Biological Sciences

SEED GERMINATION AND SEEDLING SURVIVAL OF *ASIMINA OBOVATA* IN FLORIDA SCRUB

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ABSTRACT: *Scrub pawpaw (*Asimina obovata*), is a characteristic but scattered long-lived shrub in upland Florida ecosystems. We report on results of two field germination experiments that help define its requirements for seed germination and seedling survival. In our first experiment (begun in 1999), germination rates were low (7%) and nearly all seeds germinated under shrubs (vs. in gaps). Some of these seedlings survived a 2001 fire by resprouting. A larger second experiment (2002) showed that time-since-fire and nearby canopy height were the best predictors of germination (overall rate 42%). Locations with intermediate time-since-fire or intermediate canopy height had higher germination. In both experiments, we did not find evidence that protection from mammalian herbivory by caging affected observed germination. Although most germination occurred during the first year, calculations suggest about 20% of seeds enter a persistent seed bank, 30% of seeds survive in the seed bank annually, and 30% of surviving seeds can germinate in each of the next three years. Seedling survival through 2009 from the 2002 experiment was high (usually > 80% annually) and unaffected by caging. Survival varied among sites and was highest in the long-unburned site. Although pawpaw persists at frequently burned sites by resprouting, seed germination and seedling recruitment are favored by infrequent fires.*

Key Words: scrub pawpaw, fire, herbivory, recruitment, seed dormancy

ALTHOUGH most shrubs of Florida scrub have high survival rates and are [1] able to survive fires by resprouting (Abrahamson, 1984; Menges and Kohfeldt, 1995), seedling recruitment is still necessary to compensate for mortality and may help retain genetic variation in populations (Dolan et al., 2008). Seedling recruitment, especially over several years, is also important in successful restorations (Colas et al., 2008). Nonetheless, factors affecting seedling recruitment are poorly known for most Florida scrub plants.

Florida scrub plants respond to fire in various ways. Most dominants of Florida scrub, especially those of scrubby flatwoods and oak scrub, are strong resprouters after fire (Menges and Kohfeldt, 1995; Abrahamson, 1999; Maguire and Menges, in revision). These resprouters may or may not recruit seedlings after fire. Many resprouters show strong post-fire flowering as well

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(Ostertag and Menges, 1994) and delayed post-fire seedling recruitment may result from these flowering episodes (McConnell and Menges, 2002). Other species, especially common in rosemary scrub, are killed by fire and recover from seed stored in a soil seed bank (Menges and Kohfeldt, 1995).

Scrub pawpaw (*Asimina obovata*) is a typical species of Florida scrub and sandhill ecosystems (Abrahamson et al., 1984), although it is generally not abundant enough to be analyzed in studies of species responses to fire (Abrahamson, 1984; Menges and Kohfeldt, 1995; Weekley and Menges, 2003). This large shrub is a strong post-fire resprouter which also often flowers prolifically after fires (Menges, unpublished data). Small plants are occasionally found, but it is difficult to discern whether they are seedling recruits or suppressed older plants. Small plants often suffer herbivory and grow very slowly from year to year (Menges, unpublished data).

In order to gain understanding of the role of sexual reproduction and seedling recruitment in the population biology of scrub pawpaw, we set up two germination experiments. The general goals of these experiments were to examine germination percentages as a function of microsite, burn history and protection from herbivory. We also used germination over time to infer the importance of the persistent soil seed bank. Specifically, an experiment initiated in 1999 examined the effects of scarification, microsite (open vs. under shrub), and protection from mammals on germination and seedling survival. The main objectives of a germination experiment initiated in 2002 were to determine the effects of microsite (based on several measures), burn history and protection from herbivory on pawpaw germination and seedling survival. In both experiments, we followed germination and seedling survival for approximately six years.

METHODS—*1999 Field Germination Experiment*—This germination experiment tested effects of scarification, microsite, and protection from herbivory. We collected seeds for this experiment from 25 fruits in the summer of 1999. Only seeds deemed mature (hard seeds appearing brown or tan) were retained. To avoid potential problems with seeds drying and potentially losing viability, we planted seeds into the field as soon as possible, within a few days of collection. We used 152 seeds in 16 replicates in the field; 9–10 seeds per replicate. The experiment considered three treatments (scarified vs. not, open microsite vs. under shrub, protected from mammals by exclosure vs. not) in a factorial design with 8 treatment combinations. We scarified seeds by making a short cut (about 1 cm) through the seed coat with a serrated knife. Open microsites were in gaps without litter while microsites under shrubs had leaf litter which was replaced after seeds were planted. We intended exclosures to protect seeds against mammalian seed predation; they were made from wire with a mesh size of 7mm; the exclosure size was 25cm ×25 cm ×10cm. We buried individual seeds about 1 cm beneath the soil surface and marked their locations with plastic toothpicks. Seeds were checked for germination annually in April each year through 2005 and seedlings were followed through 2010. In February, 2001, the area was burned with an accidental fire. We considered models predicting individual seed germination from seed weight, scarification, microsite, and protection. Nine candidate models were fit and their relative information evaluated using Akaike Information Criterion corrected for small sample sizes (AIC$_c$) (Burnham and Anderson, 2002).

2002 Field Germination Experiment—This germination experiment tested the effects of microsites in greater detail, as well as time-since-fire and protection from herbivory. We collected

seeds in the summer of 2002, examined them for maturity, and immediately placed them at stratified-random locations along each of three sixty meter transects in contrasting habitats at Archbold Biological Station. Sites varied in time-since-fire. The Jay Trail site was recently burned, in 2001, and the Nature Trail Sit e was burned in 1997. The site near Lake Annie was probably last burned about 1926. In the summer of 2002, we planted 180 seeds with 60 seeds per transect, each at a depth of about 1 cm, and marked seed locations with a flag and tag. We recorded microsite data: canopy height (0.1 m), litter depth (cm), and the percent cover (nearest 10% or trace, coded as 1%) for woody plants, herbaceous plants, litter, lichens, and bare sand (within a 25 cm radius circular area) at each planting location in June 2002. To protect some seeds from mammalian herbivory, we used wire exclosures similar to those used in the 1999 experiment. Transects were monitored weekly for 1.5 months, bi-weekly from September 2002 to April 2003, monthly from April 2003 through July 2003, quarterly from July 2003 through July 2005, and annually thereafter through 2009. At each census, we noted seed depredation, germination, and seedling survival. As seedlings matured, some of the wire exclosures were removed. We compared five candidate logistical regression models predicting seed germination from time-since-fire class, protection, and the various microsite variables. We again used Akaike criteria to evaluate the relative information in the candidate models. There were sufficient germinants in the 2002 experiment to analyze seedling survival patterns. We used survival analysis to compare survival curves among the three sites and for plants within or outside exclosures.

RESULTS—*1999 Field Germination Experiment*—Seed germination in the 1999 field experiment was low (10 of 152, 6.6%) and all seedlings germinated during the first year after placement (by May 2000). Nearly all (9 of 10) germinants were found beneath shrubs; higher germination occurred for scarified vs. not scarified and for protected vs. not (Figure 1). Of nine logistic regression models, three models were the most informative (lowest AIC_c; Table 1): (1) seed weight and microsite and their interaction; (2) seed weight and microsite only, and; (3) microsite only. These three models were not significantly different (model 1 vs. 2 deviance = 2.139, p = 0.144; models 1 vs. 3, deviance = 4.270, p = 0.118, models 2 vs. 3, deviance =2.131, p=0.144). The simplest (more parsimonious) model (3) has a single significant parameter, microsite (z=2.178, p = 0.0294).

Although too few seedlings germinated to analyze microsite effects on subsequent seedling survival and growth, it is worth noting that most seedlings survived the subsequent 2001 fire despite their very small size. Five of six seedlings that could be relocated resprouted post-fire and two were still alive in 2010.

2002 Field Germination Experiment—Over the course of the experiment, 75 of 180 seeds germinated (41.7%). Most (84% of the total) germination occurred during the first year (July 2002–April 2003: 63 germinants, or 35% of the seeds sown). Lesser amounts occurred in subsequent years (May 2003–April 2004: 9, May 2004–April 2005: 3). No seedlings recruited from May 2005 through April 2010. We observed very little seed predation (in 4 of 180 seeds, all in 2003). Two of the four seeds with evidence of seed predation did germinate.

Germination was markedly higher in the intermediate burn site than in recently burned or long-unburned sites (Figure 2; $\beta_{intvs\ rec}$= 1.9, P= 0.006 and $\beta_{intvslon}$= 1.1, P= 0.004). Exclosures had little effect on germination; approximately equal percentages germinated in exclosures (40.0%) vs. unprotected locations (43.3%). Germination was affected by canopy height;
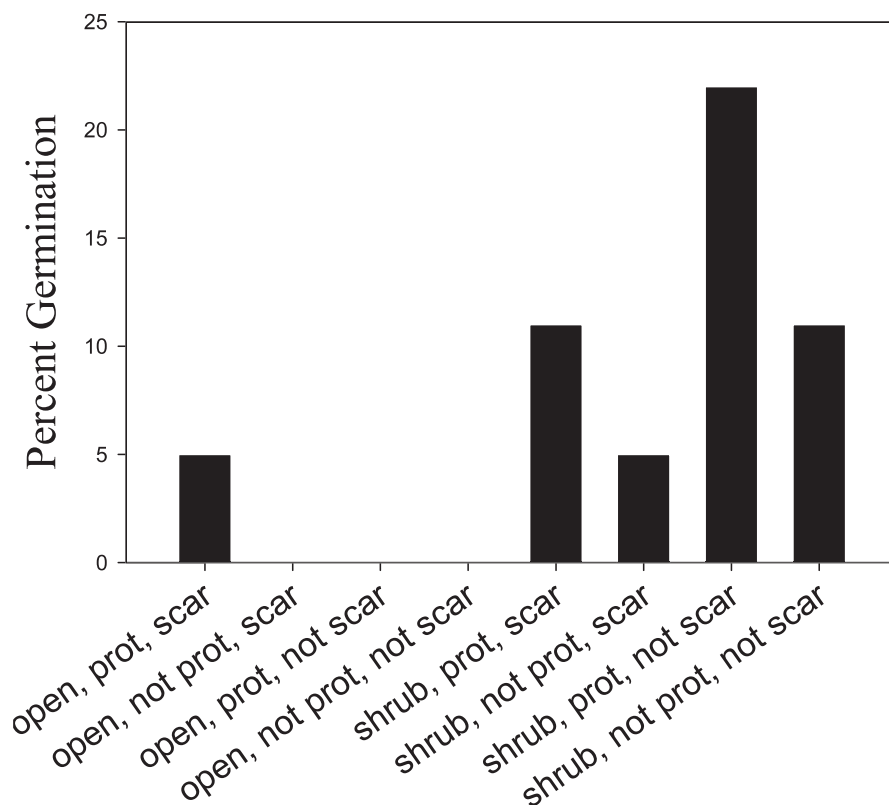
Treatment Combination

Fig. 1. Percent germination from 1999 seed germination experiment, as a function of microsite (open, shrub), protection from herbivores by caging (protected, not protected), and scarification (scarified, not scarified).

Table 1. AIC table for the 1999 seed germination experiment. M = microsite; W = seed weight; S = scarification; P = protection. AIC_c is the information criterion corrected for small sample size. w_i is the Akaike weight, measuring the strength of the model relative to others considered (Burnham and Anderson, 1992).

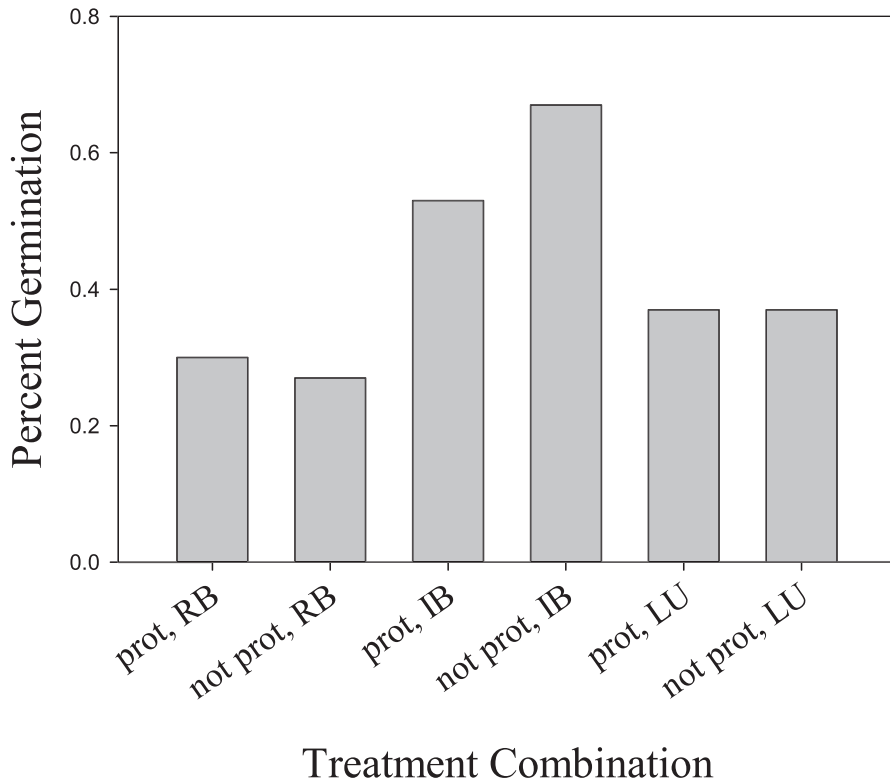| Model	AIC_c	w_i
M + W + M*W	69.69	0.213
W + M	69.72	0.210
M	69.77	0.205
M + W + (W^2)	69.96	0.186
W + S + M + P	70.91	0.116
W	73.37	0.034
W + (W^2)	73.94	0.025
P	75.88	0.010
S	77.24	0.005

Fig. 2. Percent germination from 2002 experiment by protection from herbivores (protected by exclosures, not protected) and time-since-fire (RB=recently burned [2001], IB=intermediate burn [1998], LU=long-unburned [ca. 1926]).

it was higher under canopy heights of 1–1.9 m (57%) than it was under shorter (33%) or taller (42%) canopies. Otherwise, microsite generally had little effect on germination. Ungerminated seeds were in similar microsites to germinated seeds (e.g. natural log canopy cover 0.23 vs. 0.11, litter depth 1.82 vs. 1.81, percent woody cover 55.7 vs. 55.8, all p values > 0.35 in t-tests).

Of the five AIC models examined, the model including time-since-fire and canopy height was the best (Table 2). The model that included only time-since-fire was marginally worse than the best model (deviance = 3.177, p = 0.075). Fire history was a stronger predictor than canopy height (Table 2). We have weaker evidence that germination increased with canopy height ($\beta= 0.404$, $P= 0.08$).

Estimating Seed Bank Survival—To estimate seed bank survival, we first calculated observed first year seed germination (2002 experiment, sites pooled) as 35%. The remaining 117 seeds must have an unknown mortality in the seed bank until more germinate in year 2. We assumed that this seed bank survival is constant among years, and used a spreadsheet (Table 3) to vary seed bank survival. A value of 0.3 produced< 2 seeds by year 3, which projects to zero

TABLE 2. AIC table for the 2002 seed germination experiment. F = time-since-fire; C = canopy height; LD=litter depth; LC = litter cover; P = protection; W = woody; H = herb; LICH = lichen; S = sand. AIC_c is the information criterion corrected for small sample size. w_i is the Akaike weight, measuring the strength of the model relative to others considered (Burnham and Anderson, 1992).

Model	AIC_c	w_i
F + C	236.20	0.527
F	237.28	0.309
F * C	238.58	0.156
F + P + C + LD + LC + S + LICH + W + H	244.57	0.007
C	248.28	0.001

germinants, matching the observed data for 2006. Given this 30% annual seed bank survival, second and third year germination (of surviving seeds) from the seed bank is estimated to be 0.26 and 0.38 respectively; or a median value for germination from the soil seed bank of 0.32. The proportion of seeds surviving but not germinating the first year is 35.1/180 = 0.195 (Table 3). Similar calculations were made for each of the three individual populations (not shown).

Seedling Survival in 2002 Experiment—Seedlings had consistently high survival across cohorts and years (Table 4). Across cohorts, seedling survival was high for 2002 to 2003 (0.886) and remained high for subsequent years (0.790, 0.827, 0.917, 0.870, 0.838) before dropping in 2008 to 2009 (0.512). Twenty-one seedlings were still alive in 2009, although none had flowered.

Survival analysis on the main cohort (first year seedlings in 2003) showed significant differences in survival among sites (Wilcoxon (Gehan) statistic = 7.01, df=2, p = 0.03). Cumulative survival was highest for the long-unburned Lake Annie site (52%) and was lowest for the nature trail (14%) and the Jay Trail (22%). Survival varied significantly between the Lake Annie site and both other sites (nature trail pairwise comparison F = 6.29, df=1, p =0.012; Jay Trail F = 4.50, df=1, p = 0.034). The nature trail and Jay trail did not differ in survival patterns (F = 0.01, df=1, p = 0.916).

TABLE 3. Results of iterative spreadsheet calculations to estimate seed bank survival of pawpaw based on 2002 germination experiment results (all three sites). Seed bank survival was initially unknown; we tried a range of values until we got few enough seeds in year 3 to produce the observed germination (0 germinants).

year	start # seeds	# germinants	% germ in year	# not germ	Seed bank Survival
0	180.00	63	0.35	117	0.3
1	35.10	9	0.26	26.1	0.3
2	7.83	3	0.38	4.8	0.3
3	1.45	0			

TABLE 4. Seedling survival, by cohort and (for 2002–03 cohort), by population.

ALL POPULATIONS Seedling Cohort	# germ	# surv (%) to April of next year	# surv (%) to April of second year	# surv (%) to April of third year	# surv (%) to April of fourth year
2002-3	61	55 (0.902)	44 (0.800)	37 (0.841)	29 (0.783)
2003-4	9	7 (0.778)	5 (0.714)	4 (0.800)	2 (0.500)
2004-5	3	0 (0)	—	—	—
2005-6	0	—	—	—	—
Weighted Mean %	—	**(0.886)**	**(0.790)**	**(0.837)**	**(0.783)**
LAKE ANNIE POPULATION Seedling Cohort					
2002-3	19	16 (0.842)	15 (0.938)	14 (0.933)	9 (0.643)
NATURE TRAIL POPULATION Seedling Cohort					
2002-3	27	25 (0.926)	19 (0.760)	15 (0.789)	13 (0.867)
JAY TRAIL POPULATION Seedling Cohort					
2002-3	15	14 (0.933)	10 (0.714)	8 (0.800)	7 (0.875)

Cumulative seedling survival through 2009 was similar whether outside (22%) or inside (32%) exclosures. Survival analyses indicate site differences only for plants within exclosures (statistics not shown).

DISCUSSION—Scrub pawpaw recruitment in two germination experiments was highest in more shaded sites that were not recently burned. In the 1999 experiment, germination was higher under shrubs, and in the 2002 experiment, it was highest under shrubs with an intermediate vegetation height. Germination in the latter experiment was highest in a site with an intermediate time-since-fire, and lower in recently burned and long-unburned sites.

Scrub pawpaw is one of few species studied in Florida scrub that does not prefer open microhabitats, nor has higher recruitment in recently burned sites. For example, *Dicerandra frutescens* is a specialist for open microhabitats (Menges et al., 1999) and has higher population viability in habitats that are frequently burned (Menges et al., 2006). Indeed, most rare plants in pyrogenic ecosystems in Florida have positive (or at least neutral) responses to fire (Slapcinsky et al., 2010). There are some exceptions. For example, *Asclepias curtissii* seed germination is enhanced by shade, seedlings had higher survival near shrubs than in gaps, and extant plants tended to grow close to shrubs (Mondo et al., 2010). Four scrub species that tend to occur in gaps were nonetheless not demographically favored by frequent fires (Maliakal-Witt et al., in revision).

Results from the scrub pawpaw germination experiments are consistent with field observations. Many small plants (including seedlings) of scrub pawpaw occur underneath shrub canopies (Menges, personal observation; Mark Deyrup, personal communication). Pawpaw seeds are large and ample storage reserves may allow seedlings to establish in relatively low light conditions.

Protection from mammalian herbivory by exclosures did not consistently affect scrub pawpaw germination or seedling survival in our experiments. This suggests that herbivory is not particularly intense, despite the fact that seeds are large. Foliage of other *Asimina* species contain toxic annonaceous acetogenins that deter mammals, while allowing zebra swallowtail butterflies to sequester defensive compounds (Martin et al., 1999). Unripe fruit and seeds of pawpaws also contain various annonaceous acetogenins (Pomper et al., 2009). These chemicals may also protect seeds from post-dispersal seed predation.

The fruit of scrub pawpaw becomes soft and edible as it matures. Such a large edible fruit with hard seeds may be mammal dispersed. Scarification, which may occur when seeds pass through mammalian digestive tracts, did not affect germination percentages in our experiments. Opossums and raccoons are dispersers in the current landscape (Mark Deyrup, personal communication). There may have been additional dispersal agents in the past. Several fruits from species in the family Annonaceae have been proposed as dispersed by gomphotheres in the neotropics (Janzen and Martin, 1981). There were several

large extinct herbivorous mammals (e.g. mammoths, mastodons, possibly gomphotheres) in Florida (Grayson, 1991; Koch et al., 1998) that could have dispersed fruits of scrub pawpaw. Today, scrub pawpaw is common in pastures. One factor favoring their persistence there may be dispersal by cattle, which disperse many fruits once dispersed by gomphotheres (Janzen, 1986).

These experiments have shown that scrub pawpaw seedlings have high survival, which is consistent with high survival of seedling-sized scrub pawpaw plants followed in demographic studies (Menges, unpublished data). Even small seedlings are capable of surviving fire by resprouting. This parallels the exceptionally high survival and slow growth of very small palmetto seedlings (Abrahamson and Abrahamson, 2009).

Given its high survival, resprouting ability, and persistence in long-unburned areas, scrub pawpaw will be resilient to a range of conditions, including a range of fire return intervals (including fire suppression) and to some human disturbances such as grazing. Although many species found in the Florida scrub are imperiled, many characteristic resprouting shrubs, such as scrub pawpaw, are likely to persist in remaining patches.

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