinsic factors (e.g. seed dormancy; Tieu et al. 2001; Baker et al. 2005; Long et al. 2011; Downes et al. 2014). In our study, there was no significant interaction between the impact of smoke-water treatment and light (Table 1), and the magnitude of smoke effect was similar in the light and dark at the same germination date (Fig. 1). This result does not support our hypothesis that smoke response varies with light conditions, and also contrasts with Long et al. (2011), who reported that the germination of *S. orientale* seeds experienced 1-3 months of dry after-ripening was promoted by KAR_1 only in the dark. Furthermore, in our experiment, germination was significantly higher in the dark than in the light, irrespective of smoke treatment (Table 1, Fig. 1). This result is contrary to the previous findings demonstrating a generally better germination in the presence than in the absence of light for *S. orientale* when daily average temperature exceeded 15^{o}C (Cousens et al. 1993; Chauhan et al. 2006; Long et al. 2011).

If our results are confirmed under field conditions, smoke-water applied onto the field soil at the end of summer might promote and synchronize the germination of *S. orientale*. With subsequent removal of germinants before sowing a crop, this technique could reduce the capacity of this species to produce new seeds and replenish the soil seed bank. As the seedling emergence of *S. orientale* can decline rapidly within 3-4 years in the absence of fresh seed input (Boutsalis and Powles 1998), smoke-water used in this way might improve the efficiency of weed control in the fields infested by this species. In field experiments, Stevens et al. (2007) demonstrated the ability of KAR_1 applied onto the surface of sandy soil (at 2-20 g ha^{-1}) to enhance the germination of three weed species from the soil seed bank. In Western Australia, Long et al. (2010) suggested the period just before the cropping season in autumn (April) as the best time to apply KAR_1 for triggering the synchronous germination of several weeds including *S. orientale*. However, Tormo et al. (2014) did not detect smoke-stimulated seedling establishment of *S. orientale* emerged from the soil seed bank, when applying liquid smoke to the soil at the end of summer.

In conclusion, this study demonstrates the positive effect of plant-derived smoke on the germination for *S. orientale*, which can improve our knowledge on the smoke responsiveness of this species, and might help to develop an effective weed management in agroecosystems. However, field experiments are needed to better understand the complexity of the species' smoke response in its natural environment in temperate regions. It is especially required to assess a possible seasonal variation in the influence of smoke associated with changes in the dormancy state of seeds (similar to that found for KAR_1 in Western Australia; Long et al. 2011). Furthermore, several populations of the species should be involved in order to draw more general conclusions on the applicability of smoke-water in weed management.

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Table caption

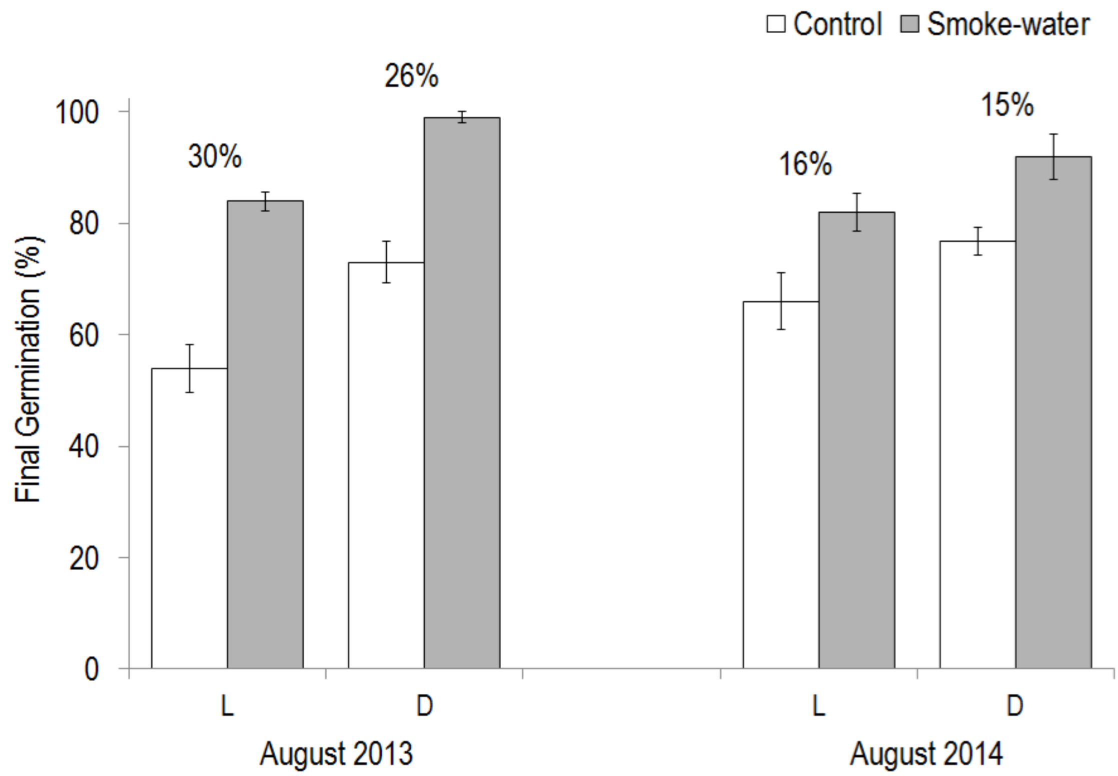
Table 1 Effect of Smoke (tap water or smoke-water), Light (dark or light conditions) and their interaction on the final seed germination of *Sisymbrium orientale* in August 2013 and 2014. For each date, results of a generalized linear model (2-way factorial ANOVA) with a binomial distribution and a logit link function are shown

	2013			2014		
Effect	Degrees of freedom	Wald Statistic	*p*	Degrees of freedom	Wald Statistic	*p*
Intercept	1	46.533	< 0.001	1	111.017	< 0.001
Light	1	12.174	< 0.001	1	6.973	0.008
Smoke	1	22.006	< 0.001	1	14.470	< 0.001
Light×Smoke	1	3.803	0.051	1	0.509	0.475

Figure caption

Fig. 1 Final germination percentage of *Sisymbrium orientale* seeds germinated with tap water (control) or smoke-water (1:2, v/v) under light (L) or dark (D) conditions in August 2013 and 2014. Mean values ± 1 SE (n = 5). Absolute differences between the treatment and control means (treatment–control) are presented above the columns

Figure 1



Control
Smoke-water
Final Germination (%)
100
80
60
40
20
0
30%
26%
16%
15%
L
D
L
D
August 2013
August 2014