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Plant-derived smoke enhances germination of the invasive common milkweed (*Asclepias syriaca* L.)

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ABSTRACT

Common milkweed (*Asclepias syriaca* L.) has become an invasive weed in Central and Eastern Europe, where human-induced fires have also taken part in forming the landscape. There is growing evidence that plant-derived smoke enhances seed germination, especially for species from fire-prone ecosystems, *via* the mechanisms of dormancy-breaking, germination stimulation or both. Hence, we hypothesized that smoke promotes seed germination for common milkweed by either or both mechanisms. To test this, germination responses of *A. syriaca* to the application of aqueous smoke solution (smoke-water) were studied in laboratory. Seeds were either cold stratified (+7°C, 16 days) in tap water (TW), smoke-water (SW) or were not stratified at all, and then were germinated with SW or with TW (encompassing 5 treatments: 0–TW, 0–SW, TW–TW, TW–SW and SW–TW, where the first abbreviation indicates stratification, the second germination condition). In line with our hypothesis, the low (5%) germination of seeds was enhanced by cold stratification with SW at a greater extent (increasing to 52%) than by cold stratification with TW (25%), indicating that SW contributed to dormancy-breaking of seeds for *A. syriaca*. In contrast, SW did not stimulate germination when it was applied during the germination phase. To our best knowledge, this is the first study demonstrating smoke-enhanced germination for common milkweed, which mechanism may help this species to successfully colonize new habitats after fire. As fire frequency is expected to increase in Europe with recent climate change, these results might contribute to a more efficient control of *A. syriaca* in areas threatened by its invasion.

Common milkweed (*Asclepias syriaca* L.) is a widespread weed native to the eastern part of the United States and Canada, where it frequently occurs on roadsides, wastelands and arable fields (Bhowmik and Baden 1976). After being introduced to Europe for horticultural and economical purposes, the species has become an invasive plant, particularly in Central and Eastern Europe, threatening natural grasslands and shrublands most frequently on sand, and also causing weed infestations in field crops, vineyards, orchards and other habitats under human impact (Weber and Gut 2005, Botta-Dukát 2008, Sárkány *et al.* 2008, Hulina 2010, Szatmari 2012). *Asclepias syriaca* propagates both by seeds and adventitious buds on rootstocks. Seeds have an inherent dormancy at maturity, and require an after-ripening period to achieve a moderate or high level of germination (Bhowmik and Baden 1976, Baskin and Baskin 1977, Bhowmik 1978).

Human-induced fires as a common management tool has long been and still is an important factor forming the European landscape (Goldammer and Bruce 2004, Niklasson *et al.* 2010, Deák *et al.* 2014). In addition, ongoing climate change is predicted to increase fire frequency throughout most of Europe in the 21st century (Pechony and Shindell 2010). Therefore, understanding the fire response of *A. syriaca* is of particular importance for predicting and controlling its spread. In North American prairie and savanna ecosystems, heterogeneous fire response of this species has been detected at population level depending on the vegetation (and habitat) type and the frequency and timing of fire (Hruska and Ebinger 1995, Johnson and Knapp 1995, Tester 1996, Towne and Kemp 2008). For example, in tallgrass prairie watersheds, the abundance of *A. syriaca* increased with the frequency of spring fires (Johnson and Knapp 1995), but decreased in response to biennial summer burning (Towne and Kemp 2008). In Europe, little is known about the effect of fire on the invasion success of this species. In an inland sand dune vegetation mosaic of Hungary, *A. syriaca* became dominant in the recovering open sand grasslands five years following the burn of pine plantations; however, in this dry and nutrient-poor habitat it did not appear to hinder the colonization of natural grassland species (Szitár *et al.* 2014). Assessing the germination response of common milkweed to fire-related cues, such as smoke derived from burning vegetation might contribute to improve our knowledge on the spreading ability of this species after fire, which may increase the efficiency of its control. Plant-derived smoke and its aqueous solution (smoke-water) have been documented to enhance seed germination as well as seedling growth of numerous wild species, particularly in fire-prone Mediterranean ecosystems (Dixon *et al.* 1995, Keeley and Fotheringham 1998, Brown *et al.* 2003, Moreira *et al.* 2010, Downes *et al.* 2014), but also in temperate regions of Europe (Adkins and Peters 2001, Daws *et al.* 2007, Måren *et al.* 2010, Mojzes and Kalapos 2014). 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).

In this study we aimed at investigating the effect of smoke-water on the seed germination of *A. syriaca*. It was hypothesized that smoke enhances germination for this species by promoting dormancy-breaking and stimulating germination. This assumption is based on the increasing literature evidence on the wide distribution of positive effects of smoke even on species not adapted to wildfires. Merritt *et al.* (2007) have pointed out that smoke appears to act as a germination stimulant rather than a dormancy-breaking agent. However, it is evidenced that smoke-derived chemicals can actively alleviate dormancy in some species (Adkins and Peters 2001, Long *et al.* 2011). In a laboratory experiment, the capacity of smoke-water for germination stimulation was tested by applying smoke-water treatment during the germination phase, while the active role of smoke-water in breaking dormancy was examined by applying smoke-water treatment during cold stratification. Previous studies showed that dormancy could be released by moist, low-temperature treatment in common milkweed seeds (Oegema and Fletcher 1972, Baskin and Baskin 1977).

About 5000 seeds from at least 15 reproductive stems of one population were collected from ruderal sand vegetation along a roadside near Fót, at the border of the Gödöllő Hills, Hungary between August 24th and September 8th 2013. Seeds were stored in paper bags at room temperature (*ca* 22°C) until used for the experiment, which was conducted in January and February 2014. Seeds that appeared viable based on colour and shape, and resisted gentle pressure were selected for the experiment. Smoke-water was prepared by burning 2 kg of dry litter from lawn grass mixture of *Festuca rubra* L. and *Lolium perenne* L., and 8 L tap water was sprinkled through the smoke 8–10 times. The smoke-water obtained by this procedure was less concentrated than that produced by other authors (*e.g.* Dixon *et al.* 1995, Brown *et al.* 2003), and is probably more similar to natural situation (*e.g.* when rainwater soaks burned vegetation). Based on our preliminary tests, an 1:2 v/v aqueous dilution of smoke-water prepared this way was the most effective in enhancing germination, thus it was used in this experiment for smoke-water treatments. Seeds were surface sterilized in 1:10 v/v aqueous dilution of cc. sodium hypochlorite solution for 20 min., then rinsed thoroughly with tap water. In each treatment, five replicates of 20 seeds (n = 5) were placed in Petri dishes on top of five layers of 8-cm diameter discs of absorbent cellulose wad (Hartmann Pehazell), which were moistened with 7 mL tap water or 1:2 v/v smoke-water solution depending on treatment. Five treatments were applied: 1) a germination test either with tap water (TW) or 2) smoke-water (SW) without pre-

Table 1. Treatments with their abbreviations used in this study. Cold stratification (if applied) was conducted preceding the germination test under +7°C and constant darkness for 16 days. For each treatment, five replicates were used (n = 5).

Cold stratification	Germination test	
	Tap water	Smoke-water
Not applied	0–TW	0–SW
Tap water	TW–TW	TW–SW
Smoke-water	SW–TW	—

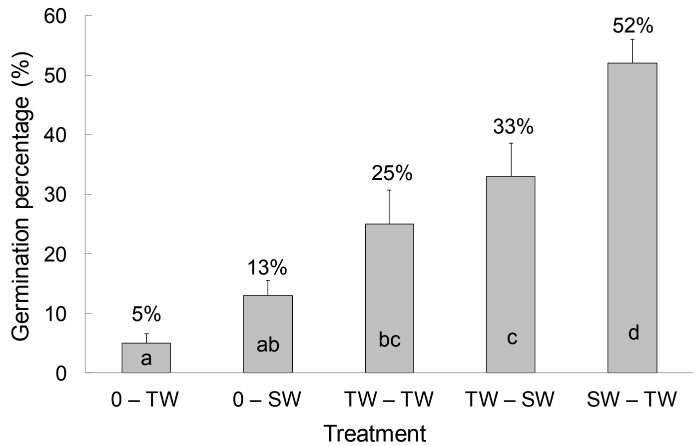

Fig. 1. Effect of 1:2 v/v smoke-water on the final germination percentage of *Asclepias syriaca*. Mean values + 1 SE (n = 5). For each treatment, the medium of cold stratification and germination test is indicated before and after a 'dash', respectively, on the x-axis. TW – tap water; SW – smoke-water; 0 – no stratification. Different letters inside columns indicate significant (P <0.05) differences among treatments. For each treatment, the final germination percentage is presented above the column.

ceding cold stratification, 3) cold stratification with tap water followed by a germination test either with tap water or 4) smoke-water, and 5) cold stratification with smoke-water followed by a germination test with tap water (Table 1). Seeds were cold stratified in the refrigerator under +7°C and constant darkness for 16 days. At the end of stratification, seeds were transferred to the germination medium, and placed under diffuse daylight (about 10-h photoperiod, midday average 35 μmol photon m^{-2} s^{-1} PPFD) at room temperature (20 ± 1°C) on the sill of the laboratory window facing North. Seeds were considered to be germinated when the radicle or plumule protruded ≥ 2 mm. Germination was scored at every 2–4 days for 21 days or until no further germination was observed for 7 days.

The effect of smoke-water treatments on final germination percentage was analyzed by using one-way ANOVA with subsequent Tukey HSD *post hoc* test. Differences were considered significant at P <0.05. The GraphPad InStat 3.05 (GraphPad Software, San Diego, California, USA) package was used for these tests.

Unstratified seeds germinated with tap water (0–TW) exhibited low germination percentage (5%), which indicated that most seeds remained dormant after *ca* 5 months of dry storage at room temperature (Fig. 1). Consistently, in other studies *A. syriaca* seeds required an 11-month period of dry after-ripening to germinate well (about 76%; Bhowmik 1978), or their germination remained below 20% even after 18 months of dry storage at room temperature (Oegema and Fletcher 1972). In our experiment, after 16 days of cold stratification with tap water, germination increased by fivefold (TW–TW: 25%; Fig. 1). This agrees with previous studies reporting that 1–9 weeks of cold stratification can be effective in breaking seed dormancy for this species (Oegema and Fletcher 1972, Baskin and Baskin 1977).

Consistent with our hypothesis, when smoke-water was applied during cold stratification (SW–TW), it enhanced the germination of seeds by 2.1-fold (52%) compared to cold stratification with tap water (TW–TW; Fig. 1). To our best knowledge, this is the first study demonstrating a positive germination response to smoke for *A. syriaca*. This result indicates that during cold stratification, smoke-water contributed to dormancy-breaking of seeds for this species, and further supports the dormancy-releasing activity of certain smoke-derived compound(s), as it was reported for *Polygonum aviculare* L. and *Veronica hederifolia* L. (Adkins and Peters 2001) or *Eragrostis curvula* (Schrad.) Nees (Long *et al.* 2011). In contrast, smoke-water treatment during the germination phase either with or without preceding stratification with tap water (TW–SW and 0–SW, respectively) did not increase significantly the germination of seeds compared to those germinated with tap water (TW–TW and 0–TW, respectively; Fig. 1). This result does not support the hypothesis that smoke-water can stimulate germination for *A. syriaca*. In our study, smoke-water appeared to act as a dormancy-breaking agent, not as a germination stimulant, opposed to several species from fire-prone habitats displaying smoke-stimulated germination after a dormancy release (*e.g.* burial or dry after-ripening: Merritt *et al.* 2007, Downes *et al.* 2014). Our results suggest that burning of habitats invaded by common milkweed in autumn, when a high number of ripened, dormant seeds are dispersed, might contribute to enhanced germination of this species due to the dormancy-breaking activity of smoke. Previous studies have shown that *A. syriaca* produces the highest seedling emergence from the upper soil layer (0.5–2 cm) with poor germination on the soil surface and at depths below 4 cm in sandy loam soil (Bhowmik 1978, Yenish *et al.* 1996), and does not maintain a persistent soil seed bank (Csontos *et al.* 2009). However, in an artificial burial experiment (in 65 cm depth), common milkweed seeds expressed considerable longevity, and had high (≥ 85%) germination rate when unburied and exposed to germination tests, throughout 6 years, which showed its ability to form a long-term persistent seed bank (Csontos 2001). Considering these data, our results suggest that the promotive agent(s) of smoke leaching through the soil profile (*e.g.* after heavy rainfall) might increase the germination of *A. syriaca* seeds even buried in the deeper soil layers caused by natural burying processes (*e.g.* wind, rain) or soil cultivation (Yenish *et al.* 1996), particularly in sandy soil. In field experiments, Stevens *et al.* (2007) demonstrated the ability of karrikinolide applied onto the sandy soil surface (at 2–20 g ha^{-1}) to penetrate into the soil and improve the germination of three weed species from the soil seed bank.

In conclusion, this study demonstrates the positive effect of plant-derived smoke on the germination of common milkweed, which – in addition to its high seed production (Bhowmik and Baden 1976, Csontos *et al.* 2009) and efficient wind seed dispersal – may help this species to successfully colonize new habitats after fire. If these results are confirmed under field conditions, they should also be considered when making a decision on the application of burning for land management or nature conservation purposes (Goldammer and Bruce 2004, Deák *et al.* 2014) in order to prevent seedling establishment in areas threatened by the invasion of this species.

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