



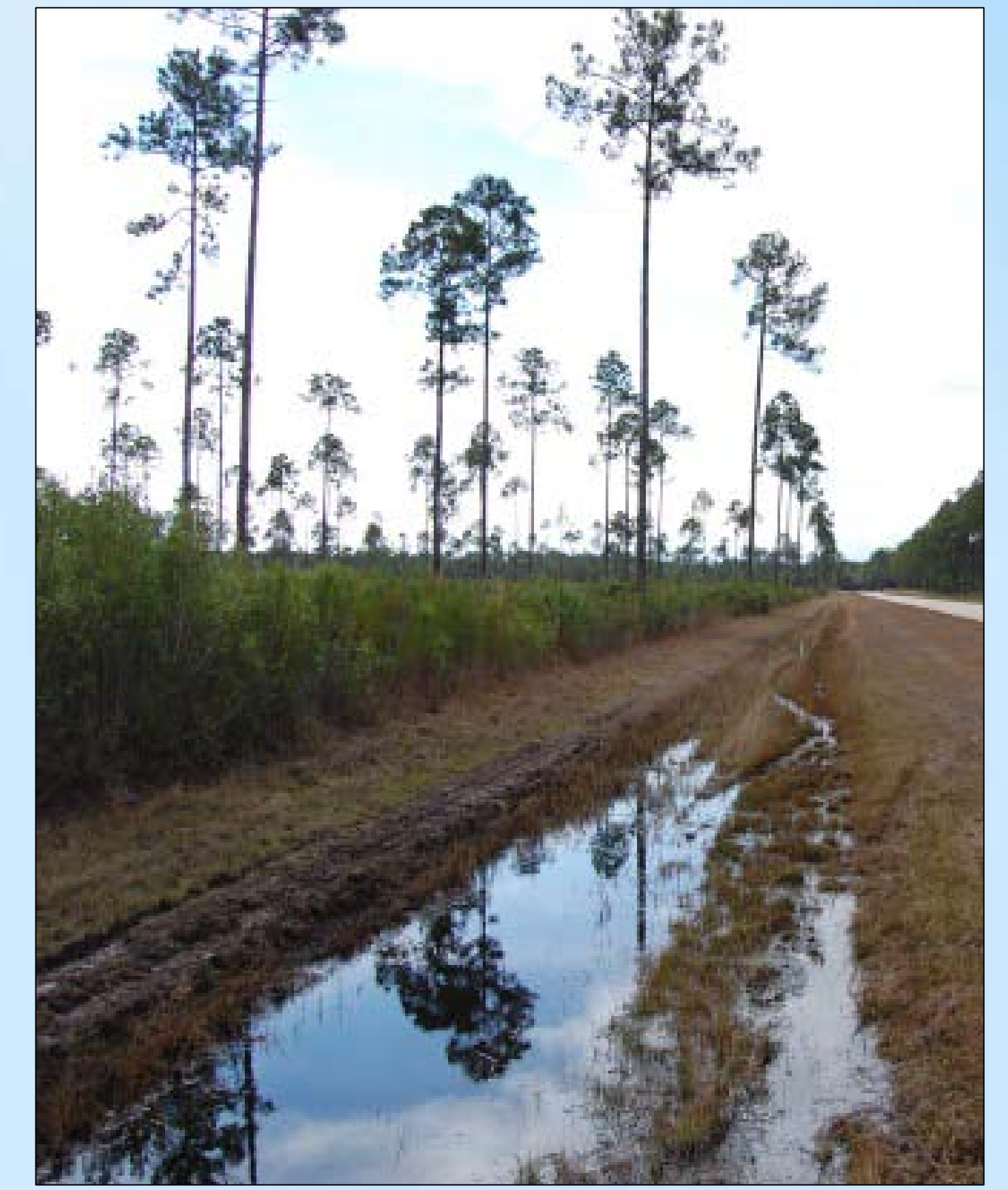
*Pinguicula ionantha* flowering

# Seed Ecology of Federally Threatened *Pinguicula ionantha* (Godfrey's Butterwort)

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Flooded roadside ditch next to longleaf pine savanna – A common location for *Pinguicula ionantha*

## Introduction

*Pinguicula ionantha* G. (Lentibulariaceae) is a federally threatened carnivorous herb with a limited range in the lower Apalachicola River region of the Florida Panhandle (Kesler *et al.* 2008). This species occurs specifically within lowland bog habitats embedded in longleaf pine savannas (Godfrey and Stripling 1961). This type of habitat is defined as a fire-dependent community (Kesler *et al.* 2008). The U.S. Fish and Wildlife Service released a 5-year review in 2009 with research recommendations for future delisting of the species. Their recommendations include studies on seed viability and timing of germination as an aid to potential reintroduction.

## Objective

Determine the most efficient method to germinate *Pinguicula ionantha* seeds.

## Materials and Methods

In late March through mid-April 2012, eleven collections of *Pinguicula ionantha* seeds were made from natural populations within the Florida Panhandle. All *P. ionantha* seeds were stored at 4°C. Germination trials began in July 2012. All germination trials took place in a Percival Scientific seed germination chamber with fluorescent lights with a 16-hour photoperiod at  $28 \pm 9 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  at  $25.0 \pm 0.0$  °C. Seeds were checked every other day for germination for 8 weeks, then kept in the germination chamber for 15 weeks for final counts. Media for seed treatments included moistened filter paper, 1% agar, or filter paper on top of 1% agar. Means and standard errors for percent germination were calculated. Percent germination was transformed with arcsine before data analysis was performed. A one-way analysis of variance was calculated with SPSS, followed by post hoc tests using a Duncan's Multiple Range Test at 5% level.

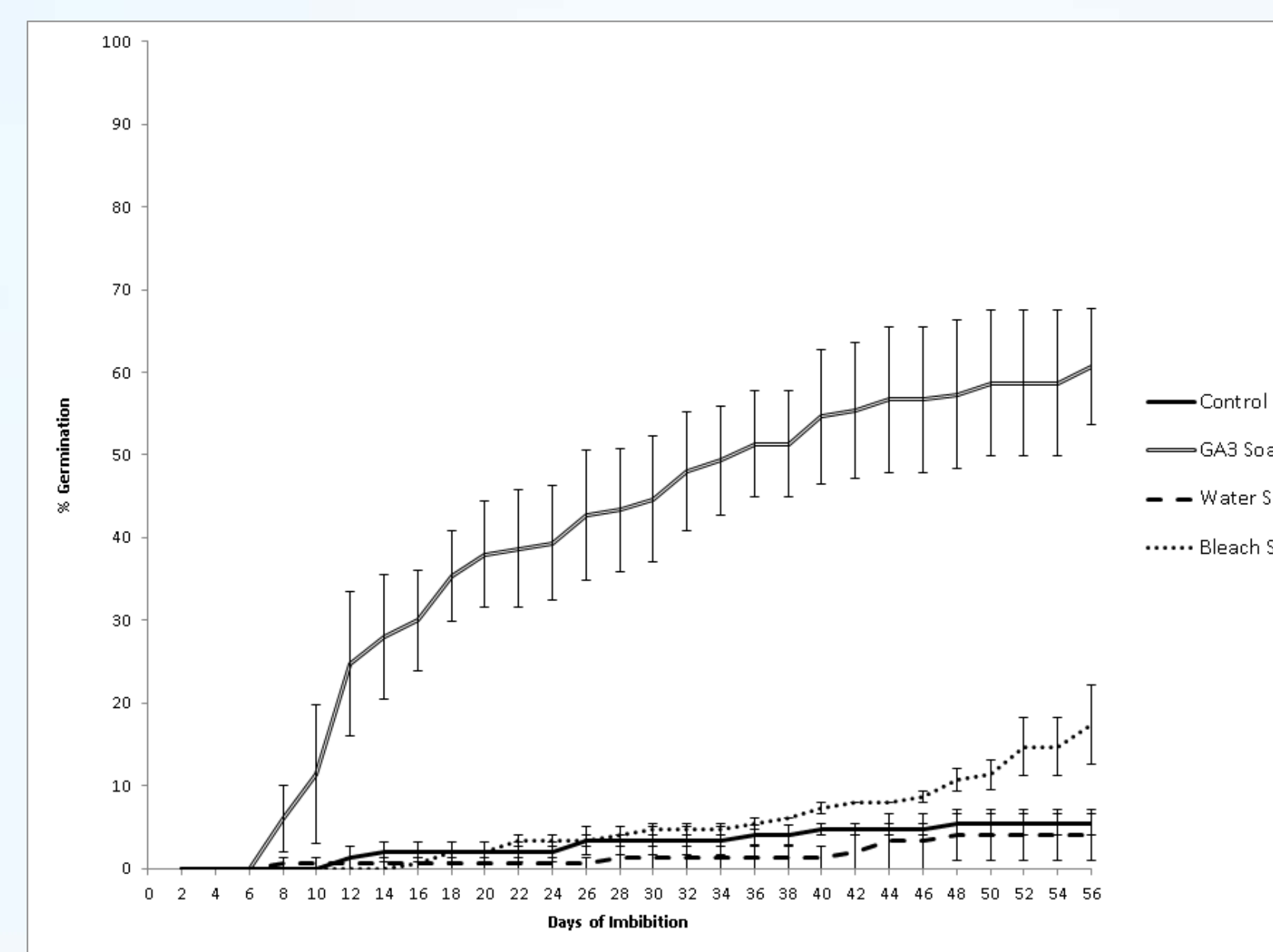
### Seed Treatments:

- **Stratification:** Seeds were placed into a moistened mixture of sphagnum moss and sand at 4 °C for 1 or 2 months.
- **Liquid Smoke Solutions (Smoke Seasoning):** Smoke solutions were created by diluting Wright's® All Natural Hickory Smoke Seasoning to 1:100 and 1:500 concentrations (mL smoke seasoning/mL distilled water).
- **Liquid Smoke Solution (Plant Debris):** Smoke solutions were created by burning plant debris collected from 2 *Pinguicula ionantha* populations (Site 1 and Site 2). Smoke solutions were prepared at 1:5, 1:100 and 1:500 concentrations (mL concentrated smoke solutions/mL distilled water).
- **Plant Growth Regulators (PGRs):** Three treatments using plant growth regulators were tested on 1% agar medium supplemented with 2.5 μM Benzyladenine purine (BA), 7.5 μM Indole-3-butyric acid (IBA), or a combination of 2.5 μM and 5 μM IBA. Seeds were placed directly on the solidified media.
- **Murashige and Skoog (MS) Media:** Two treatments were tested using 1% agar supplemented with Murashige and Skoog solutions diluted to 1/2 (100 mL MS/100 mL distilled water) and 1/4 (50 mL MS/100 mL distilled water) concentrations. Two types of containers were used for treatments: plastic petri dishes and 25 mm culture tubes. A 1% agar solution was used for the control.
- **Gibberellic Acid (GA<sub>3</sub>, Bleach, and Water Presoaks):** Seeds were soaked for 24 hours in a GA<sub>3</sub> solution (500 mg/L) or water, or seeds were soaked for 10 minutes in a 10% bleach solution.

## Abstract

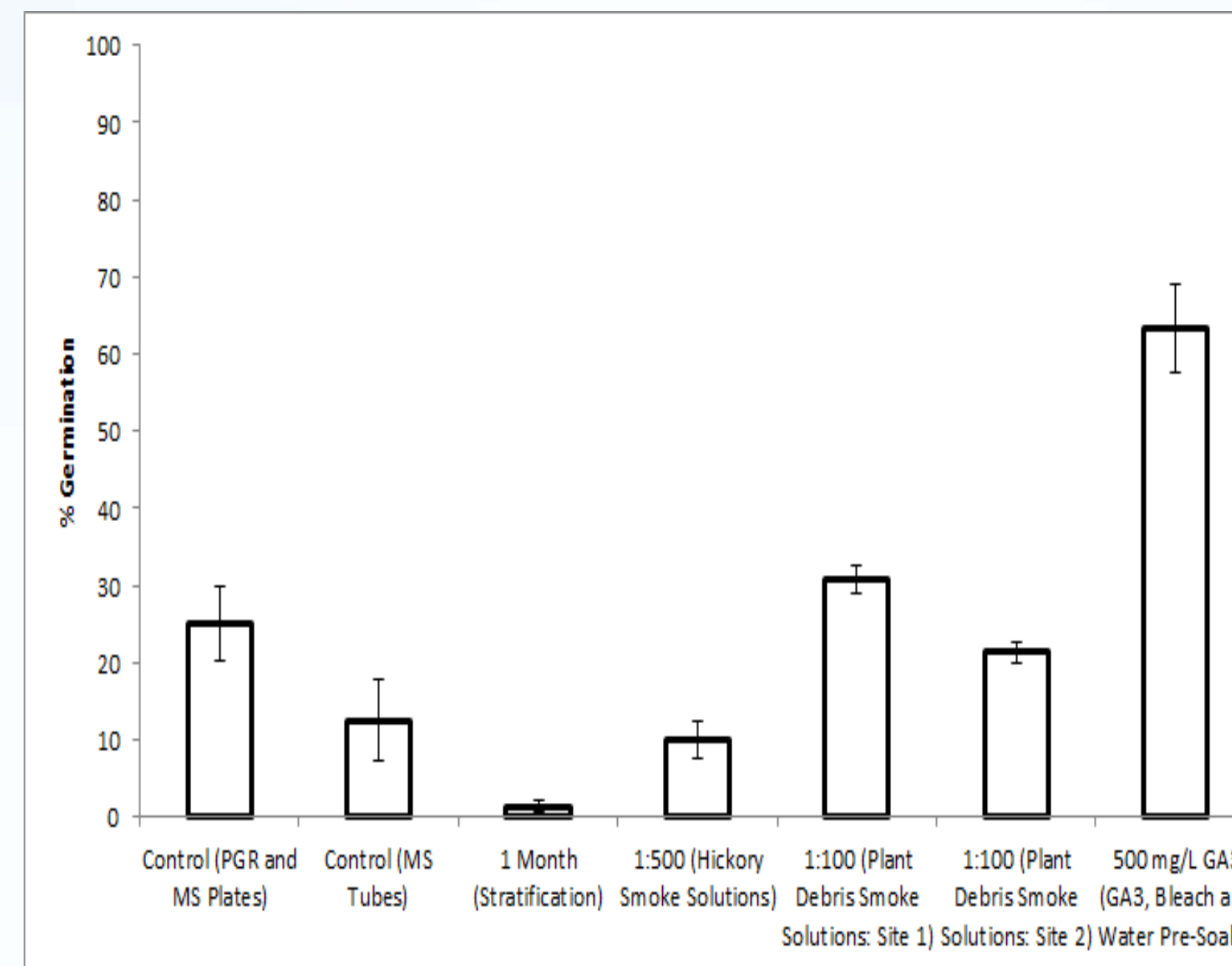
*Pinguicula ionantha* (Godfrey's butterwort) is a carnivorous plant occurring in historically fire-dependent bogs of longleaf pine savannas within the Florida Panhandle. This species is listed as federally threatened due largely to habitat loss. The objective of this study was to determine the most efficient method to germinate *P. ionantha* seeds. Seed treatments included stratification (4 °C for 1 or 2 months), Wright's® All Natural Hickory Smoke Seasoning (dilutions of 1:100 and 1:500), plant debris smoke solutions (dilutions of 1:5, 1:100 and 1:500), plant growth regulators (Benzyladenine purine--BA, Indole-3-butyric acid--IBA or a combination), Murashige and Skoog (MS) media (1/2 and 1/4 concentrations), gibberellic acid presoak (500 mg/L), bleach presoak (10%), and water presoak. All germination trials occurred in chambers (16 hours light) at 25°C with germination counted daily. Seed treatments were on moistened filter paper, 1% agar, or filter paper on top of agar. Highest germination percentages were achieved with GA<sub>3</sub> presoak, but bleach also significantly increased germination compared to the control. Stratification, all smoke solutions, MS treatments in tubes, and BA or BA + IBA treatments produced similar germination to control. IBA and MS treatments in plates inhibited germination. Control plates with agar were as high or higher in germination than those with only filter paper. Future studies might focus on combining different concentrations of gibberellic acid presoaks with seeds sown directly on 1% agar.

## Germination Rates – GA<sub>3</sub>, Bleach & Water Presoaks



Means standard errors.

## Highest Percent Germination Within Each Treatment



Means standard errors.

## % Germination – All Treatments

Treatment		% Germination
Stratification	Control	1.0 ± 0.3 <sup>1</sup> a <sup>2</sup>
	1 Month	1.3 ± 0.4 a
	2 Months	0.0 ± 0.0 a
Wright's® Hickory	Control	6.7 ± 0.7 a
	Smoke Seasoning	1:100 9.3 ± 4.7 a 1:500 10.0 ± 2.3 a
Plant Debris	Control	16.7 ± 2.7 a
	Smoke Solutions	1:5 12.0 ± 9.2 a Site 1 1:100 30.7 ± 1.8 a 1:500 22.7 ± 3.3 a
Plant Debris	Control	16.7 ± 2.7 a
	Smoke Solutions	1:5 16.0 ± 3.1 a Site 2 1:100 21.3 ± 1.3 a 1:500 10.7 ± 4.1 a

Treatment		% Germination
Plant Growth Regulators	Control	27.0 ± 4.7 <sup>3</sup> a
	BA	24.0 ± 4.5 a
	IBA	2.0 ± 1.3 b
	BA + IBA	22.0 ± 4.2 a
Murashige and Skoog Media (Plates)	Control	27.0 ± 4.7 <sup>3</sup> a
	1/4 MS	10.0 ± 3.3 b
	1/2 MS	8.0 ± 3.9 b
Murashige and Skoog Media (Tubes)	Control	12.5 ± 5.3 a
	1/4 MS	10.0 ± 3.8 a
	1/2 MS	5.0 ± 3.3 a
GA <sub>3</sub> , Bleach, & Water Presoaks	Control	8.0 ± 2.3 c
	500 mg/L GA <sub>3</sub>	63.3 ± 5.8 a
	10% Bleach	33.3 ± 5.5 b
	Water	4.7 ± 2.9 c

<sup>1</sup>Means + standard errors.

<sup>2</sup>Means followed by a different letter are significantly different within a treatment.  
<sup>3</sup>Control plates with 1% agar produced higher germination compared to all other control media.

## Summary

**Plant Growth Regulators (PGRs):** The IBA treatment significantly inhibited germination compared to other PGR treatments and the control.

**Murashige and Skoog (MS) Media:** In plates, both MS treatments significantly inhibited germination compared to the control.

**Gibberellic Acid (GA<sub>3</sub>), Bleach, and Water Presoaks:** Gibberellic acid presoaks at 500 mg/L produced significantly highest germination compared to all other presoaks and the control, as well as all other treatments in this study. The 10% bleach soak produced significantly higher germination compared to the water soak and the control.

## Significance

This study demonstrates an efficient method to propagate *P. ionantha* by seed using a 500 mg/L presoak of gibberellic acid. This documented study is the first to produce a specific method to increase germination in this species. This information may be used for potential reintroduction of this species into areas where populations are declining or extirpated.

## Acknowledgements

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## Works Cited

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Kesler, H.C., L. Trusty, S.M. Hermann and C. Guyer. 2008. Demographic responses of *Pinguicula ionantha* to prescribed fire: a regression-design LTRE approach. *Oecologia* 156: 545-557.

United States Fish and Wildlife Service. 2009. 5-Year Review: *Pinguicula ionantha* (Godfrey's butterwort). Panama City, Florida.



Source: "Atlas of Florida Vascular Plants: Institute for Systematic Botany" (<http://florida.plantatlas.usf.edu/>)



*Pinguicula ionantha*: Broken capsule with seeds



*Pinguicula ionantha* seedling transplanted from petri dishes to native soil/sphagnum mix.

## Germination & Growth of *Pinguicula ionantha*

