LARVAE OF SARCOPHAGIDAE (INSECTA: DIPTERA) AND

THEIR RELATIONSHIP WITH THE PITCHER PLANTS

(SARRACENIACEAE: *SARRACENIA*) OF

SOUTHEASTERN U.S. BOGS

Except where reference is made to the work of others, the work described in this thesis is my own or was done in collaboration with my advisory committee. This thesis does not include proprietary or classified information.

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Angela Marie Spano Underwood

Certificate of Approval:

_____	_____
Wayne E. Clark	Debbie R. Folkerts, Chair
Professor	Assistant Professor
Entomology	Biological Sciences
_____	_____
Robert Lishak	George Flowers
Associate Professor	Acting Dean
Biological Sciences	Graduate School

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Angela Marie Spano Underwood

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Angela Marie Spano Underwood

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Signature of Author

_____

Date of Graduation

VITA

Angela Marie (Spano) Underwood is the daughter of Earl and Beverly May and Bill and Melanie Spano. She earned a Bachelor of Arts degree in Biology and Psychology from Huntingdon College in 2001, graduating Magna Cum Laude with Honors in Biology. Married in November 2007 to Will Underwood, she accepted a position with Weeks Bay National Estuarine Research Reserve in January 2008. Angela is passionate about her love of nature and enjoys spending time outdoors with her husband. They reside in Magnolia Springs, Alabama and spend their free time botanizing, photographing nature, and bird watching.

THESIS ABSTRACT

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Larvae of diptera of the family Sarcophagidae from bogs in Mississippi, Alabama, and Florida were collected, reared, and identified to species. Additionally, the effect of sarcophagid larvae on nutrient uptake in white-topped pitcher plant leaves (*Sarracenia leucophylla*) was examined at Crawford bog in south Alabama. The influence of sarcophagid larvae on nutrition of pitcher plants has not been previously recorded in the literature.

I reared *Sarcophaga sarraceniae*, *Fletcherimyia abdita* , and *F. celarata* from pitchers of *S. leucophylla*. In Alabama, all three species occurred at Crawford Bog while

only *S. sarraceniae* and *F. abdita* occurred at Splinter Hill Bog. I reared *F. abdita* from pitchers of *S. alata* occurring in Desoto West Bog in Mississippi. Two species of sarcophagids, *F. rileyi* and *F. jonesi*, were reared from pitchers of *S. flava.* Both occurred at Sumatra Bog in Florida, while only *F. rileyi* was found at Crawford Bog. I also calculated sarcophagid larval frequencies in *S. leucophylla* and *S. flava* at Crawford Bog during 2004 and 2005. In 2004, 70% of *S. leucophylla* leaves surveyed contained at least one sarcophagid larva. In 2005, only 58% of leaves contained larvae, but among these, four were occupied by multiple larvae. Eighty-six percent and 94% of *S. flava* leaves in 2004 and 2005 respectively were occupied by at least one larva. During both years, 4 of the occupied pitchers contained multiple larvae.

Nutrition field experiments were conducted at Crawford Bog during the summers of 2004 and 2005. I demonstrated a strong positive relationship between the addition of prey and leaf concentrations of macronutrients (N, P, and K). In each experimental group, pitchers supplemented with prey and with prey plus larvae contained significantly higher nutrient levels than the other experimental conditions (except 2004 potassium control). Additionally, I demonstrated that larvae do not negatively affect plant nutrition. Pitchers supplemented with prey plus sarcophagid larvae showed a strong trend towards higher nutrient levels than pitchers with prey only.

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INTRODUCTION

Carnivory in plants is defined by the ability of a plant to attract, capture, and digest prey items, as well as absorb nutrients from the decomposed prey mass (Lloyd 1942). Carnivorous plants are found in every region of the world except the high Arctic, Antarctica, and extreme deserts (Givnish 1989), where they tend to inhabit areas characterized by sunny, open environments with moist, nutrient poor soils (Givnish et al. 1984, Plummer 1963). Soils in areas suitable for carnivorous plants are mostly acidic (Chandler & Anderson 1976, Juniper et al. 1989, Plummer 1963, Roberts and Oosting 1958), although a few carnivorous plants are found in habitats where soils are neutral to basic (Mandossian 1965, Wherry 1929). Scientists hypothesize that the evolution of carnivory in plants was an adaptation to these low nutrient levels and unfavorable soil moisture and pH conditions (Givnish et al. 1984). According to Givnish (1989), carnivory has arisen independently at least six times with there being 538 or more carnivorous plant species found in 18 genera and eight families.

Early investigators were disinclined to believe that plants could capture and consume animal matter. In reference to pitcher plants, these investigators felt that the lid of the pitcher was only a mechanism to help conserve water in the plant (Lloyd 1942). Others felt that pitchers were refuges for insects trying to escape the weather and other animals (Lloyd 1942, Schnell 2002). Even William Bartram (1791), who realized that insects were caught and trapped by pitchers, felt it doubtful that caught insects would

"serve for aliment or support to these kinds of plants." Through detailed experimentation using *Drosera rotundifolia* L. (Roundleaf Sundew), Charles Darwin (1875) was the first to show that plants did actually trap and digest invertebrates and absorb the resulting nutrients. A few years later, Francis Darwin (1878) demonstrated that captured prey enhanced growth and seed production in *Drosera rotundifolia*.

Since then, several studies have shown that prey provide an alternative source of nutrients for carnivorous plants. Experiments by Hepburn et al. (1920) demonstrated the absorption of nitrogenous compounds and phosphorous in pitcher plants. Plummer and Kethley (1964) used radioisotopes to show that the leaves of *Sarracenia flava* L. (Yellow Pitcherplant). not only absorb sulfur and phosphorous but also translocate these elements to other parts of the plant, thus they concluded these elements are of importance to the plant. Williams (1966) used C^{14} - labeled fruit flies to verify the absorption of animal proteins by *Sarracenia purpurea* L. (Purple Pitcherplant). Christensen (1976) found that prey capture led to significantly higher tissue concentrations of nitrogen and phosphorous in *Sarracenia flava*, although addition of insects had no effect on concentrations of potassium, calcium, and magnesium. Schulze et al. (1997) found that insect derived nitrogen was an important resource for pitchers of *Darlingtonia californica* Torr. (California Pitcherplant). Additionally, recent studies have shown that the presence of prey increases growth, reproduction, and root nutrient uptake in carnivorous plants (Adamec 2002, Hanslin and Karlsson 1996, Thoren and Karlsson 1998, Thum 1988), allowing these plants to survive where soil nutrient concentrations may be low.

Within North America, diversity of carnivorous plants is highest in pitcher plant bogs of the southeastern United States (Folkerts 1982). Pitcher plant bogs are so named

because they are visually dominated by pitcher plants of the genus *Sarracenia* (Sarraceniaceae), although grasses and sedges are often more abundant. Eight species of pitcher plants are typically recognized in the genus (Bell 1949, McDaniel 1971) although up to eleven may be distinguished, depending on taxonomic opinion. One species, *S. purpurea*, occurs in deep sphagnum bogs from British Columbia, Canada, south to the Coastal Plain of South Carolina and Georgia as well as in pitcher plant bogs of the Gulf Coastal Plain. Seven additional species occur in pitcher plant bogs within the Atlantic and Gulf Coastal Plain of the southeastern United States.

Even though pitcher plant habitats in the southeast may be classified into eleven habitat types (Folkerts 1991), they have many characteristics in common. Most bogs where *Sarracenia* occur are open and sunny and contain sandy to loamy soils that are saturated for at least a portion of the year. High amounts of water passing through the soil causes leaching of nutrient cations, resulting in nutrient poor, acidic soils (Platt 1999).

A distinct assemblage of plants is associated with bogs and is adapted to frequent, moderate growing-season fire (Platt 1999). Natural occurrence of fire is caused by lightning strikes during summertime storms. These regularly occurring fires reduce the fuel load, causing cooler burns. Fire within these ecosystems helps remove old growth and competition from woody species, as well as opens up bare soil for seed germination.

Pitcher plants of the genus *Sarracenia* are herbaceous, rhizomatous plants that possess funnel-shaped leaves which act as passive pitfall traps to capture a variety of prey (Lloyd 1942). Prey are attracted to the traps by odor, coloration, and nectar (Juniper et al. 1989, Lloyd 1942, Slack 1979). Differences in these characteristics along with variation

of leaf morphology within the genus make species of pitcher plants attractive to different types of insects (Folkerts 1999). In *S. minor* Walt. (Hooded Pitcherplant) and *S. rubra* Walt. (Red Pitcherplant), nectaries along the frontal wing help direct ants from the ground into the pitcher (Fish 1976, Harper 1918, Schnell 2002, Slack 1979). The smaller, decumbent pitchers of *S. psittacina* Michx. (Parrot Pitcherplant) specialize in capturing crawling insects. Other species, such as *S. flava* and *S. leucophylla* Raf. (White-topped Pitcherplant), have evolved large, flower-like pitchers that contain combinations of UV-absorbing dark centers, radiating stripes and peripheral dots which advertise nectaries around the hood of the pitcher and tend to be attractive to flying insects, especially Hymenoptera (Biesmeijer et al. 2005, Slack 1979) and Lepidoptera (Folkerts, 1999). Escape of prey is prevented by downward pointing hairs, a smooth, waxy surface that provides no footing, and in some species by an intoxicating chemical, coniine (Mody et al. 1976), which is released by the leaf. Captured prey material is digested by a combination of enzymatic (Hepburn et al 1927), bacterial (Plummer and Jackson 1963), and commensal activity (Bradshaw and Creelman 1984).

Not only do leaves of pitcher plants act as traps for prey, but they also function as microhabitats for a variety of arthropod associates. *Sarracenia purpurea* has a short-form, open-topped pitcher that holds a watery phytotelm (plant held water) harboring a number of aquatic insects. Pitchers of the tall-form species have a hood which covers the pitcher orifice, and normally hold a moist mass of decomposing prey in a phytotelm that is rarely watery. Arthropod associates of pitcher plants include many endemic mite species, lepidopteran herbivores, a facultatively nesting wasp, and members of several dipteran families, notably several species of sarcophagid flies in the genera *Fletcherimyia*

(formally *Blaesoxipha*) and *Sarcophaga* (Dahlem and Naczi 2006, Folkerts 1999, Forsyth and Robertson 1975, Hepburn and Jones 1919, Jones, 1904, 1907, 1908, 1918, 1920, 1921, Judd 1959, Swales 1969, 1972, Rymal and Folkerts 1982, Wray and Brimley 1943).

Members of the family Sarcophagidae vary greatly in their lifestyles. The majority of sarcophagid species are parasites of invertebrates (Aldrich 1914, 1915), while others scavenge on dead animals (Aldrich 1914, 1915, Forsyth and Robertson 1975) or parasitize vertebrates (Kamal 1958). Interestingly, one species in the genus *Sarcophaga* and several species in the genus *Fletcherimyia* are obligately associated with pitcher plants in North America (Aldrich 1916, Dahlem and Naczi, 2006, San Jean 1957). A similar relationship is observed in the tropics of Asia and Australia between sarcophagid species and the old world pitcher plants of the genus *Nepenthes* L. (Beaver 1979, Lever 1956, Shinonaga and Beaver 1979, Souza Lopes 1958, Yeates et al. 1989). These pitcher inhabiting species are the only members of the family considered to have truly aquatic larvae (Johannsen 1935). The large, whitish maggots of these species can be found feeding within the prey mass in pitchers or floating at the surface of the fluid that may be contained by the leaves. These larvae are able to live in pitchers and escape digestion by producing antiproteases that protect them from digestive enzymes secreted by the plant (Hepburn and Jones 1919). Typically only one larva is found per pitcher (Farkas and Brust 1986, Fish and Hall, 1978, Hardwick and Giberson 1996), due to aggression and cannibalism among larvae developing in the same pitcher (Forsyth & Robertson 1975). This behavior may be an adaption to limited food supply, and according to Beaver (1979), a similar behavior occurs in the sarcophagid species *Pierretia urceola* Shinonaga

& Beaver, the larvae of which live in *Nepenthes* pitchers in Malaysia. This behavior is unique among sarcophagids, although competition for resources occurs among non-pitcher dwelling species and has been shown to reduce the size of larvae, pupae, and adults, as well as population sizes (Beaver 1973, Kamal 1958). Although single larvae are typical, on occasion, multiple larvae have been observed in pitchers (Yanoviak and Folkerts 1991, Yeates et al. 1989, personal observation). Frequency and number of sarcophagid larvae in pitchers varies over time and geographically. In 1955, Judd (1959) observed only 3% of *S. purpurea* pitchers surveyed were inhabited by larvae and no pitchers contained multiple larvae, whereas in 1956, 32% of leaves contained larvae, with 6 of these leaves containing multiple larvae. Forsyth and Robertson (1975) observed 93.2% of 106 occupied *S. purpurea* pitchers contained a single larva, with multiple larvae per pitcher occurring during peak density. Fish (1976) reported that 64% of *S. minor* leaves were occupied by single larvae.

Mature larvae crawl from the pitchers and pupate in the soil at the base of the plant. Late season larvae appear to overwinter in the soil (Farkas and Brust 1986, Yanoviak and Folkerts 1991). Only the larvae inhabit *Sarracenia* leaves, while adults rarely enter pitchers. Adult flies prefer new leaves (Fish and Hall 1978, Forsyth and Robertson 1975) and larger leaves (Krawchuck and Taylor 2003) for larviposition and usually larviposit from the edge of the orifice, although they may roost in the flower heads of pitcher plants (Argo 1964, Jones 1908, Krawchuck and Taylor 1999, Swales 1972). Jones (1908) reported up to four adult flies of *Sarcophaga sarraceniae* Riley crowding into blossoms of *Sarracenia flava*, indicating their possible role as pollinators.

However, research has shown that large-flowered species of pitchers plants are pollinated by queen bumblebees (Schnell 1983, G. Folkerts pers. comm.).

Recent researchers have emphasized the dynamics of phytotelm communities in *S. purpurea* (Hamilton and Duffield 2002, Heard 1994, Kneitel and Miller 2003, Miller et. al., 2002, 2002b, Rango 1999b), but few authors have investigated the sarcophagid species specifically (Dahlem and Naczi 2006, Krawchuk and Taylor 1999, Rango 1999) and rarely has the system been investigated in the Gulf Coastal Plain (Fish 1976) or in tall-form pitcher plants. Moreover, it has not been shown whether consumption of prey by sarcophagids is detrimental to pitchers. Fish (1976) estimated that larvae may consume up to 50% of prey material and felt this to be detrimental to the host plant. However, Folkerts (1999) observed that most prey-consumers could occur in pitchers without any noticeable damage to the plants. In fact, inhabitants may have beneficial effects on pitchers.

As early as 1882 Schimper (quoted by Hepburn et al. 1927) reasoned that "innumerable worms" in the leaves "possibly participate in the transformation of the animal bodies into soluble components". Additionally, Hubbard (1896) said the following about maggots of *Sarcophaga saraceniae* Riley:

> "… (they) are so uniformly present and so abundant in every species of pitcher-plant which I have examined from the swamps of Lake Superior to the bay-heads of Florida that I am constrained to think they have a more intimate connection with the economy of the plant than has been assigned to them. They certainly aid materially in disintegrating the mass of accumulated insects in the pitchers, and I see no reason for considering that they rob the plant of its proper food, since they

must add their own excreta to the macerated digestive material, and this may serve the needs of the plant well, or even better, than the disintegration of the animal matter produced by its own fluid".

More recently, Bradshaw and Creelman (1984) showed that the degradation of prey by midge and mosquito larvae is beneficial to *S. purpurea*. Could degradation of prey by sarcophagid larvae also be beneficial to the plants?

Little direct information is known about the relationship of sarcophagid flies with tall-form pitcher plants. Therefore, my study focused on the ecological relationship of sarcophagid larvae inhabiting pitcher plants in pitcher plant bogs in southwest Alabama and elsewhere in the southeastern United States. The objectives of the study included: 1) Measuring the frequency of sarcophagid larvae inhabiting pitchers of *S. leucophylla* and *S. flava*; 2) Identifying species of sarcophagids associated with species of *Sarracenia* in several bogs across the southeastern United States 3) Determining the average nutrient content of white-topped pitcher plant leaves; 4) Evaluating the effects of sarcophagid larvae on nutrient uptake in white-topped pitcher plant leaves.

METHODS

STUDY SITE

The primary study site, Crawford Bog, is located in Conecuh National Forest in Covington County, Alabama. It is a seepage bog (as defined by Folkerts 1991), approximately 5.67 hectares in size, characterized by open, sunny habitat and dominated by herbaceous plant species. Carnivorous species include 3 species of pitchers plants (S. *leucophylla, S. flava, and S. psittacina*), two species of sundews (*Drosera tracyi* Macfarlane and *D. capillaris* Poir.), and two species of bladderworts (*Utricularia cornuta* Michx. and *U. subulata* L.). At Crawford Bog, different varieties of *S. leucophylla* and *S. flava* were found, as well as hybrids between the two species. Although *S. purpurea* has not been discovered at the site, possible hybrids between *S. purpurea* and *S.leucophylla* have been found indicating either the past presence of *S. purpurea* at Crawford Bog or close proximity to a bog containing *S. purpurea*. Other herbaceous species include grasses (*Aristida beyrichiana* Trin. & Rupr, Ctenium *aromaticum* [Walt.] Wood and *Panicum* spp.), sedges (*Dichromena latifolia* Baldw.), asters (*Chaptalia tomentosa* Vent., *Liatris*., *Balduina*., *Helenium* and *Bidens* spp.), terrestrial orchids (*Calopogon pallidus* Chapman , *C. tuberosus* [L.] B.S.P, *Cleistes bifaria* [Fern.] Catling & Gregg, and *Pogonia ophioglossoides* [L.] Ker-Gawl), milkweed (*Asclepias lanceolata* Walt. and *A. longifolia* Michx.), goldencrest (*Lophiola americanum* [Pursh] Wood), milkworts

(*Polygala lutea* L. and *P. cruciata* L.), meadowbeauties (*Rhexia alifanus* Walt. and *Rhexia lutea* Walt.), hatpins *(Eriocaulon* and *Lachnocaulon* spp.), groundpine (*Lycopodium alopecuroides* L.), and *Sphagnum*. The study site is bordered on the north by a silvicultural clear-cut. Examination of aerial photographs and soil profiles suggests that the bog at one time extended beyond its present boundary. The upslope vegetation on the east and south side of the bog consists of an upper canopy of mature slash and longleaf pine (*Pinus elliottii* Engelm. and *P. palustris* P.Mill.) with an understory of small gallberry (*Ilex glabra* [L.] Gray), blueberries (*Vaccinium* spp.), huckleberry (*Gaylussacia* sp.) and gopher apple (*Licania michauxii* Prance) among other species. The west portion of the bog transitions from open, bog habitat into a dense bay swamp that occupies the floodplain of a nearby creek. A small stream bisects the site and is surrounded by a shrub bog consisting mainly of *Ilex glabra*, sweetbay (*Magnolia virginiana* L.), wax myrtle (*Myrica cerifera* L.), and black titi (*Cliftonia monophylla* [Lam.] Britt. ex Sarg). The bog proper contains few scattered woody species (*I. glabra* and *P. elliottii*) and is maintained by the National Forest Service using frequent, prescribed fire, averaging a burn every 2 to 3 years (Michael Heard, FMO USFS Conecuh National Forest, pers. comm.).

LARVAL FREQUENCY AND SPECIES IDENTIFICATION

In order to determine the natural level of use of pitchers by sarcophagid larvae, arbitrarily selected pitchers of *S. leucophylla* and *S. flava* were split open with a razor and examined for the presence of sarcophagid larvae. During the two year study, 100 pitchers of each species were examined, for a total of 200 pitchers. The percentage of pitchers

occupied by larvae on a yearly basis was calculated by dividing the number of occupied pitchers by the total number of pitchers sampled in that year.

For identification, larvae were collected each year from pitchers of *S. leucophylla* and *S. flava* at Crawford Bog and reared to adult in the lab. Twenty larvae were taken from pitchers of each plant species each year for a total of 80 larvae. Additionally, I opportunistically collected larvae from three other sites in the southeastern U.S.

Each larva was placed in an individual clear polystyrene vial with distilled water and freeze-killed crickets. Vials were then placed in individual plastic jars containing bog soil for pupation and covered with No-See-Um© netting to prevent escape of specimens. Rearings were conducted in an environmental chamber at 26º and 70% humidity. Although specimens were kept in a controlled environment, pupal mortality was high. Dahlem and Naczi (2006) felt that pupal mortality was high in their study due to insufficient humidity in the rearing containers. In my experience, soil moisture was difficult to maintain even in a climate controlled rearing chamber and pupae often desiccated before eclosion. In another study, pupal survival was better achieved by loosely placing a lid over the rearing jars, thus helping to maintain humidity and soil moisture (G. Folkerts pers. comm.).

Adult specimens were freeze-killed and pinned. The genitalia of male sarcophagids were spread using a method detailed by Dahlem and Naczi (2006). Female sarcophagids were dissected allowing for the examination of sternites 6, 7 and 8. Specimens were then identified using the keys and figures found in Aldrich (1916), Dahlem and Naczi (2006), and Roback (1954).

NUTRITION STUDY

A field study was conducted during the spring and summer of 2004 and 2005 at Crawford Bog in order to detect the effect of sarcophagid larvae on nutrition in pitchers. Data were collected from April 10-June 24, 2004 and May 17-July 9, 2005. The study site was inspected each year in the early spring for newly emerging leaves of *Sarracenia leucophylla*. As leaves of pitcher plants develop, the orifice remains closed, excluding all prey items until the pitcher reaches a mature height. In 2004, 50 nearly opened pitchers of relatively equal size were bagged with insect exclusion netting and cotton, while in 2005, only 40 pitchers were bagged due to the elimination of one of the experimental treatments. The insect exclusion bags were made out of a fine, No-See-Um© (0.3mm mesh) and tied with cotton drawstrings. Bags were placed over the top portion of the pitcher. The opening of the bag was lined with cotton batting to cushion the leaves and increase the effectiveness of the seal as the drawstrings were closed. The bags were tied to numbered wire flags for identification and to support the leaves. After opening, each leaf was arbitrarily subjected to one of several experimental treatments: 1) Control: bagged, no prey or larvae added; 2) Larvae (2004 season only): bagged, larvae added; 3) Prey: bagged, prey added; 4) Prey and larvae: bagged, prey and larvae added; 5) Unmanipulated: not bagged, naturally captured prey and larvae (potentially) present. Larvae used in the experiment were approximately 2nd instars and were taken from other pitchers of *S. leucophylla* at the study site. Prey consisted of pre-weighed crickets. In 2004, 10 freeze-killed crickets weighing 4.0g +/- 0.1 g were placed into each pitcher receiving prey, while in 2005, 5 crickets weighing 2.0 g +/- 0.1g were placed in pitchers.

The lower amount of prey in the second year more accurately mimicked natural conditions as judged in the first year of the study.

After approximately 10 weeks, leaves were cut off at ground level, placed in plastic bags, and transported to the lab in coolers. In the lab, each pitcher was cleaned with distilled water and then taken to the Auburn University Soils Laboratory. At the soils lab, levels of calcium (Ca), potassium (K), magnesium (Mg), phosphorous (P), aluminum (Al), boron (B), barium (Ba), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), sodium (Na), nickel (Ni), lead (Pb), and zinc (Zn) in leaves were analyzed using inductively coupled plasma (ICP) emission spectroscopy and were reported in parts per million (ppm). Percent nitrogen (N) and carbon (C) were determined using a combustion method.

I tested two hypotheses: 1) If the addition of prey has an effect on nutrition within pitchers, then we will see either an increase or decrease in nutrient levels; I predicted that the addition of prey would increase the nutrient content of leaves. 2) If consumption of prey by sarcophagid larvae has an effect on nutrient levels within pitchers, then nutrient content will differ between the treatment groups; I predicted that the addition of larvae to pitchers with prey would increase nutrient content of leaves.

STATISTICAL ANALYSIS

Multivariate Analysis of Variance (MANOVA) was used to examine the overall effect of experimental manipulations on the uptake of nutrients by pitcher plants. This approach allowed for a comparison of the effects of experimental manipulation on a number of nutrient values without compounding the error associated with individual

ANOVA's. Roy's Greatest Root, which is a conservative estimate of variance and is robust for small sample sizes, was used to determine the significance of overall effects. Differences in means between treatment groups for each nutrient were evaluated at $\alpha = 0.05$. All statistical analyses were conducted using SAS (v 9.1).

Analysis was conducted in a two tier approach. In the first tier of the analysis, data from each year were examined to determine the influence of the aforementioned experimental treatments on the following nutrients: Nitrogen (N), Phosphorous (P), Calcium (Ca), Magnesium (Mg), and Potassium (K). These nutrients were identified from the primary literature as being limited in availability in acidic soils (Chapin and Pastor 1995, Christensen 1976, Plummer 1963). The second tier of the analysis was purely exploratory in nature. MANOVA was used to evaluate the effects of the experimental treatments on all of the measured nutrient levels. Micronutrient levels in pitcher plant leaves are poorly understood and little studied. My exploratory analysis provides baseline levels for measurable nutrients and allows for comparison of trends among the effects of experimental treatments on these nutrient levels. I will draw few conclusions from these exploratory analyses; rather I provide these data to spur further research into this area.

RESULTS

LARVAL FREQUENCY IN PITCHERS

In 2004, 70% of *S. leucophylla* leaves surveyed at Crawford Bog contained at least one sarcophagid larva. Of these pitchers, only one contained multiple larvae. In 2005, the percentage of leaves occupied by at least one larva dropped to 58%, but out of these leaves, four were occupied by multiple larvae, with one leaf containing 5 larvae. Eighty-six percent and 94% of *S. flava* leaves in 2004 and 2005 respectively were occupied by at least one larva. During each year, 4 of the occupied pitchers contained multiple larvae.

SPECIES OF SARCOPHAGIDAE

I reared *Sarcophaga sarraceniae*, *Fletcherimyia abdita* Pape , and *F. celarata* Aldrich from pitchers of *S. leucophylla*. All three species occurred at Crawford Bog in Covington County, AL while only *S. sarraceniae* and *F. abdita* occurred at Splinter Hill Bog in Baldwin County, AL. Two species of sarcophagids were reared from pitchers of *S. flava. Fletcherimyia. rileyi* Aldrich and *F. jonesi* Aldrich both occurred at Sumatra Bog in Appalachicola, FL, while only *F. rileyi* was found at Crawford Bog (Table 1). I reared *F. abdita* from pitchers of *S. alata* occurring in Desoto West Bog in Desoto National Forest, MS. I saw no overlap in the fly species inhabiting the syntopic pitcher plants, *S. leucophylla* and *S. flava*; however, one of the fly species found *in S. leucophylla* was also found in *S. alata* at a site where no *S. leucophylla* occurs.

NUTRITION STUDY

2004 First Tier Analysis

The MANOVA for the 2004 analysis revealed an overall significant effect of the experimental treatments on mean nutrient levels (Roy's Greatest Root = 1.60, DF 5,44, $p < 0.001$). Figure 1 shows the mean comparisons for nitrogen in 2004. Pitchers containing prey (treatment 3) and those containing prey plus larvae (treatment 4) did not significantly differ from each other but had significantly higher levels of nitrogen than pitchers in the other treatment groups. Figure 2 shows mean comparisons for phosphorous in 2004. Pitchers containing prey and larvae (treatment 4) and those containing prey only (treatment 3) did not differ from each other but contained significantly higher phosphorous levels than pitchers in the other treatment groups. Calcium levels were highest in pitchers supplemented with prey (treatment 3) and those with prey and larvae (treatment 4), but none of the treatments differed significantly from the control group (treatment 1) (Figure 3). Magnesium levels were highest in pitchers that were supplemented with prey (treatment 3), but no group differed significantly from control pitchers (treatment 1) (Figure 4). Potassium levels were highest in the unmanipulated pitchers (treatment 5) and those containing both prey and larvae (treatment 4), but none of the groups differed significantly in potassium levels compared to control condition (treatment 1) (Figure 5).

2005 First Tier Analysis

MANOVA results for 2005 indicated that there was a significant overall effect of the treatment groups on nutrient levels in the leaves of white-topped pitcher plants (Roy's Greatest Root 3.65, DF 5,33, $p < 0.0001$). Mean percent nitrogen was significantly higher in pitchers subjected to supplemental feeding (treatment 3) and those with both larvae and supplemental food (treatment 4) than in control pitchers (treatment 1) and unmanipulated pitchers (treatment 5) (Figure 1). Similarly, mean phosphorous levels did not differ between pitchers fed and with larvae (treatment 4) and those fed only (treatment 3), but did differ significantly from both control (treatment 1) and unmanipulated pitchers (treatment 5) (Figure 2). Calcium levels in unmanipulated pitchers (treatment 5) were significantly higher than those in control pitchers (treatment 1), but otherwise no mean differences were significant (Figure 3). Mean magnesium levels differed significantly between the unmanipulated group (treatment 5) and the prey and larvae group (treatment 4), but no other differences were significant (Figure 4). Mean potassium levels where highest in the prey and larvae (treatment 4) and prey group (treatment 3), while unmanipulated (treatment 5) and control group (treatment 1) did not differ from each other (Figure 5).

Second Tier Analysis

Results of the second tier analysis are shown in Table 4 and Table 5. It is interesting to note that in both years, Zn and Na show a similar pattern to N, P, and K in my first tier analysis. Additionally, there was a pattern for the control treatment (treatment 1) to contain significantly higher levels of metallic elements (Ni, Cd 2004 only

and Cr both years) than the other experimental groups, not including unmanipulated pitchers.

DISCUSSION

Published data on the flesh flies inhabiting pitcher plants have been rare and confusing. Dahlem and Naczi (2006) published a comprehensive literature review and revision of the sarcophagid flies associated with pitcher plants of North America. This work helped guide my identifications and also provided a basis for comparison with my data.

From the four sites sampled, I was able to identify one fly species in the genus *Sarcophaga* and four species in the genus *Fletcherimyia*. *Sarcophaga sarraceniae* is considered a generalist (Rymal and Folkerts 1982) and is the most common pitcher plant sarcophagid from sites in the southeastern U.S. (Dahlem and Naczi 2006, Yanoviak and Folkerts 1991). In my study it was the most abundant species found in *S. leucophylla* and *S. alata*, followed by *F. abdita*. Additionally, *F. celarata* was reared only from pitchers of *S. leucophylla*. *Fletcherimyia celarata* is considered to be only associated with *S. leucophylla* (Dahlem and Naczi 2006) and may be the third most common sarcophagid species collected in bogs of Mississippi, Alabama, and the Florida Panhandle (Yanoviak and Folkerts 1991).

In agreement with other authors, I found *F. jonesi* and *F. rileyi* in pitchers of *S. flava.* These flies are generally associated with pitchers of *S. flava* (Aldrich 1916, Dahlem and Naczi 2006, Hepburn and Jones 1919) and *S. minor* (Aldrich 1916, Fish 1976), the latter of which does not occur within my study area. I did not rear any *S.*

sarraceniae from pitchers of *S. flava* although previous published studies indicate that the species uses *S. flava* as a host pitcher (Aldrich 1916, Dahlem and Naczi 2006, Jones 1904, 1908). While I had high pupal mortality among larvae sampled from *S. flava*, it is possible that my failure to find *S. sarraceniae* in these pitchers could be attributed to a host preference for pitchers of *S. leucophylla* over *S. flava* when they occur syntopically.

One objective of my study was to determine whether sarcophagid larvae influence the dynamics of nutrition within the leaves of pitcher plants. It is one matter to determine whether a single sarcophagid larva may affect nutrition within a single pitcher, but in order to determine whether or not fly larvae influence a significant portion of a population, I needed to determine the frequency of sarcophagid flies inhabiting my main study area, Crawford Bog. Previous research has concentrated on the frequency of fly species in northern and eastern bogs (Forsyth and Robertson 1975, Hamilton and Duffield 2002, Hardwick and Giberson 1996, Judd 1959, Rango 1999b). However, published data on larval densities in southern bogs are limited (Fish 1976), and none have been reported concerning the pitcher plant species *S. leucophylla* and *S. flava*.

In 2004 and 2005 respectively, 70% and 58% of *S. leucophylla* leaves surveyed at Crawford Bog contained larvae. Within the same years, 86% and 94% of *S. flava* leaves were occupied. Research in northern bogs has shown that larviposition does not begin until early June (Forsyth & Robertson 1975) and peak larval density occurs between late July and August (Forsyth & Robertson 1975, Hardwick & Giberson 1996, Rango 1999b). During peak larval density in northern bogs, 67%-85% of pitchers surveyed contained larvae (Forsyth & Robertson 1975, Hardwick & Giberson 1996, Rango 1999b). Because I surveyed for larvae only once during each field season, peak larval density and the onset

and duration of larviposition cannot be determined from my work. However, suitable pitchers for larviposition are available over a much larger period of time because of the extended growing season in the southern U.S. As a result, larviposition most likely begins earlier and ends later in southern bogs, and there may be multiple (Yanoviak and Folkerts 1991) and overlapping generations of flies during the season. The larval densities recorded at Crawford Bog equal or exceed those reported for the peak larval densities in northern bogs and lead to the conclusion that the majority of pitchers in southern bogs are occupied and potentially affected by the presence of sarcophagid larvae at some point during the growing season.

My final objectives in this study were to: 1) Document nutrient levels in pitchers; 2) Evaluate the influence of prey on nutrient content of pitcher leaves; and 3) Examine the interaction of sarcophagid larvae with pitcher plants. There are few data in the literature documenting nutrient levels in the leaves of pitcher plants in the southeastern United States. I sampled ten unmanipulated leaves of *S.leucophylla* each year of the study and analyzed them to provide baseline nutrient data. As would be expected, nutrient content within these pitchers was variable, likely due to the fact that pitchers contained variable amounts of prey (Table 6.).

Next, I was interested in determining whether the addition of supplemental prey produces a detectable effect on the nutrition of pitchers and whether the presence of sarcophagid larvae is detrimental or beneficial to the plants. I performed the study over a two year period and examined the results independently because of the evolution of my experimental methods and differences in my sampling period. My results indicate a strong positive relationship between the addition of prey and concentrations of

macronutrients (N, P, and K) in the sampled leaves. In each of these groups, pitchers supplemented with prey (treatment 3) and with prey plus larvae (treatment 4) contained significantly higher nutrient levels than in the other experimental conditions (except 2004 potassium control). This complements previous studies which demonstrated the benefits of carnivory to plant nutrition (Adamec 2002, Christensen 1976, Hanslin and Karlsson 1996, Hepburn et al.1920, Schulze et al. 1997, Thoren and Karlsson 1998, Thum 1988, Williams 1966). It is interesting to note that when the amount of prey was decreased by half in the second year of the study, the trend for increased nutrient levels was still strongly evident.

More importantly, this study provides novel data indicating that the presence of sarcophagid larvae is not detrimental to the nutrition of the plant. In my study, pitchers supplemented with prey plus larvae (treatment 4) showed a strong trend toward higher nutrient levels than pitchers with prey only (treatment 3). As demonstrated by Bradshaw and Creelman (1984) in the phytotelm holding pitcher plant species, *S. purpurea*, the addition of larval associates may increase the rate of break down of prey, as well as, increase nitrogen availability to the plant due to nitrogen compounds, mainly ammonia, found in the excreta of the larva. Additionally, the movement of larvae among the prey mass may increase colonization by bacteria and stimulate the secretion of additional digestive compounds, thus causing prey to decompose more rapidly. Perhaps a larger sample size would have allowed for statistical differentiation between these two groups (prey only and prey plus larvae). Moreover, my evaluation of nutrient uptake in leaves was conducted through an indirect approach of measuring overall leaf nutrient content. It may be valuable to conduct further research involving a similar experimental setup but

using a more direct approach of measurement, such as tracing radioisotopes as done in previous studies (Plummer and Kethley 1964, Williams 1966). As it is, my data provide a clear indication that the presence of sarcophagid larvae does not lower the nutrients available from prey to pitcher plants. The trend for increase in nutrient uptake in the presence of larvae is less clear.

CONCLUSIONS

In this study, I find evidence to strengthen the argument that carnivory is beneficial to the nutrition of pitcher plants. As has been shown in previous studies, N, P, and K appear to be the major macronutrients absorbed by leaves of plants, reflecting their limited availability within the habitat. Moreover, initial evidence is provided that shows sarcophagid fly species associated with pitcher plants do not cause harm to the plants. In fact, there is a trend for increased nutrition in pitcher leaves that are occupied by these fly larvae. An understanding of the ecology of pitcher associates is crucial in the effort to preserve these endangered ecosystems.

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Figure 1. Least square mean differences in nitrogen content showing 95% CL of leaves of *Sarracenia leucophylla* subjected to experimental treatments during the spring of 2004 & 2005. All samples were collected from Crawford Bog in the Conecuh National Forest (Covington Co., AL). All leaves, except unmanipulated, were bagged prior to leaf opening in the spring to exclude prey and plant associates. Control leaves were bagged prior to opening and never subjected to additional manipulation. In 2004, larva indicates leaves in which one sarcophagid fly larva was added after leaf opening. Unmanipulated leaves were not bagged, representing a natural population. In 2004, 4g prey indicates 4 grams of crickets added after leaf opening. In 2005, 2g prey indicates 2 grams of crickets added after leaf opening. In 2004, 4g prey & larva indicates leaves in which 4 grams of crickets and one sarcophagid larva were added after leaf opening. In 2005, 2g prey & larva indicates leaves in which 2 grams of crickets and one sarcophagid larva were added after leaf opening.

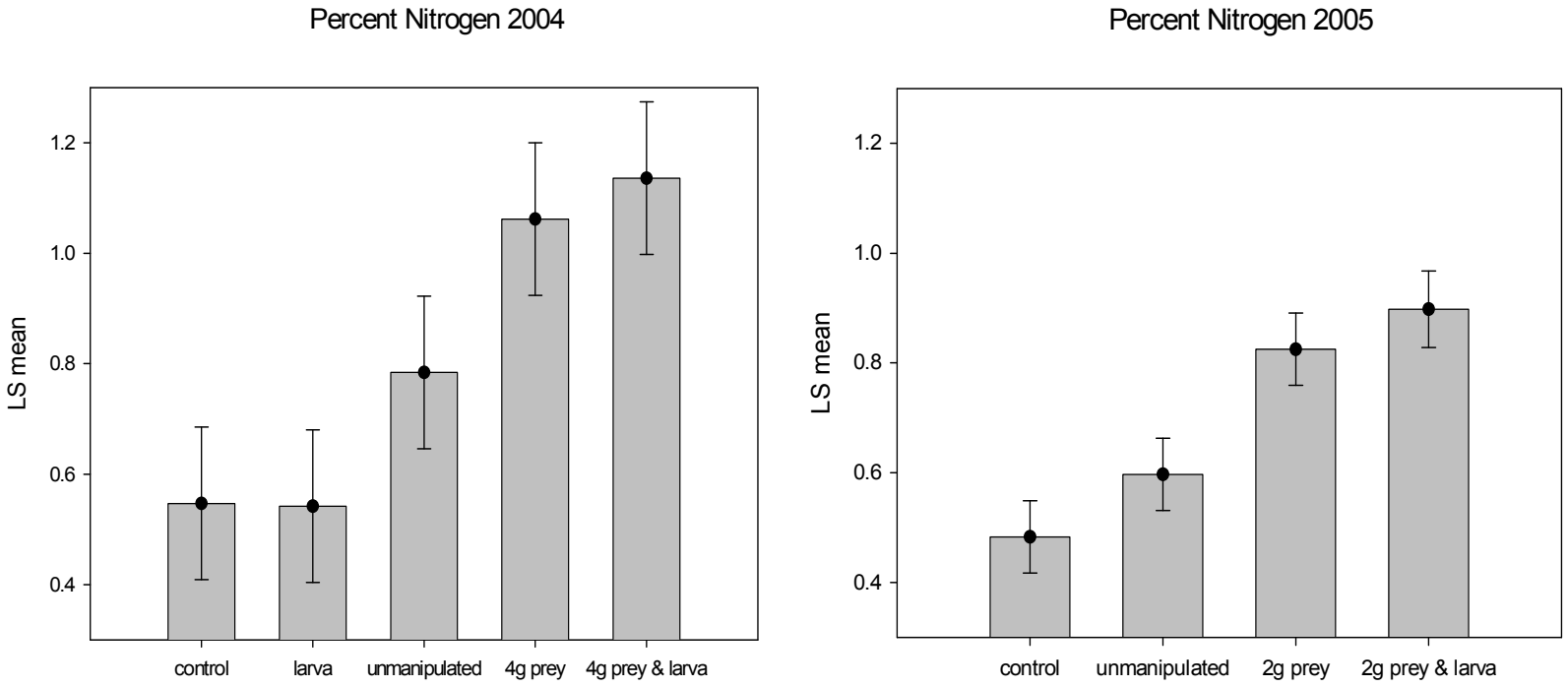


Figure 2. Least square mean differences in phosphorus content showing 95% CL of leaves of *Sarracenia leucophylla* subjected to experimental treatments during the spring of 2004 & 2005. All samples were collected from Crawford Bog in the Conecuh National Forest (Covington Co., AL). All leaves, except unmanipulated, were bagged prior to leaf opening in the spring to exclude prey and plant associates. Control leaves were bagged prior to opening and never subjected to additional manipulation. In 2004, larva indicates leaves in which one sarcophagid fly larva was added after leaf opening. Unmanipulated leaves were not bagged, representing a natural population. In 2004, 4g prey indicates 4 grams of crickets added after leaf opening. In 2005, 2g prey indicates 2 grams of crickets added after leaf opening. In 2004, 4g prey & larva indicates leaves in which 4 grams of crickets and one sarcophagid larva were added after leaf opening. In 2005, 2g prey & larva indicates leaves in which 2 grams of crickets and one sarcophagid larva were added after leaf opening.

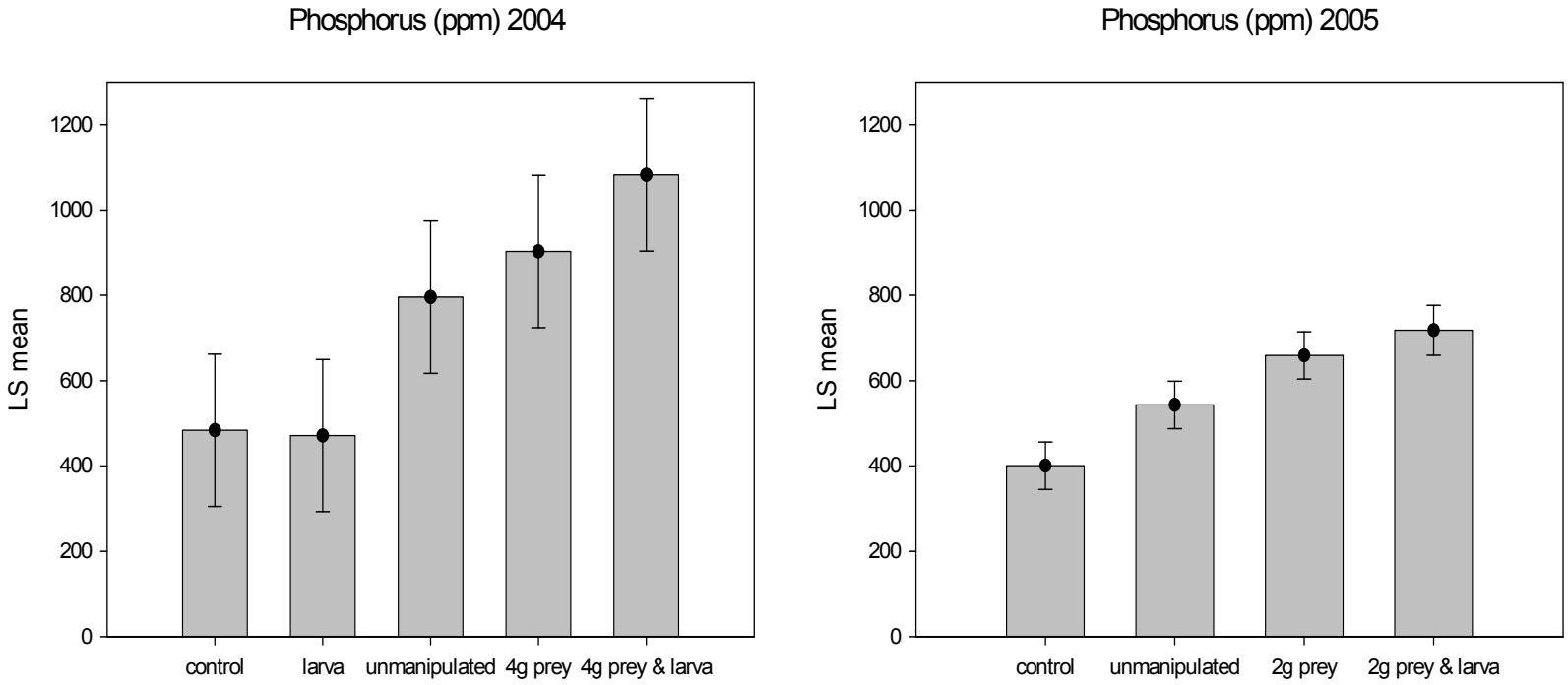
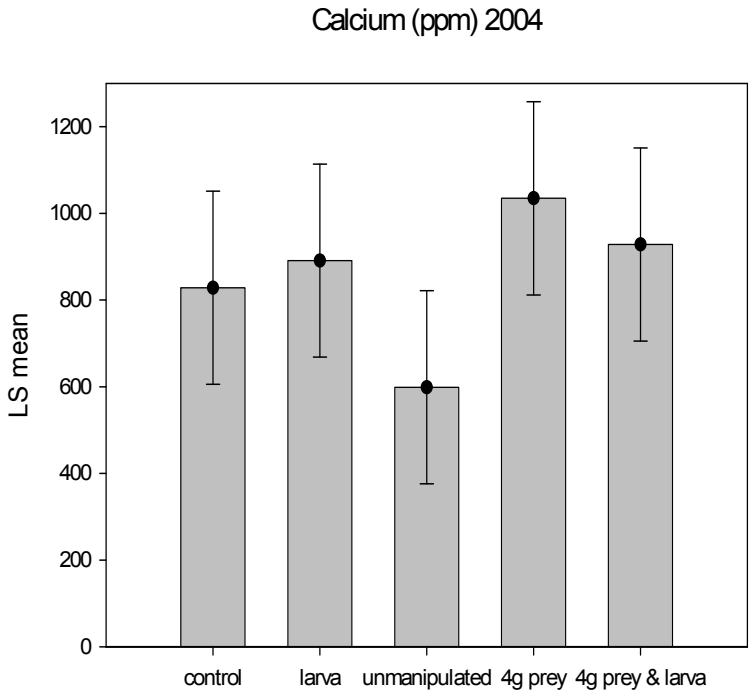


Figure 3. Least square mean differences in calcium content showing 95% CL of leaves of *Sarracenia leucophylla* subjected to experimental treatments during the spring of 2004 & 2005. All samples were collected from Crawford Bog in the Conecuh National Forest (Covington Co., AL). All leaves, except unmanipulated, were bagged prior to leaf opening in the spring to exclude prey and plant associates. Control leaves were bagged prior to opening and never subjected to additional manipulation. In 2004, larva indicates leaves in which one sarcophagid fly larva was added after leaf opening. Unmanipulated leaves were not bagged, representing a natural population. In 2004, 4g prey indicates 4 grams of crickets added after leaf opening. In 2005, 2g prey indicates 2 grams of crickets added after leaf opening. In 2004, 4g prey & larva indicates leaves in which 4 grams of crickets and one sarcophagid larva were added after leaf opening. In 2005, 2g prey & larva indicates leaves in which 2 grams of crickets and one sarcophagid larva were added after leaf opening.



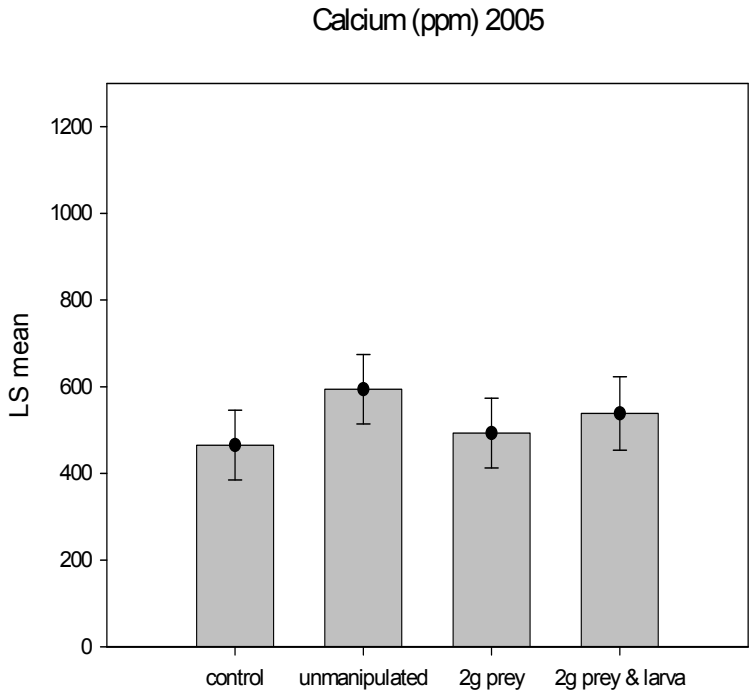


Figure 4. Least square mean differences in magnesium content showing 95% CL of leaves of *Sarracenia leucophylla* subjected to experimental treatments during the spring of 2004 & 2005. All samples were collected from Crawford Bog in the Conecuh National Forest (Covington Co., AL). All leaves, except unmanipulated, were bagged prior to leaf opening in the spring to exclude prey and plant associates. Control leaves were bagged prior to opening and never subjected to additional manipulation. In 2004, larva indicates leaves in which one sarcophagid fly larva was added after leaf opening. Unmanipulated leaves were not bagged, representing a natural population. In 2004, 4g prey indicates 4 grams of crickets added after leaf opening. In 2005, 2g prey indicates 2 grams of crickets added after leaf opening. In 2004, 4g prey & larva indicates leaves in which 4 grams of crickets and one sarcophagid larva were added after leaf opening. In 2005, 2g prey & larva indicates leaves in which 2 grams of crickets and one sarcophagid larva were added after leaf opening.

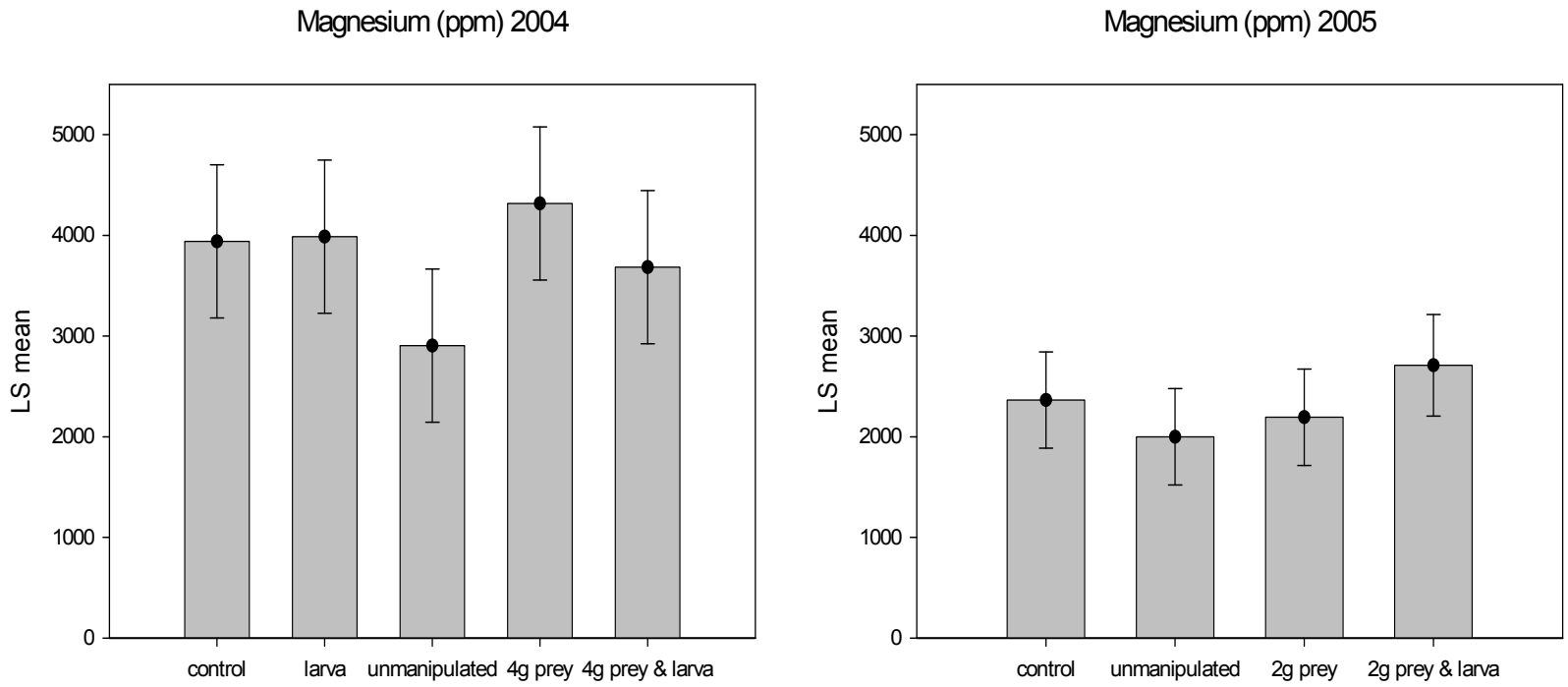


Figure 5. Least square mean differences in potassium content showing 95% CL of leaves of *Sarracenia leucophylla* subjected to experimental treatments during the spring of 2004 & 2005. All samples were collected from Crawford Bog in the Conecuh National Forest (Covington Co., AL). All leaves, except unmanipulated, were bagged prior to leaf opening in the spring to exclude prey and plant associates. Control leaves were bagged prior to opening and never subjected to additional manipulation. In 2004, larva indicates leaves in which one sarcophagid fly larva was added after leaf opening. Unmanipulated leaves were not bagged, representing a natural population. In 2004, 4g prey indicates 4 grams of crickets added after leaf opening. In 2005, 2g prey indicates 2 grams of crickets added after leaf opening. In 2004, 4g prey & larva indicates leaves in which 4 grams of crickets and one sarcophagid larva were added after leaf opening. In 2005, 2g prey & larva indicates leaves in which 2 grams of crickets and one sarcophagid larva were added after leaf opening.

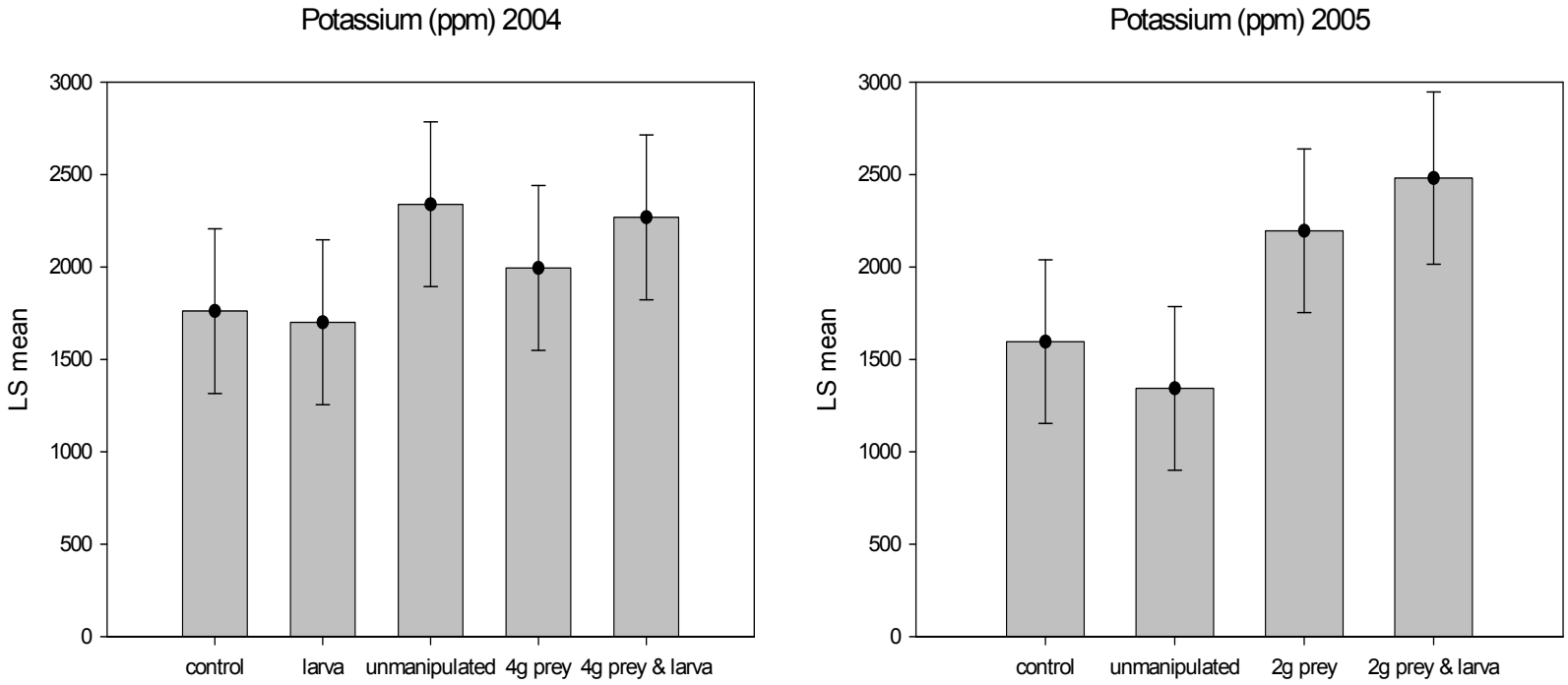


Table 1. Pitcher plants and associated sarcophagid fly species from four bogs in the southeastern U. S.

	Crawford Bog Covington Co., AL	**Splinter Hill Bog Baldwin Co., AL**	**Sumatra Bog Liberty Co., FL**	**Desoto West Bog Harrison Co., MS**
Sarracenia leucophylla	*Sarcophaga sarraceniae* (3 ♂, 5 ♀) *Fletcherimyia abdita* (2 ♂) *Fletcherimyia celarata* (1 ♂)	*Sarcophaga sarraceniae* (8 ♂, 4 ♀) *Fletcherimyia abdita* (5♂, 2♀)		
Sarracenia flava	*Fletcherimyia rileyi* (3♂, 1♀)		*Fletcherimyia rileyi* (1♂, 1♀) *Fletcherimyia jonesi* (1♀)	
Sarracenia alata				*Sarcophaga sarraceniae* (4♂, 3♀) *Fletcherimyia. abdita* (1♂, 1♀)

Table 2. Least squares mean comparisons for 2004 first tier nutrient analysis of *S. leucophylla*. Significant differences ($p < 0.05$) are indicated with *.

Mean Nitrogen (%)		**Larvae**	**Prey**	**Prey and Larvae**	**Unmanipulated**
0.55	Control	0.9591	*<0.0001	*<0.0001	*0.0185
0.54	Larvae		*<0.0001	*<0.0001	*0.0163
1.06	Prey			0.4492	*0.0063
1.14	Prey and Larva				*0.0007
0.78	Unmanipulated				
Mean Phosphorus (ppm)					
483.87	Control	0.9214	*0.0017	*<0.0001	*0.0165
471.44	Larvae		*0.0012	*<0.0001	*0.0129
902.93	Prey			0.1595	0.3973
1082.11	Prey and Larva				*0.0271
795.89	Unmanipulated				
Mean Calcium (ppm)					
828.52	Control	0.6904	0.1939	0.5272	0.1491
891.24	Larvae		0.3635	0.8142	0.0682
1034.88	Prey			0.4991	*0.0078
928.24	Prey and Larva				*0.0409
598.80	Unmanipulated				
Mean Magnesium (ppm)					
3939.97	Control	0.9304	0.4844	0.6333	0.0591
3986.93	Larvae		0.5402	0.5727	*0.0489
4316.66	Prey			0.2421	*0.0113
3683.33	Prey and Larva				0.1524
2905.48	Unmanipulated				
Mean Potassium (ppm)					
1761.20	Control	0.8468	0.4599	0.1119	0.0715
1700.39	Larvae		0.3524	0.0761	*0.0473
1994.43	Prey			0.3857	0.2771
2268.51	Prey and Larva				0.8236
2338.67	Unmanipulated				

Table 3. Least squares mean comparisons for 2005 first tier nutrient analysis. of *S. leucophylla*. Significant differences ($p < 0.05$) are indicated with *.

Mean Nitrogen (%)		**Prey**	**Prey and Larvae**	**Unmanipulated**
0.48	Control	*<0.0001	*<0.0001	*0.0181
0.83	Prey		0.1323	*<0.0001
0.90	Prey and Larva			*<0.0001
0.60	Unmanipulated			
Mean Phosphorus (ppm)				
400.48	Control	*<0.001	*<0.0001	*0.0008
659.26	Prey		0.1459	*0.0048
718.26	Prey and Larva			*<0.0001
543.10	Unmanipulated			
Mean Calcium (ppm)				
465.32	Control	0.6226	0.2128	*0.0273
493.12	Prey		0.4374	0.0792
538.30	Prey and Larva			0.3368
594.32	Unmanipulated			
Mean Magnesium (ppm)				
2363.73	Control	0.6139	0.3202	0.2823
2193.93	Prey		0.1416	0.5637
2709.36	Prey and Larva			*0.0458
1999.48	Unmanipulated			
Mean Potassium (ppm)				
1595.99	Control	0.0596	*0.0084	0.4173
2195.88	Prey		0.3751	*0.0090
2480.29	Prey and Larva			*0.0010
1343.03	Unmanipulated			

Table 4. Least squares mean comparisons for 2004 second tier nutrient analysis of *S. leucophylla*. Significant differences ($p < 0.05$) are indicated with *.

Mean Aluminum (ppm)		**Larvae**	**Prey**	**Prey and Larvae**	**Unmanipulated**
55.16	Control	0.4292	0.1825	0.4459	0.3423
67.98	Larvae		0.5809	0.9772	0.0856
76.91	Prey			0.5616	*0.0253
67.51	Prey and Larva				0.0907
39.73	Unmanipulated				
Mean Boron (ppm)					
7.76	Control	0.2562	0.0546	0.6567	0.1297
9.20	Larvae		*0.0031	0.1171	*0.0099
5.28	Prey			0.1340	0.6693
7.20	Prey and Larva				0.2789
5.82	Unmanipulated				
Mean Barium (ppm)					
18.09	Control	0.1657	0.3145	0.1558	0.2437
23.07	Larvae		*0.0193	*0.0065	*0.0129
14.50	Prey			0.6719	0.8705
12.99	Prey and Larva				0.7942
13.92	Unmanipulated				
Mean Cadmium (ppm)					
0.35	Control	0.0785	0.2102	0.6642	*0.0243
0.48	Larvae		*0.0036	*0.0302	*0.0002
0.25	Prey			0.4086	0.2951
0.31	Prey and Larva				0.0647
0.17	Unmanipulated				
Mean Cobalt (ppm)					
0.08	Control	0.6944	0.3016	0.9776	0.1207
0.09	Larvae		0.1566	0.6738	0.0542
0.04	Prey			0.3147	0.5941
0.08	Prey and Larva				0.1273
0.02	Unmanipulated				
Mean Chromium (ppm)					
5.45	Control	*0.0078	*0.0089	*0.0106	*0.0011
1.85	Larvae		0.9614	0.9088	0.4907
1.92	Prey			0.9437	0.4610
2.00	Prey and Larva				0.4222
0.95	Unmanipulated				

Mean Copper (ppm)		**Larvae**	**Prey**	**Prey and Larvae**	**Unmanipulated**
16.96	Control	0.8412	0.5062	0.7450	0.3558
17.53	Larvae		0.3880	0.9005	0.4683
15.07	Prey			0.3239	0.1159
17.88	Prey and Larva				0.5477
19.59	Unmanipulated				
Mean Iron (ppm)					
87.26	Control	0.0853	0.3604	0.7788	*0.0080
54.82	Larvae		0.4078	*0.0470	0.3155
70.22	Prey			0.2339	0.0708
92.47	Prey and Larva				*0.0038
36.11	Unmanipulated				
Mean Manganese (ppm)					
97.78	Control	0.0700	0.1450	0.9379	0.2397
135.69	Larvae		0.7110	0.0822	*0.0039
128.07	Prey			0.1670	*0.0104
99.38	Prey and Larva				0.2107
73.44	Unmanipulated				
Mean Sodium (ppm)					
811.03	Control	0.2992	*0.0044	*0.0007	0.9131
1019.28	Larvae		0.0579	*0.0134	0.3519
1405.19	Prey			0.5341	*0.0060
1529.43	Prey and Larva				*0.0010
832.79	Unmanipulated				
Mean Nickel (ppm)					
5.12	Control	*0.0085	*0.0054	*0.0190	*0.0020
2.14	Larvae		0.8622	0.7528	0.5967
1.96	Prey			0.6255	0.7217
2.49	Prey and Larva				0.3999
1.57	Unmanipulated				
Mean Lead (ppm)					
0.55	Control	0.6654	0.1845	0.7018	0.6406
0.43	Larvae		0.3680	0.9603	0.3700
0.20	Prey			0.3425	0.0762
0.45	Prey and Larva				0.3969
0.67	Unmanipulated				

Mean Zinc (ppm)		**Larvae**	**Prey**	**Prey and Larvae**	**Unmanipulated**
19.42	Control	0.9157	*0.0040	*<0.0001	0.0679
18.60	Larvae		*0.0030	*<0.0001	0.0541
42.75	Prey			*0.0335	0.2504
59.60	Prey and Larva				*0.0016
33.80	Unmanipulated				
Mean Carbon (%)					
45.44	Control	0.4580	0.8068	0.3957	0.2199
45.22	Larvae		0.3253	0.1153	0.0524
45.51	Prey			0.5440	0.3236
45.68	Prey and Larva				0.7009
45.79	Unmanipulated				

Table 5. Least squares mean comparisons for 2005 second tier nutrient analysis of *S. leucophylla*. Significant differences ($p < 0.05$) are indicated with *.

Mean Aluminum (ppm)		**Prey**	**Prey and Larvae**	**Unmanipulated**
34.85	Control	0.4135	0.5953	*0.0319
41.46	Prey		0.7892	0.1683
39.25	Prey and Larva			0.1102
52.7	Unmanipulated			
Mean Boron (ppm)				
5.71	Control	0.4569	0.1761	0.7070
6.37	Prey		*0.0418	0.2656
4.46	Prey and Larva			0.3186
5.38	Unmanipulated			
Mean Cadmium (ppm)				
0.02	Control	0.6354	0.4896	0.4779
0.03	Prey		0.2524	0.2398
0.01	Prey and Larva			1.0000
0.01	Unmanipulated			
Mean Chromium (ppm)				
0.95	Control	0.0895	0.0733	*0.0158
0.42	Prey		0.8844	0.4340
0.37	Prey and Larva			0.5368
0.17	Unmanipulated			
Mean Copper (ppm)				
14.16	Control	0.1032	0.4231	0.2611
7.48	Prey		*0.0199	*0.0079
17.48	Prey and Larva			0.7651
18.71	Unmanipulated			
Mean Iron (ppm)				
1.33	Control	0.1111	0.1331	0.3279
8.66	Prey		0.9579	0.5247
8.41	Prey and Larva			0.5708
5.78	Unmanipulated			
Mean Manganese (ppm)				
81.05	Control	0.4288	0.7621	0.6903
62.60	Prey		0.6384	0.2373
73.83	Prey and Larva			0.4909
90.31	Unmanipulated			

Mean Sodium (ppm)		**Prey**	**Prey and Larvae**	**Unmanipulated**
551.82	Control	*<0.0001	*<0.0001	0.6432
1294.74	Prey		0.4367	*0.0003
1429.12	Prey and Larva			*<0.0001
629.50	Unmanipulated			
Mean Nickel (ppm)				
1.86	Control	0.3337	0.6944	0.6219
2.46	Prey		0.5804	0.6323
2.11	Prey and Larva			0.9303
2.16	Unmanipulated			
Mean Lead (ppm)				
0.11	Control	0.5702	*0.0204	0.8765
0.78	Prey		0.0803	0.6794
2.92	Prey and Larva			*0.0340
0.29	Unmanipulated			
Mean Zinc (ppm)				
13.07	Control	0.0638	*0.0017	0.2155
18.44	Prey		0.1315	0.5184
22.89	Prey and Larva			*0.0361
16.61	Unmanipulated			
Mean Carbon (%)				
45.79	Control	0.3903	0.3562	0.8036
45.61	Prey		0.9300	0.2702
45.59	Prey and Larva			0.2464
45.84	Unmanipulated			

Table 6. Least square means of nutrient levels for unmanipulated pitchers of *S. leucophylla* in 2004 and 2005 with 95% confidence limits (CL). Cells marked with * indicate values not measured.

Nutrient	**LS Mean**		**Lower CL**		**Upper CL**	
	2004	**2005**	**2004**	**2005**	**2004**	**2005**
Nitrogen (%)	0.78	0.60	0.65	0.53	0.92	0.66
Phosphorus (ppm)	795.89	543.08	617.48	487.65	974.29	598.51
Calcium (ppm)	598.80	594.32	375.95	513.96	821.65	674.69
Magnesium (ppm)	2905.48	1999.48	2144.56	1520.60	3666.40	2478.36
Potassium (ppm)	2338.67	1343.03	1893.05	900.69	2784.29	1785.37
Aluminum (ppm)	39.73	52.70	16.85	41.23	62.62	64.16
Boron (ppm)	5.82	5.38	4.04	4.12	7.61	6.63
Barium (ppm)	13.92	*	8.89	*	18.95	*
Cadmium (ppm)	0.17	0.01	0.06	0.00	0.28	0.03
Cobalt (ppm)	0.02	*	0.00	*	0.07	*
Chromium (ppm)	0.95	0.17	0.00	0.00	2.80	0.61
Copper (ppm)	19.59	18.71	15.57	12.99	23.61	24.44
Iron (ppm)	36.11	5.78	9.85	0.00	62.36	12.21
Manganese (ppm)	73.44	90.31	44.35	57.22	102.53	123.39
Sodium (ppm)	832.79	629.50	550.43	390.84	1115.15	868.15
Nickel (ppm)	1.57	2.16	0.03	1.28	3.11	3.03
Lead (ppm)	0.67	0.29	0.30	0.00	1.04	1.95
Zinc (ppm)	33.80	16.61	22.85	12.58	44.74	20.63
Carbon (%)	45.80	45.84	45.39	45.54	46.20	46.15