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The Role of Red Leaf Coloration in Prey Capture for Pinguicula planifolia

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Abstract - Anthocyanins in the leaves of carnivorous plants are suggested to play a role in prey capture. In this study, we investigated the role of red leaf coloration (an indicator of anthocyanins) on prey capture using *Pinguicula planifolia* (Chapman's Butterwort). Overall, red leaves had less prey (i.e., Collembola) than green leaves, suggesting that red coloration does not enhance prey capture for Chapman's Butterwort. However, the frequent presence of Collembola on leaves suggests that this plant species could be relying on other cues to attract prey (e.g., olfactory cues).

Introduction

Anthocyanins are prominent in the vegetative tissues of several evolutionarily distinct carnivorous plant families, and an emerging argument suggests that these pigments serve a role in prey capture (Joel 1988, Jürgens et al. 2015, Moran et al. 1999). Schaefer and Ruxton (2008) demonstrated that the markedly red coloration on the pitchers of *Nepenthes* (tropical pitcher plants) species may enhance prey capture compared to pitchers without red coloration. Ichiishi et al. (1999) suggested that anthocyanins present in the trapping leaves of *Dionaea muscipula* J. Ellis (Venus Fly-trap) and *Drosera spatulata* Labill. (Spoon-leaved Sundew) are produced when the plants become nitrogen deficient, and that this pigment production subsequently increases prey attraction in both species, providing a means to regain nutrients lacking in the substrate. Jürgens et al. (2015) proposed that anthocyanins reduce the risk of pollinator–prey conflict in sundew species because the pigments tended to deter pollinating insects while still attracting insect prey, but the red-pigmented leaves also lowered total prey-capture. The debate associated with the involvement of red pigmentation in prey capture is due partly to the widely accepted argument that insects' color vision is poor in the red spectrum of light (Bennett and Ellison 2009, Chittka et al. 2001). Although it is unlikely that color is the only determining factor in attracting insect prey by carnivorous plants, these studies do present more questions than answers in terms of how these red pigments are beneficial for plant taxa such as Butterwort species that rely on captured prey for nutrients (Ademec and Pavlovič 2018, Legendre 2000). The focus of this study was to determine how red leaf coloration affects prey capture for Chapman's Butterwort—a species in which leaf color in natural populations ranges from green

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to red (Gluch 2005). In addition, Annis (2016) verified the presence of anthocyanins associated with red coloration for this species.

Materials and Methods

We studied a population of Chapman's Butterwort at the St. Joseph Bay State Buffer Preserve in Gulf County, FL. In mid-March 2015, we marked 20 red plants and 20 green plants by placing bamboo skewers near plants, and measured and recorded the length and width of the sampled leaves. We placed 2 wooden insect-traps (3.5 cm x 5.0 cm x 0.5 cm wood palette) within a 15-cm radius of each marked plant; traps were secured to the ground with metal sod staples. Regardless of the color of the focal plant, 1 trap was painted red, 1 was painted green, and both were smeared with TangleTrap Sticky Coating (The Tangle Company, Grand Rapids, MI). We chose specific paint colors for the green and red traps by using a color palette and matching the closest available paint color to natural leaf colors of Chapman's Butterwort observed in the field. Although we did our best to match wooden traps and leaf colors, we did not use a spectroradiometer to verify the reflectance and absorption spectra of the traps and leaves (Horner et al. 2018). We set traps for 3 d chosen when no rain was in the forecast and then collected, covered with wax paper, and stored them in plastic bags. We carefully applied white electrical tape to the marked leaf, peeled it off, and placed leaf samples in 70% ethanol on same day as we collected traps. We repeated this prey-capture procedure for the same plants (different leaves) after 2 weeks. We used leaf areas (calculated using dimensions $A = \pi ab$ where A = area, a = width, b = length) to obtain number of prey captured/cm^2. We performed a two-way analysis of variance to determine the effects of trap type (wooden or leaf) and trap color (red or green) on prey capture. We identified to the arthropod-order level and determined abundance for each prey order on leaves and wooden traps. Based on this information, we determined taxa percentages caught per trap and per leaf. We conducted all statistical analyses in SPSS Statistics Version 22.

Results

A 2-factor analysis of variance showed a significant effect of trap color (F_1 = 5.3, $P \leq 0.05$)] and trap type (F_1 = 10.7, $P \leq 0.05$) on prey capture, but no significant interaction occurred between trap color and trap type (F_1 = 0.0, $P > 0.05$). Green coloration captured more prey than red coloration, and painted wood blocks captured more prey than leaves (Table 1). All trap colors and types captured a large percentage of Collembola; wooden traps also captured Nematocera followed by Brachycera, whereas leaves captured Arachnida followed by Nematocera and Brachycera (Fig. 1).

Discussion

In contrast to arguments that certain carnivorous plants utilize anthocyanins to attract prey (Ichiishi et al. 1999, Schaefer and Ruxton 2008), our results do not support that contention; green leaves of Chapman's Butterwort caught more prey than red leaves, suggesting that red leaf coloration could be a deterrent to prey. Similar results have been found for several sundew species (Foot et al. 2014, Horner et al. 2018, Jurgens et al. 2015). Perhaps differences found for prey preference to color by different species of carnivorous plants relates to differences in the type of

Table 1. Number of arthropods captured by color (green vs. red) and trap type (wooden blocks vs. leaves) on or near Chapman's Butterwort plants. Mean ± SE are reported and means within trap color or trap type with different superscript letter differ based on ANOVA ($P \leq 0.05$). n = sample size.

Variable	n	Arthropods/cm^2
Trap color		
Green	59	0.6 ± 0.1^A
Red	60	0.4 ± 0.1^B
Trap type		
Artificial	79	0.7 ± 0.0^A
Leaf	40	0.4 ± 0.1^B

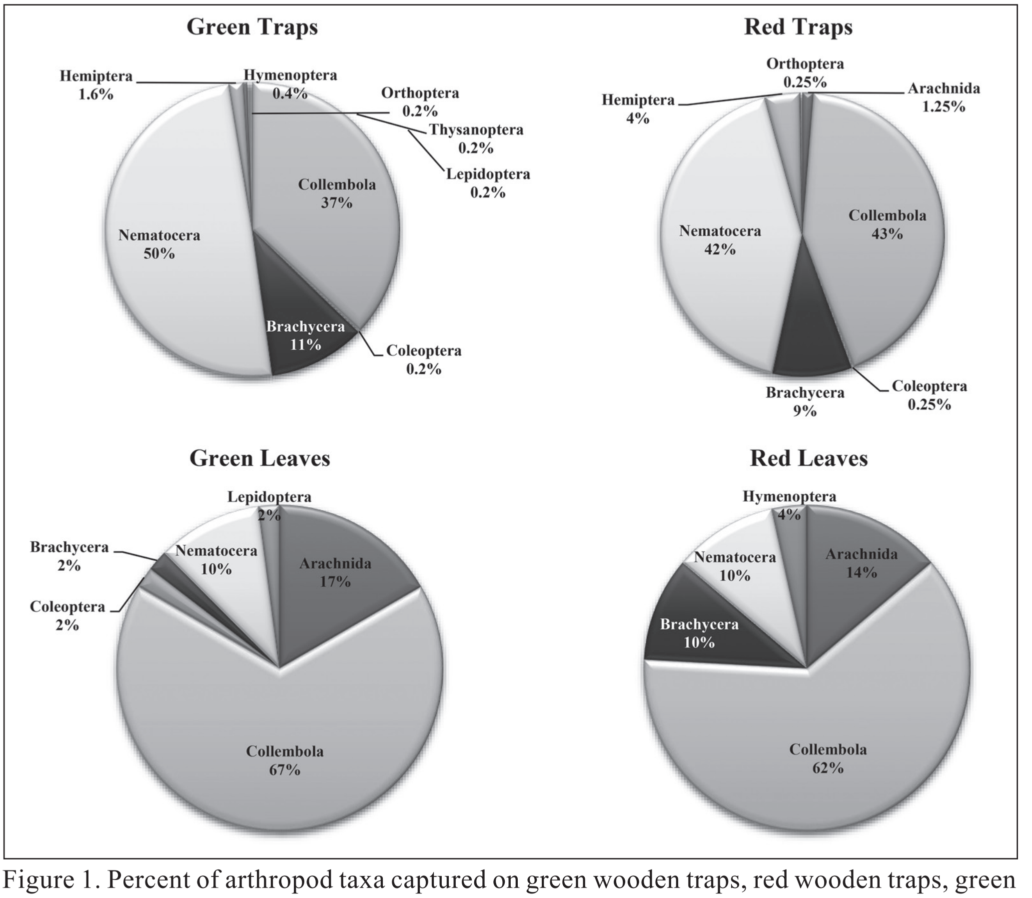

Figure 1. Percent of arthropod taxa captured on green wooden traps, red wooden traps, green leaves, and red leaves on or near Chapman's Butterwort plants. All percent values have been rounded.

prey captured. The prey capture by Chapman's Buttwort is similar to other butterwort species in major taxa captured (i.e., Collembola and Arachnida), but different in other taxa (Diptera) and in the percentage of each taxon captured. Zamora (1990) found that the major prey group of the European *Pinguicula nevadensis* (H. Lindb.) Casper (Grasilla) was Diptera (62.2%), followed by Arachnida (17.1%), and Collembola (7.6%). Pavón et al. (2011) found a similar pattern in Central Mexico for the butterwort *Pinguicula moranensis* Kunth (53.6% Diptera, 29.1% Collembola, and 13.2% Arachnida). For Chapman's Butterwort, we found that Collembola was the top prey (Fig. 1).

Anthocyanins do not seem to play a role in attracting prey for Chapman's Butterwort, and Joel et al. (1985) reported that no obvious UV patterns are displayed by butterworts to attract insects; thus, we hypothesize that Chapman's Butterwort could be relying on nonvisual cues, such as olfactory cues, to attract prey. For example, Lloyd (1942) and Fleischmann (2016) mentioned that butterwort mucilage emits a "delicate fungus-like odor" that may attract insects. The majority of prey captured on red (62%) and green (67%) Chapman's Butterwort leaves was Collembola. The large majority of most Collembola species' diet consists of fungal hyphae (Newell 1984), and studies show that these soil arthropods are attracted to the odor of fungi (Bengtsson et al. 1988, Hedlund et al. 1995). Although anthocyanins do not seem to play a role in attracting prey in Chapman's Butterwort, they could be performing a photoprotection role (Horner et al. 2018).

Perspective

Future studies should address the: (1) role of olfactory cues to attract prey, possibly including amounts or consistency of mucilage; (2) role of environmental factors (i.e., temperature, humidity, and rainfall) on prey availability; (3) seasonal patterns of prey capture and availability; and (4) the cues that attract different prey as captured by different species of insectivorous plants. These studies could provide a better understanding of prey-capture dynamics associated with this species and other carnivorous plant species.

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