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## AN EFFECTIVE SYSTEM TO PRODUCE SMOKE SOLUTIONS FROM DRIED PLANT TISSUE FOR SEED GERMINATION STUDIES<sup>1</sup>

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- *Premise of the study:* An efficient and inexpensive system was developed to produce smoke solutions from plant material to research the influence of water-soluble compounds from smoke on seed germination.
- *Methods and Results:* Smoke solutions (300 mL per batch) were produced by burning small quantities (100–200 g) of dried plant material from a range of species in a bee smoker attached by a heater hose to a side-arm flask. The flask was attached to a vacuum water aspirator, to pull the smoke through the water. The entire apparatus was operated in a laboratory fume hood.
- *Conclusions:* Compared with other smoke solution preparation systems, the system described is easy to assemble and operate, inexpensive to build, and effective at producing smoke solutions from desired species in a small indoor space. Quantitative measurements can be made when using this system, allowing for replication of the process.

**Key words:** seed germination; smoke compounds; smoke solution system.

Fire is an important natural element of many ecosystems ranging from forests to grasslands, and is recognized as an important ecosystem management tool. Several aspects of fire, such as heat, quick release of nutrients from burnt plant tissue, and compounds in ash, have been studied for their influence on seed germination and/or plant growth. Since 1990, focus has been directed on the importance of smoke compounds in breaking seed dormancy and enhancing germination for some species. DeLange and Boucher (1990) were the first to report that compounds found in plant smoke stimulated seed germination. Since their initial work, many other studies have shown that specific water-soluble compounds found in the smoke from burning plant tissue play an important role in breaking seed dormancy (Dixon et al., 1995; Brown and van Staden, 1997; Blank and Young, 1998; Keeley and Fotheringham, 1998; Tieu et al., 1999; Landis, 2000; Pennacchio et al., 2007; Jefferson et al., 2008; Lindon and Menges, 2008). Investigations of smoke compounds focused on plant species from shrubland in South Africa, Florida, and Western Australia; chaparral in California; and tallgrass prairie in the midwestern United States (DeLange and Boucher, 1990; Brown, 1993a, 1993b; Dixon et al., 1995; Keeley and Fotheringham, 1998; Tieu et al., 1999; Cochrane et al., 2002; Pennacchio et al., 2007; Jefferson et al., 2008; Lindon and Menges, 2008). Reports show that some of the most widely

described stimulants in smoke are karrikins (a class of butenolide derivatives), water-soluble, naturally occurring compounds that are created from burning plant tissue and are involved with breaking seed dormancy (Flematti et al., 2004, 2008, 2009; Pennacchio et al., 2007). Recently, cyanohydrins, also found in smoke, have been identified as influencing seed germination and seedling growth (Chiwocha et al., 2009; Nelson et al., 2012).

For those interested in investigating the influence of smoke on seed germination, various methods have been reported for exposing seeds to smoke compounds. These methods include the application of commercial smoke preparations, direct fumigation of seeds, and the application of smoke solutions. Three currently available commercial preparations are: “Seed Starter–Australian Smoky Water” from the Friends of Kings Park and Botanic Garden in Perth, Western Australia; “Kirstenbosch Instant Smoke Plus” seed primer from the National Botanical Institute in Cape Town, South Africa; and Cape “Super Smoke Plus” Seed Germinator Primer from Seedman.com. Commercial preparations are convenient, but have disadvantages. For instance, they may contain additional seed germination enhancers such as gibberellic acid, which is known to break plant embryo dormancy and stimulate germination in some species (Brown and van Staden, 1997; Baskin and Baskin, 1998; Keeley and Fotheringham, 1998; Cochrane et al., 2002). Separating the effects of smoke compounds from other additives would not be possible when using commercial preparations. In addition, commercial preparations do not allow researchers to control the species of plant materials burned to produce smoke solutions, and chemicals are known to vary within different assemblages of plants. Although butenolides are formed when cellulose is burned and all plants contain cellulose, other compounds yet to be discovered may be involved in breaking seed dormancy and these may depend on the species of plants burned

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(Landis, 2000; Flematti et al., 2004, 2009). Therefore, producing smoke—whether for fumigation of seeds or for smoke solutions—using plant species from the ecosystem being studied may be the most appropriate method to produce smoke compounds that would occur naturally (Baxter et al., 1995). Standardized smoke solutions also could be produced from burning cellulose filter paper (Flematti et al., 2004). Many studies use direct fumigation of seeds to enhance seed germination (DeLange and Boucher, 1990; Brown et al., 1993; Baxter et al., 1995; Dixon et al., 1995; Blank and Young, 1998; Keeley and Fotheringham, 1998; Pennacchio et al., 2007; Jefferson et al., 2008; Lindon and Menges, 2008). Direct fumigation of seeds is effective for promotion of germination of some species, but because the identified active compounds are known to be water soluble, they could naturally be carried into the soil by rainwater or snowmelt. Hence, the use of smoke solutions also is effective and may be more convenient due to ease of storage and application. Smoke solutions have therefore become a common method to investigate the influence of smoke compounds on seed germination (Brown, 1993a; Brown and van Staden, 1997; Keeley and Fotheringham, 1998; Tieu et al., 1999; Landis, 2000; Flematti et al., 2004). Previous reports have described systems used to prepare smoke solutions, although descriptions often lack details of construction and/or have limitations such as being expensive to build or not being suitable for indoor use (Brown, 1993a; Baxter et al., 1994; Brown and van Staden, 1997; Keeley and Fotheringham, 1998; Tieu et al., 1999; Landis, 2000; Flematti et al., 2004).

Because of these limitations, we developed an effective system to prepare small volumes of smoke solutions from burning plant tissues to investigate the effects of smoke compounds on seed germination. Our system is easy to assemble and use,

compact, effective, and inexpensive. In addition, the amount of plant tissue added to the smoker, the length of time for combustion, and the amount of ash remaining after combustion can be quantified.

## METHODS AND RESULTS

**Smoke solution system**—The smoke system (Fig. 1) was constructed in a fume hood so that any excess smoke could be evacuated safely from the laboratory. The sample plant material is burned inside a stainless steel bee smoker with attached bellows (10 cm diameter × 23.5 cm high; model 15239, GloryBee Foods, Eugene, Oregon, USA) (Fig. 1-1). The smoke flows from the smoker through a heat-resistant rubber heater hose (80 cm long, 2.7 cm inner diameter [ID], and 3.4 cm outer diameter [OD]; HBD/Thermoid, Bellefontaine, Ohio, USA) clamped to the spout of the smoker (Fig. 1-2,3). The other end of the rubber hose is placed below the surface of 300 mL of water in a 1000-mL side-arm flask with a 3.6-cm ID mouth (Corning Pyrex Vista filter flask; Corning Life Sciences, Tewksbury, Massachusetts, USA) (Fig. 1-4). The dimensions of the hose and flask are chosen so that the outer diameter of the tubing will fit snugly into the inner diameter of the mouth of the flask. The flask is clamped to a ring stand for stability (Fig. 1-5). A water aspirator is attached to the side arm of the flask (Fig. 1-6,7). The vacuum generated by the aspirator is used to help draw the smoke from the smoker into the water.

**Plant tissue preparation**—After collection, material from each plant species or a mixture of species is placed into individual paper bags. The woody species are chipped; the forbs and grasses are cut into small pieces. Each sample is mixed thoroughly to maximize homogeneity and allowed to air dry at room temperature. The drying time will vary depending on moisture content of original material, size of pieces, and ambient temperature and humidity. Dried samples are stored at room temperature in paper bags.

**Smoke solution preparation**—Burns are initiated by placing 50 g of plant material into the bee smoker and igniting the material with a refillable Bernz-Omatic butane micro torch (Worthington Cylinder Corporation, Columbus, Ohio,



Fig. 1. Smoke solution system. The completed burning system including: (1) bee smoker with bellows, (2) hose clamp, (3) heater hose, (4) side-arm flask, (5) ring stand with clamp, (6) vacuum tubing, and (7) water aspirator. The working system was constructed and used in a fume hood.

USA). The material is allowed to burn with the smoker open for 10–20 s, after which time the smoker lid is closed and the tubing is clamped to the opening of the smoker. The smoke is drawn through the water in the flask, dissolving the water-soluble compounds. Bellows on the bee smoker are pumped for the duration of the process to keep the plant material burning and to increase the amount of smoke entering the water in the flask. As the plant material burns, more is added to the bee smoker until the total sample is burned. During the burning process, caution must be exercised because the smoker gets very hot. Once the plant material has finished burning, the system is allowed to cool for approximately 15 min. After the cooling process, the ash contents in the smoker are removed and weighed. The smoke-water solution in the side-arm flask then is available to use in its concentrated form or diluted as needed for analysis or seed treatments. This apparatus could be used repeatedly, but because considerable residue remains in the smoker, heater hose, and flask, these components may need to be replaced for each plant species. The cost for each of these items is minimal. The smoker, heater hose, and flask are labeled so that they can be reused when the same species is burned repeatedly.

**Results**—The smoke solution system described offers several advantages compared to systems described previously, including ease of construction and use. The materials used to construct the system are readily available (Table 1) and the system is easy to assemble, requiring no special tools. The system is compact, being able to fit inside a fume hood (1.3 m width × 0.7 m depth × 1.1 m height). With caution, our system can be used safely, presenting minimal fire hazard or exposure to smoke fumes, because it is located in a fume hood that drafts excess smoke from the building. The bee smoker does become very hot so caution is required, including use of heat-resistant gloves. Regardless of weather conditions, the system can be used year round. The system allows smoke solutions to be quantified because the mass of plant material is weighed initially, the mass of ash is weighed after burning is completed, and the volume of water in the flask also can be measured. These measurements would allow others to create similar solutions and replicate the trials. In addition, the ash that is collected could be used to test whether it affects germination or other parameters. In contrast to using commercial smoke solutions where species and other components may be unknown, this system also allows the investigator to select which species to use for creation of customized smoke solutions.

This smoke system also saves money and time, being economical, quick to assemble, and easy to use. The materials to build the system are inexpensive, totaling only US\$270 (2013) to assemble initially, provided one has access to a fume hood (Table 1). Of the materials listed, several are available in most laboratories. Another advantage for this system is its efficient use of time. The system is very easy to operate with minimal training (usually less than an hour) required for staff, saving time as well as money. The system also requires minimal time to create a smoke solution. Depending on the original starting plant material (i.e., what species, how coarse, how dry, how much), burning required 1–5 h for 100–200 g plant material and resulted in 5–87 g ash (Tables 2 and 3). More research is needed to understand the variation in burn times and quantity of ash produced for different samples.

TABLE 1. Suppliers and costs for items needed to create the smoke solution system.<sup>a</sup>

Item	Suppliers	Approximate cost <sup>b</sup>
Bee smoker	GloryBee Foods Inc.	\$25
Hose clamp	Hardware	\$10
Heater hose (80 cm long)	Automotive parts	\$5
Side-arm flask (1000 mL)	Fisher Scientific	\$80
Ring stand with clamp	Fisher Scientific	\$90
Vacuum tubing	Fisher Scientific	\$25
Water aspirator	Fisher Scientific	\$15
Butane micro torch	Hardware	\$20
Total		\$270

<sup>a</sup> Standard laboratory equipment required: fume hood.

<sup>b</sup> US dollars, 2013.

This system has been used to support a variety of different projects in our research program. Smoke solutions were created from burning 13 individual species (Table 2), including both herbaceous and woody. In addition, we created smoke solutions using mixtures of species from four longleaf pine savanna sites in Florida (Table 3). Smoke solutions created using this system were used for germination studies showing the effects of smoke solutions from buffelgrass (an invasive species in Arizona) on native species of the Sonoran Desert (Maruszak and Coons, 2013). Smoke solutions from longleaf pine savannas were also tested on two federally threatened species (*Pinguicula ionantha* R. K. Godfrey and *Scutellaria floridana* Chapm.) (Annis, 2013; O'Brien, 2013).

## CONCLUSIONS

A need existed to develop a system to create plant smoke solutions for which more variables could be controlled (Landis, 2000). Many earlier reports were unclear about how the smoke solution was created, and thus procedures are not easily reproducible. Others have used smoke solutions created from commercially prepared products (Dixon et al., 1995; Cochrane et al., 2002), but this method does not allow the investigator to select specific plant species for creation of a smoke solution. Two important components of our system are a bee smoker and a heater hose, which were used in earlier studies to create aerosol smoke but not smoke solutions (Baxter et al., 1995; Lindon and Menges, 2008). We believe that our system provides greater control of variables while offering several additional advantages over previous systems. Our system is more compact, allowing

TABLE 2. Burning information for tallgrass prairie, woody, and other species used in this study.

Scientific name	Common name	Mass of plant material (g)	Mass of ash (g)	Burn hours
<b>Tallgrass prairie species</b>				
<i>Andropogon gerardii</i> Vitm.	Big bluestem	200	47	5.0
<i>Asclepias tuberosa</i> L.	Butterfly milkweed	200	22	1.0
<i>Baptisia alba</i> (L.) Vent.	White indigo	197	30	2.8
<i>Eryngium yuccifolium</i> Michx.	Rattlesnake master	200	28	1.8
<i>Liatris spicata</i> (L.) Willd.	Prairie blazing star	200	29	2.0
<i>Monarda fistulosa</i> L.	Wild bergamot	200	25	3.0
<i>Ratibida pinnata</i> (Vent.) Barnhart	Pinnate prairie coneflower	200	87	4.8
<i>Ruellia humilis</i> Nutt.	Wild petunia	200	22	2.0
<i>Schizachyrium scoparium</i> (Michx.) Nash	Little bluestem	200	33	2.0
<i>Silphium terebinthinaceum</i> Jacq.	Prairie dock	200	76	4.0
<b>Woody species</b>				
<i>Carya ovata</i> (Mill.) K. Koch	Shagbark hickory	200	54	1.8
<i>Quercus alba</i> L.	White oak	200	43	1.5
<b>Other species</b>				
<i>Pennisetum ciliare</i> (L.) Link	Buffelgrass	100	21	1.3
<i>Pennisetum ciliare</i>	Buffelgrass	100	22	1.4



TABLE 3. Burning information for mixtures of species from longleaf pine savannas in Florida.

Site	Mass of plant material (g)	Mass of ash (g)	Burn hours
1	100	10	1.5
2	100	21	1.5
3	100	6	1.5
4	100	5	1.5

for creation of a smaller volume (300–750 mL) of liquid in a more confined space than some systems (Tieu et al., 1999; Flematti et al., 2004). Unlike systems where water was simply exposed to smoke, our system increases the opportunity for water-soluble compounds to dissolve by bubbling the smoke through water (Keeley and Fotheringham, 1998). In our system, a vacuum is created by an inexpensive and easy-to-use water aspirator to pull smoke through water, in contrast with some systems that are described as using suction or a pump (Tieu et al., 1999; Flematti et al., 2004). Among our system's numerous advantages are ease of obtaining materials, assembling the system, and training staff in its use. In addition, the system is safe to use indoors as it is small enough to fit into a fume hood. Some systems are quite large (DeLange and Boucher, 1990; Brown, 1993b), and are used outdoors where heat can be troublesome, making it best to create smoke on cooler days (Brown et al., 1993). The system is inexpensive to construct and to operate, and requires a short amount of time for its creation and use. Perhaps most important of all, this system offers a potential advance in technology for creation of smoke solutions because one can quantify how the smoke solution was created so studies can be described in detail and reliably repeated. One improvement for the described system would be to develop an automated way to pump the bellows on the bee smoker. Overall, this smoke-water production system is very effective for preparation of smoke solutions to use for seed germination studies.

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APPENDIX 1. Construction and operation of the smoke solution collection system.

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Supplies needed: Paper bags for sample collection, bee smoker (10 cm diameter × 23.5 cm high; model 15239, GloryBee Foods Inc., Eugene, Oregon, USA), rubber heater hose (80 cm long, 2.7 cm inner diameter [ID], and 3.4 cm outer diameter [OD]; HBD/Thermoid, Bellefontaine, Ohio, USA), hose clamp, 1000-mL side-arm flask with a 3.6-cm ID mouth (Corning Pyrex Vista filter flask; Corning Life Sciences, Tewksbury, Massachusetts, USA), ring stand and 3-pronged clamp, vacuum tubing, water aspirator, and refillable Bernzomatic butane micro torch (Worthington Cylinder Corporation, Columbus, Ohio, USA).

**Collect plant material**

Step 1: Collect herbaceous or woody plants and place in individual paper bags as required. After collection, cut or chip the plants, mix thoroughly into small pieces, and allow to air dry with the bags open. We allow the samples to dry to touch (approximately 2 wk). Times will vary depending on ambient humidity and temperature.

Step 2: After the plant material dries sufficiently, weigh samples (we used 100–200 g), and place into smaller paper bags for storage until ready to burn.

**Construct smoke solution system**

Step 3: Place the bee smoker apparatus in a fume hood (see Fig. 1). The burning compartment of the system is contained in a metal bee smoker with an attached bellows. Clamp the heater hose to the spout of the smoker with a hose clamp, although if the hose fits snugly enough this clamp may not be necessary.

Step 4: Stabilize a 1-L side-arm flask with a ring stand and 3-pronged clamp around the neck of the flask. Put the unattached end of the heater hose into the mouth of the flask holding at least 300 mL of deionized water, although more water could be used. The heater hose should fit snugly into the mouth of the flask and the end should be positioned below the level of the water in the flask so that the smoke will bubble into the water. Attach a water aspirator to the side arm of the flask to help draw the smoke into the water.

**Create the smoke solution**

Step 5: Caution: The bee smoker gets very hot during the burning, so care is needed when the burn is in progress and until the smoker cools after the burn is finished. Heat-resistant gloves should be worn as required.

Step 6: Load the plant material into the bee smoker through the open lid and ignite the sample using a butane micro torch. Once the plant material is ignited, close the lid of the bee smoker and use the bellows to provide air to sustain the fire and to help push the smoke through the hose and into the water. The bellows should be pumped throughout the burn.

Step 7: Add more material to the bee smoker until the entire sample is burned. We usually burn and collect smoke from a total 100–200 g of plant material for each species. The burning for each 100–200 g sample lasts approximately 1–5 h.

Step 8: Once the sample has burned completely, allow the system and the ash to cool. Remove the ash and weigh it. The smoke water can be used immediately or frozen for later use or analysis.

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