SEED GERMINATION OF THE NORTHERN PITCHER PLANT, *SARRACENIA PURPUREA*

SYBIL G. GOTSCH AND AARON M. ELLISON *

ABSTRACT - We present data on dormancy-breaking requirements and germination rates of the northern pitcher plant, *Sarracenia purpurea* var. *terrae-novae* de la Pylaie. Upon dispersal from the parent plant, embryos are only partially developed. Seeds require a prolonged moist chilling period for after-ripening and maturation of the embryo but scarification prior to stratification is not required for germination. Exposure to light stimulates germination. Eighty-five percent of seeds moist chilled for 6 weeks germinated when placed into ambient sunlight, as compared with only 53% of seeds moist chilled for 4 weeks and then placed in ambient sunlight. Fewer than 10% of seeds germinated when placed in darkness following moist chilling. Germination rate in the light was more rapid among seeds stratified for 6 weeks than those moist chilled for 4 weeks. These data suggest that *S. purpurea* seeds are dispersed in a state of morphological dormancy.

INTRODUCTION

Carnivorous plants have attracted attention from ecologists, evolutionary biologists, horticulturalists, and amateur collectors for centuries. The carnivorous habit as an adaptation to low nutrient conditions, and the morphological variations attendant to carnivory in plants have received a great deal of attention from evolutionary ecologists since Darwin (1875) published one of the first synthetic accounts of these plants (see also Arber 1941; Givnish et al. 1984; Heslop-Harrison 1978; Macfarlane 1908). One group of carnivorous plants, the pitcher-plants (families Sarraceniaceae and Nepenthaceae), has been studied intensively because they host inquiline communities dominated by larval dipterans (reviews in Bradshaw 1983; Fish 1983; Higley 1885). However, comparatively little scientific attention has been focused on the autecology of pitcher-plants themselves (e.g., Barker and Williamson 1988; Bradshaw and Creelman 1984; Burr 1979; Mandossian 1965; Weiss 1980).

As part of a larger, on-going study of the population dynamics of the northern pitcher plant *Sarracenia purpurea* L. in western Massachusetts, we experimentally investigated the dormancy properties and germination characteristics of its seeds. While horticulturalists and carnivorous-plant collectors and hobbyists generally recommend cold storage and 4-6 week

* Department of Biological Sciences, Mount Holyoke College, 50 College Street, South Hadley, MA 01075-6418

moist chilling of *Sarracenia* seeds for optimal germination (on-line Carnivorous Plant Archive at http://randomaccess.unm.edu/www/cp/cparchive.html), the rationale for this (other than individual growers' personal experiences) is unknown. Germination of *S. purpurea* seeds was examined as part of larger studies by Mandossian (1965) and Burr (1979), and they reported highest germination frequencies following prolonged moist chilling.

The seed bank of any given habitat is made up of seeds representing a diversity of germination strategies (Baskin and Baskin 1988, 1989). According to these authors, germination of mesic temperate-zone herbs frequently occurs only following: an after-ripening period allowing for completion of embryo development (*morphological dormancy*); a physiological trigger (*physiological dormancy*); or both an after-ripening period and a physiological cue (*morphophysiological dormancy*) (terminology of Baskin and Baskin 1988). Based on horticultural experience, we focused on embryo morphology and after-ripening requirements in this study.

STUDY SPECIES AND COLLECTION SITE

Sarracenia purpurea L. (Sarraceniaceae) occurs in *Sphagnum* dominated peatlands, and on sandy and marly shores on the eastern coast of North America from Newfoundland south to northern Florida (Gleason and Cronquist 1991). For our germination studies, we used seeds of *S. purpurea* var. *terrae-novae* de la Pylaie (following nomenclatural clarification of Reveal 1993; equivalent to *S. purpurea* var. *purpurea* Wherry in Gleason and Cronquist 1991) collected from Hawley Bog, located 543 m above sea level at 42° 16' N, 72° 29' W in the Berkshire Plateau of western Massachusetts (Moizuk and Livingston 1966).

MATERIALS AND METHODS

Mature seeds were collected on 22 September 1996 from nine individual *S. purpurea* plants growing at Hawley Bog. To determine if seeds could germinate immediately (i.e., were dispersed ripe and non-dormant), we planted 10 seeds from each parent immediately after collection. These seeds were surface-sterilized (10% bleach solution, followed by triple-rinse with distilled water), then surface-sown on moist, sterile milled *Sphagnum* and placed on a warm (20° C) bench in high light (> 1,000 µmol m^{-2} s^{-1}) in the Mount Holyoke College greenhouse. Ten additional seeds were dissected to examine the state of the embryo upon dispersal. Dissected embryos were stained with tetrazolium to determine viability (Lakon 1949).

The other seeds were stored dry at 5° C until February 1997. We then established five germination treatments: (1) seeds cold chilled at 4° C for four weeks, then placed in normal March light conditions in the greenhouse at 20° C; (2) four weeks cold chilling then placed in the greenhouse in total darkness; (3) six weeks cold chilling followed by normal light in the greenhouse; (4) six weeks cold chilling then dark conditions in the greenhouse; (5) scarification with 1M H_2SO_4 (30 seconds) followed by a triple-rinse with distilled water, then four weeks cold stratification, followed by normal light in the greenhouse. Treatments 1-4 tested the need only for an after-ripening period, while treatment 5 tested for the impermeability of the seed coat.

For cold chilling, seeds were: (1) surface sterilized for 1 min in a 10% bleach solution; (2) triple-rinsed in distilled water; (3) placed in sterile petri plates between two sheets of filter paper moistened with 3 ml sterile distilled water; (4) placed in a dark refrigerator at 4° C. All work was done aseptically under a laminar-flow hood. For each treatment, we used five replicate plates of ten seeds each from each parent plant. Sterile distilled water was added to each plate as needed (normally 2 ml every 4-8 d) to prevent seeds from drying out.

After cold chilling, plates were placed on greenhouse benches either in full sun (maximum 1,500 µmol m^{-2} s^{-1}) or under several layers of shade cloth that reduced light availability to less than 0.01 µmol $m^{-2}s^{-1}$. Temperature (20° C) and relative humidity (70%) were maintained by computer controllers. Temperatures within petri plates were not monitored, although they probably exceeded ambient temperature. Once in the greenhouse, 2-3 mls of distilled water were added to each plate every 3-4 days to maintain moisture levels. Plates were examined every three days for one month for evidence of radicle emergence. Seeds in the dark treatments were examined in the unlit head-house attached to the greenhouse. All plates were monitored for one month, by which point no change in percentage germination had been observed for at least two weeks.

Data were analyzed using ANOVA and regression procedures in Systat ver. 7 (SPSS, Inc., Evanston, IL). Throughout, results are presented as means ± one standard error. Comparisons among treatments were done in two ways. First, we compared total number of seeds germinated after 32 days using ANOVA. Second, we compared differences in germination rates (number of seeds germinated per day) among treatments using Model II regression (Sokal and Rohlf 1995). We plotted the percent of seeds that had germinated in one treatment at observation time t versus the percent of seeds that had germinated in a different treatment at the same time t, for all observation times. A regression line through these observations would have slope = 1 if the seeds in the two treatments germinated at identical rates, and a slope ≠ 1 otherwise (Ellison et al. 1993).

RESULTS

We did not observe any germination of seeds planted immediately following collection. All ten seeds dissected had live embryos, as indicated by positive (red) tetrazolium staining. Dissected embryos were creamy white in color, linear in shape, filled one-third to one-half of the seed, and had obvious cotyledons and a hypocotyl. Differentiated

Table 1. Results of ANOVA illustrating effects of duration of cold chilling (HRS), greenhouse light environment (LIGHT), and parent plant (PARENT) on % germination at the end of the experiment. Data were arcsin-square root transformed prior to analysis to eliminate heteroscedasticity. Parent plant 3 was deleted from this analysis.

Source	df	MS	F	P
HRS	1	2.30	16.64	0.001
LIGHT	1	28.03	202.78	< 0.001
LIGHT*HRS	1	1.11	8.02	< 0.001
PARENT*LIGHT*HRS	7	0.10	0.84	0.553
Error	157	0.14		

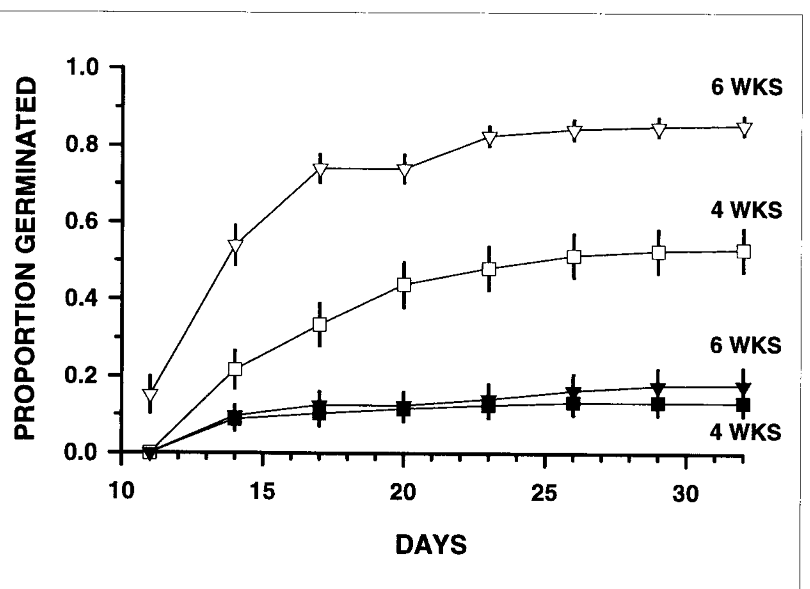

Figure 1. Germination trajectories of seeds in the four treatments. Open symbols are seeds in natural light; filled symbols are seeds in the dark. Values shown are means ± 1 SE.

plumules were not apparent. Endosperm accounted for one-half to two-thirds of the total seed volume.

No differences were observed in overall germination frequency or number of seeds germinating per day between seeds placed in the light following four weeks cold chilling and those seeds placed in the light following scarification and four weeks cold chilling (germination percentage — cold chilled only: 53 ± 6%; scarified + cold chilled: 48 ± 7%; $t_{66} = 0.61$, $P = 0.54$; germination rate — slope of Model II regression = 1.0). Therefore, subsequent analysis focused on differences among cold chilling and light treatments.

Upon removal from stratification, seeds germinated rapidly in the light, but very little germination was observed in the dark (Fig. 1; P <

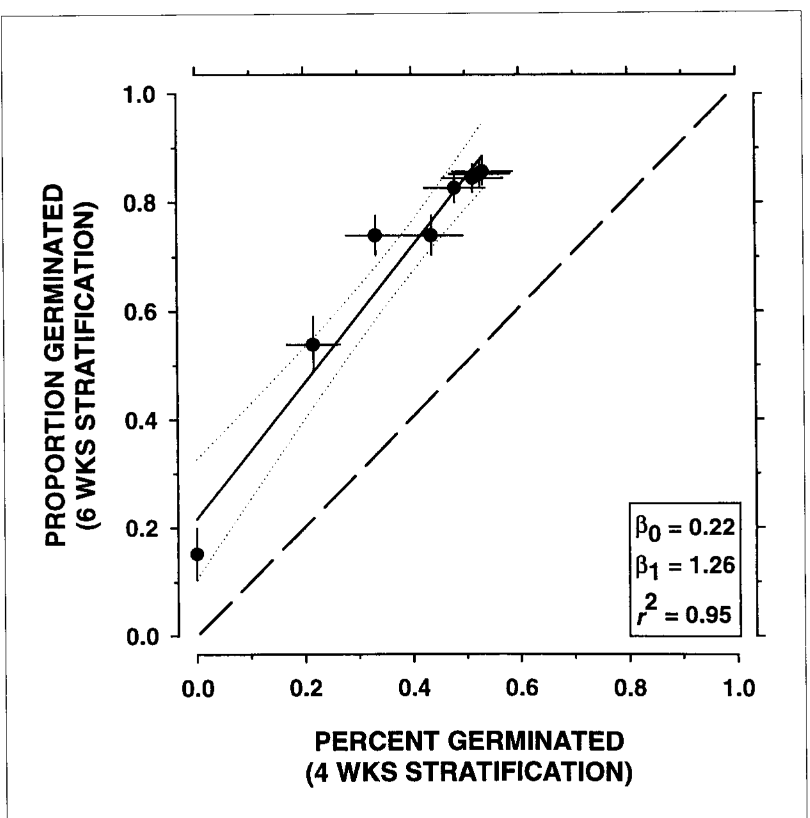

Figure 2. Differences in germination rates of seeds stratified for 4 vs. 6 weeks (mean % ± 1 SE; time proceeds from bottom left to top right). The solid line is the best-fit Model II regression line with 95% confidence intervals. The dashed line ($\beta_1 = 1$) is the expectation if seeds in the two treatments germinate at identical rates.

0.001, LIGHT effect, Table 1). Germination slowed after two weeks and leveled off within three to four weeks in all treatments (Fig. 1). In natural light, more seeds germinated following six weeks of stratification than those seeds stratified for only four weeks (Fig. 1; LIGHT*HRS effect, Table 1) and the former germinated significantly more rapidly as well (slope of Model II regression = 1.26; Fig. 2).

Although initial ANOVA analysis suggested that parent plant significantly affected germination frequency ($P = 0.02$), examination of coplots (Cleveland 1993) showed that this effect was due to one 'aberrant' parent plant with an unusually high frequency of germination (data not shown). Removal of this plant from the analysis also removed the significant effect of the PARENT*LIGHT*HRS term in the ANOVA (Table 1).

DISCUSSION

The lack of observed difference in germination frequency of rates between seeds stratified for four weeks and seeds scarified before stratification suggests that *S. purpurea* seed dormancy is not caused by an impermeable seed coat. Like Burr (1979) and Mandossian (1965), we observed that increasing stratification time resulted in increased germination success, once seeds are placed into the light. In the absence of stratification, Mandossian (1965) reported no germination following scarification with sulfuric acid for 1 or 5 minutes (concentration not reported). She attributed that result to destruction of the embryo, but it is possible that her seeds failed to germinate because they did not have fully developed embryos, as she may have scarified *S. purpurea* seeds that had not after-ripened. Concordant with our observations that *S. purpurea* seeds are dispersed with underdeveloped embryos, DeBuhr (1975) also reported that *Sarracenia* embryos (species not specified) are not fully developed at time of seed dispersal.

In her dissertation study of germination properties of *S. purpurea* in Michigan, Mandossian (1965) similarly found that stratification was necessary for germination. Even with three months of stratification, however, Mandossian (1965) reported only 30% germination at 22° C in long-day conditions (16 hours light, 8 hours darkness). Mandossian (1965) had higher germination rates (55%) with a combined light and thermoperiod (16 hours light at 22° C; 8 hours dark at 5° C) independent of stratification duration (2 weeks through three months). As with our study, Mandossian (1965) found little germination in total darkness at 22° C, although she found that germination increased modestly in total darkness at 28° C. Using two months stratification, Burr (1979) reported germination percentages of 40-80% for both selfed and outcrossed *S. purpurea*.

In sum, our illustrating the need for cold, wet stratification and subsequent exposure to light are concordant with the data of both Mandossian (1965) and Burr (1979). The germination data, along with our observations of embryo morphology, suggest strongly that *S. purpurea* seeds are morphologically dormant (*sensu* Baskin and Baskin 1988) at the time of dispersal.

Despite centuries of interest in these botanical curiosities, there are few controlled, experimental data on germination properties of carnivorous plants. Such data would be useful not only to expand existing catalogues of dormancy and germination characteristics of a wide variety of herbaceous plants (e.g., Baskin and Baskin 1988), but also would allow for a broader understanding of the evolution and diversification of dormancy mechanisms in plants with well-known habitat requirements and patterns of geographic diversity. The data presented here would be well-complemented by a broad, comparative study of germination in the Sarraceniaceae.

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