Seasonal germination responses of *Liatris punctata* to heat and smoke

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Introduction

Germination, a critical stage in the life cycle of plants, encompasses the processes starting from imbibition to radicle protrusion through the testa. The timing of germination ultimately determines survival success (Wolny et al., 2018). Seed germination is primarily regulated by naturally occurring hormones, namely gibberellins (GAs) (Carrera-Castaño, 2020). Environmental factors often influence the biosynthesis of these hormones. Fire-associated cues, such as heat shock and smoke, have been identified as key triggers capable of stimulating germination (Bradshaw et al. 2011, Keeley et al, 2011). Smoke specifically has emerged as a potent germination stimulant in over 1,000 plant species worldwide, as it is rich in compounds known as karrikins, or butenolide molecules (Moyo et al., 2022).

Liatris punctata, or dotted gayfeather, is a native aster common in dry prairies and plains as well as the foothills of the Rocky Mountains in Colorado (Menhusen, 1972). Although non-threatened, *L. punctata* plays a critical role as the primary nectar source of the threatened Pawnee montane skipper butterfly (*Hesperia leonardus montana*). This butterfly subspecies is endemic to the South Platte River system in Colorado, specifically near Deckers and Cheesman Reservoir in dry, open ponderosa pine woodlands (Ellis et al., 2023). Despite the importance of *L. punctata* to the butterfly's preservation, the plant's germination requirements remain poorly understood. Data from a previous study found exogenously applied gibberellins, specifically GA4+7, increased germination but not as pronounced as with butenolide (Figure 1). Thus, more research on the germination and establishment of *L. punctata* is critical to the butterfly's recovery.

As low intensity fires in the ponderosa forest habitats where *L. punctata* occurs are an occasional yet essential part of the ecology, fire may be important to the germination physiology of this species. This study examines the role of gibberellins 4 and 7 (GA4+7) in germination following different combinations of smoke and heat exposure on *L. punctata* seeds in early and late spring conditions. We aim to identify and interpret the effects of low-grade fire on germination, exploring individual factors such as smoke, gibberellins, and heat. [note: unreported data indicated that *L. punctata* prefers GA4+7 slightly over GA3]

Methods

L. punctata seeds were obtained from Prairie Moon Nursey, Winona, MN and stratified at 4°C in the dark for three weeks. After, seeds were washed with distilled water, dried, and placed on standard filter papers or smoke papers containing butenolide in 1% agar plates. Prior to washing, a subset of seeds was heat-treated in a pre-heated oven at 70°C for an hour to mimic low-grade fire residence times in montane ponderosa pine savannas (Massman et al., 2003).

In total, 272 plates were created (8 replicate plates with 8 seeds, 13 treatments per season). Two incubators simulated early and late spring conditions modeled after temperature data from Cheesman Reservoir (USNO, 2009) and the germination requirements of *Liatris squarossa* (Baskin & Baskin, 1989): early spring 20°C day/5°C night and late spring 28°C day/15°C night, with a 12/12 hour photoperiod.

Germination success was recorded at 3, 7, and 10 days. Viability was assessed by checking seed firmness with forceps, with non-viable seeds excluded from the germination count.

Table 1. Seeds were subject to 13 different germination treatments. Treatments used 3mL of the respective solution. The chemical uniconazole was used here to inhibit GA biosynthesis. 10^-3M of GA4+7 was used.

[note: dH2O = distilled water. Solvent = 2% ethanol in distilled water solution.]

ID	Season	Temperature Treatment	Smoke	Treatment
Solvent Control	Early Spring Late Spring	22°C	No Smoke	3mL Solvent
Water Control	Early Spring Late Spring	22°C	No Smoke	3mL dH2O
GA Blocker	Early Spring Late Spring	22°C	No Smoke	1.5mL Uniconazole 1.5mL Solvent
GA4+7	Early Spring Late Spring	22°C	No Smoke	1.5mL Uniconazole 1.5mL GA4+7
Smoke	Early Spring Late Spring	22°C	Smoke	3mL Solvent
Smoke + GA Blocker	Early Spring Late Spring	22°C	Smoke	1.5mL Uniconazole 1.5mL Solvent
Smoke + GA4+7	Early Spring Late Spring	22°C	Smoke	1.5mL Uniconazole 1.5mL GA4+7
70C	Early Spring Late Spring	70°C	No Smoke	3mL Solvent
70C + Smoke	Early Spring Late Spring	70°C	Smoke	3mL Solvent
70C + GA Blocker	Early Spring Late Spring	70°C	No Smoke	1.5mL Uniconazole 1.5mL Solvent
70C + Smoke + GA Blocker	Early Spring Late Spring	70°C	Smoke	1.5mL Uniconazole 1.5mL Solvent
70C + GA4+7	Early Spring Late Spring	70°C	No Smoke	1.5mL Uniconazole 1.5mL GA4+7
70 + Smoke + GA4+7	Early Spring Late Spring	70°C	Smoke	1.5mL Uniconazole 1.5mL GA4+7

Results

Figure 1. Germination Frequency Of Treatments Over 10 Days

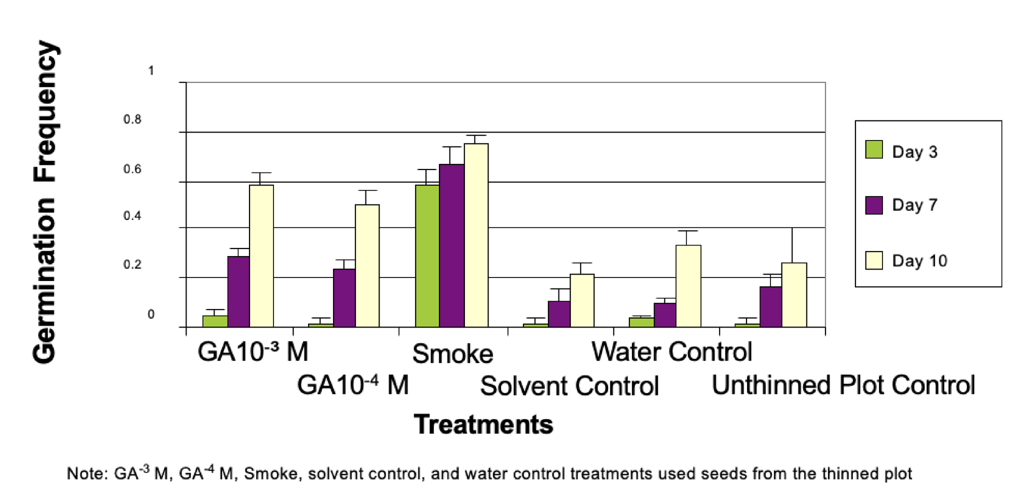

Figure 1. Germination frequency of smoke and GA4+7 treatments over days 3, 7, and 10 in late spring conditions. This previous study used wild-collected seeds. The results indicated that smoke treatment significantly enhanced germination success, while the addition of GA4+7 did not significantly enhance germination compared to smoke (Ragan et al., 2008).

Figures 2-5 report germination responses to various experimental treatments comparing simulated early spring and late spring growing conditions. Fire significantly enhanced germination in early spring but not as significantly in late spring.

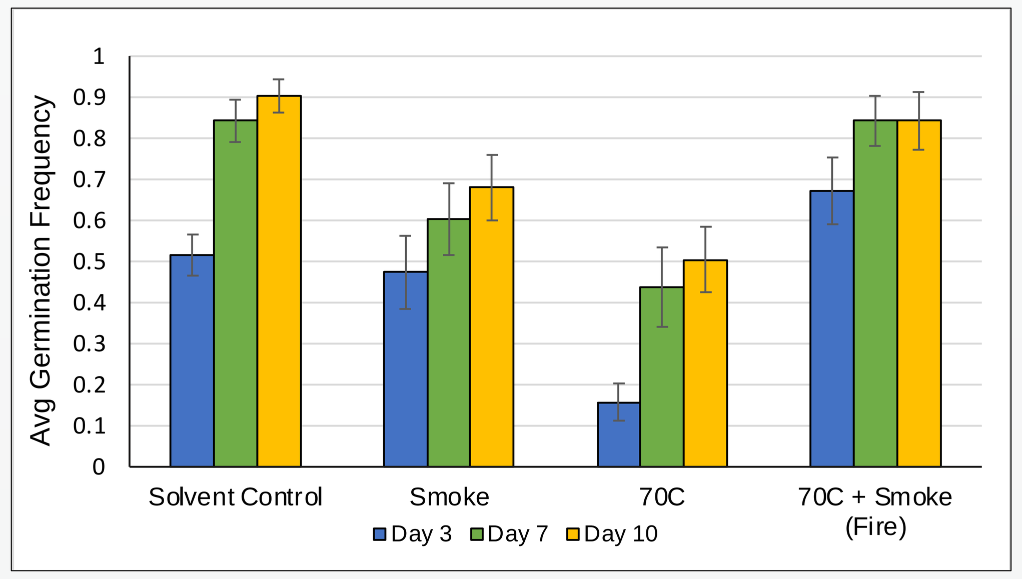

Figure 2. Mean (± SE) germination frequencies for smoke treatments over days 3, 7, and 10 in early spring.

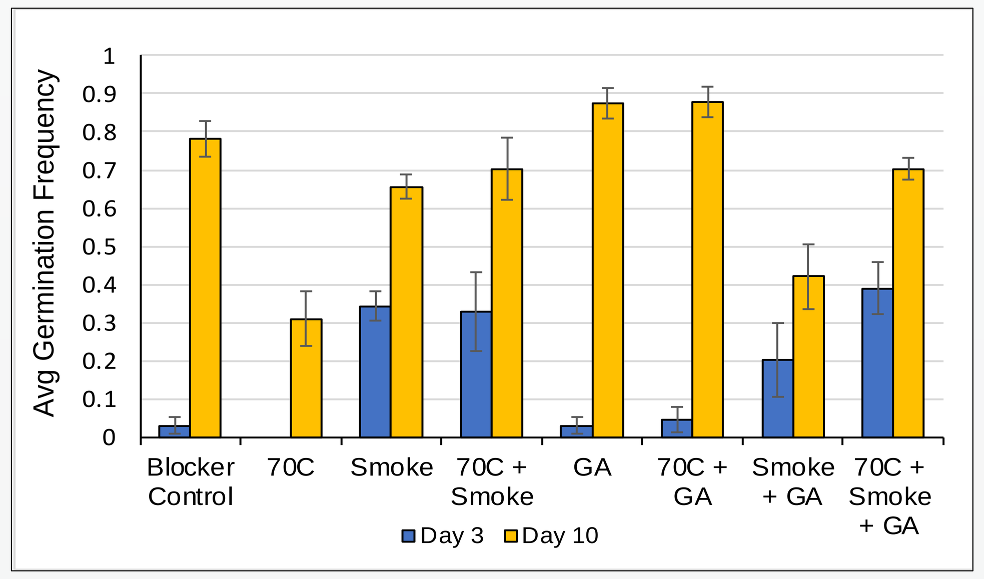

Figure 4. Mean (± SE) germination frequencies for uniconazole (Blocker) treatments over days 3 and 10 in early spring. All treatments include uniconazole.

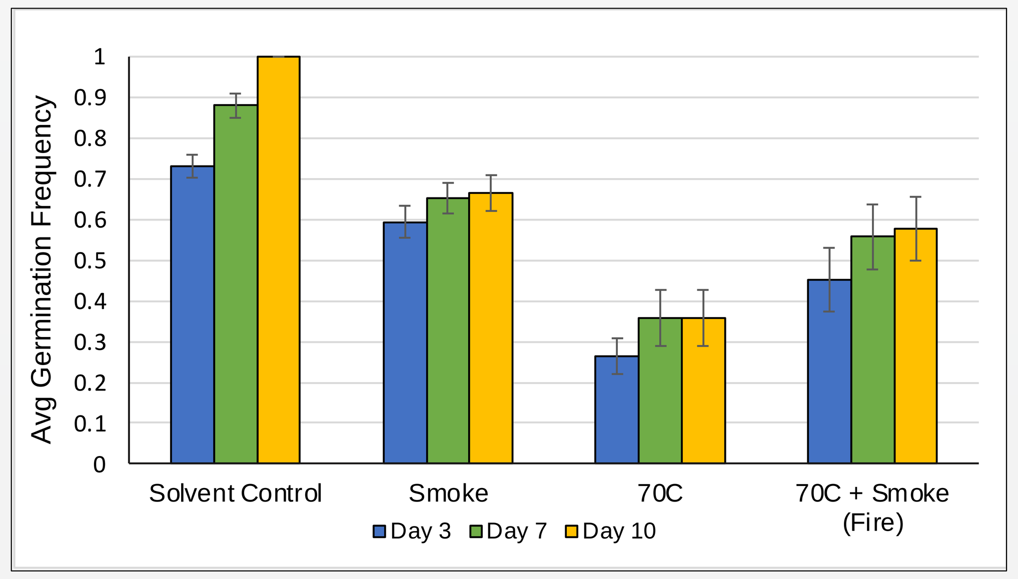

Figure 3. Mean (± SE) germination frequencies for smoke treatments over days 3, 7, and 10 in late spring.

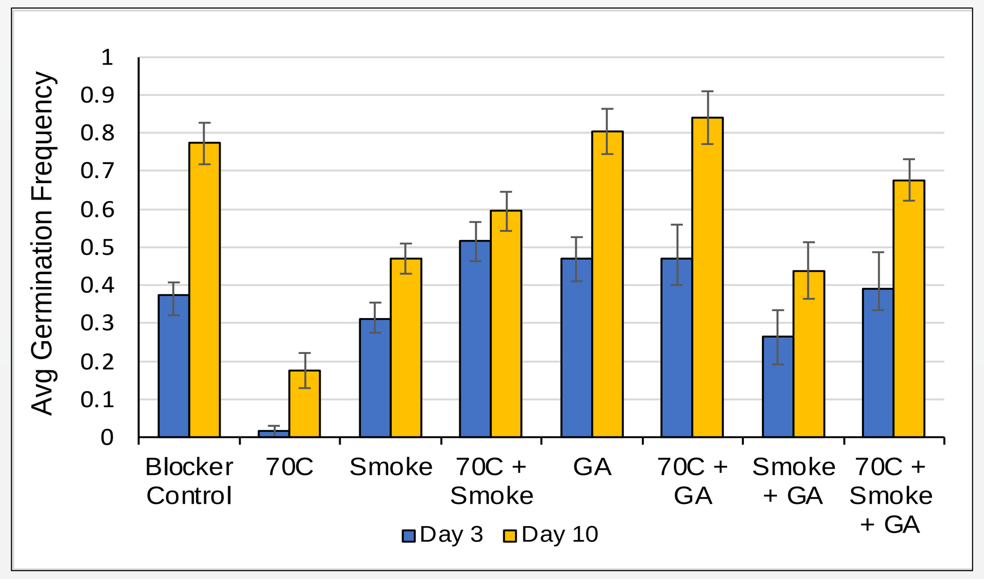

Figure 5. Mean (± SE) germination frequencies for uniconazole (Blocker) treatments over days 3 and 10 in late spring. All treatments include uniconazole.

Table 2. ANOVA for smoke treatment and temperature (Temp) on germination frequency in early and late spring. F and p values are reported: *** p<0.001; ** p<0.01; * p<0.05; + p<0.10.

Effect	Early Spring Day 3	Early Spring Day 10	Late Spring Day 3	Late Spring Day 10
	F Values			
Smoke	49.0737***	0.2336***	6.9183**	0.5125
Temp	0.0346	0.1867	0.7626	2.735
Smoke * Temp	5.3590*	6.8975**	4.2875*	9.3736**

Table 3. ANOVAs for temperature and treatment type (Trt) and for smoke and treatment type (Trt) on germination frequency in early and late spring. F and p values are reported: *** p<0.001; ** p<0.01; * p<0.05; + p<0.10.

Effect	Early Spring Day 3	Early Spring Day 10	Late Spring Day 3	Late Spring Day 10
	F Values			
Temp	0.0377	0.0028	2.375	7.1235**
Trt	18.3508***	7.1501*	14.9546***	11.7680***
Temp * Trt	1.2265	1.5357	4.7010*	9.4771***

Effect	Early Spring Day 3	Early Spring Day 10	Late Spring Day 3	Late Spring Day 10
	F Values			
Smoke	60.7672***	0.1152	4.0064*	3.4304+
Trt	26.1516***	6.6944**	13.2526***	8.6277**
Smoke * Trt	0.8669	11.8144***	12.7184***	5.9134**

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Conclusions

How do heat and smoke impact germination of *Liatris punctata* seeds?

- In early spring, heat and smoke together (fire) increased germination frequency, particularly early in germination. (Figure 2). In late spring, germination frequency was not significantly improved by fire (Figure 3).
- Heat treatment alone was never a significant factor in germination in either spring conditions. On the other hand, smoke had a significant role in promoting early spring germination, but its role in late spring germination became less pronounced later into germination (Table 2).
- Germination timing in spring may play a role in the heat and smoke-induced germination response.

What is the role of GA4+7 in germination responses to heat and smoke? Does the role of GA4+7 differ between heat or smoke-induced *Liatris* germination?

- Germination frequencies of smoke and/or heat-treated seeds were reduced by uniconazole, which inhibits GA biosynthesis (GA blocker) (Figures 4 & 5).
- In both springs, Smoke+GA treatments reduced germination, indicating that smoke may negatively interact with GA4+7 in *Liatris* germination response. However, GA4+7 rescued heat-treated seeds in both seasons. Interestingly, GA4+7 had no significant impact on fire-treated seeds (Figures 4 & 5).
- Exogenously applied GA4+7 increased germination (Figures 4 & 5).

In comparison with past data (Figure 1), smoke treatments on *Liatris* seeds did not induce the same degree of germination. Here, germination frequency of the smoke treatment was lower compared to the solvent control (Figure 3), and GA4+7 significantly increased germination frequency relative to the smoke treatment (Figure 5). Because our study uses farm-collected seeds, there may be a genetic component to these different responses.

Low-grade fires may be a major factor in signaling germination in *L. punctata*, particularly in early spring. GA4+7 may have a secondary role in its smoke-induced germination response, potentially negatively interacting with smoke.

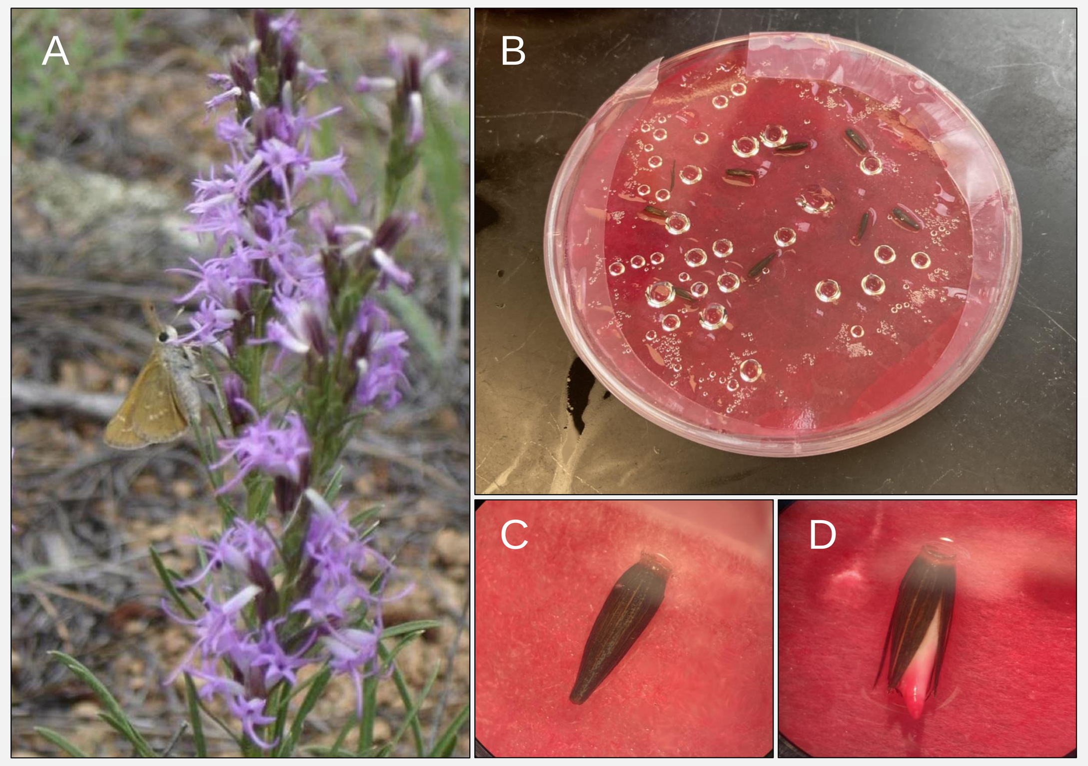

Figure 6. a) Pawnee montane skipper (*Hesperia leonardus montana*) female nectaring on dotted gayfeather (*Liatris punctata*), Source: Boyce A. Drummond; b) Experimental replicate containing *L. punctata* seeds and smoke paper with butenolide; c) A *L. punctata* seed that has not germinated; d) A germinated *L. punctata* seed. Both c and d were photographed through a light microscope.

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