EFFECTS OF SMOKE WATER ON GERMINATION OF A CARNIVOROUS PLANT

by
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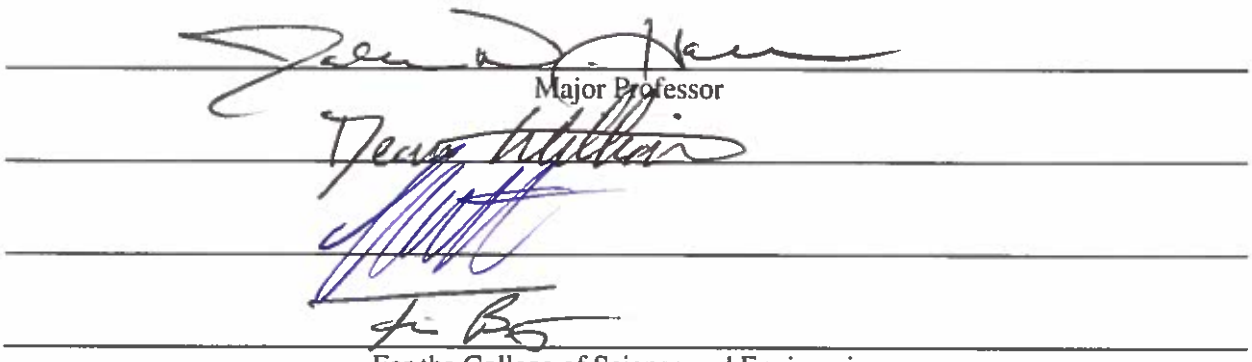
Thesis approved:



Major Professor

For the College of Science and Engineering

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Introduction

Low-intensity fires play an important role in revitalizing ecosystems globally (Bond and Keeley 2005; Heim et al. 2021). The frequency of low-intensity fires can vary from once every few years, such as in longleaf pine savannas (Stambaugh et al. 2011), to once every few thousand years in arctic tundra (Higuera et al. 2011). Low-intensity fires burn quickly at low temperatures, creating a natural disturbance, with minimal long-term damage (Keeley 2009). While fire removes the understory, plant litter, and competing plants, it also increases light and creates space for seeds to germinate. In addition, fire helps return essential nutrients to the soil, including nitrogen and phosphorous, leading to an increase in germination of early-successional species (Wan et al. 2001). These environmental changes can trigger germination in dormant seeds in the seed bank (Baskin and Baskin 2014).

Smoke is also known to affect germination in seeds (Light and van Staden 2004). When plant cellulose burns, it generates a group of chemicals called karrikinolides (Chiwocha et al. 2009). These chemicals resemble the plant growth hormone gibberellic acid, a compound naturally found in plants that stimulates growth and germination. This chemical trigger is believed to aide certain species in recovery from fire events (Khatoon et al. 2020).

Smoke is known to drastically change the environments in which it is introduced. After smoke is wafted over a landscape, and the environmental conditions are right, large amounts of seeds have been observed to germinate in synchrony (van Staden et al. 2000). This change in germination has been linked to chemicals, such as karrikinolides, which trigger environmental cues to cause dormant seeds to germinate (Chiwocha et al. 2009; Tuckett et al. 2010). Once dormancy had been broken, some species experience an increase in percent germination following treatments of either gibberellic acid or karrikinolides (Tuckett et al. 2010). Over 1,300 species of plants have

been tested for their germination responses to smoke (Jefferson et al. 2014). These studies are largely focused on Mediterranean-type climates, as these have a well-known frequent fire regime.

Not all species have a notable germination response to smoke (Dixon et al. 1995). For example, within the genus *Drosera* there are varied germination responses amongst the different species which include no response, decreased germination, and mild increased germination (Dixon et al. 1995; Roche et al. 1997). Inconsistency of germination response from species within the same genera is important to note as it shows interspecific responses to smoke. Smoke is also known to cause asynchronous germination response in some species, meaning that the seeds do not all germinate at the same time despite being in the same environment (Zirondi et al. 2019).

Lack of fire in ecosystems that once had a regular fire regime can cause altered germination and ecosystem function. Fire suppression causes drastic ecosystem shifts, leading to a reduction in fire prone ecosystems (Stambaugh et al. 2011). Instead of experiencing periodic disturbance and renewal from a fire, systems absent from fire will continue the natural progression to late-successional ecosystems (Bond and Keeley 2005), such as wetlands progressing to forests stands (Heim et al. 2021). This type of impact can alter nutrient cycling and species composition (Jefferson et al. 2014). Wetlands are particularly threatened by fire suppression because over time the wetlands will fill in since there is no longer a mechanism to remove plant litter (Heim et al. 2021).

As a result of this discovery, land managers are re-introducing fires to fire-suppressed ecosystems with the intent of restoring the ecosystem function. They attempt to replicate the historic fire frequency as determined by burn scars in tree rings and charcoal deposits in soil

(Stambaugh et al. 2011). Through these restoration efforts, conservationists are not only examining the benefits of fire, but how smoke interplays in these environments.

The longleaf pine ecosystems of the southern U.S. are one of many ecosystems in which fire reintroduction efforts are being employed. Many of the longleaf pine ecosystems of the southern U.S. are wet pine savannas that historically experienced fires every two to five years (Brewer 2001). This ecosystem illustrates the importance of fire because it contains 187 rare plants, 16 of which are threatened or endangered. Many of these species have reached this level of rarity due to fire suppression activities (Stambaugh et al. 2011). Due to fire suppression the seeds of the longleaf pine are caught in the plant litter along the forest floor, preventing the seeds from reaching the soil and germinating. This ecological change has led to the endangerment of the species (Farjon 2013).

Another example of a fire-dependent species from the longleaf pine habitat is the North American Pale Pitcher Plant, *Sarracenia alata*. This species is currently listed under International Union for Conservation of Nature and Natural Resources Red List (IUCN) as near threatened (Schnell et al. 2000). Anthropogenic changes to the environment, such as decreasing fire frequency, converting wetland habitat for agricultural purposes, pollution, and poaching have all lead to the decline of *S. alata* along with other species of *Sarracenia* (Cross et al. 2020). Understanding *S. alata*'s ecology will not only aid in the conservation of this species, but will also help other vulnerable and critically endangered species such as *S. leucophylla* and *S. oerophila* as they have similar morphologies (Brewer 2001).

Sarracenia alata is susceptible to competitive displacement by other plant species as a result of a diminished fire regime (Brewer 2001). Prescription burns that occur in *S. alata* habitat usually take place in the winter or early spring (Sheridan and Karowe 2000; Brewer 2001). Flowers

from *S. alata* are not impacted by early spring fires (Barker and Williamson 1988). *Sarracenia alata* exhibits increased growth and seedling establishment following a fire event (Barker and Williamson 1988). Though it is known that heat from fire is not needed to overcome dormancy or stimulate germination (Ellison 2001), the effects of smoke on germination have not been studied.

Each *S. alata* plant produces on average a single flower which produces hundreds of seeds (Horner et al. 2014). Seeds of are dormant and require cold stratification, or overwintering, to germination (Ellison 2001). These seeds will germinate the following growing season after they have overwintered. Seeds from *S. alata* experience increased germination when exposed to plant growth hormone gibberellic acid (Hopkins and Gravatt 2019).

The purpose of this study was to 1) examine the effect of smoke water on seed germination in *S. alata* and 2) evaluate whether seeds exposed to smoke water during different times in relation to cold stratification yield different germination responses. For the first part, we hypothesized that since *S. alata* responds with increased germination rate to treatments of gibberellic acid (Hopkins and Gravatt 2019), that applications of smoke water (that may contain karrikinolides that mimic gibberellins) would also result in an increased germination rate. For the second part, we proposed that as differences in response suggests the potential for seasonal preference in fire regime (Brewer 2001), that smoke exposure before cold stratification mimics fall fire, smoke exposure during cold stratification mimics winter fire, and smoke exposure after cold stratification mimics spring early fire. To determine different responses in germination under various smoke and stratification conditions, we collected the proportion viable, percent, rate, and synchrony of seeds germinated. We hypothesize that germination of *S. alata* will be affected by both the different concentrations of smoke water as different concentrations of smoke have be found to have

varying germination responses in other species. We also expect that the timing of exposure to smoke will have an effect on germination as early spring fires have been linked to increases in seedlings (Barker and Williamson 1988).

Materials and methods

Study organism

Sarracenia alata is a rhizomatous, perennial carnivorous plant that ranges from Alabama to eastern Texas (Fig. 1). It resides in nutrient-poor bogs and uses a modified leaf as a pitfall trap to capture insects. These insects are digested by enzymes and bacteria, found in the pitcher, for their nutrients (Green and Horner 2007; Horner et al. 2011). The pitchers are photosynthetic and require full sun exposure. Flowers are produced in March or early April, and shortly after the pitchers begin to emerge from the plant's rhizome (Carmickle and Horner 2019; Winer 2019). New pitchers are produced throughout the growing season (Winer 2019).

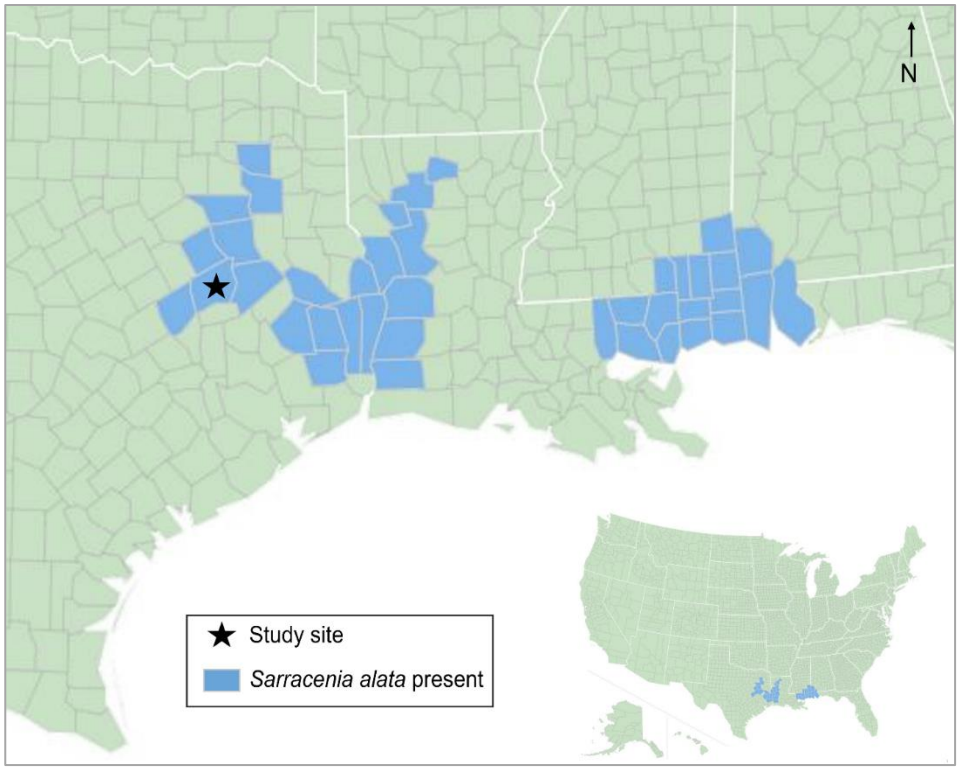

Figure 1: ***Range map Sarracenia alata. Counties (and parishes in LA) where S. alata has been found are indicated by blue polygons. The black star indicates the location from which S. alata ovaries were collected in October 2020 and November 2021. Map sourced from Tom's***

After pollination, seeds mature until mid-October. Seeds are then released from the ovaries around in the late summer and early fall. Seeds of *S. alata* are known to germinate at high rates (96%) under laboratory conditions (Hopkins and Gravatt 2019). The seeds of *S. alata* are hydrophobic and are believed to be dispersed by skimming across the water surface (Ellison and Parker 2002).

Study Site and Seed Collection

Our study site is a hillside seepage bog located in Leon County, Texas, USA (Fig. 1) on privately owned land. The dominant species are post oak and loblolly pine, and secondary species are willow, maple, and graminoids (Carmickle and Horner 2019; Winer 2019). Within the bog

itself, there are densely populated clusters of *S. alata* interspersed with other species of herbaceous plants and graminoids. Other carnivorous species in the bog are sundews (*Drosera* spp.) and bladderworts (*Utricularia* spp.) (Carmickle and Horner 2019; Winer 2019). Currently, these bogs are burned every two to four years by the landowner, typically in the late fall or early spring.

Sarracenia alata ovaries were collected in mid-October 2019 and mid-November 2021 from our study site. Seeds from 2019 were used in the preliminary study. Thirty ovaries were visually inspected that had minimal herbivore damage, were engorged with seeds, and had not fully dehisced. These ovaries were stored in paper bags at 21℃. Ovaries were then dissected, and seeds removed in December of 2019. Seeds were then stored in manila coin envelopes at 21℃ until used in experiments. Approximately 1,600 seeds were randomly sampled from 30 ovaries and mixed together for the experiments.

Seeds collected on November 10th, 2021, were used for the primary study. During collection, *S. alata* ovaries were visually inspected and 43 were collected from the field following the same method as above. The following day, the ovaries and their seeds were shipped in Ziploc™ bags to Eugene, OR where the seeds were removed from the ovaries. Six ovaries were noted to have mold and/or herbivore damage and were discarded from the study. Seeds from the remaining 37 ovaries were mixed together.

Positive Control

For a positive control, we purchased *Lactuca sativa* variety Grand Rapids Lettuce seeds from Eden Brothers in April of 2021 and stored them at 21℃. *Lactuca sativa* was selected as a control because smoke-derived chemicals are known to increase the germination of seeds (Flematti et al.

2004). Cold stratification is not required for germination of *L. sativa* seeds. In the primary study, *L. sativa* seeds were assigned to either a control treatment (water) or concentrations of smoke water that were determined by the second preliminary study, which tested for the effects of different concentrations of smoke water on the germination of *S.* alata seeds. Each treatment had eight replicates, each of which consisted of 20 randomly selected seeds. Seeds were plated in 30 X 15 mm sterile petri dish on a sheet of Whatman No. 1 filter paper. Once exposed to moisture, *L. sativa* completes germination within 24 hours. The number of seeds germinated was recorded every 6 hours during a 24-hour period.

Preliminary Study: The effects of smoke water derived from different woods on the germination of *Lactuca sativa* seeds (Positive Control)

This preliminary study was conducted to test the germination response of *L. sativa* to smoke derived from two different vegetative sources to determine if the germination response was the same or different. If the germination response is different, then it means that the smoke types have different chemical compositions. Smoke derived from different vegetative materials produce different effects on various species (Ren and Bai 2016). Therefore, we initially used two smoke types, hickory and mesquite, from the same brand, Wrights All-Natural Liquid Smoke®, to determine whether the smoke solutions would have the same effect on *L. sativa,* our positive control species.

We tested eight different concentrations each of the two smokes against a control of water. The concentrations that we selected were 1) 1:100, 2) 1:1,000 (hereafter 1:1K), 3) 1:10,000 (1:10K), 4) 1:100,000 (1:100K), 5) 1:1,000,000 (1:1M), 6) 1:10,000,000 (1:10M), 7) 1:100,000,000 (1:100M), and 8) 1:1,000,000,000 (1:1B). These concentrations were chosen to show a range of germination responses for *L. sativa*. This range was based on information from Calabrese and

Agathokleous (2021), who found that certain concentrations of smoke water can create a hormetic dose responses, meaning that low concentrations of a substance cause stimulation and high concentrations cause inhibition. Smoke solutions were diluted with 0 total dissolved solids (TDS) filtered water using a ZeroWater® filter. Each treatment had eight replicates with 25 randomly selected seeds assigned to each replicate, thus a total of 64 sets of seeds were tested. Each set of seeds were plated on 60 X 15 mm sterile petri dish on a sheet of Whatman No. 1 filter paper.

The preliminary trial revealed there to be a significantly higher percent germination of seeds at 12 hours after exposure to hickory smoke compared to mesquite (F = 21.71, df = 1, P = 0.000; Fig. 2). These results demonstrated that the positive control seeds germinated faster when hickory smoke was applied. Subsequently, for our primary experiment (described below), we selected to use hickory smoke. Moreover, our preliminary trial revealed there to be a significant difference in the percent germination of L. sativa seeds when exposed to different concentrations of hickory smoke (F = 39.93, df = 7, P = 0.000; Fig. 2A). Specifically, we found a higher percent germination of seeds at all concentrations, with the exception of 1:100, compared to the control. As a result of this, we conducted a second preliminary study to determine how different concentrations of hickory smoke influenced the percent germination of S. alata to further inform the primary experiment.

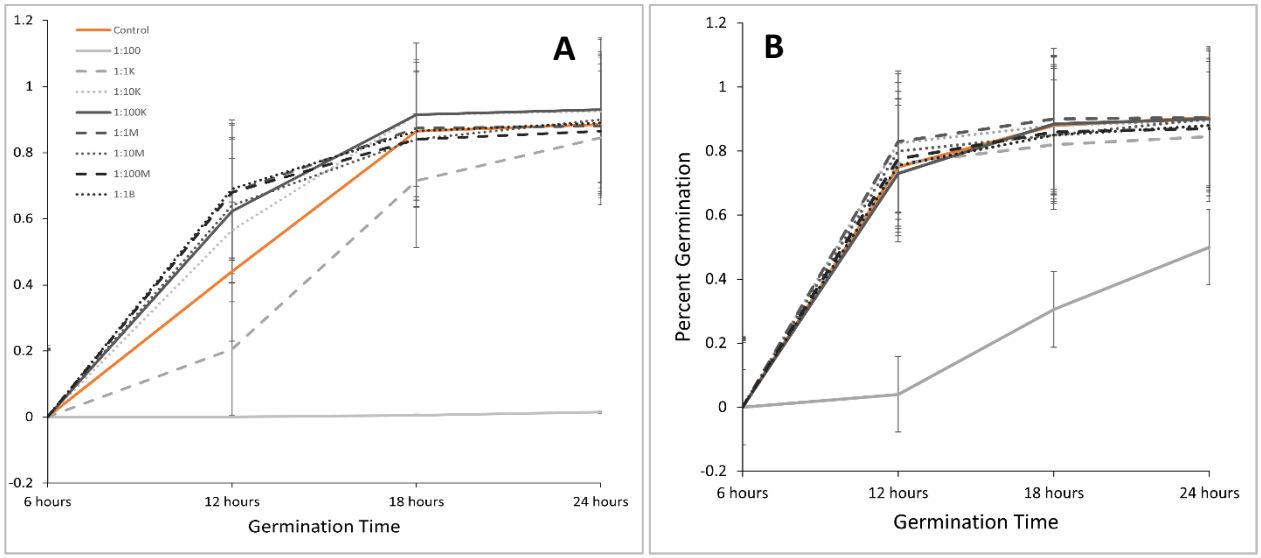

Figure 2: Percent germination of Lactuca sativa from smoke derived from different vegetative materials (A) Hickory and (B) Mesquite at varying concentrations of smoke water for a 24-hour duration. Concentrations of smoke water are 1:100, 1:1K, 1:10K, 1:100K, and 1:1M, 1:10M, 1:100M, and 1:1B.

Preliminary Study: The effects of different concentrations of smoke water on germination of *Sarracenia alata* seeds

Our second preliminary study was conducted to determine which concentrations of hickory smoke have the greatest variation in their germination response to seeds from *S. alata*. For this study we examined a range of smoke-water concentrations and used hickory smoke based on results from our first preliminary study with *L. sativa*. The study analyzed eight concentrations of smoke water that ranged from 1:100 to 1:1B and increased by factors of 10, as well as a control of water.

Seeds collected in 2019 were used for this study. Each treatment had eight replicates which consisted of 20 randomly selected seeds. Seeds were surface sterilized by constantly agitating the seeds for 10 minutes in a 10% liquid bleach solution (Ellison 2001; Hopkins and Gravatt

2019). Afterwards, seeds were strained from the solution using Whatman No. 1 filter paper followed by three rinses of filtered water. The seeds were then randomly assigned to their treatment groups and plated on a sheet of Whatman No. 1 filter paper in a 30 X 15 mm sterile petri dish and moistened with 2.5 ml of 0 TDS water. Seeds were then cold stratified in a refrigerator at 4°C for 21 days. After cold stratification, the seeds were then set to germinate in an incubator that maintained a temperature of 20°C and had a 14-hour photoperiod under 32-watt LED lights (Ellison 2001).

Seeds were monitored daily for signs of germination and counted as germinated when the radicle (root) protruded past the split seed coat. After thirty days of monitoring, seeds that did not germinate were scored with a razor blade treated with a 1% solution of tetrazolium chloride to determine seed viability. Tetrazolium chloride stains respirating tissues a bright red, making viable seeds relatively easy to decipher.

The results of our second preliminary trial found that hickory liquid smoke did have an impact on the germination of *S. alata* (Fig. 3). This impact to germination was found in the synchrony of germination ($F = 2.353$, df = 7, $P = 0.035$). The greatest differences in the synchronization of germination lies between the treatment groups 1:100K and 1:10K ($p = 0.031$) and 1:100K and 1:100m ($p = 0.023$). All treatment groups germinated asynchronously. The treatment group with the lowest synchrony was 1:100M (synchrony = 0.15), and the treatment with the highest synchrony was 1:100K (synchrony = 0.33). The rate of germination was not impacted by the different concentrations of smoke water ($F = 0.777$, df = 7, $P = 0.607$).

Percent germination was found using data from day 14 (Fig. 3) as we observed the greatest variation in germination response on this day. The greatest variation in germination from the control were concentrations 1:100, 1:1K, and 1:1M ($F = 3.104$, df = 3, $P = 0.043$). Based on

these observations we have chosen to use concentrations 1:100, 1:1K, and 1:1M for the primary study.

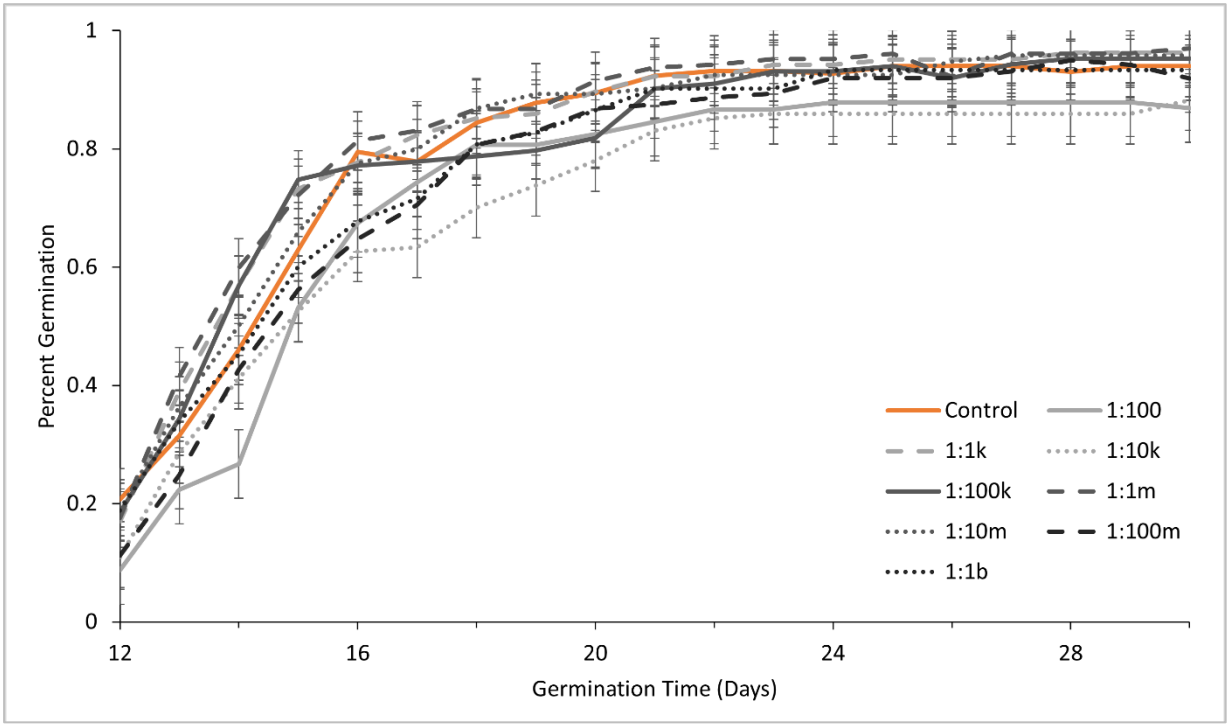

Figure 3: Percent germination of Sarracenia alata from exposure to varying concentrations of hickory smoke water. Seeds cold stratified and then exposed to smoke solutions for 30 days. Concentrations of smoke water are 1:100, 1:1K, 1:10K, 1:100K, and 1:1M, 1:10M, 1:100M, and 1:1B. Bars are standard error.

Smoke solutions

Based on the results from our first preliminary study, we used an aqueous solution of culinary hickory smoke, which is cost-effective and readily available at most grocery stores. Similarly, the three smoke solutions that we used were 1:100, 1:1K, and 1:1M were based on the results from our second preliminary study. In addition, we used a control group which received filtered water as its treatment. These three concentrations were selected because they had the most varied germination response compared against the germination response of the control.

The smoke solutions were prepared at the beginning of the experiment and stored at 4 ºC in between applications of the smoke solution. The solutions were contained in 100ml beakers with parafilm covering the opening to prevent evaporation. Each solution was agitated and thoroughly mixed before applying them to the seeds.

Exposure to smoke in relation to cold-stratification

Seeds were exposed to different smoke treatment groups (TG) before, during, and after a cold stratification. This experiment was undertaken to determine whether discernable germination responses are correlated to seasonal variation in fires. In other words, the exposure time of smoke for each of the treatment groups was selected to simulate the timing of fire in relation to seed germination (i.e., seasonal preference).

The first group (known as TG1) demonstrates how seeds would germinate in years without a fire and with a normal winter season (cold stratification). TG1, therefore, represented a control (with cold stratification, but no smoke exposure). The second group, TG2, tested smoke exposure before a cold stratification to replicate a fall fire before a winter cold snap (i.e., smoke exposure before cold-stratification). The third group, TG3, tested smoke exposure during a cold stratification to demonstrate how the seeds responded to a fire during the winter months (i.e., smoke exposure during cold-stratification). The last group TG4, tested smoke exposure after a cold stratification to demonstrate how seeds responded to smoke exposure after a spring fire (i.e., smoke exposure after cold-stratification).

Primary study

We paired smoke concentrations and exposure time with the intention of highlighting differences in germination response that may have been driven by the combination of the two variables.

This combination resulted in 10 treatment variables, with each of the three smoke concentrations in each treatment group (TG2-TG4), excluding our control (TG1) which did not receive smoke exposure. This study also used *L. sativa* as a positive control and used the methods outlined above for the treatment of seeds. For this study, each treatment had 20 randomly selected seeds per plate (Ellison 2001), with 10 replicates per treatment, totaling 100 plates.

All seeds were surface-sterilized by stirring the seeds for 10 minutes in a 10% liquid bleach solution (Ellison 2001; Hopkins and Gravatt 2019) and then pouring the seeds into a fine mesh metal kitchen sieve followed by three rinses with filtered water (Ellison 2001). After surface-sterilization, the seeds were randomly assigned to their treatment groups. Seeds in each group were then placed on a sheet of Whatman No. 1 filter paper in a 30 X 15 mm sterile petri dish and moistened with 2.5 ml of filtered water or smoke solution if seeds were in TG2. Filtered water was added to the filter paper every as needed to prevent drying. The petri dishes were placed in the incubator (20°C and 14- hour photoperiod) for seven days. All petri dishes were wrapped with parafilm to prevent desiccation.

After the seven days in the incubator, all petri dishes were replated with new filter paper and placed in a refrigerator at 4°C for 11 days. After this, all petri dishes were replated, during which the seeds in TG3 were administered 2.5 ml of smoke solution. All treatment groups were then returned to the refrigerator for another seven days, totaling an 18-day cold stratification. The treatments were then removed from the refrigerator and replated. At this time, the seeds in TG4 received 2.5 ml of smoke solution. All the treatment groups were placed in the incubator to start germination and after seven days, all the petri dishes were replated, hydrated with filtered water, and then placed back in the incubator for the remainder of the experiment (36 days).

Seeds were monitored daily for 36 days to examine for signs of germination and determine germination rates (Ellison 2001). Signs of germination were seed coat splitting and radicle (root) protruding. This information was used to determine germination proportion viable, percent, rate, and synchrony of germination.

To determine the proportion viable seeds, the number of viable seeds needed to be determined and then subtracted from the number of nonviable seeds. To find viability, seeds that did not germinate during the experiment were scored with a razer blade and treated with a 1% solution of tetrazolium chloride to determine if tissue was respiring (Lakon 1949). Tetrazolium chloride is a chemical that stains respirating tissues bright red. Once the tetrazolium chloride solution was added to the seeds, the seeds were then stored in a dark cabinet for 48 hours. Afterwards, the seeds were then examined under a dissecting microscope to determine if the tetrazolium chloride had stained the seeds. Seeds that were stained red were deemed viable seeds; the rest were considered not viable. Percent germination was determined from daily seed counts and subtracted from the proportion of viable seeds. These comparisons were done for each day of the experiment

Germination rate was measured using the equation in Ranal and de Santana's (2006). This is expressed as *Rate = (number of seedlings)/ (days to first count) + … + (number of seedlings)/ (last day of experiment)*. Once germination rate was determined for each of the replicates it was then compared against the germination rates of all the other treatments.

The percent germination was then determined from the percent of total viable seeds that germinated. Total viability was determined as the sum of total germinated seeds on day-36 and the number of seeds that were found viable with the tetrazolium chloride treatment. This percentage was then subtracted from the average percent germination of the control group.

Finally, synchronicity of germination was tested using Z index analysis as outlined in Ranal and de Santana (2006), which determined the amount of overlap each treatment has in germination rates (Ranal and de Santana 2006). This equation is expressed as $Z = \Sigma C_{ni,2}/N$, where $\Sigma C_{ni,2} = n_i (n_i - 1)/2$; $N = \Sigma n_i (\Sigma n_i - 1)/2$; and n_i is the number of seeds germinated in time i. $C_{ni,2}$ is the sum of the seeds germinated in *i*. Z shows the level of synchrony with 1 as synchronous and 0 as asynchronous (two seeds or more seeds germinating).

Statistical Analysis

Each response variable (proportion of viable seeds, percent of seeds germinated, germination rate, and synchronicity) was analyzed separately using two sets of analyses. The first analysis is an analysis of variance (ANOVA) to calculate differences of the TG2-4 against TG1 (control) while factoring the differences of exposure time and the smoke concentrations.

The analysis for the proportion of viable seeds was taken from the number of viable seeds resulting from the tetrazolium chloride test minus the number of seeds that were not viable. This number was corrected with our control by subtracting the TG proportion viable from the proportion of viable seeds from the control. Analysis was done on whether the concentrations of smoke and/or the timing of exposure to smoke had an effect on the viability of seeds in any of the treatment groups.

We examined the percent germination of viable seeds on day 20 of the experiment across the varying treatment groups minus the average percent germination of the control. For percent germination, a univariate general linear model was used to analyze the significance of the different treatment groups and the percent germination. This analysis was done with both the percent germination viable seeds across the varying treatment groups, and the percent

germination viable seeds across the varying treatment groups minus the average percent germination of the control.

The analysis for rate of germination was calculated by using the rate of germination equation as described above and by using a univariate general linear model to compare the differences in germination rates between the different treatment groups of smoke concentration and exposure time.

The analysis for synchrony of germination was calculated by using the Z index as outlined above and by using a univariate general linear model to compare the differences in synchronization of germination time between the different treatment groups of smoke concentration and exposure time.

Results

Seeds from *L. sativa*, our positive control, showed significant differences ($F = 2.353$, df = 7, $P = 0.03$) in percent of seeds germinated in all treatment groups during the sampling at 12 hours (Fig. 4). Seeds exposed to smoke concentration 1:100 had a 0% germination at hour 12, which demonstrated inhibition to germination from the smoke (Fig. 4). By hour 24, seeds in all treatment groups reached 83% total germination.

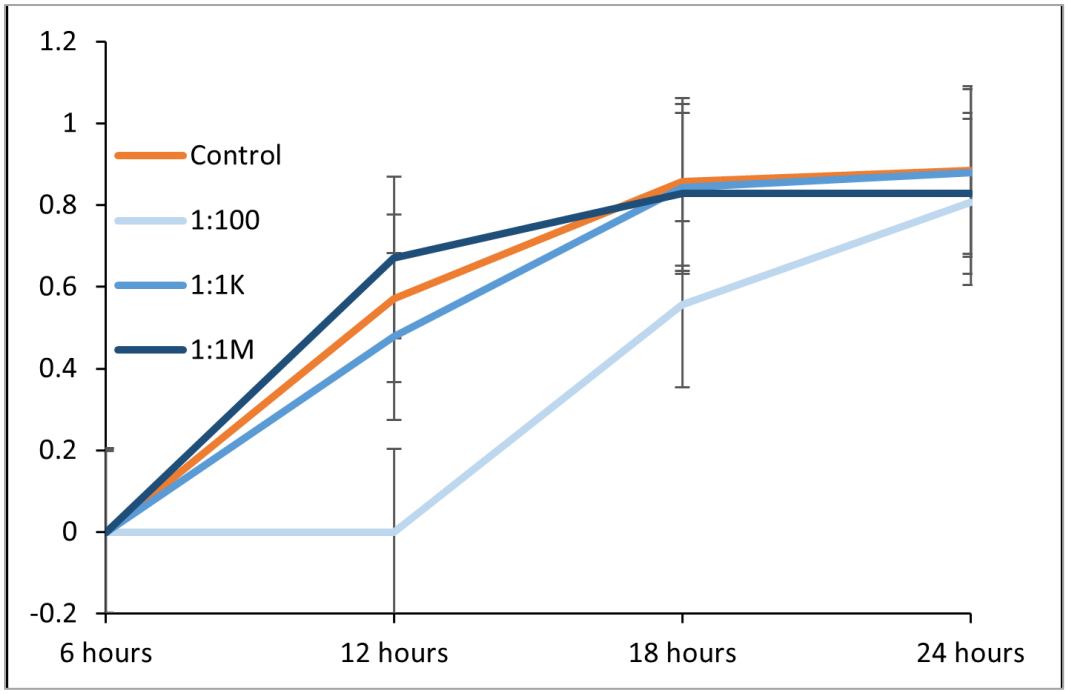

Figure 4: Percent germination of Lactuca sativa from exposure to 24 hours in response to different concentrations of smoke with standard error bars. Different concentrations of smoke are 1:100, 1:1K and 1:1M.

Seeds of *S. alata* from 2021 were found to have a 59% viability. During the germination trials from December 8th, 2021, to January 12th, 2022, 71% of viable seeds germinated (Fig. 5A). There was significance in the proportion of viable seeds that germinated that were in different smoke exposure treatment groups ($F = 0.234$, df = 2, $P = 0.050$). Posthoc analysis found a lower viability of seeds in the during treatment (mean difference for before treatment: -0.07, $P = 0.097$; mean difference for after treatment: -0.08, $P = 0.075$) in comparison with the seeds from the other exposure treatments. Smoke concentration did not have a significant effect on the proportion of viable seeds ($F = 0.234$, df = 2, $P = 0.792$).

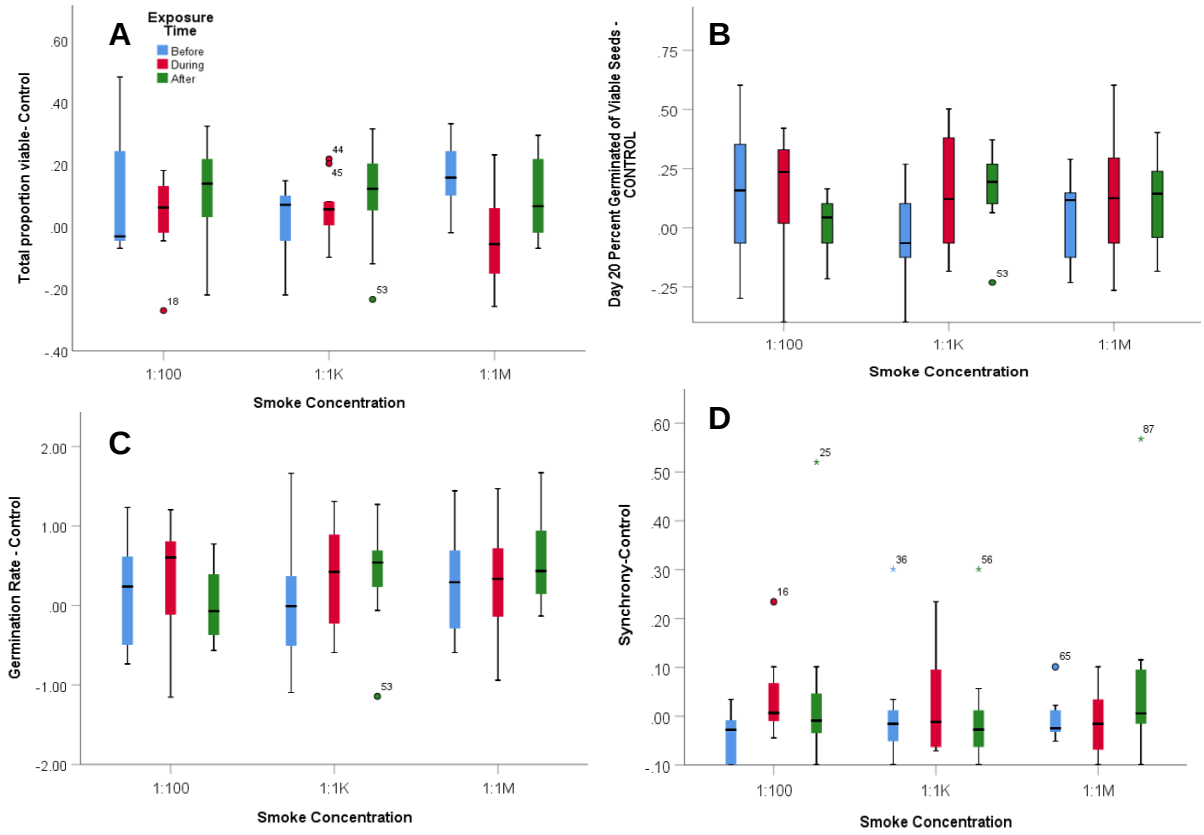

Figure 5: Effects of various smoke concentrations and exposure times on the germination of Sarracenia alata seeds. Smoke concentrations are 1:100, 1:1K, and 1:1M. Exposure times are before, during, and after. (A) Effects of smoke solutions and exposure times on the proportion of viable seeds minus the proportion of viable seeds for the control. (B) Effects of smoke solutions and exposure times on the percent germination of viable seeds on day 20 minus the percent of viable seeds for the control. (C) Effects of smoke solutions and exposure times on the rate of germination seeds minus the rate of germination seeds for the control. (D) Effects of smoke solutions and exposure times on the synchrony of germination seeds minus the synchrony of germination seeds for the control.

There was no significant difference of smoke concentrations (F = 1.423, df = 2, P = 0.247) or the time of exposure (F = 0.075, df = 2, P = 0.928) on the percent germination of viable seeds (Fig. 5B). Percent germination of viable seeds was found on day 20 as this was the day with the greatest variation in germination response (Fig. 6).

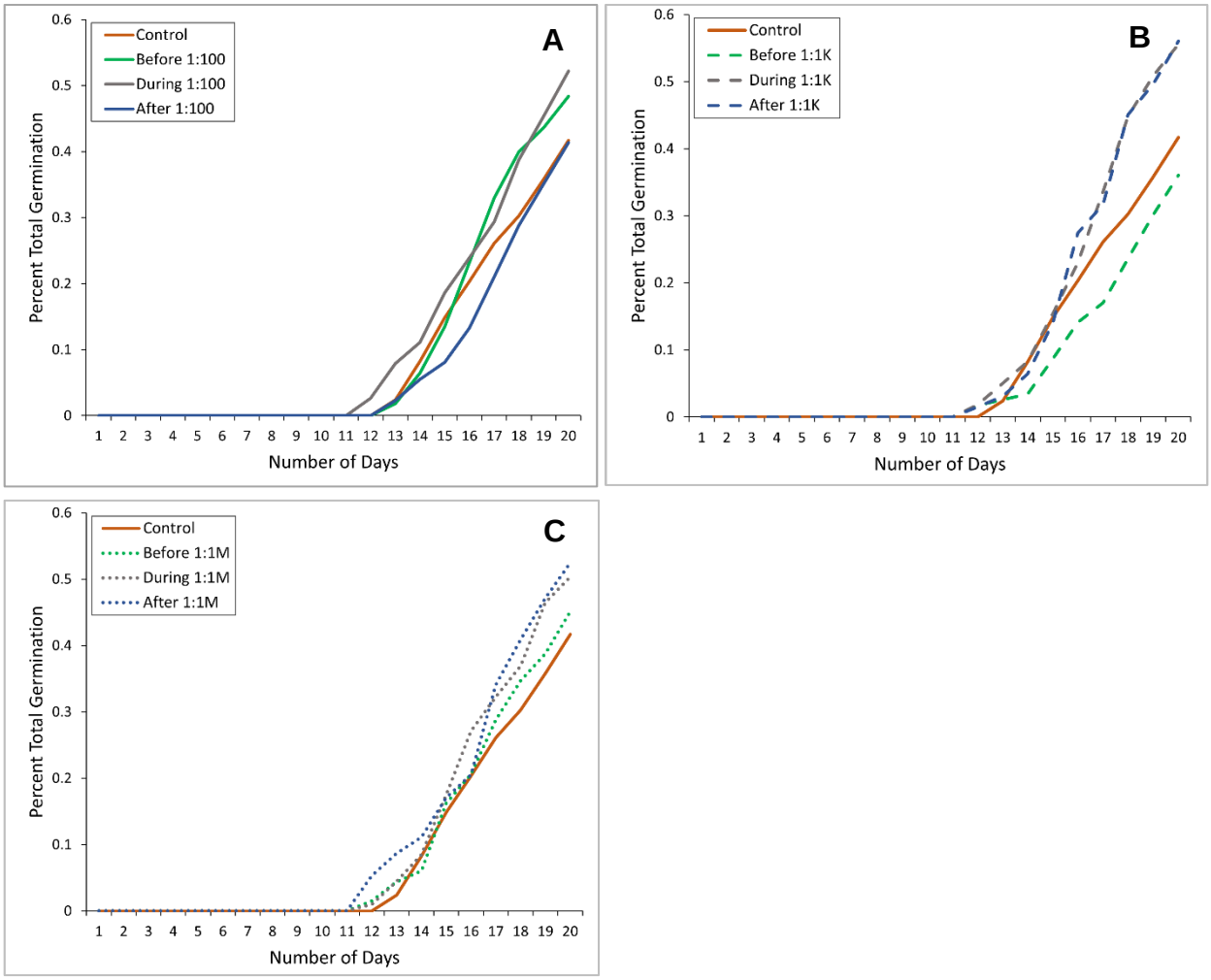

Figure 6: Daily total percent germination of S. alata from day 1 to 20 in response to different concentrations of smoke and timing of exposure (before, during, and after). Different concentrations of smoke are (A) 1:100, (B) 1:1K, and (C) 1:1M.

Our analysis found no significance on the influence of smoke concentrations (F = 0.432, df = 2, P = 0.651) nor the exposure time (F = 0.661, df = 2, P = 0.519) on the rate of germination of the seeds (Fig. 5C). We also found no significance on the influence of the smoke concentrations (F = 0.011, df = 2, P = 0.989) nor the exposure time (F = 1.455, df = 2, P = 0.239) on the synchronization of germination time of the seeds (Fig. 5D).

Discussion

We found that the germination of seeds from *S. alata* were affected by exposure to smoke. The proportion of viable seeds from *S. alata* was impacted by the exposure time of when seeds were exposed to smoke water solutions. The percent, rate, and synchrony of germination were not affected by either the smoke treatment nor the time of exposure in this experiment.

In this study, the concentrations of smoke water did not have an impact on the germination of *S. alata* seeds. Yet in our preliminary study, we found evidence that different concentrations of smoke water have an effect on the synchrony of germination and lead to asynchronous germination. The potential reason for these differing results between our primary and preliminary study was the length of the exposure to the concentrations of smoke. Suggesting that in order to have a noticeable germination effect, seeds from *S. alata* may require an exposure to smoke water that is longer in duration than 7 days. This hypothesis is plausible due to the hydrophobic properties of the seed coat, which may prevent smoke water from penetrating the seed within the timeframe of the exposure.

Exposure time of smoke in relation to cold-stratification was found to have a lower proportion of viable seeds when seeds were exposed to smoke solutions during cold-stratification in comparison with seeds exposed to smoke before or after cold. This difference in germination response around smoke exposure may signify that fires during certain times of the year yield different germination responses from *S. alata*. Our finding suggest that seeds exposed to smoke before or after cold-stratification will have increased seed germination in comparison with seeds exposed during cold-stratification.

This study showed that hickory smoke does have an effect on the germination of *S. alata* seeds in certain conditions. One of the conditions that was not examined in this study was the effects of smoke derived from vegetation endemic to the same bogs as *S. alata* on the germination of *S. alata* seeds. We know that chemical compositions of plant derived smokes are as varied as the plants used to derive the smoke (Khatoon et al. 2020). We can gather from this statement that seeds from *S. alata* are likely to have different germination responses to spokes derived from different species of plants. Further studies will be needed to test the effects of different smoke sources on the germination of *S. alata*.

Future research should also address questions pertaining to smoke and its interactions within the bog environment. Knowing how long smoke persists in a wetland environment and how the hydrology of the wetland changes the concentrations smoke water will better inform conservationists around prescriptions of controlled burns. Additionally, understanding how chemicals from smoke may impact the flora of the bog, specifically species of *Sarracenia*, will improve conservation management plans for *S. alata* as well as other more imperil species of *Sarracenia*.

From this study we found that smoke exposure during cold-stratification results in a lower percent germination compared with smoke applications before and after cold-stratification. Our second preliminary study also showed that long exposures to different concentrations of smoke water result in changes to the synchrony of germination of *S. alata*. This study on the impacts of aqueous smoke on the germination of *S. alata* was intended to provide a starting point for future examination of the impacts of smoke on *S. alata* and other bog-inhabiting species.

Conclusion

This study demonstrates that the timing of smoke exposure does have an impact on the germination of *S. alata*. While the results of our study were inconclusive as to the effects of concentrations of smoke, further investigation is required to confirm the effects of smoke on the germination of *S. alata*. Thus, understanding how fire dependent wetland species interact with fire is important as prescription burns become a more common conservation management tool. Knowledge of species response to fire would allow development of conservation strategies to better suit these species requirements. Fires help to increase seedling production (Ellison 2001). Synchronization of germination is reduced by smoke, potentially resulting in less resource competition and increased seed survivorship (Gioria et al. 2018).

We encourage further research of germination response to controlled burns for other species of *Sarracenia* and species endemic to longleaf pine bogs. Further research will only increase our understandings of fire ecology in southeastern North America and aid in better conservation of these habitats. Thus, we recommend further research to increase our scientific knowledge in fire ecology to help with protection and conservation of fire dependent environments.

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Abstract

EFFECTS OF SMOKE WATER ON GERMINATION OF A CARNIVOROUS PLANT

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Many ecosystems are fire adapted, and conservationists often utilize prescription fires as a restoration tool. These burns have been linked to an increase in germination. Smoke is one of the driving factors for increased germination as it is chemically similar to a plant hormone (gibberellic acid). The purpose of this study was to examine the effect of smoke on seed germination of a fire adapted species, *Sarracenia alata*. *Sarracenia alata* is a carnivorous plant species found in bogs and seeds require a cold stratification to germinate. We examined germination response of seeds to treatments that combined exposure of seeds to varying concentrations of smoke water (1:100, 1:1K, and 1:1M) and cold-stratification (before, during, and after). Daily germination was used to calculate the percent, rate, and synchrony of germination. Timing of exposure to Smoke water had a significant impact on the germination of *S. alata*.