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Breaking Physical Seed Dormancy of Three *Baptisia* Species with Chemical Scarification

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Abstract: Prior research has demonstrated that acid scarification is effective at breaking physical seed dormancy. Exposing seeds to concentrated base solutions should have similar effects to acid scarification due to their corrosive properties, but such treatments are rarely investigated. Consequently, we tested acid and base scarification in three species in the wild indigo (*Baptisia*) genus: *Baptisia alba*, *B. australis*, and *B. bracteata*. Seeds were scarified in either concentrated 98% sulfuric acid or 1M NaOH for different time intervals (20, 40, 60, and 90 min) and seedling emergence recorded 21 days after exposure. Species, chemical treatment, and time of exposure were all highly significant predictors ($p < 0.0001$) of emergence. We found evidence of an interaction between species identity and time of exposure ($p < 0.01$). *Post hoc* tests suggested acid was the most effective treatment overall, but both acid and base treatments promoted higher emergence compared to controls ($p < 0.1$). We recommend higher than tested chemical concentrations or longer exposure times for *B. alba* and *B. bracteata* seeds due to their demonstrated resilience to physical damage. To reliably break dormancy, we recommend acid exposure times for 135, 40, and 60 minutes for *B. alba*, *B. australis*, and *B. bracteata*, respectively. Our data support prior findings regarding the efficacy of acid scarification, and confirm that chemical scarification can quickly and reliably overcome strong physical dormancy mechanisms.

Introduction

Baptisia (Fabaceae) species found in eastern North America are reliably associated with native plant communities (Taft *et al*. 1997). Their propagation for prairie restorations and native gardens has generated interest due to their showy inflorescences and value to native pollinators (Ault 2003; Gardner 2011; Padmanabhan et al. 2017). Like many prairie forbs, *Baptisia* species possess dormancy mechanisms to prevent premature seed germination (Sorensen and Holden 1974). Commonly, cold moist stratification is used to overcome seed dormancy for propagating forbs (Bratcher *et al*. 1993). However, past research has indicated that *B. australis* can be more difficult to germinate compared to other forbs (Hitchmough *et al*. 2004). Other *Baptisia* species may have stronger dormancy mechanisms than *B. australis*, taking years to germinate and emerge under natural conditions (Gardner 2011).

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This difficulty in promoting germination within *Baptisia* is caused by physical dormancy due to high water impermeability of the seed coat, a dormancy type characteristic of the Fabaceae family (Baskin *et al*. 2000). Physical dormancy in Fabaceae is caused by a dense layer of palisade layers containing Malphigian cells on the seed coat along with the presence of substances impermeable to water; these must be damaged for water to reach the embryo and facilitate germination (Baskin 2003). While natural conditions such as freezing, thawing, fire, and animal digestion can increase the permeability of the seed coat, artificially damaging seeds can similarly increase seed coat permeability (Baskin and Baskin 2014). Mechanical scarification is one way to overcome physical dormancy, but it can be time consuming for large seed numbers and potentially damage seeds (Baskin and Baskin 2014). Moreover, standardizing the degree of damage to the seed coat can be difficult. Because acid scarification can damage seeds efficiently and homogenously, and because past studies have utilized acid scarification to break the dormancy of other native species (Stewart and McGary 2010), we tested chemical scarification on native *Baptisia* seed.

Previous studies have used chemical scarification in *Baptisia*, including *B. australis* (Boyle and Hladun 2005), *B. hirsuta* (Thetford 1999), and *B. tinctoria* (Voss *et al*. 1994). However, *B. alba* and *B. bracteata*, currently

lack published studies on proper chemical scarification protocols. Since Boyle and Hladun (2005) demonstrated effective acid scarification treatments for *B. australis*, we expanded on their work and applied their protocols to two additional *Baptisia* species: *B. alba* and *B. bracteata.* We also retested *B. australis* to verify we could replicate results from Boyle and Hladun (2005). Additionally, we tested another type of chemical scarification, base scarification, on all three species. Base scarification used corrosive sodium hydroxide (NaOH). Using bases as chemical scarifying agents has been performed (Yeo and Dow 1978), but using strong acids seems to be far more prevalent for promoting germination and emergence. In our experiment, we measured seedling emergence after chemical scarification as a conservative proxy of germination.

Our objectives were to: 1) determine the relative efficacy of acid and base scarification for overcoming seed dormancy, 2) compare exposure-emergence responses among the species, and 3) establish ideal chemical scarification treatment times for each of the three species for potential commercial or private use.

Materials and Methods

Species selection

Baptisia species are potential target species for restoration or horticultural uses because of their showy racemes and ecological value to wildlife. The three study species have wide geographic ranges throughout the eastern half of the United States. In selecting species, we chose species native to midwestern tallgrass prairies that were also commercially available. Nomenclature follows Mohlenbrock (2013). *Baptisia alba* (L.) Vent (white wild indigo), also known as *B. lactea* or *B. leucantha*, is a 1-1.5m high herbaceous perennial with white blossoms on erect racemes (Rickett 1966; Hilty 2017). *Baptisia alba* is found in prairies to open woods from Ohio to Minnesota and Nebraska, and southward to Mississippi and Texas. *Baptisia australis* (L.) Vent (blue wild indigo) is an herbaceous perennial up to 1.5m tall that inhabits open areas from Pennsylvania to Indiana and southward to Georgia and Tennessee. Racemes of blue-purple blossom in late spring and are a popular food source for bumblebees. *Baptisia australis* is also used by the horticultural industry for producing *Baptisia* hybrids and cultivars (Ault 2003). *Baptisia bracteata* (Ell.) Vent (cream wild indigo) is a stout herbaceous legume at 0.3-1m tall with a sprawling growth form. It has racemes of cream-colored flowers which bloom earlier than *B. alba* and *B. australis*. *Baptisia bracteata* is found in high quality prairie and savanna remnants and produces low seed sets compared to *B. alba* despite occupying similar habitats (Peterson *et al*. 2013). It ranges from Michigan to Minnesota and Nebraska, and south to Kentucky, Louisiana, and Texas. Due to these characteristics, *B. bracteata* seed is expensive, even by prairie forb standards; seed prices are commonly over $1,000/lb, making consistent germination rates desirable.

Seed source

Unscarified seeds of all three species were purchased from Prairie Moon Nursery (Winona, MN) in May 2016. Seeds were stored indoors at room temperature until experimental trials began in June 2016.

Scarification procedure

Seeds were exposed to a stock solution of 98% sulfuric acid (i.e. 18M H_2SO_4) or a prepared solution of sodium hydroxide (1M NaOH) for various time intervals and then planted in seedling trays to determine emergence. Scarification trials were conducted by species with individual species receiving treatment on different days. On a given day, approximately 80 seeds of a species were counted and randomly assigned into one of nine treatment groups. These treatment groups included both acid and base treatments over four exposure times (20min, 40min, 60min, or 90min) as well as a control that soaked seeds in tap water for 60min. To each of our four 100mL beakers, 30 mL additions were made of 98% H_2SO_4 for acid treatments, 1M NaOH for base treatments, and tap water for the control. A follow-up trial was conducted for *B. alba* seeds only, due to a linear increase in emergence rates from the initial trial times (see Results) using the same general procedures. This subsequent trial included extended acid scarification exposure times (45min, 90min, 135min, or 180min).

Beakers containing each solution and seeds were placed on magnetic stir plates with stir bars and run on low-medium speed to expose the seeds to the treatment solution. After each treatment, seeds were removed from the beaker, put into a metal sieve, and rinsed in tap water for three to five minutes. Seeds were then removed from the tap water wash and planted within three hours of treatment. Scarification procedures were run in June 2016 through July 2016. The follow-up trial for *B. alba* was run in September 2016.

Planting protocol

Planting trays with 72 cells (3.9cm width $\times$ 7.6cm depth) were purchased from A.M. Leonard Horticultural Supply Company (Piqua, OH). Cells were filled tightly with Miracle Grow® garden soil. Each tray contained seed from just one species for all of its nine treatments. For *B. australis*, ten trays were planted overall (total *B. australis* seeds planted = 715). Fifteen trays were planted for *B. alba* (total seeds planted = 1050), ten trays for the first trial and five trays for the

Table 1: Effect of 18M sulfuric acid scarification exposure on emergence of three *Baptisia* species. Note that the values for the control and acid 90min for *B. alba* were pooled for the two trials. *Bolded emergence percentages indicate the highest recorded emergence for each species across all treatments.

Acid treatment	Number emerged/ total treated for *B. alba*	% emerged for *B. alba*	Number emerged/ total treated for *B. australis*	% emerged *B. australis*	Number emerged/ total treated for *B. bracteata*	% emerged *B. bracteata*
Control	6/116	5.2	14/80	17.5	2/48	4.2
20min	8/80	10.0	65/79	82.3	9/46	19.6
40min	14/80	17.5	72/80	**90.0***	13/44	29.5
45min	10/37	27.0	-	-	-	-
60min	16/80	20.0	64/78	82.1	23/47	**48.9***
90min	42/116	36.2	56/79	70.9	17/44	38.6
135min	14/37	**37.8***	-	-	-	-
180min	9/35	25.7	-	-	-	-

second trial (see Table 1). *Baptisia bracteata* had six trays planted (total *B. bracteata* seeds planted = 411); less seed was available for this species due to cost. Within each planted tray, eight seeds from each of the nine treatments (i.e. eight treatment replicates per tray, seed availability permitting) were randomly assigned to cell numbers. One seed was then planted in each corresponding cell at a 2cm depth and covered lightly with soil. The different treatments were marked using toothpicks coded using colored tape. Trays were labelled and put in indirect sunlight in a temperature-controlled greenhouse (approximately 18-24°C) at the University of Illinois at Springfield (39.7287° N, 89.6174° W) and gently watered. Trays were manually watered with tap water every one to two days to keep soil consistently moist over the trial period.

Data collection

Emergence rates were measured by counting the number of seedlings that emerged until 21 days after planting. Care was taken to watch the cells in order to record seedlings which emerged prior to the final 21-day count. Seedlings found dead (e.g. root penetration visible but cotyledons not present) were assumed to be a positive emergence event and were included in the final 21-day count.

Data analysis

Data analysis was conducted using R v. 3.4.1 (R Core Team 2017). Binomial logistic regression was performed with species, solution type (acid versus base), and exposure time as factors, as well as their respective interaction terms. An analysis of deviance was conducted on the logistic regression model using a χ^2 test to determine which factors were significantly associated with emergence. For this model, the control solution treatments were excluded because of the standardized time of exposure (60min) to the water solution. We then performed a second logistic regression across solution types and species for 60-minute exposure times to compare all three solution types across species. This model was followed by Tukey's HSD to differentiate the efficacy of solution types and control for experiment wise type I error. Treatment groupings were then generated using the *emmeans* package to interpret the Tukey's HSD results.

RESULTS

Acid scarification treatments were generally more reliable at promoting emergence than base scarification, with intermediate exposure times showing the highest emergence rates (Figs. 1-3, Tables 1 and 2). Maximum emergence was observed within acid solution treatments, with 90% being the highest proportion of emerged seedlings from *B. australis*. In contrast, *Baptisia alba* and *B. bracteata* had comparatively modest maximum emergence percentages at 37.8% and 48.9%, respectively. The second trial for *B. alba* suggested the extended acid scarification exposure times had equal or greater success at promoting emergence compared to shorter exposure times (Fig. 1). Our logistic regression identified predictors of seed emergence (Table 3): species identity, solution type, and time of exposure to the solution were highly significant ($p < 0.001$, Table 3). We found no significant interaction of species identity*solution type or solution type*time of exposure ($p > 0.1$, Table 3). Our model however did identify a significant interaction of species identity*time of exposure ($p < 0.001$, Table 3). The separate logistic regression, run for 60-minute exposure times only, indicated a marginally significant effect of a species

Table 2: Effect of 1M NaOH scarification exposures on the emergence of three *Baptisia* species. Note the higher sample sizes for *B. alba* due to pooling results of its two trials.

Base treatment	Number emerged/ total treated for *B. alba*	% emerged for *B. alba*	Number emerged/ total treated for *B. australis*	% emerged *B. australis*	Number emerged/ total treated for *B. bracteata*	% emerged *B. bracteata*
Control	6/116	5.0	14/80	17.9	2/48	4.2
20min	4/113	5.0	50/80	62.5	6/43	14.0
40min	7/117	6.0	52/80	65.0	5/47	10.6
60min	9/119	7.6	50/80	62.5	6/48	12.5
90min	17/120	14.2	55/79	69.6	4/44	9.1

Table 3: Summary of the logistic regression model for all treated seeds of the three study species. "***" indicates significance at $p < 0.001$.

Factor	Df	Deviance	Residual df	Residual deviance	Pr(>Chi)
NULL			1852	2418.0	
Species identity	2	597.02	1850	1821.0	<0.001***
Solution	1	75.53	1849	1745.5	<0.001***
Time	1	15.35	1848	1730.1	<0.001***
Species identity*Solution	2	1.64	1846	1728.5	0.440
Species identity*Time	2	22.52	1844	1705.9	<0.001***
Solution*Time	1	0.13	1843	1705.8	0.719

identity*solution type interaction ($p < 0.1$, Table 4). Treatment groupings from the HSD tests for solution type indicated a greater efficacy of both chemical scarification treatments compared to the control for promoting emergence, with acid having the greatest effect (Table 4).

Discussion

Chemical scarification promoted reliable germination and emergence in these three *Baptisia* species. The responses toward chemical scarification exposure times varied among the species, as indicated by our significant

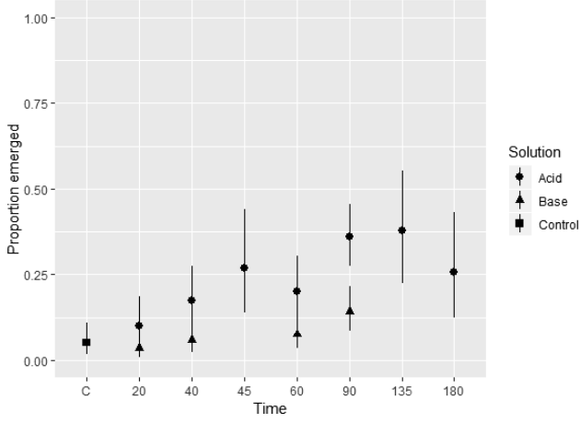

Figure 1. Emergence of *B. alba* following chemical scarification at various exposure times. Error bars are 95% confidence intervals.

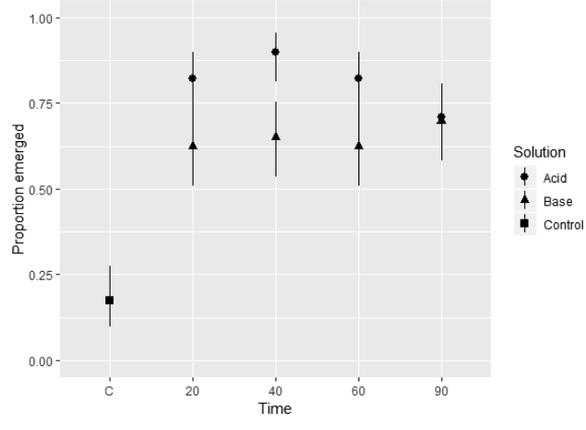

Figure 2. Emergence of *B. australis* chemical scarification at various exposure times. Error bars are 95% confidence intervals.

Table 4: Summary of the logistic regression model for seeds which were exposed to different solution types for 60 minutes. We separated the effects of solution type by running a post-hoc Tukey test. Different letters in Tukey's HSD groupings indicate those solution types had significantly different ($p < 0.05$) effects to emergence across all three species at the 60 minute exposure time. "***" indicates significance at $p < 0.001$; "." indicates significance at $p < 0.1$.

Factor	Df	Deviance	Residual df	Residual deviance	Pr(>Chi)	Solution type	Proportion overall emerged ± standard error	Tukey's HSD groupings
NULL			695	816.00		Control	0.0895±0.018	a
Species identity	2	135.31	693	680.69	<0.001***			
Solution	2	109.87	691	571.51	<0.001***	Base	0.2752±0.025	b
Species identity* Solution	4	9.05	687	562.46	0.060.	Acid	0.5033±0.032	c

species identity*time of exposure interaction. Upon further investigation, this result appears to be driven by a unique response by *B. alba*. *Baptisia australis* and *B. bracteata* emergence proportions both levelled off or even decreased with increasing exposure times, whereas *B. alba* emergence increased linearly across the same range of exposure times (20, 40, 60, and 90min exposure times). Species-specific exposure responses are not surprising considering Baskin and Baskin (2014) provide a list of congeners, many of which have varying exposure times in sulfuric acid to yield maximum germination. For example, Baskin et al. (1998) found a differing response to sulfuric acid scarification for breaking the physical dormancy between two Caesalpinoid legumes: *Senna marilandica* and *Senna obtusifolia*. Similar to differences found between *B. alba* and *B. australis* in our study, *Senna marilandica* benefited from double the exposure time for maximum germination compared to *S. obtusifolia*.

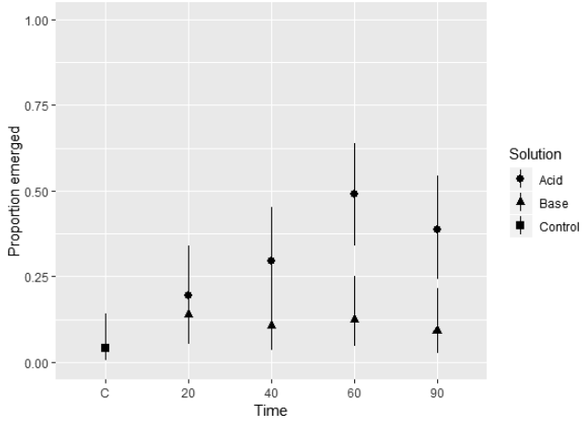

Figure 3. Emergence of *B. bracteata* chemical scarification at various exposure times. Error bars are 95% confidence intervals.

Our treatments had greater success of promoting emergence with *B. australis* compared to the other two species. Results for the acid scarification treatment in *B. australis* were similar to those found by Boyle and Hladun (2005), indicating that we successfully replicated their findings. We had more success with promoting germination and emergence in *B. australis*, with emergence being particularly high (>80%) after acid scarification for 20, 40, and 60 minutes. Our results also suggest that acid scarification can bypass the weeks of cold moist stratification needed to break the dormancy of *B. autralis* (see Bratcher *et al*. 1993). Overall, our data support the conclusions of Boyle and Hladun (2005) that sulfuric acid scarification can promote the rapid and uniform germination of large numbers of *B. australis* seeds.

We found that *B. alba* and *B. bracteata* responded less favorably to our chemical scarification treatments compared to *B. australis*; these two species did not yield emergence above 50%. Although emergence percentages in these two species were lower than for *B. australis*, they were still much higher than published values *B. hirsuta*, which only yielded 9% germination after 3 weeks following 15, 20, and 25 minutes exposure to sulfuric acid (Thetford 1999). Hence, breaking physical dormancy of *B. alba* and *B. bracteata* with sulfuric acid has intermediate success compared to other studied *Baptisia* species. Since Voigt (1977) found mechanical scarification to yield 100% germination among forty seeds of *B. alba*, chemical scarifications may be inferior treatments for this species. The low emergence responses observed in *B. alba* and *B. bracteata* could be due to higher testa lignification or lower seed viability compared to

B. australis (Boyle and Hladun 2005). Chemical scarification could have also caused embryo mortality among the study species, especially considering how emergence levelled off or declined in our acid scarification results.

Emergence for our study species might have improved if we had paired additional and different treatments to the chemically scarified seeds. For example, other studies have paired chemical and mechanical scarification with cold moist stratification in their study species (Stewart and McGary 2010; Gardner 2011; Pipinis *et al*. 2011). Regardless of the proximate cause for the lower emergence proportions in *B. alba* and *B. bracteata*, acid scarification was still fairly reliable at breaking dormancy. Other studies suggest that prairie species with strong physical dormancy mechanisms may have below 50% germination, regardless of treatment types (Stewart and McGary 2010).

The base solution promoted less emergence across all species compared to the acid. While base scarification promoted relatively high emergence in *B. australis*, the solution of base was not as concentrated as the sulfuric acid treatment (1M vs 18M) and failed to yield consistent emergence in *B. alba* and *B. bracteata*. We recommend either drastically increasing base concentrations or increasing exposure times if base scarification is used for these two species. Nonetheless, both chemical scarification methods were superior to the control. To optimally overcome the study species' physical dormancy, we recommend *B. alba* be treated in 98% H_2SO_4 for 135 minutes; *B. australis* should be treated for 40 minutes; and *B. bracteata* should be treated for 60 minutes.

Conclusion

Emergence varied among species, among the type of chemical treatment, and from the duration of exposure to the chemical agent. For *B. australis*, both acid and base methods yielded a large proportion of emerged seedlings. However, our treatments for *B. alba* and *B. bracteata* had more modest success. This demonstrates the importance of performing such tests across multiple species in a genus due to variation of a method's efficacy within a given genus. Chemical scarification is a viable option to break physical dormancy in *Baptisia* species and can be used for fast and consistent results from a large number of seeds in commercial or private use.

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