

## LIMITS TO REPRODUCTIVE SUCCESS OF *SARRACENIA PURPUREA* (SARRACENIACEAE)<sup>1</sup>

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Plant biologists have an enduring interest in assessing components of plant fitness and determining limits to seed set. Consequently, the relative contributions of resource and pollinator availability have been documented for a large number of plant species. We experimentally examined the roles of resource and pollen availability on seed set by the northern pitcher plant *Sarracenia purpurea*. We were able to distinguish the relative contributions of carbon (photosynthate) and mineral nutrients (nitrogen) to reproductive success. We also determined potential pollinators of this species. The bees *Bombus affinis* and *Augochlorella aurata* and the fly *Fletcherimyia fletcheri* were the only floral visitors to *S. purpurea* that collected pollen. Supplemental pollination increased seed set by <10%, a much lower percentage than would be expected, given data from noncarnivorous, animal-pollinated taxa. Seed set was reduced by 14% in plants that could not capture prey and by another 23% in plants whose pitcher-shaped leaves were also prevented from photosynthesizing. We conclude that resources are more important than pollen availability in determining seed set by this pitcher plant and that reproductive output may be another “cost” of the carnivorous habit.

**Key words:** *Augochlorella aurata*; *Bombus affinis*; carnivorous plants; *Fletcherimyia fletcheri*; nitrogen; pollination; reproduction; *Sarracenia purpurea*.

Successful seed set defines plant fitness, and as a consequence botanists and plant ecologists have an enduring interest in determining limits to plant reproductive success (see reviews in Bierzychudek, 1981; Willson and Burley, 1983; Wiens, 1984; Haig and Westoby, 1988; Burd, 1994; Larson and Barrett, 2000; Ashman et al., 2004; Knight et al., 2005). Although early studies considered the impact of pollen supply and resource availability in isolation, some degree of interaction of these two factors generally determines seed set in a wide variety of taxa (reviews in Willson and Burley, 1983; Casper and Niesenbaum, 1993). Resources that interact with pollen supply to limit reproductive success include both carbon (Niesenbaum, 1993; Campbell and Halama, 1993; Juenger and Bergelson, 1997; Griffin and Barrett, 2002; Asikainen and Mutikainen, 2005) and mineral nutrients (de Jong and Klinkhamer, 1989; Lawrence, 1998; Huang and Guo, 2002). But the acquisition of mineral nutrients also has a cost: roots or other nutrient-absorbing structures are constructed from photosynthetically derived carbon. Thus, observed reductions in seed set in low-light conditions or when herbivores remove leaf tissue may derive from a direct reduction in carbon or an indirect reduction in mineral nutrients. Separating these possibilities is difficult because nutrient-gathering structures (e.g., roots) are spatially separated from photosynthesizing leaves.

Here, we take advantage of the fact that carnivorous plants derive most of their mineral nutrients from prey captured by modified leaves or leaf parts (Ellison and Gotelli, 2001) to begin to disentangle the relative contributions of carbon and mineral nutrients, along with pollen availability, on plant

reproductive success. Just as the allocation of carbon into root production reduces carbon allocation to other structures, the modification of leaves into structures for prey capture and nutrient absorption comes with its own photosynthetic cost (Givnish et al., 1984). Further, it is relatively easy to measure allocation to nutrient-gathering structures (i.e., leaves modified into arthropod traps) at the same time as reproductive output is measured, and it is also relatively easy to manipulate supply of mineral nutrients (as prey or inorganic nutrients). The amount of arthropod prey captured limits plant growth across all major carnivorous plant taxa (reviewed by Ellison, 2006), and captured prey also has been shown to limit seed set in *Drosera* and *Pinguicula* species (Stewart and Nilsen, 1992; Thorén et al., 1996; Thorén and Karlsson, 1998; Anderson et al., 2002; Méndez and Karlsson, 2005). Neither the effects of pollen limitation alone nor the interactive effects of pollen and resource limitation on reproductive success, however, has been studied in carnivorous plants.

Here we examine limits to reproductive success in the pitcher plant *Sarracenia purpurea* L. (Sarraceniaceae). Our primary goal was to experimentally test, in the field, the relative importance of pollen and resources (both carbon and mineral nutrients) on the reproductive success of this carnivorous plant. We also asked if we could identify trade-offs between production of structures dedicated to nutrient uptake (through prey capture) on the one hand and seed set on the other. As a correlate of this experimental study, we identified potential pollinators of *S. purpurea*. There is considerable disagreement in the literature regarding pollinators of *Sarracenia* (Mandossian, 1965; Burr, 1979; Schnell, 1983), and while we do not conclusively resolve this issue, we do provide detailed information about pollinators from a geographic region in which *Sarracenia* pollination has not been explored previously.

### STUDY SPECIES

We studied *Sarracenia purpurea* L. var. *purpurea* (Raf.) Wherry (sensu Gleason and Cronquist, 1991; see Ellison, 2001,

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for a discussion of the disputes over the nomenclature of this species), a long-lived (30–50 years), rosette-forming, perennial carnivorous plant that grows in *Sphagnum* bogs, poor fens, and seepage swamps of the northeastern United States and Canada (Schnell, 2002). This variety ranges northward in the coastal plain of the eastern United States from Maryland and Virginia and grows throughout New England and the northern United States westward through the Great Lakes region and all of Canada east of the Continental Divide (Schnell, 2002; Buckley et al., 2003; Ellison et al., 2004). The southern variety *S. purpurea* var. *venosa* (Raf.) Fernald occurs on the coastal plain south of New Jersey to northern Florida. The recently recognized *S. rosea* Naczi, Case & Case (formerly *S. purpurea* subsp. *venosa* var. *burkii* Schnell) is endemic to the Florida panhandle.

**Reproductive biology of *Sarracenia***—Flower morphology of *S. purpurea* is similar to that of the other 11 species of *Sarracenia* (McDaniel, 1971). The inflorescence is a single-flowered scape that extends as high or higher than the pitcher-shaped leaves (Fig. 1). Each pendulous flower is subtended by three bracts and normally has five sepals and five red-to-maroon petals. There are many (often >80) stamens in 10 fascicles. The gynoecium has five carpels, and the five-locular ovary has a simple apical style. This style is expanded into an umbrella-shaped disk, which is held upside-down in the open flower. The stigmas are under each of the notched lobes at the upward-pointing ends of the style. The downward-hanging petals and the upward-pointing styler lobes form a curtain through which pollinators must pass to obtain nectar and to reach the anthers and the stigmas. Nectar is secreted not only by the nectaries distributed throughout the wall of the ovaries, but also by extrafloral nectaries around the pitcher lip (Vogel, 1998). These extrafloral nectaries provide food for foraging arthropods, some of which end up as prey drowned in the pitchers (Deppe et al., 2000).

*Sarracenia* individuals are self-compatible, which has been associated with inbreeding depression in some populations (Sheridan and Mills, 1998; Sheridan and Karowe, 2000). Although agamospermy is unknown from, and self-pollination (autogamy) rarely occurs in, native populations of *S. purpurea* (Mandossian, 1965; Burr, 1979; Thomas and Cameron, 1986), populations introduced from single individuals do establish and grow rapidly (Schwaegerle and Schaal, 1979; Taggart et al., 1990; Parisod et al., 2005). The lack of autogamy in natural populations of either *S. purpurea* (Burr, 1979) or *S. flava* L. (Schnell, 1983) does not result from dichogamy (via protandry), but rather appears to be a consequence of the spatial separation of anthers and stigmas within the flower and the structural need for direct transfer of pollen by insects.

As for most carnivorous plants, there is scant information on pollinators of *S. purpurea*. Hypothesized pollinators include bumblebees (*Bombus* spp.) (Mandossian, 1965; Burr, 1979; Schnell, 1983) and the sarcophagid flies *Sarcophaga sarraceniae* Riley and *Fletcherimyia fletcheri* (Aldrich) (Mandossian, 1965; Burr, 1979). Adult *F. fletcheri* frequently roost overnight in *S. purpurea* flowers (Krawchuk and Taylor, 1999; and see data in Results: *Floral visitors*), while their larvae are important components of the aquatic food web that inhabits the leaves of *S. purpurea* and that decomposes captured prey, releasing nutrients for uptake by the plant (Forsyth and Robertson, 1975; Fish and Hall, 1978; Bledzki and Ellison, 1998; Gotelli and Ellison, 2006).

## MATERIALS AND METHODS

**Study sites**—Field work was conducted during the summer of 2005 at two bogs, Tom Swamp (42.50° N, 72.80° W), a pond-margin bog (Kearsley, 1999) at the Harvard Forest Long-Term Ecological Research Site in Petersham, Massachusetts, and Swift River Bog (42.27° N, 72.34° W), a glacial kettle-hole bog (Kearsley, 1999) in Belchertown, Massachusetts, 50 km south of Tom Swamp. Both sites have typical acidic bog vegetation, dominated by *Chamaedaphne calyculata* L. and other ericaceous shrubs (Swan and Gill, 1970; Gotelli and Ellison, 2002). Thousands to tens of thousands of *S. purpurea* individuals grow throughout the *Sphagnum* mats of both bogs. *Sarracenia purpurea* flowers were open, shedding pollen, and had receptive stigmas between 31 May and 10 June 2005 at Swift River Bog and from 4 June to 13 June 2005 at Harvard Pond.

**Identification of potential pollinators**—Pollinator activity was observed at Harvard Pond and Swift River Bog by two of us (G. Ne'eman and R. Ne'eman) between 0800 and 1700 hours daily from 7 June to 12 June 2005. A total of 32 observations of 10-min duration were made. All visiting insects were recorded, and visit frequency was calculated as number of visits per flower per hour. Because *F. fletcheri* has been reported to roost in flowers (Krawchuk and Taylor, 1999), we carefully examined a total of 90 *S. purpurea* flowers over three mornings at 0600 hrs to verify this behavior, which may lead to pollen transfer among flowers. Representative specimens of all potential pollinators were collected for identification. Nomenclature of bumblebees follows Michener (2000); halictid bees follows Coelho (2004); and sarcophagid flies follows Pape (1990) and Dahlem and Naczi (2006). Vouchers are stored in the Harvard Forest insect collection (Harvard Forest Biota database ID nos. 001254–001263).

**Does resource availability alter reproductive success?**—To determine whether carbon (photosynthesis) and nutrient acquisition (via prey capture) altered reproductive success of *S. purpurea*, we manipulated the ability of flowering plants to photosynthesize and capture prey. At Tom Swamp, we haphazardly located 20 1-m<sup>2</sup> plots, each with 2–5 flowering plants. The distance between plants within a plot was ~50 cm, and the distance between adjacent plots was always >1 m. Plants with visible buds in each plot were assigned at random to one of the following three treatments on 25 May 2005: (1) pitchers totally covered (PTC) by *Sphagnum*, so they were neither able to capture potential prey nor able to photosynthesize; (2) pitchers' mouth covered (PMC) with netting, so that the pitchers were able to photosynthesize but unable to capture prey; (3) a control, with pitchers open (PO), photosynthesizing, and able to catch prey. After assigning plants to treatments and before the start of flowering, the contents of all pitchers were emptied using a suction aspirator (Nastase et al., 1991) and refilled with filtered (0.5-mm mesh) water from the adjacent Harvard Pond. At the time this experiment was set up, new pitchers had not yet been produced and only pitchers from the previous year were present. Flowering plants, however, had more pitchers ( $7.6 \pm 0.95$  [SE]) than did nonflowering plants ( $4.5 \pm 0.42$ ). Prey was removed weekly with a suction aspirator, preserved in alcohol (70%) and identified to lowest taxonomic level possible. Pitchers were produced during the course of the experiment and by peak flowering (6 June 2005), we were able to sample new and old pitchers separately. Pitcher production and prey capture was monitored further at weekly intervals through mid-July. In addition, we monitored prey capture in nonflowering plants with unmanipulated pitchers (PONF) that were similar in size to those of flowering plants.

Unripe fruits were harvested on 2 August 2005, by which point developing (fertilized) seeds and unfertilized ovules could be distinguished. We counted both for each fruit with a dissecting microscope and determined the ratio of the number of seeds set to the actual number of ovules available. This ratio is an established indicator of limits to reproductive success (Wiens, 1984; Larson and Barrett, 2000). The absence of a significant correlation between number of seeds and number of ovules is a reliable indicator that resources and/or pollen are likely to be strong factors limiting seed set, whereas the presence of a significant correlation between these two variables would suggest that regardless of treatment, resources and/or pollen availability only weakly limit seed set.

**Manipulations of pollen and nutrient supply**—We examined the potential for nutrient and pollen (co-)limitation of reproductive success at Swift River Bog. There, we haphazardly selected 200 plants in bud on 26 May 2005. These plants were assigned randomly to one of five treatment groups. The first three



Fig. 1. Photograph of *Sarracenia purpurea* with flowers at Tom Swamp. Note the spatial separation between the flowers and the new pitchers. Photograph by A. Ellison.

treatment groups replicated the treatments at Tom Swamp (treatments PTC, PMC, and PO). Pitchers in the remaining two treatments were left uncovered and visible and were fed weekly with 5 ml of either 1 ppm (F1) or 5 ppm (F5)  $\text{NH}_4\text{NO}_3$  in distilled-deionized water. *Sarracenia purpurea* receives 10–20% of its nutrients from captured prey (Chapin and Pastor, 1995; Ellison and Gotelli, 1991). In New England populations, much of the balance of the plant's nitrogen budget is attributable to  $\text{NH}_4^+$  or  $\text{NO}_3^-$  excreted by rotifers and deposited by rainfall (Bledzki and Ellison, 1998; Ellison and Gotelli, 2002). In our previous work with *S. purpurea* in Massachusetts, we have found that  $\text{NH}_4\text{NO}_3$  is a good surrogate for prey and can increase leaf production and reproductive output in the short term (Ellison and Gotelli, 2002; Ellison, 2006). All plants were left open to pollinators.

To examine whether or not pollen availability (co-)limited reproductive success, one-half of the plants in each treatment group was hand-pollinated twice at 4-day intervals, using a camel-hair brush and xenogamous pollen, when pollen was dehiscent and viable, and stigmas were receptive (as determined with MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide; Dafni et al., 2005]). Unripe fruits from all plants were harvested on 30 July 2005, and the percentage of fertilized ovules was determined for each fruit. The effect of supplemental pollination relative to open pollination at Swift River was quantified as  $1 - (P_o/P_s)$ , where  $P_o$  is the proportion of seed set (seeds/ovules) of open-pollinated flowers and  $P_s$  is the proportion of seed set of flowers that received supplemental pollination. This measure is equal to the "pollen limitation index" ( $L$ ) of Larson and Barrett (2000) and the "effect size" ( $e$ ) of supplemental pollination of Ashman et al. (2004). In fruits that developed

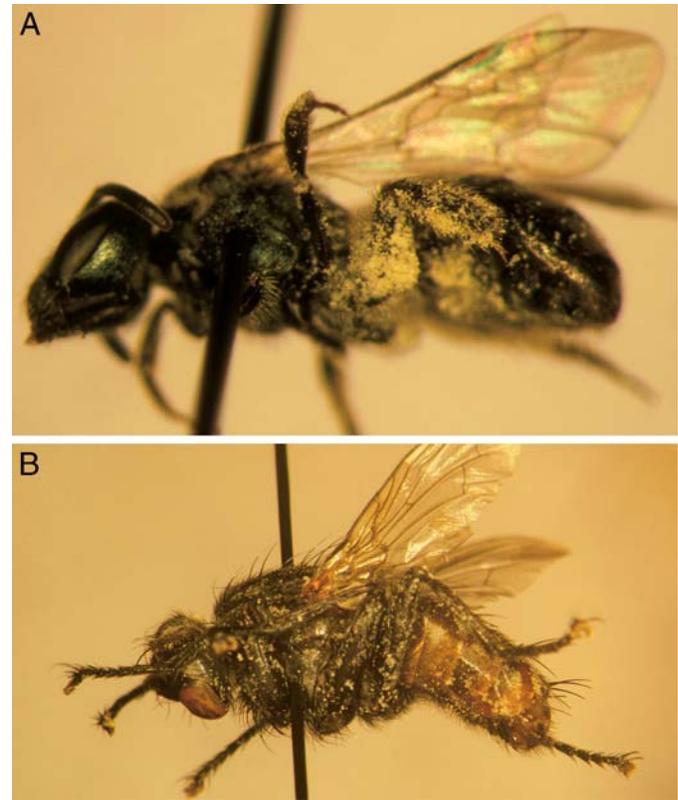


Fig. 2. Pollinators of *Sarracenia purpurea*. (A) *Augochlorella aurata*. Note dense pollen on legs and abdomen. (B) *Fletcherimyia fletcheri*. Note pollen on legs, dorsal surface of thorax, and abdomen. Photographs by G. Ne'eman.

on hand-pollinated plants, we also distinguished between unripe, but full seeds, and unripe, but empty (nonviable) seeds (i.e., aborted seeds). Finally, although other authors have found that *S. purpurea* rarely self-pollinates and cannot reproduce apomictically (Mandossian, 1965; Burr, 1979), we tested this for our populations using a set of 10 plants for which the flowers were bagged and emasculated. A seed predator common in southern populations of *S. purpurea*, larvae of the tortricid moth *Endothenia daeckiana* (Kearfott), was not observed in our populations.

## RESULTS

**Floral visitors**—In 19 (60%) of the 10-min observation units, insects were observed visiting flowers. Three insect species were observed collecting pollen from *S. purpurea* flowers at Tom Swamp. The small solitary bee *Augochlorella aurata* (Smith) (Fig. 2a) entered the flower heads to collect pollen and nectar ( $1.5 \pm 0.48$ , range = 0–12, visits  $\cdot$  flower $^{-1} \cdot$  h $^{-1}$ ). The bumblebee *Bombus affinis* Cresson demonstrated a high degree of floral fidelity and visited a number of *S. purpurea* flowers during a single observation bout ( $0.5 \pm 0.25$ , range = 0–6 visits, flower $^{-1} \cdot$  h $^{-1}$ ). This bumblebee would enter a flower and leave a few seconds later carrying virtually all the available pollen. The sarcophagid fly *Fletcherimyia fletcheri* flew rapidly among flowers ( $0.3 \pm 0.18$ , range = 0–5 visits, flower $^{-1} \cdot$  h $^{-1}$ ), and collected specimens were covered with pollen (Fig. 2b). Visitation rates of all three of these potential pollinators were distributed as Poisson random variables ( $P > 0.25$ , all cases).

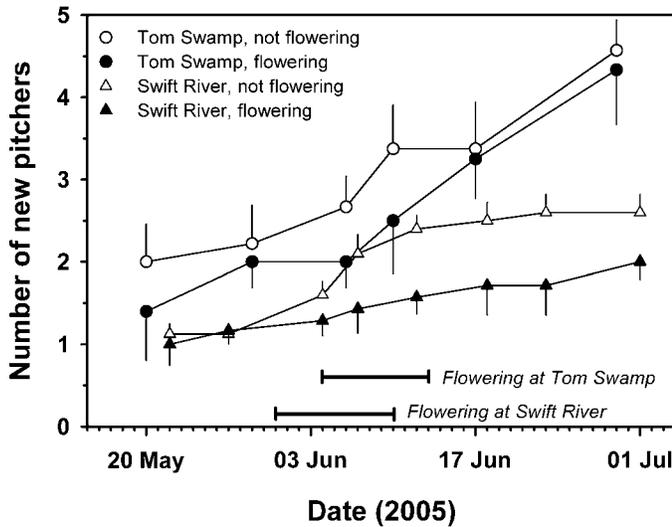


Fig. 3. Time-course of pitcher production in control flowering (white symbols) and nonflowering (black symbols) plants of *Sarracenia purpurea* at Tom Swamp (circles) and Swift River Bog (triangles) from prior to the onset of flowering (mid-May) until 1 month after flowering had ceased (30 June). The lines at the bottom of the plot indicate the duration of flowering at the two sites. See Results: *Prey capture and reproductive effort* for statistical analysis of these pitcher production data.

Forty-eight *F. fletcherii* flies also were found roosting in *S. purpurea* flowers during our early morning censuses. Of 90 flowers sampled, most had either no flies (57 flowers) or one fly (23 flowers) roosting in them, but seven had two flies, three had three flies, and one had five flies in them. As with the rate of visitors to flowers, the rate of fly roosting was indistinguishable from a Poisson random variable ( $P = 0.8$ ). Roosting flies were totally covered with pollen.

*Sarracenia purpurea* flowers secreted large amounts of thick nectar (about 5–10  $\mu$ l with sugar content >50%, as measured with a refractometer). The high viscosity of the nectar precluded accurate measurements of volume with our calibrated microcapillary tubes. Nectar was secreted at the base of the flower among the anthers and on the inner concave part of the style. This part of the style continued secreting nectar even after shedding the anthers and petals, and white sugar crystals could be easily seen.

**Prey capture and reproductive effort**—Both control flowering (PO) and nonflowering (PONF) plants at Tom Swamp had more larger pitchers than plants at Swift River ( $8 \pm 0.5$  vs.  $6 \pm 0.4$  old pitchers, respectively; site effect  $F_{1,26} = 11.2$ ,  $P = 0.002$ ). At both sites, flowering plants had significantly more old pitchers (produced in 2004) than did nonflowering plants (Tom Swamp:  $9 \pm 1.0$  vs.  $7 \pm 0.5$ ; Swift River:  $6 \pm 0.7$  vs.  $5 \pm 0.5$ ;  $F_{1,26} = 5.3$ ,  $P = 0.03$ ). New pitcher production was delayed by flowering plants relative to nonflowering plants and by midsummer, flowering plants had significantly fewer pitchers than did nonflowering ones ( $F_{1,23} = 4.9$ ,  $P = 0.03$ ; Fig. 3). There was no significant site  $\times$  flowering status interaction in either initial plant size ( $F_{1,26} = 0.6$ ,  $P = 0.45$ ) or number of new pitchers produced by midsummer ( $F_{1,23} = 0.3$ ,  $P = 0.60$ ).

At Tom Swamp, where we monitored prey capture by both flowering and nonflowering plants, somewhat more prey was captured by flowering plants ( $9 \pm 2.6$  insects, range: 0–47)

than by nonflowering plants ( $5 \pm 0.8$  insects, range: 1–11), but the difference was not significant ( $t_{30} = 1.2$ ,  $P = 0.24$ ). Prey was captured by both old and new pitchers of both flowering and nonflowering plants. Among flowering plants, new pitchers captured  $3 \pm 0.9$  (range 0–14) insects, and old pitchers captured  $6 \pm 2.2$  (range 0–36) insects during the sampling period ( $t_{18} = 1.5$ ,  $P = 0.15$ , paired  $t$  test). Among nonflowering plants, new pitchers captured  $2 \pm 0.8$  (range 0–9) insects and old pitchers captured  $4 \pm 0.9$  (range 0–11) insects during the sampling period ( $t_{12} = 1.3$ ,  $P = 0.23$ , paired  $t$  test). Prey included amphipods, ants, aquatic beetles, weevils and other terrestrial beetles, dragonfly larvae and adults, small-bodied flies, larval and adult mosquitoes, spiders, and small wasps. Floral visitors were never collected as prey.

Extrafloral nectaries around the lip of pitchers secreted thick nectar with sugar content >50%. On flowering plants, new pitchers did not secrete nectar during flowering, but began to secrete nectar only after the fruits began to mature. In contrast, new mature pitchers on nonflowering plants were secreting nectar throughout the growing season. Old pitchers, which had been produced in 2004, were never observed to secrete nectar from their extrafloral nectaries.

**Effects of prey exclusion on seed set**—Among flowering plants at both Tom Swamp and Swift River, the average number of ovules per flower did not vary significantly among the three treatment groups (PTC, PMC, PO; Table 1). At Tom Swamp, plants whose pitchers were completely obscured by moss (PTC) produced significantly fewer seeds than control (PO) plants (Table 1). The percentage of fertilized ovules was 14% higher among PO plants relative to plants whose pitchers were visible but prevented from capturing prey (PMC), and 37% higher relative to PTC plants. Seed number was not correlated with ovule number in PTC or PMC ( $r = -0.026$ ,  $P = 0.919$ ;  $r = 0.325$ ,  $P = 0.174$ , respectively). In contrast, ovule number and seed number were significantly correlated in control plants ( $r = 0.675$ ,  $P = 0.002$ ).

**Effects of nutrient addition**—At Swift River, plants in all five treatments (PTC, PMC, PO, and the two inorganic nutrient addition treatments F1 and F5) had similar numbers of ovules per flower (Table 1). Unlike at Tom Swamp, however, treatment did not significantly affect seed production at Swift River (Table 1). Seed number and ovule number were correlated only in the plants that received 5 ppm  $\text{NH}_4\text{NO}_3$  ( $r = 0.69$ ,  $P = 0.002$ ). Correlations between seed and ovule number in all other treatment groups were  $\leq 0.23$  ( $P \geq 0.43$ ).

**Supplemental pollination**—Even though plants in the high-nutrient (F5) supplemental pollination group produced more ovules than flowers in other treatment groups (Table 1), supplemental hand-pollinations did not significantly affect seed production by those plants at Swift River (Table 1). The overall increase in seed set due to supplemental pollination (Larson and Barret's [2000] *L* or Ashman et al.'s [2004] *e*) was quite small: 6% for control plants, 9% for plants with obscured and blocked pitchers (PTC), 0% for plants with visible but blocked pitchers (PMC), 1% for pitchers fed 1 ppm  $\text{NH}_4\text{NO}_3$ , and 4% for pitchers fed 5 ppm  $\text{NH}_4\text{NO}_3$ . ANOVA also revealed no interactive effects between prey or nutrient treatments and pollination treatments ( $F_{4,145} = 1.47$ ,  $P = 0.22$ ); however, seed and ovule number were strongly correlated in all treatment groups that received supplemental hand pollinations ( $r \geq 0.75$ ,

TABLE 1. Reproductive effort (number of ovules) and reproductive success (number of seeds produced) by *Sarracenia purpurea* in plants with prey, nutrient, and pollinator manipulation treatments. At both Tom Swamp and Swift River, treatments were PO: control pitchers open to prey capture and exposed to the sun, PMC: pitchers visible to pollinators but blocked by netting so they could not capture prey, and PTC: pitchers completely covered by moss not exposed to the sun and not able to capture prey. At Swift River Bog, we also added supplemental nutrients once a week to all open pitchers, either 5 ml of a 1 ppm  $\text{NH}_4\text{NO}_3$  (F1) or 5 ml of a 5 ppm  $\text{NH}_4\text{NO}_3$  (F5) solution. One-half of the plants in each treatment at Swift River were hand-pollinated (supplemental pollination).

Treatment	Tom Swamp						Swift River			
	Open pollinated			Open pollinated			Supplemental hand-pollinations			
	N	No. ovules <sup>a</sup>	No. seeds <sup>b</sup>	N	No. ovules <sup>c</sup>	No. seeds <sup>d</sup>	N	No. ovules <sup>e</sup>	No. seeds <sup>f</sup>	% aborted <sup>g</sup>
PO (Control)	20	1369 ± 68.6	866 ± 82.9 <sup>B</sup>	16	1144 ± 75.8	877 ± 66.1	15	1003 ± 68.2 <sup>A,B</sup>	833 ± 62.3	12 ± 3.2
PMC	19	1396 ± 74.6	760 ± 91.9 <sup>A,B</sup>	16	1126 ± 67.7	812 ± 69.1	16	929 ± 42.9 <sup>A</sup>	748 ± 39.8	12 ± 1.7
PTC	18	1312 ± 63.7	529 ± 74.6 <sup>A</sup>	12	1176 ± 67.7	824 ± 67.8	11	923 ± 63.6 <sup>A</sup>	764 ± 53.9	17 ± 4.8
F1	—	—	—	13	1091 ± 89.6	920 ± 81.0	21	1098 ± 49.8 <sup>A,B</sup>	936 ± 46.4	9 ± 1.3
F5	—	—	—	19	1117 ± 75.9	875 ± 48.6	16	1178 ± 67.6 <sup>B</sup>	959 ± 64.9	—

<sup>a</sup> No differences among ovules among treatments ( $F_{2,54} = 0.6$ ,  $P = 0.54$ ).

<sup>b</sup> Overall effect of treatment at Tom Swamp:  $F_{2,54} = 4.23$ ,  $P = 0.019$ ; different superscripts indicate significant treatment effects ( $P < 0.05$ ), Tukey's honest significant difference test for post-hoc comparison among means.

<sup>c</sup> No differences among ovules among treatments ( $F_{4,71} = 0.1$ ,  $P = 0.98$ ).

<sup>d</sup> No differences in seed set among treatments ( $F_{4,71} = 0.15$ ,  $P = 0.96$ ).

<sup>e</sup> Plants in the high fertilization treatment (F5) began with a significantly greater number of ovules than plants in either the prey manipulation treatments (PMC or PTC) ( $F_{4,74} = 3.45$ ,  $P = 0.01$ ). Different superscript letters indicate significant differences ( $P < 0.05$ ) Tukey's HSD post-hoc test for comparisons among means.

<sup>f</sup> No differences in seed set among treatments ( $F_{4,74} = 2.19$ ,  $P = 0.08$ ).

<sup>g</sup> No differences in percentage of aborted seeds among treatments ( $F_{3,54} = 1.85$ ,  $P = 0.15$ ; analysis on arcsin square-root transformed data. No data were taken on high fertilization treatment [F5] plants).

$P \leq 0.002$ ). The percentage of postfertilization seed abortion in supplementary pollinated flowers decreased gradually from plants with pitchers that could not catch prey to plants fed inorganic nutrients, but the differences were not significant among treatments (Table 1).

None of the plants from which we excluded pollinators and emasculated flowers set seed.

## DISCUSSION

Our results provide new observations on potential pollinators of *Sarracenia purpurea*. Our work also suggests new insights into the relative importance of availability of resources and pollen on the reproductive success of this pitcher plant. These results can be fruitfully compared with results from other studies of reproductive success in noncarnivorous plants.

**What pollinates *Sarracenia purpurea*?**—Although pitcher plants are self-compatible, they are not apomicts and rarely self-pollinate (Mandossian, 1965; Burr, 1979; Schnell, 1983; Thomas and Cameron, 1986). Bumblebees (*Bombus* spp.) have been found to be significant pollinators in all previous field studies of pollination of *S. purpurea* (Mandossian, 1965; Burr, 1979) and *S. flava* (Schnell, 1983). *Bombus affinis* was not the most frequent visitor to *S. purpurea* flowers at our study sites, and when it did visit a flower, it removed virtually all available pollen. Our observations of pollen-covered sarcophagid flies (Fig. 2) corroborate those of Mandossian (1965), and these observations, together with the frequency with which *Fletcherimyia fletcheri* roosts in *S. purpurea* flowers (see also Krawchuk and Taylor, 1999) suggests that these flies can be significant pollinators of *S. purpurea*. The relative contribution of large bees, smaller bees (such as *Augochlorella aurata*), and flies to *Sarracenia* pollination could best be resolved by

selective *Bombus* exclosures, which await the efforts of future researchers.

**Pollen availability and seed set by *Sarracenia purpurea***—Among 241 noncarnivorous, animal-pollinated plants in 74 families, Larson and Barrett (2000) reported an average 40% (range 11–68%) increase in seed set following supplemental pollination. Similarly, Ashman et al. (2004) reported an average of 42% increase in seed set among 85 additional noncarnivorous species in 14 families. In contrast, the average increase in seed set by *S. purpurea* was <10% in all treatments, well below the range reported by Larson and Barrett (2000) and outside the 95% confidence interval reported by Ashman et al. (2004). These data suggest that although pollen availability may have a modest effect on seed set in *S. purpurea*, resources are more likely to (co-)limit reproductive success in this carnivorous plant, just as resources significantly limit growth of most carnivorous plants (Ellison, 2006).

**Resource limitation to seed set by *Sarracenia purpurea***—Our data suggest that both carbon and nitrogen availability limit seed set by *S. purpurea*. In open-pollinated plants at both sites, seed and ovule numbers were correlated only in the highest nutrient treatments—plants able to capture prey at Tom Swamp and plants receiving 5 ppm  $\text{NH}_4\text{NO}_3$  at Swift River. At Tom Swamp, plants that could neither capture prey nor photosynthesize (PTC) set 23% fewer seeds than did plants that were photosynthetically active but from which prey were excluded (PMC). At Swift River, ovule abortion rate of plants receiving supplemental pollen was 5% higher in PTC plants than PMC plants. These two results suggest an additional “cost of carnivory” beyond reduction in growth due to photosynthetic inefficiency of carnivorous organs (Givnish et al., 1984). Photosynthate is clearly needed for seed set, and we can add a

“cost of reproduction” to the photosynthetic inefficiency of carnivorous plants.

Our experiments were conducted only in a single season, however, and these plants may be able to mobilize resources stored in roots, rhizomes, and pitchers produced in previous years (J. L. Butler and A. M. Ellison, unpublished data) if prey capture and photosynthesis provides insufficient resources for seed set. Measured photosynthetic rates of *S. purpurea* are low ( $\sim 2.5 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ; Small, 1972; Ellison and Gotelli, 2002; Wakefield et al., 2005), and the contribution of stored reserves and retranslocated nutrients to growth and reproduction is likely to be substantial. Other carnivorous plant species rapidly mobilize stored reserves during flowering and fruiting (Thorén et al., 1996; Eckstein and Karlsson, 2001). Further, initiation of flower buds in *S. purpurea* occurs in the season prior to flowering (Shreve, 1906) and likely depends on prey capture in that season, as it does in some species of *Pinguicula* (Worley and Harder, 1996, 1999).

**Relative importance of pollen and resource limitation on seed set by *Sarracenia purpurea***—We can separate the effects of resources from those of pollinators only at Swift River, where we directly manipulated both pollen supply and resource availability. There, seed and ovule numbers were correlated in all treatments that received supplemental pollen. Thus, our data suggest that not only resources but also pollen availability contribute to reproductive success in *S. purpurea*. The additional effect of supplemental pollination was <10% in all treatments, however, and so we conclude that pollen availability is less important than resource availability via prey capture in determining seed set in this pitcher plant.

Analysis of the reproductive biology of carnivorous plants provides an interesting contrast to general studies of reproductive success in flowering plants. Whether a plant is carnivorous or not, seed set generally is limited by a combination of resource and pollen availability (Willson and Burley, 1983; Haig and Westoby, 1988; Ashman et al., 2004). Among resources, light rarely limits photosynthesis by carnivorous plants (Givnish et al., 1984; Benzing, 1987, 2000), but photosynthetic rates of carnivorous plants are generally low (Small, 1972; Ellison, 2006). Carbon budgets of carnivorous plants have not been studied directly (Ellison, 2006), although theory predicts substantial, quantifiable trade-offs in allocation of carbon to leaves, carnivorous traps, and seeds (Givnish et al., 1984). The degree of nutrient limitation by carnivorous plants is directly associated with the ability of plants to successfully capture prey (Adamec, 1997; Wakefield et al., 2005). Nutrient availability also contributes to photosynthetic efficiency because N and P are both needed for photosynthesis. Thus, an understanding of limits to reproduction in carnivorous plants demands an appreciation of exactly what resource is limiting—carbon, mineral nutrients, or pollinators—a conclusion that is not limited only to these botanical curiosities (Willson and Burley, 1983; Haig and Westoby, 1988; Ashman et al., 2004).

#### LITERATURE CITED

- ADAMEC, L. 1997. Mineral nutrition of carnivorous plants: a review. *Botanical Review* 63: 273–299.
- ANDERSON, B., J. J. MIDGLEY, AND B. A. STEWART. 2002. Facilitated selfing offers reproductive assurance: a mutualism between a hemipteran and carnivorous plant. *American Journal of Botany* 90: 1009–1015.
- ASHMAN, T.-L., T. M. KNIGHT, J. A. STEETS, P. AMARASEKARE, M. BURD, D. R. CAMPBELL, M. R. DUDASH, M. O. JOHNSTON, S. J. MAZER, R. J. MITCHELL, M. T. MORGAN, AND W. G. WILSON. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* 85: 2408–2421.
- ASIKAINEN, E., AND P. MUTIKAINEN. 2005. Pollen and resource limitation in a gynodioecious species. *American Journal of Botany* 92: 487–494.
- BENZING, D. H. 1987. The origin and rarity of botanical carnivory. *Trends in Ecology and Evolution* 2: 364–369.
- BENZING, D. H. 2000. Bromeliaceae: profile of an adaptive radiation. Cambridge University Press, Cambridge, UK.
- BIERZYCHUDEK, P. 1981. Pollinator limitation of plant reproductive effort. *American Naturalist* 117: 838–840.
- BLEDZKI, L. A., AND A. M. ELLISON. 1998. Population growth and production of *Habrotrocha rosa* Donner (Rotifera: Bdelloidea) and its contribution to the nutrient supply of its host, the northern pitcher plant, *Sarracenia purpurea* L. (Sarraceniaceae). *Hydrobiologia* 385: 193–200.
- BUCKLEY, H. L., T. E. MILLER, A. M. ELLISON, AND N. J. GOTELLI. 2003. Reverse latitudinal trends in species richness of pitcher-plant food webs. *Ecology Letters* 6: 825–829.
- BURD, M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Botanical Review* 60: 83–139.
- BURR, C. A. 1979. The pollination ecology of *Sarracenia purpurea* in Cranberry Bog, Weybridge, Vermont (Addison County). M.S. thesis, Middlebury College, Middlebury, Vermont, USA.
- CAMPBELL, D. R., AND K. J. HALAMA. 1993. Resource and pollen limitations to lifetime seed production in a natural plant population. *Ecology* 74: 1043–1051.
- CASPER, B. B., AND R. A. NIESENBAUM. 1993. Pollen versus resource limitation of seed production: a reconsideration. *Current Science* 65: 210–214.
- CHAPIN, C. T., AND J. PASTOR. 1995. Nutrient limitation in the northern pitcher plant *Sarracenia purpurea*. *Canadian Journal of Botany* 73: 728–734.
- COELHO, B. W. T. 2004. A review of the bee genus *Augochlorella* (Hymenoptera: Halictidae: Augochlorini). *Systematic Entomology* 29: 282–323.
- DAFNI, A., P. G. KEVAN, AND B. C. HUSBAND. 2005. Practical pollination biology. Enviroquest, Cambridge, Ontario, Canada.
- DAHLEM, G. A., AND R. F. C. NACZI. 2006. Flesh flies (Diptera: Sarcophagidae) associated with North American pitcher plants (Sarraceniaceae), with descriptions of three new species. *Annals of the Entomological Society of America* 99: 218–240.
- DE JONG, T. J., AND P. G. L. KLINKHAMER. 1989. Limiting factors for seed production in *Cynoglossum officinale*. *Oecologia* 80: 167–172.
- DEPPE, J. L., W. J. DRESS, A. J. NASTASE, S. J. NEWELL, AND C. S. LUCIANO. 2000. Diel variation of sugar amount in nectar from pitchers of *Sarracenia purpurea* L. with and without insect visitors. *American Midland Naturalist* 144: 123–132.
- ECKSTEIN, R. L., AND P. S. KARLSSON. 2001. The effect of reproduction on nitrogen use-efficiency of three species of the carnivorous genus *Pinguicula*. *Journal of Ecology* 89: 798–806.
- ELLISON, A. M. 2001. Interspecific and intraspecific variation in seed size and germination requirements of *Sarracenia* (Sarraceniaceae). *American Journal of Botany* 88: 429–437.
- ELLISON, A. M. 2006. Nutrient limitation and stoichiometry of carnivorous plants. *Plant Biology* 8: in press [DOI: 10.1055/s-2006-923956].
- ELLISON, A. M., H. L. BUCKLEY, T. E. MILLER, AND N. J. GOTELLI. 2004. Morphological variation in *Sarracenia purpurea* (Sarraceniaceae): geographic, environmental, and taxonomic correlates. *American Journal of Botany* 91: 1930–1935.
- ELLISON, A. M., AND N. J. GOTELLI. 2001. Evolutionary ecology of carnivorous plants. *Trends in Ecology and Evolution* 16: 623–629.
- ELLISON, A. M., AND N. J. GOTELLI. 2002. A fine-scale indicator of nitrogen saturation in northern ecosystems. *Proceedings of the National Academy of Sciences, USA* 99: 4409–4412.
- FISH, D., AND D. W. HALL. 1978. Succession and stratification of aquatic

- insects inhabiting the leaves of the insectivorous pitcher plant *Sarracenia purpurea*. *American Midland Naturalist* 99: 172–183.
- FORSYTH, A. B., AND R. J. ROBERTSON. 1975. K reproductive strategy and larval behaviour of the pitcher plant sarcophagid fly, *Blaesoxipha fletcheri*. *Canadian Journal of Zoology* 53: 174–179.
- GIVNISH, T. J., E. L. BURKHARDT, R. E. HAPPEL, AND J. D. WEINTRAUB. 1984. Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist nutrient-poor habitats. *American Naturalist* 124: 479–497.
- GLEASON, H. A., AND A. CRONQUIST. 1991. Manual of vascular plants of northeastern United States and adjacent Canada. New York Botanical Garden, Bronx, New York, USA.
- GOTELLI, N. J., AND A. M. ELLISON. 2002. Biogeography at a regional scale: determinants of ant species density in bogs and forests of New England. *Ecology* 83: 1604–1609.
- GOTELLI, N. J., AND A. M. ELLISON. 2006. Food web models predict species abundances in response to habitat change. *PLoS Biology*: 44: e324.
- GRIFFIN, S. R., AND S. C. H. BARRETT. 2002. Factors affecting low seed : ovule ratios in a spring woodland herb, *Trillium grandiflorum* (Melanthiaceae). *International Journal of Plant Sciences* 163: 581–590.
- HAIG, D., AND M. WESTOBY. 1988. On limits to seed production. *American Naturalist* 131: 757–759.
- HUANG, S. Q., AND Y. H. GUO. 2002. Variation of pollination and resource limitation in a low seed-set tree, *Liriodendron chinense* (Magnoliaceae). *Botanical Journal of the Linnean Society* 140: 31–38.
- JUENGER, T., AND J. BERGELSON. 1997. Pollen and resource limitation of compensation to herbivory in scarlet gilia, *Ipomopsis aggregata*. *Ecology* 78: 1684–1695.
- KEARSLEY, J. 1999. Non-forested acidic peatlands of Massachusetts: a statewide inventory and vegetation classification. Massachusetts Natural Heritage & Endangered Species Program, Westborough, Massachusetts, USA.
- KNIGHT, T. M., J. A. STEETS, J. C. VAMOSI, S. J. MAZER, M. BURD, D. R. CAMPBELL, M. R. DUDASH, M. O. JOHNSTON, R. J. MITCHELL, AND T.-L. ASHMAN. 2005. Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution, and Systematics* 36: 467–497.
- KRAWCHUK, M. A., AND P. D. TAYLOR. 1999. Roosting behaviour by *Fletcherimyia fletcheri* (Diptera: Sarcophagidae) in *Sarracenia purpurea* (Sarraceniaceae). *Canadian Entomologist* 131: 829–830.
- LARSON, B. M. H., AND S. C. H. BARRETT. 2000. A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society* 69: 503–520.
- LAWRENCE, W. S. 1998. Resource and pollen limitation: plant size-dependent reproductive patterns in *Physalis longifolia*. *American Naturalist* 141: 296–313.
- MANDOSSIAN, A. J. 1965. Some aspects of the ecological life history of *Sarracenia purpurea*. Ph.D. dissertation, Michigan State University, East Lansing, Michigan, USA.
- MCDANIEL, S. 1971. The genus *Sarracenia* (Sarraceniaceae). *Bulletin of the Tall Timbers Research Station* 9: 1–36.
- MÉNDEZ, M., AND P. S. KARLSSON. 2005. Nutrient stoichiometry in *Pinguicula vulgaris*: nutrient availability, plant size, and reproductive status. *Ecology* 86: 982–991.
- MICHENER, C. D. 2000. The bees of the world. Johns Hopkins University Press, Baltimore, Maryland, USA.
- NASTASE, A. J., C. L. DE LA ROSA, AND S. J. NEWELL. 1991. A comparison of three methods for collecting dipteran insect larvae which inhabit the northern pitcher plant (*Sarracenia purpurea*). *American Midland Naturalist* 125: 356–359.
- NIESENBAUM, R. A. 1993. Light or pollen—seasonal limitations on female reproductive success in the understory shrub *Lindera benzoin*. *Journal of Ecology* 81: 315–324.
- PAPE, T. 1990. Revisionary notes on American Sarcophaginae (Diptera: Sarcophagidae). *Tijdschrift voor Entomologie* 133: 43–74.
- PARISOD, C., C. TRIPPI, AND N. GALLAD. 2005. Genetic variability and founder effect in the pitcher plant *Sarracenia purpurea* (Sarraceniaceae) in populations introduced into Switzerland: from inbreeding to invasion. *Annals of Botany* 95: 277–286.
- SCHNELL, D. E. 1983. Notes on the pollination of *Sarracenia flava* L. (Sarraceniaceae) in the Piedmont province of North Carolina. *Rhodora* 85: 405–420.
- SCHNELL, D. E. 2002. Carnivorous plants of the United States and Canada. Timber Press, Portland, Oregon, USA.
- SCHWAEGERLE, K. E., AND B. A. SCHAAL. 1979. Genetic variability and founder effect in the pitcher plant *Sarracenia purpurea* L. *Evolution* 33: 1210–1218.
- SHERIDAN, P. M., AND D. N. KAROWE. 2000. Inbreeding, outbreeding, and heterosis in the yellow pitcher plant, *Sarracenia flava* (Sarraceniaceae), in Virginia. *American Journal of Botany* 87: 1628–1633.
- SHERIDAN, P. M., AND R. R. MILLS. 1998. Genetics of anthocyanin deficiency in *Sarracenia* L. *HortScience* 33: 1042–1045.
- SHREVE, F. 1906. The development and anatomy of *Sarracenia purpurea*. *Botanical Gazette (old series)* 42: 107–126.
- SMALL, E. 1972. Photosynthetic rates in relation to nitrogen recycling as an adaptation to nutrient deficiency in peat bog plants. *Canadian Journal of Botany* 50: 2227–2233.
- STEWART, C. N., AND E. T. NILSEN. 1992. *Drosera rotundifolia* growth and nutrition in a natural population with special reference to the significance of insectivory. *Canadian Journal of Botany* 70: 1409–1416.
- SWAN, J. M., AND A. M. GILL. 1970. The origins, spread, and consolidation of a floating bog in Harvard Pond, Petersham, Massachusetts. *Ecology* 51: 829–840.
- TAGGART, J. B., S. F. McNALLY, AND P. M. SHARP. 1990. Genetic variability and differentiation among founder populations of the pitcher plant (*Sarracenia purpurea* L.) in Ireland. *Heredity* 64: 177–184.
- THOMAS, K., AND D. M. CAMERON. 1986. Pollination and fertilization in the pitcher plant (*Sarracenia purpurea* L.). *American Journal of Botany* 73: 678.
- THORÉN, L. M., AND P. S. KARLSSON. 1998. Effects of supplementary feeding on growth and reproduction of three carnivorous plant species in a subarctic environment. *Journal of Ecology* 86: 501–510.
- THORÉN, L. M., P. S. KARLSSON, AND J. TUOMI. 1996. Somatic cost of reproduction in three carnivorous *Pinguicula* species. *Oikos* 76: 427–434.
- VOGEL, S. 1998. Remarkable nectaries: structure, ecology, organophyletic perspectives. II. Nectarioles. *Flora* 193: 1–29.
- WAKEFIELD, A. E., N. J. GOTELLI, S. E. WITTMAN, AND A. M. ELLISON. 2005. The effect of prey addition on nutrient stoichiometry, nutrient limitation, and morphology of the carnivorous plant *Sarracenia purpurea* (Sarraceniaceae). *Ecology* 86: 1737–1743.
- WIENS, D. 1984. Ovule survivorship, brood size, life history, breeding systems, and reproductive success in plants. *Oecologia* 64: 47–53.
- WILLSON, M. F., AND N. BURLEY. 1983. Mate choice in plants: tactics, mechanisms, and consequences. Princeton University Press, Princeton, New Jersey, USA.
- WORLEY, A. C., AND L. D. HARDER. 1996. Size-dependent resource allocation and costs of reproduction in *Pinguicula vulgaris* (Lentibulariaceae). *Journal of Ecology* 84: 195–206.
- WORLEY, A. C., AND L. D. HARDER. 1999. Consequences of preformation for dynamic resource allocation by a carnivorous herb, *Pinguicula vulgaris* (Lentibulariaceae). *American Journal of Botany* 86: 1136–1145.