

ABSTRACT

HAMON, LAURA ELIZABETH. The Pollination Ecology of the Venus Flytrap (*Dionaea muscipula*) and a Status Survey of its Native Populations (Under the direction of Rebecca Irwin and Clyde Sorenson).

Venus flytrap (*Dionaea muscipula* J. Ellis) is a carnivorous plant native only to North and South Carolina. Numerous studies have focused on the physiology of *D. muscipula* traps. However, little is known about the reproductive biology of *D. muscipula*. This is concerning because *D. muscipula* is rare in the wild, and inadequate pollination is frequently correlated with reduced population viability in rare plants. In this thesis, I present the results of three studies that examine factors affecting the pollination ecology of *D. muscipula*, specifically addressing the following questions: 1) Does burn history affect the pollination success of *D. muscipula*? 2) Do pollen limitation and conspecific density interact to affect *D. muscipula* reproduction? And 3) Is *D. muscipula* reproduction more limited by pollen or resource limitation? I also present the results of a range-wide survey of *D. muscipula* populations conducted in collaboration with the North Carolina Natural Heritage Program (NCNHP).

Like many co-occurring plants in its habitat, *D. muscipula* depends on frequent fire to limit the growth of other light competitors. We investigated the effect of time since burn on the pollination of *D. muscipula* by supplementing pollen to plants in sites of varying burn histories. We found that *D. muscipula* is pollen limited, but the degree of pollen limitation did not differ between sites. This finding suggests that *D. muscipula* can be pollen-limited, but the pollination services to this species are potentially consistent up to nearly four years following a burn.

Given the finding that *D. muscipula* can be pollen-limited, we were then interested to understand whether the degree of pollen limitation differs between sites of differing quality. To investigate this, we examined sites with differing conspecific densities across four managed

areas. We observed strong pollen limitation of seed set across sites, with supplemental hand-pollination increasing seed set per fruit by 27% on average relative to open-pollinated controls. Seed set per fruit increased with conspecific density local to individual experimental plants, but the degree of pollen limitation did not differ by conspecific density measured at the site level. We also did not observe a significant relationship between floral visitor abundance, visitation rates, or trapping rates by conspecific density, pointing to the likelihood that an alternative covariate of density and pollen limitation affects seed set per fruit across the range of this species.

Carnivorous plants depend on arthropods as both pollinators and prey. To compare whether *D. muscipula* reproduction is limited by both pollen and prey resources in the wild, we supplemented pollen and prey to plants in a two-way factorial experiment. Seed set did not differ significantly between pollen and prey supplementation treatments, but plants that were given supplemental prey in spring produced significantly more flowers. These findings point to the ability of *D. muscipula* to quickly mobilize surplus resources to potentially boost reproduction at the whole-plant level.

Dionaea muscipula is under review as a candidate for listing under the Endangered Species Act in 2016. As part of an effort to inform the listing decision, I co-coordinated a status survey of *D. muscipula* populations in collaboration with the NCNHP. We counted and estimated nearly 880,000 individuals and 69 extant populations of *D. muscipula*. Most populations contain 500 or fewer individuals, and we frequently observed evidence of fire suppression, land use change, and drought threatening smaller populations.

Throughout these studies, we observed that *D. muscipula* reproduction is frequently limited by adequate pollination, as well as receipt of insect prey. As pollinators face ongoing

threats from habitat loss, climate change, and other pressures, these results highlight the importance of considering insects as part of a holistic conservation management scheme for this species.

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The Pollination Ecology of the Venus Flytrap (*Dionaea muscipula*) and a Status Survey of its
Native Populations

by
Laura Elizabeth Hamon

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DEDICATION

To Elizabeth Helen Hamon, Nicholas Mark Hamon, my sister Katherine Eleanor Hamon, my brother Michael Christopher Hamon, and my “sister” Holly Hamon. You rescued me when I was stuck in the mud, both literally and figuratively.

BIOGRAPHY

I was born in North Carolina, but my roots are not local. My parents and siblings moved from Essex in the United Kingdom to Cary, North Carolina shortly before I was born. From ages 5-10, my family lived in a wooded location in northwest New Jersey, adjacent to public game lands. I consider those five years a formative part of my relationship with nature. My father Nick is an entomologist and encouraged my interest in all things that crawled, swam, and flew. My mother Elizabeth is an enthusiastic gardener and instilled in me a love of growing things.

I graduated from Carrboro High School in 2012. My first job was as at an animal boarding facility and veterinary clinic. I enrolled at the University of Carolina at Chapel Hill later that year. In 2013, I volunteered as a field assistant in Keith Sockman's lab under the direction of Susan Lyons, where I assisted with a study on songbird behavior in Silverton, Colorado. In 2014, I joined Joel Kingsolver's lab, where I assisted with insect colony care and experiments concerning phenotypic plasticity of insects in a changing climate. I also began to collect butterfly observations at a local preserve as part of an independent research project under the advisement of Dr. Kingsolver and Allen Hurlbert. Later, this project developed into a citizen science project in collaboration with Kati Moore and Elizabeth Moore.

In 2014, I visited a longleaf pine savanna for the first time as part of a class on North Carolina flora taught by Alan Weakley. I was awestruck by the biodiversity and surreal beauty of the pine savanna, and officially turned towards a career path in ecology. When searching for a graduate school program, I knew that I was interested in plant-insect interactions, with an increasing focus on the plant perspective. I joined the Irwin and Sorenson labs in the summer of 2017. It has been incredibly humbling to live and work in the environment that inspired me to pursue a career in ecology, and to spend five years with this celebrity plant.

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Michael Kunz and Johnny Randall of the N.C. Botanical Garden provided the materials to conduct germination trials in 2018 and were continuously gracious with their time and expertise.

Field work in 2017 was possible thanks to the assistance and experience of Madeline Adams, Sara June Connon, Jacqueline Fitzgerald, Alison Fowler, Jonathan Giacomini, Daniel Marulanda, Francesca Romero, and Michael Stemkovski.

Part of the present work includes a status survey of *D. muscipula* conducted for the North Carolina Natural Heritage Program, which was a multi-agency collaborative effort. As such, I thank Misty Buchanan, Stephanie Horton, and Dan Hannon for their support, and for allowing me to include the survey as part of my dissertation. The survey was funded by the United States Fish and Wildlife Service under Cooperative Agreement Award F18AC00111. Dale Suiter and Mike Wicker of the Raleigh Ecological Services Office initiated the survey and guided the survey goals. Summer Lauder (N.C. Agricultural & Technical State University, NCNHP intern), Ryan Martin (State of North Carolina intern), John McLaughlin (University of North Carolina at Wilmington, State of North Carolina intern), and Libby Seay (N.C. State University, NCNHP intern) comprised the core team of field assistants and assisted with data entry. The survey would not have been possible without their hard work and fortitude.

Multiple other status survey teams provided data between 2019 and 2021. Justin Bashaw (U.S. Army Corps of Engineers), Keith Bradley (South Carolina Department of Natural Resources), Keleigh Cox (U.S. Army Corps of Engineers), Laurel Davis (U.S. Army Corps of Engineers), James “Trapper” Fowler (South Carolina Department of Natural Resources), Alicia Jackson (Dr. J.H. Carter III & Associates. Inc.), Janice Johnson (U.S. Army Corps of Engineers), Teresa Russell (U.S. Army Corps of Engineers), Dale Shew (University of North Carolina-

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CHAPTER 1: Pollination ecology and morphology of Venus flytrap in sites of varying time since last fire.

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ABSTRACT

Management of natural habitats is an important strategy for rare plant conservation. One common tool for managing natural habitats is the use of controlled fire. Rare plants in fire-dependent ecosystems often rely on frequent fires to increase nutrient availability, initiate germination, and limit cover from light competitors. Fire can also alter arthropod communities, including the pollinator communities upon which many flowering plants rely for sexual reproduction. However, it remains unclear how fire affects the pollination ecology of rare plants in fire-dependent ecosystems. Here we studied sites of varying burn history to examine the role of time since last fire on the morphology, flower visitor community, and degree of pollen limitation of seed production of Venus flytrap (*Dionaea muscipula*). The area occupied by blooming *D. muscipula* and number of traps per individual decreased with increasing time since burn. Though flower visitor richness and evenness were highest in sites of intermediate time post-burn, we found no differences in the composition of the flower visitor community in sites of different burn histories. Hand-pollinated flowers produced 8.3% more seeds per fruit than open-pollinated flowers, indicating that *D. muscipula* was pollen-limited, but burn history did not affect the magnitude of pollen limitation. Though we found no clear effect of burn history on the pollination ecology of *D. muscipula*, differences in blooming area and trap number suggest that burn history influences its distribution and growth, and affirms the benefits of frequent fires to its persistence.

Keywords: Venus flytrap, *Dionaea*, pollination ecology, fire ecology, rare plants

INTRODUCTION

Plant conservation is becoming increasingly urgent, with approximately 20% of global plant species threatened with extinction (Brummitt et al. 2015). The risk of extinction is expected to be particularly high for plant species with small populations and restricted ranges (Matthies 2004). Maintenance of natural habitat is a common conservation practice that can be used to protect vulnerable species and prevent species loss (Marrero-Gómez et al. 2003, Pressey et al. 2007). For effective site management, it is important to understand the biotic and abiotic factors that limit reproductive success in rare plants, as these have repercussions for the long-term sustainability of rare plant populations.

Pollination ecology is an example of a biotic factor that plays a vital role in plant reproduction. Over 85% of flowering plant species depend on animal vectors to transport pollen for sexual reproduction (Ollerton et al. 2011). Both low pollinator visitation and inadequate delivery of compatible pollen to the stigma can result in pollen limitation of fruit or seed set (Knight et al. 2005), potentially reducing plant abundance and population growth for species that are seed-limited (Jennersten 1988, Lennartsson 2002). Pollen limitation has been observed in many plant species (Burd 1994, Ashman et al. 2004), including in rare plants (Pavlik et al. 1993, Schemske 1994). In the case of rare plants, this is concerning because plant-pollinator interactions can be disrupted in species with restricted ranges or scattered populations, potentially exacerbating pollen limitation (Karron 1987, Kunin 1997, Lennartsson 2002). Climate change and habitat fragmentation are expected to further interrupt plant-pollinator

interactions (Jennersten 1988, Hegland et al. 2009), highlighting an increasing need to understand the contribution of pollinators to the persistence of rare plants. However, despite evidence that pollination ecology is a potentially crucial component of plant conservation (e.g., Pavlik et al. 1993, Sipes and Tepedino 1995, Dutra et al. 2009), habitat management for rare plants is often performed without knowledge of the identity or habitat requirements of important pollinating species.

One tool for habitat management is controlled fire. It is well established that changes in fire history and intensity can alter the density and community composition of both plants (Brockway and Lewis 1997) and insects (Bailey and Whitham 2002, Potts et al. 2003, Peralta et al. 2017). In communities that experience frequent fire, many plants depend on fire to increase available nutrients in the soil, limit light competition from taller plants, or initiate seed germination (Gray et al. 2003). Consequently, many studies have pointed to the benefits of fires for maintaining high plant diversity and rare plant communities (Brockway and Lewis 1997, Gray et al. 2003). There is also evidence that diversity in fire intensity and frequency influences the diversity of plant-pollinator interactions (Ponisio et al. 2016), pollinator visitation (Van Nuland et al. 2013), and the abundance of floral resources available to pollinators (Potts et al. 2003). However, the effects of fire management on pollinator communities and pollination success are still poorly understood. Furthermore, it remains unclear how the intensity and frequency of controlled fires affect pollen limitation and pollination ecology in rare plants.

The longleaf pine (*Pinus palustris* Miller) savannas of the southeastern US are home to numerous rare plants, many of which are fire-dependent. Among these species is the Venus flytrap (*Dionaea muscipula* J. Ellis), a carnivorous plant whose native range is restricted to the wet pine savannas of southeastern North Carolina and northeastern South Carolina (Luken 2005,

Schafale 2012). The soil at these sites is frequently water-saturated and has low available nitrogen and phosphorus, and *D. muscipula* supplements its nutrient intake by using its modified leaves, which form a snap-trap mechanism to capture and digest prey. *Dionaea muscipula* is dependent on regular fires to limit cover from taller plants, which can impede access to both light and insect prey (Schulze et al. 2001, Luken 2007). Fire has also been shown to stimulate flowering and seedling establishment in *D. muscipula* (Roberts and Oosting 1958, Luken 2007). Though the importance of fire to the persistence of *D. muscipula* is apparent, there is surprisingly little research on the pollination ecology of *D. muscipula* in its native range (Youngsteadt et al. 2018), and the influence of fire on its pollination ecology is unknown.

The purpose of this study was to investigate the role of time since fire on the morphology, pollinator community, pollen limitation, and germination success of *D. muscipula*. We used field observations and an experiment to address the following predictions: more recently burned sites would (1) have larger, denser populations of flowering *D. muscipula*, (2) attract the most abundant and diverse pollinator communities, (3) experience lower pollen limitation of seed production, and (4) produce the highest quality seeds, measured in terms of seed weight and probability of germination. This study provides insight into the pollination ecology of the rare plant *D. muscipula* and the consequences of fire management for both rare plants and the pollinators upon which they depend.

METHODS

Study system and sites

Dionaea muscipula (Droseraceae) is a long-lived perennial species that flowers from mid-May to early July. The white flowers are actinomorphic with 5 petals, a unilocular style, and a bifurcated, papillose stigma. The inflorescence is a cymous raceme with 8-12 flowers, which is isolated 15-35 cm above the rosette of traps by an elongated scape (Smith 1929; Roberts and Oosting 1958). *Dionaea muscipula* is reported to be self-incompatible and dependent on pollinators for sexual reproduction (Roberts and Oosting 1958). The flower is visited by a suite of generalist pollinators that include sweat bees and beetles, though it is not known which flower visitors are its most efficient pollinators (Youngsteadt et al. 2018). Fruits mature in June and July (Smith 1929), and seedlings typically take approximately 3 years to reach the flowering stage (Roberts and Oosting 1958). Following fire, *D. muscipula* rapidly re-sprouts from an underground rhizome (Gao et al. 2015).

This study took place at eight sites in a managed natural area in Pender County, NC. Sites in this area are regularly burned to reflect an average fire return interval of 3.0 to 3.5 years. The primary goals of these burns are to decrease the risk of catastrophic fires, improve game and non-game animal habitat, and promote high plant diversity. Burns typically take place from January to March, but often occur later in the spring and summer as a means of controlling hardwood tree abundance. *Dionaea muscipula* was abundant at all sites, represented by hundreds to thousands of flowering stems. Sites ranged in size from 131-14860 m² and were separated from each other by 0.75-5.0 km. Our sites varied in the amount of time elapsed since the last controlled burn, with the most recent burn occurring 2 months prior to the study and the oldest burn occurring 4 years prior. Of the eight sites, two pairs of sites were last burned on the same

date, yielding a total of six unique burn dates. The number of days since last burn (hereafter referred to as time since burn) for each site was calculated relative to May 1, 2017 (Table 1), which approximates the start of the flowering season in the year of our study.

Field methods

Site variables — To determine the size of each site as estimated by bloom area, we used GPS to map the shortest perimeter that included all flowering *D. muscipula* and calculated the area of the resultant polygon. Within a 50 x 1 m transect at each site, we counted the number of plants of all species flowering during *D. muscipula* bloom and identified them to the lowest taxonomic resolution possible, typically species or genus, using standard references (e.g., Porcher and Rayner 1995, Thornhill et al. 2014). We also counted the number of inflorescences or flowers on up to 10 plants per flowering species per transect. For taxa with compact inflorescences—specifically, *Polygala lutea* (Polygalaceae), *Lachnocaulon* spp. (Eriocaulaceae), and plants in the Asteraceae family—we counted inflorescences, hereafter also referred to as “flowers”. For each species, we multiplied the mean number of flowers per individual by the total number of individuals within the transect and divided by 50 to estimate the flower density per m² of *D. muscipula* and heterospecific species at each site. The relative abundance of *D. muscipula* was calculated by dividing the average number of *D. muscipula* flowers per m² by the average number of all flowers per m².

Plant traits — We haphazardly selected 20 flowering *D. muscipula* per site (N = 160 plants total across all sites). We selected plants that had only one rosette and one floral scape per individual. On all plants, we recorded the flower scape height (from the ground to the base of the lowest flower pedicel, measured to the nearest 0.1 cm) and counted the number of buds, flowers, and developing and aborted fruits as well as the number of traps at the time of flowering. The

total flower production per plant for the year of study was calculated as the sum of buds, flowers, and developing and aborted fruits.

Floral visitors — We sampled flower visitors from the eight sites over five dates in 2017 (May 17, May 19, May 26, June 1, and June 9; Table 1). To sample flower visitors, we searched the sites and collected any arthropods observed landing or resting on *D. muscipula* flower parts. Specimens were either netted and transferred into vials or collected directly into vials. Collection was performed on days with fair weather between 1048 and 1802 hrs. Each time we visited a site, we collected for 26-140 person-minutes, with a total of 1347 person-minutes of collecting. To determine the possibility of nocturnal visitors, we also collected between 2045-2136 hrs for 110 person-minutes over three sites. Evening sampling was conducted while wearing red LED headlamps. Collected flower visitors were sacrificed, transported on dry ice, and stored at -30 °C until processing.

Flower visitors were swabbed for pollen with fuchsin gel (Kearns and Inouye 1993). The gel was mounted on a microscope slide and examined under a compound microscope at 200X magnification to determine the presence of *D. muscipula* pollen and heterospecific pollen based on a pollen reference collection from the study area (as in Youngsteadt et al. 2018). We counted the number of *D. muscipula* and heterospecific pollen grains in five fields of view on each slide that contained *D. muscipula* pollen (one in the center and four in the periphery of the stained sample). The sum of the pollen grains from these five fields of view was used as a measure of *D. muscipula* and heterospecific pollen load. We identified flower visitors to the lowest taxonomic level possible (typically species or morphospecies within genus) using standard references (e.g., Mitchell 1980, Gibbs 2011, Evans 2014, Faust 2017 and references therein) and comparison to specimens in the NC State University Insect Museum, where pinned specimens will be archived.

Pollen limitation — We hand-pollinated flowers on three dates in 2017 (May 9, May 19, and May 26). At each of the eight sites, we used the same 20 plants that were also used to measure flower and trap production (N = 160 plants across all sites). On each of these plants, we used two flowers of similar bloom stage with receptive stigmas. One flower was randomly assigned to the pollen-supplementation treatment and the other flower served as an open-pollinated control. To hand-pollinate flowers, we collected dehiscing anthers from at least 5 pollen donor plants growing within the same site, and we rubbed dehiscing anthers against receptive stigmas to augment pollen loads. We handled the open-pollinated control flower but did not add pollen to the stigma. We marked hand-pollinated and control flowers with either green or black thread tied around the pedicel. To prevent color stimulus bias, we alternated the thread color assigned to each treatment between sites. When we collected flower visitors (see *Floral visitors*) we did not collect from plants in the hand-pollination experiment.

We collected mature fruits on June 1 and June 9. We then counted and weighed (to the nearest 0.1 μg) the seeds in each fruit. To calculate average seed weight, we divided the seed weight per fruit by the number of seeds in that fruit. One plant was omitted from statistical analyses because the control flower was missing at the time of seed collection.

Germination success — Seeds were stored in coin envelopes at room temperature for 7 months prior to germination. To determine germination success as a function of seed weight, we haphazardly selected and weighed individual seeds (to the nearest 0.1 μg) with representation from all eight sites and both pollination treatments. For each site, we selected seeds from at least eight maternal plants. Of the eight sites, five had a relatively narrow range of seed weights (approximately 140 – 240 μg), and we selected 36-39 seeds from each of these five sites. The remaining three sites had a much broader range of seed weights (approximately 160 – 460 μg),

and we selected 53-55 seeds from these sites to better compare germination as a function of variation in seed weight. In total, we weighed 347 individual seeds to be included in the germination trial.

Seeds were placed in petri dishes on filter paper moistened with 2 mL of H₂O. In each dish, we arranged 20-25 seeds. Petri dishes were then placed in a germination chamber under conditions of 12 hr light at 30 °C and 12 hr dark at 20 °C. We recorded seeds as germinating when the radicle broke through the seed coat. We recorded germinations until 7 days had elapsed since the last recorded germination.

Statistical analyses

All statistical analyses were performed using R v.3.3.3 within R studio v.1.0.143 (R Core Team 2017; RStudio Team 2016). To compare the flowering plant community between sites, we calculated the rarefied flower species richness (rarefied to 21 individuals) using the package ‘vegan’ (Oksanen et al. 2017). As a measure of species evenness, we also calculated Hurlbert’s probability of interspecific encounter (PIE) using the package ‘benthos’ (Walvoort 2017). This index describes the probability that two randomly sampled individuals from a population will be the same species and has a value between 0 and 1, where 1 represents a perfectly even sample (Hurlbert 1971). We used this index because it is relatively robust against varied sample size (Olszewski 2004). We then used linear regression models to examine whether *D. muscipula* bloom area and *D. muscipula* relative abundance and flower density differed with time since burn. To determine whether rarefied flowering species richness and evenness differed with time since burn, we compared linear regression models and second degree polynomial models. We did not include site ID as a random effect in these models because there was only one measurement per site.

To examine trends in phenotypic variation for *D. muscipula*, we calculated pairwise correlation coefficients for morphological traits (scape height, flower production, and number of traps per plant) within and across sites using the package ‘Hmisc’ (Harrell 2017). In the correlation analyses, we also included seed set per fruit and average seed weight from open-pollinated flowers as estimates of seed investment. We tested for homogeneity of the covariance structure of the variables across sites using the ‘boxM’ function in the package ‘biotools’ (da Silva et al. 2017). In addition, we used linear mixed-effects models to determine whether number of traps per plant, number of flowers per plant, and scape height were correlated with time since burn. In these models, we included site as a random variable. All linear mixed-effects models were constructed using the package ‘lme4’ (Bates et al. 2017) and tested using the ‘Anova’ function within the package ‘car’ (Fox and Weisberg 2011).

To compare flower visitor richness between sites, we rarefied 5 sites to a sample size of 20 individuals and extrapolated the remaining 3 sites to a sample size of 20 individuals using the package ‘iNEXT’ (Hsieh et al. 2016). We also determined flower visitor species evenness using Hurlbert’s PIE. We then compared linear regression models and second degree polynomial regression models to determine whether rarefied flower visitor species richness and evenness differed with time since burn. To visualize differences in the flower visitor community between sites, we used nonmetric multidimensional scaling (NMDS) in 2 dimensions with 20 random starting configurations, including only those flower visitors that were observed in more than one site. We then performed a nonparametric multivariate analysis of variance (‘adonis’) using pairwise distance matrices calculated using the Bray-Curtis method and tested using a permutation test with 999 random permutations.

To determine whether *D. muscipula* was pollen limited and whether time since burn affected the magnitude of pollen limitation, we constructed a linear mixed-effects model in which seed set per fruit was the response, and hand-pollination treatment (hand-pollinated vs. control), time since burn, and their interaction were included as predictors. To test whether pollen supplementation affected investment in seed size, we constructed a similar linear mixed-effects model in which average seed weight was the response, and treatment, time since burn, and their interaction were predictor variables. To account for our paired treatments (hand-pollinated vs. control) over eight sites, both site and individual nested within site were included in these models as random effects.

To examine whether seed weight influenced germination success, we constructed a generalized linear mixed-effects model with binomial error distribution and logit link function, where germination success (Yes/No) was the categorical response variable, and seed weight, hand-pollination treatment, time since burn, and the interactions between seed weight with hand-pollination treatment and seed weight with time since burn were the predictor variables. Site and plant nested within site were again included in the model as random effects.

RESULTS

Site variables

Dionaea muscipula bloom area decreased with increasing time since burn ($F_{1,6} = 7.51$, $R^2 = 0.56$, $P = 0.03$; Fig. 1). We found no relationship between *D. muscipula* flower density ($F_{1,6} = 0.003$, $R^2 = 0.0006$, $P = 0.96$; Supp. Fig 1a) or flowering *D. muscipula* relative abundance ($F_{1,6} = 0.89$, $R^2 = 0.13$, $P = 0.38$; Supp. Fig. 1b) and time since burn. We observed 2-9 flowering species

per site, representing 13 plant genera and 9 plant families across sites. Flowering species evenness ranged from 0.09-0.74. We found no significant linear relationship between rarefied flowering species richness ($F_{1,6} = 1.53$, $R^2 = 0.20$, $P = 0.26$; Supp. Fig. 1c) or evenness ($F_{1,6} = 0.94$, $R^2 = 0.14$, $P = 0.37$; Supp. Fig. 1d) and time since burn, nor a significant non-linear relationship ($F_{2,5} = 1.61$, $R^2 = 0.15$, $P = 0.29$ and $F_{2,5} = 1.98$, $R^2 = 0.22$, $P = 0.23$, respectively).

Plant traits

Dionaea muscipula plants had 5-22 flowers and 0-11 traps, with flowers on a scape 17.0-40.5 cm above the traps. There was a significant relationship between number of traps per plant and time since burn ($F_{1,6} = 6.58$, $R^2 = 0.14$, $P = 0.04$; Fig. 2), with trap number decreasing with increasing time since burn. However, we found no significant relationship between scape height ($F_{1,6} = 0.24$, $R^2 = 0.01$, $P = 0.64$; Supp. Fig. 2a) or number of flowers ($F_{1,6} = 0.01$, $R^2 = 0.0001$, $P = 0.95$; Supp. Fig. 2b) and time since burn. Phenotypic correlations among traits were site-specific (Supp. Table 1), indicated by unequal covariance matrices ($X^2 = 201.91$, $df = 105$, $P < 0.0001$). The majority (but not all) of the correlations were positive, but often not significantly so. Looking at the statistically significant correlations only, all were positive except for two (Supp. Table 1). The one set of consistent patterns was that average seed weight was positively correlated with both scape height and number of traps across all eight sites, albeit not significantly so (Supp. Table 1).

Flower visitor community

We collected 251 flower visitors (10 to 48 per site). Overall, 153 visitors (61%) carried pollen, 108 of which (71%) carried *D. muscipula* pollen. Of the visitors that carried *D. muscipula* pollen, 21 (19%) carried only *D. muscipula* pollen and 87 (81%) carried a mix of *D. muscipula*

and other pollen. Across all sites, the most frequent pollen-carrying insect was a Cerambycid beetle, *Typocerus sinuatus* (Table 2). When examining changes in flower visitor species richness and evenness with days since last burn, second-degree polynomial models fit significantly better compared to linear regression models, with a significant non-linear relationships between species richness ($F_{2,5} = 19.36$, $R^2 = 0.84$, $P = 0.004$; Fig. 3a) and evenness ($F_{2,5} = 20.76$, $R^2 = 0.85$, $P = 0.004$; Fig. 3b) and time since burn. However, we found no significant differences in flower visitor community composition between sites of different burn dates ($F_{1,6} = 0.86$, $R^2 = 0.13$, $P = 0.58$). The stress value for the two-dimensional ordination plot was 0.11, indicating a reasonable representation of the relationship between points (Supp. Fig. 3).

Pollen limitation

Of the 318 fruits included in the experiment, 307 (97%) set seed. Fruits contained an average of 20.2 ± 8.7 seeds. We found a significant effect of pollination treatment on seed set per fruit ($F_{1,157} = 4.56$, $P = 0.03$), with hand-pollinated flowers producing approximately 8.3% more seeds per fruit than control flowers (Fig. 4). Seed set per fruit did not vary with time since burn ($F_{1,6} = 1.21$, $P = 0.27$), and the interaction between hand-pollination treatment and time since burn was not statistically significant ($F_{1,157} = 0.02$, $P = 0.89$), suggesting time since burn did not affect the magnitude of pollen limitation. There was no significant effect of the hand-pollination treatment on average seed weight ($F_{1,150} = 0.70$, $P = 0.40$), nor did seed weight vary by time since burn ($F_{1,6} = 0.32$, $P = 0.59$) or by the interaction between treatment and time since burn ($F_{1,150} = 1.03$, $P = 0.31$).

Seed weight and germination success

For seeds weighed individually, the average seed weight was $252.4 \pm 5.4 \mu\text{g}$, (mean \pm SE) with a range of 49.5 - 501.3 μg . There was a significant effect of seed weight on germination success, with higher likelihood of germination success for heavier seeds ($X^2 = 14.02$, $df = 1$, $P = 0.0091$; Fig. 5). There were no significant effects of hand-pollination treatment ($X^2 = 0.49$, $df = 1$, $P = 0.48$), time since burn ($X^2 = 0.0086$, $df = 1$, $P = 0.93$), interaction between seed weight and hand-pollination treatment ($X^2 = 0.72$, $df = 1$, $P = 0.40$), and interaction between seed weight and time since burn ($X^2 = 0.019$, $df = 1$, $P = 0.89$) on germination success.

DISCUSSION

In fire-dependent habitats, frequent fire disturbance plays a critical role in maintaining ecological communities. In this study, we recorded floral traits, collected floral visitors, and hand-pollinated flowers to assess how the morphology and pollination ecology of *D. muscipula* are influenced by time since burn. We demonstrate that an increase in time since burn is correlated with decreases in bloom area and number of traps per plant. Moreover, *D. muscipula* can exhibit pollen-limitation, but the magnitude of pollen limitation did not vary with time since burn. Taken together, these data suggest that *D. muscipula* is likely dependent on its pollinators for increased seed set, but that time since burn may have a relatively low impact on its pollinator community. Nonetheless, this study also adds to the body of knowledge that demonstrates the importance of fire to the persistence of *D. muscipula* (Roberts and Oosting 1958, Luken 2007).

Longleaf pine ecosystems are considered fire-dependent habitats, with fire typically increasing plant density and species diversity (Brockway and Lewis 1997, Provencher et al.

2003). Consequently, we expected the bloom area and density of *D. muscipula* flowers and flowering species richness to be highest in sites that had been most recently burned. Furthermore, since fire can promote an increase in floral resources, which can attract insects (Potts et al. 2001, Van Nuland et al. 2013), we expected greater flower visitor diversity in sites that had been more recently burned. Our result for bloom area of *D. muscipula* supported our prediction, with higher bloom area in more recently burned sites. This is likely because fire produces gaps in shrub and other vegetation cover (Menges and Kimmich 1996), allowing *D. muscipula* to persist (Luken 2005). As time elapses, these gaps fill in with developing vegetation (Hawkes and Menges 1995), reducing the size of suitable patches and eventually outcompeting *D. muscipula* for light, and limiting access to the large insect prey that promote flowering (Schulze et al 2001). We note that site S2 exhibited a relatively small *D. muscipula* bloom area, despite being recently burned. This is likely because this site was bordered on one side by dense pocosin, which is burned at a longer fire return interval than longleaf pine savanna.

Counter to our predictions, we found no associations between *D. muscipula* flower density or relative abundance and time since burn. We only recorded flowering plants of *D. muscipula*; thus, we do not know whether total *D. muscipula* flower density correlates with total density of flowering and non-flowering plants. In previous studies, *D. muscipula* in plots where vegetation was clipped or mowed exhibited a higher percentage of flowering individuals than in control plots (Luken 2005, 2007). This points to the likely role of light availability in flowering success of *D. muscipula*, and we cannot rule out the possibility that burn history differentially affects factors that drive total density (via seedling establishment and plant persistence) versus flowering (Menges and Hawkes 1998, Luken 2005). In a similar vein, we also found no relationship between time since burn and species richness or evenness in co-flowering species.

Again, we recorded only plants that were flowering at the time of this study, rather than all plant species, so it is possible that phenological differences between sites or sampling dates obscured differences in underlying vegetative diversity. Future studies are needed that measure the density of flowering and non-flowering *D. muscipula* and co-occurring plant species to assess the generality of this result. One caveat about these results is that we did not control (or have information about) the intensity of the burns. Studies that directly manipulate both the frequency and intensity of burns on the density of *D. muscipula* and plant and insect species richness may yield additional management insight.

Fire can alter community composition in insects (Potts et al. 2003, Peralta et al. 2017). We found a significant non-linear relationship between flower visitor richness and evenness and time since burn, with the highest flower visitor richness and evenness at sites of intermediate amount of time post-burn. These results support predictions made by the intermediate disturbance hypothesis, with species diversity highest following an intermediate amount of time since the last disturbance (Connell 1978, Collins et al. 1995). Despite this pattern, we found no significant differences in flower visitor community composition between sites in this study. Among the most frequent visitors of *D. muscipula* are beetles, chiefly *Typocerus sinuatus* (Cerambycidae) and *Trichodes apivorus* (Cleridae) (Youngsteadt et al. 2018). We report similar flower visitor communities here, though flower visitor collection in the study by Youngsteadt et al. (2018) was more intensive (68-670 person-minutes per site, compared to 26-140 person-minutes per site in the present study) and was conducted at some, but not all, of the same sites used in this study. Studies that have examined the effect of fire on pollinator communities have largely focused on bees (Ponisio et al. 2016, Peralta et al. 2017) while studies of the effects of fire on beetle pollinators are scant (Holliday 1991, Sullivan et al. 2003). In one study in a

grassland ecosystem, Bulan and Barrett (1971) found that post-fire herbivorous beetle diversity was significantly reduced compared to carnivorous beetle diversity. Thus, different functional groups of beetles may respond differently to fire, but the comprehensive effects of fire on beetle pollinators remains unclear. Our study suggests that the *D. muscipula* flower visitor community may be robust against fire disturbance, and that they quickly return to sites post-fire. In a similar vein, several studies have reported rapid recovery of bee pollinators just a few years post-fire (Potts et al. 2003, Peralta et al. 2017).

Of the morphological traits we assessed, the only plant trait that was significantly correlated with time since burn was the number of traps per plant, and there was no relationship between time since burn and flower production. In a qualitative study of *D. muscipula* growth in burned versus unburned plots, Roberts and Oosting (1958) found that *D. muscipula* grew faster in recently burned sites compared to unburned sites, likely due to the increase in light and nutrient availability. It has been proposed that carnivory is too energetically expensive to persist under low-light conditions, and that carnivorous plants will decrease investment in prey capture in response to light-limitation (Givnish et al. 1984), although experimental support for this theory is varied (Thorén et al. 2003, Brewer 2005). Fire has also been shown to advance flowering phenology (Roberts and Oosting 1958) and increase flowering rate in *D. muscipula* populations (Luken 2007), suggesting a stimulating effect by fire on *D. muscipula* flowering. We therefore expected that, among plants that did flower, their individual investment in flowering would also decrease with increasing time since burn. However, we did not observe a correlation in flowers per plant with time since burn. This potentially indicates that individual investment in flowering may not be limited by post fire light and nutrient availability to the same degree as the leaves within the context of the burn histories that we studied.

Although *D. muscipula* was pollen limited during this study, we found no evidence of an association between pollen limitation and time since burn. We also found no evidence to suggest that the current fire regimes at these sites significantly affect the pollinator community of *D. muscipula*. Therefore, pollinators are likely a lower priority concern when planning the fire management of this species. Nonetheless, the presence of pollen limitation in *D. muscipula* indicates that adequate pollinator visitation may be a limiting factor of seed set. Although the magnitude of pollen limitation was small, given the conservation status of *D. muscipula*, any factors that limit seed production are of concern, especially if seed production is linked to species abundance and population growth, as occurs in other species (Ågren 1996, Ward and Johnson 2005). One caveat about interpretation of our pollen limitation results is that we worked in relative densely flowering *D. muscipula* sites, with density ranging from 0.14-9.05 flowers/m². We cannot rule out the possibility that decreases in *D. muscipula* flower density and bloom area could have repercussions for its pollination ecology and increase pollen limitation, since small, disjunct patches of flowers may attract fewer pollinators (Sih and Baltus 1987, Sowig 1989, Aguilar et al. 2006) and experience reduced movement of pollen between populations (Lennartsson 2002). Understanding how pollinator visitation and pollen limitation vary across populations of various size and density within a demographic framework will yield additional ecological insight.

Fire is an important management tool in fire-dependent ecosystems to maintain the health of individual species and the diversity of the community (Brockway and Lewis 1997, Gray et al. 2003). In this study, we found that time since burn was negatively correlated with number of traps per individual and the area of blooming individuals in *D. muscipula*, further affirming the necessity of regular fires to the conservation of this species. We also provide evidence that *D.*

muscipula can be pollen-limited, demonstrating the role of pollinators in its reproduction and success. Though we found no differences in the pollinator community or pollen limitation in sites of different burn histories in this species, the fact that fire history influenced blooming area points to potential repercussions for pollinator visitation in *D. muscipula* and other species dependent on gaps in shrub cover following fire. More work is required to parse how fire heterogeneity alters bloom area and influences pollinator visitation to rare plants.

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Table 1.1. Summary of site variables. Bloom area, density, and relative abundance refer to *Dionaea muscipula*. Time since burn was calculated as the number of days since the most recent burn from 1 May 2017. Site ID numbers correspond to increasing time since last burn.

Site ID	Burn date	Time since burn (days)	Bloom area (m ²)	Density	Relative abundance	Rarefied flowering species richness (with 95% CI)	Evenness index (PIE)
S1	29 Mar 2017	33	12252	1.236	0.96	8.96 (6.42, 11.50)	0.72
S2	22 June 2016	313	317	4.160	0.19	12.14 (6.29, 17.88)	0.96
S3	22 June 2016	313	14860	8.064	0.87	12.72 (9.99, 15.44)	0.94
S4	20 June 2016	315	12164	2.900	0.27	14.42 (10.18, 18.65)	0.94
S5	2 Feb 2016	453	8914	3.456	0.45	13.53 (11.56, 15.50)	0.94
S6	10 Mar 2014	1148	131	9.048	0.74	12.34 (10.17, 14.51)	0.93
S7	10 Mar 2014	1148	687	0.140	0.03	10.61 (7.50, 13.73)	0.88
S8	13 May 2013	1449	695	1.976	0.37	7.11 (5.05, 9.18)	0.76

Table 1.2. Top 11 most abundant *Dionaea muscipula* pollen-carrying insects across all eight sites.

Species	Family	Order	Number	Pollen grain count (mean \pm SE)
<i>Typocerus sinuatus</i>	Cerambycidae	Coleoptera	19	10.2 \pm 3.8
<i>Mordella atrata</i>	Mordellidae	Coleoptera	15	4.0 \pm 1.6
<i>Cryptocephalus venustus</i>	Chrysomelidae	Coleoptera	7	2.0 \pm 0.5
<i>Trichodes apivorus</i>	Cleridae	Coleoptera	7	8.3 \pm 1.8
<i>Bombus pensylvanicus</i>	Apidae	Hymenoptera	6	33.5 \pm 14.2
<i>Augochlorella gratiosa</i>	Halictidae	Hymenoptera	5	38.6 \pm 19.8
<i>Lasioglossum creberrimum</i>	Halictidae	Hymenoptera	5	20.2 \pm 13.8
<i>Bombus impatiens</i>	Apidae	Hymenoptera	4	81.0 \pm 72.4
<i>Ceratina floridana</i>	Apidae	Hymenoptera	2	1.5 \pm 0.5
<i>Megachile brevis</i>	Megachilidae	Hymenoptera	2	12.0 \pm 9.0
<i>Photinus cooki</i>	Lampyridae	Coleoptera	2	6.5 \pm 4.5

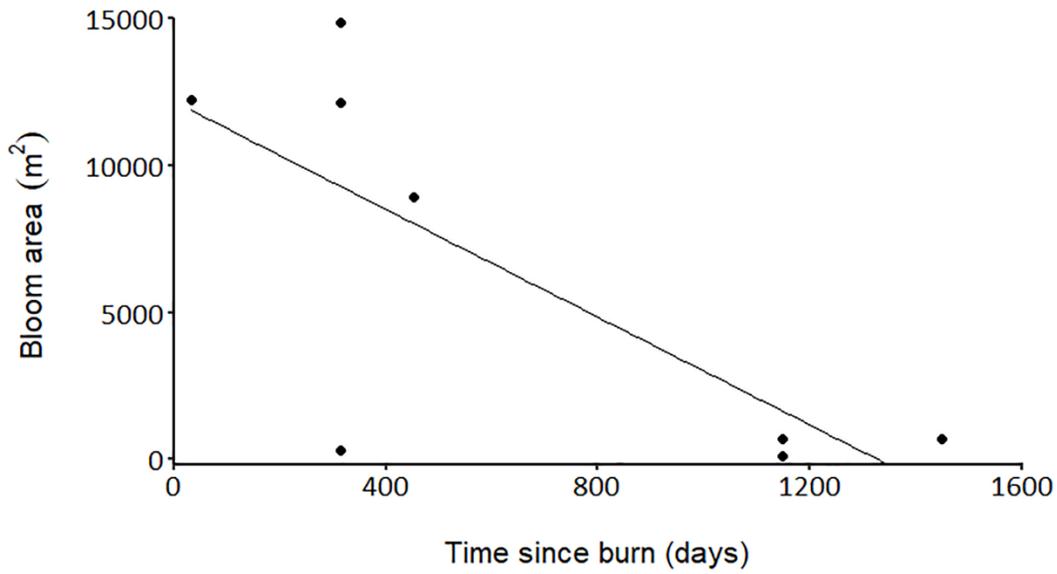


Figure 1.1. *Dionaea muscipula* bloom area by time since burn. Bloom area decreased significantly with increasing number of days since burn. Each point represents a site.

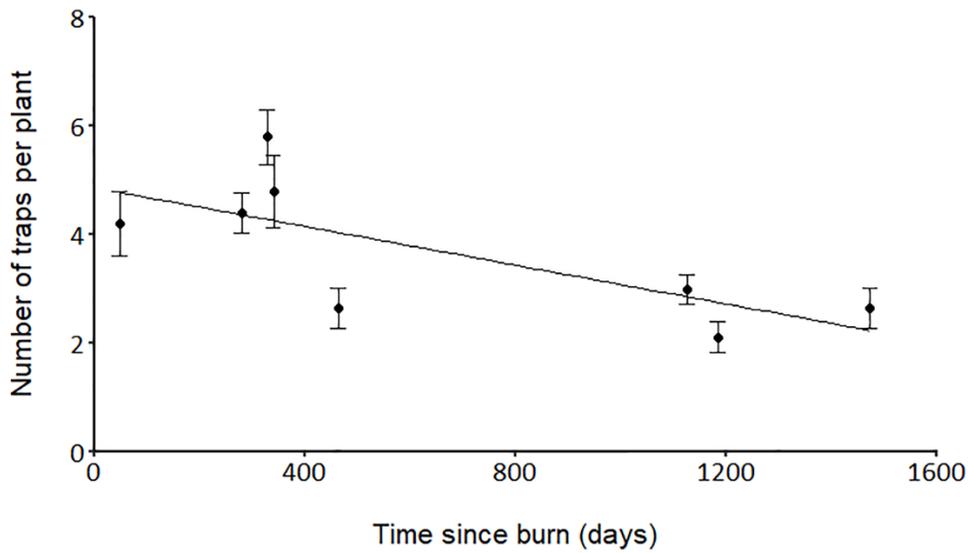


Figure 1.2. Mean number of traps per plant by time since burn. Traps per plant decreased significantly with increasing time since burn. Points are site means \pm SE. Points are jittered for clarity.

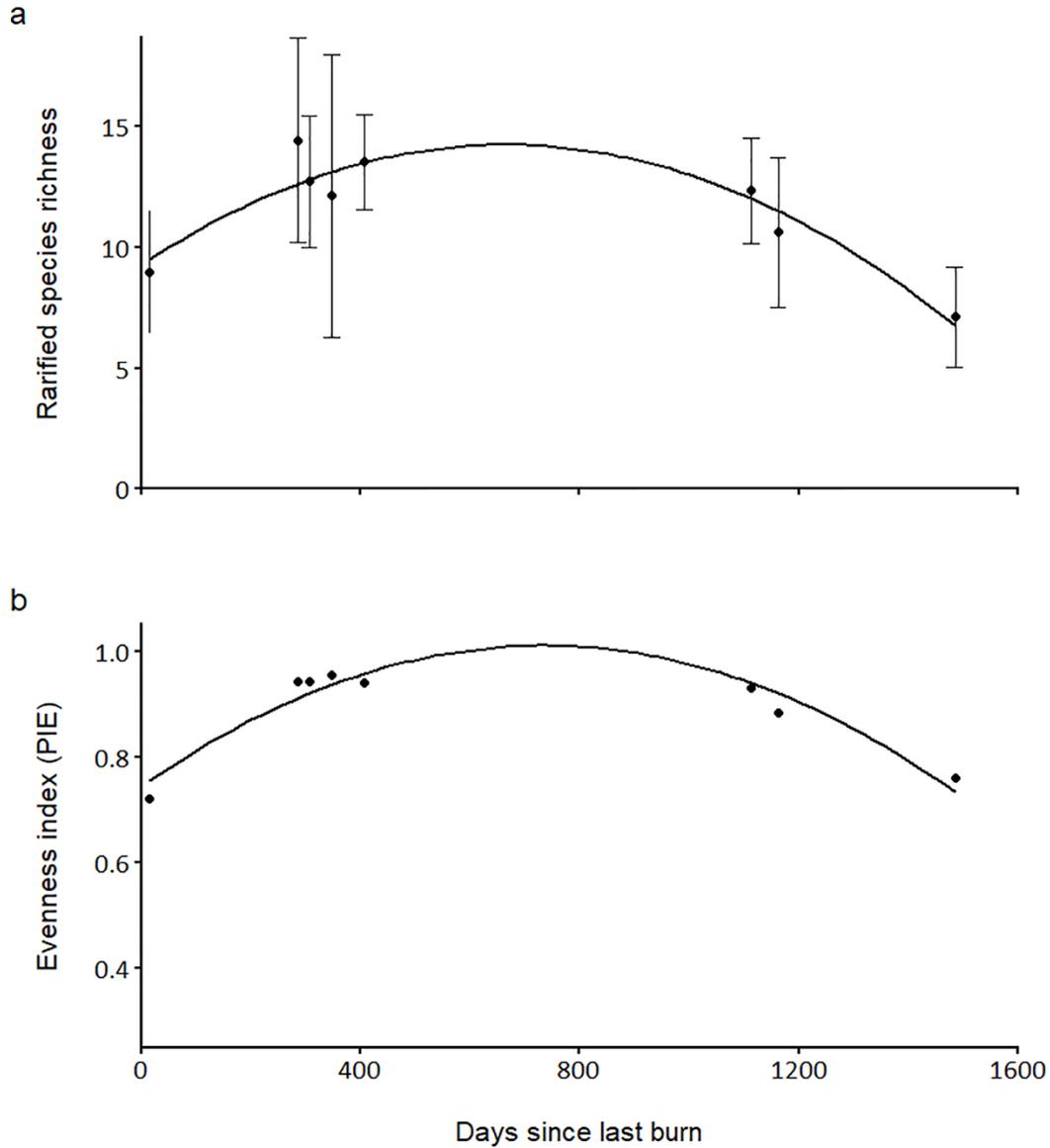


Figure 1.3. There was a non-linear relationship with significant second-degree polynomial terms between (a) rarefied/extrapolated richness with 95% confidence intervals of pollen-carrying insect species and (b) evenness of pollen-carrying insect species and time since burn. Each point represents a site. Points are jittered for clarity.

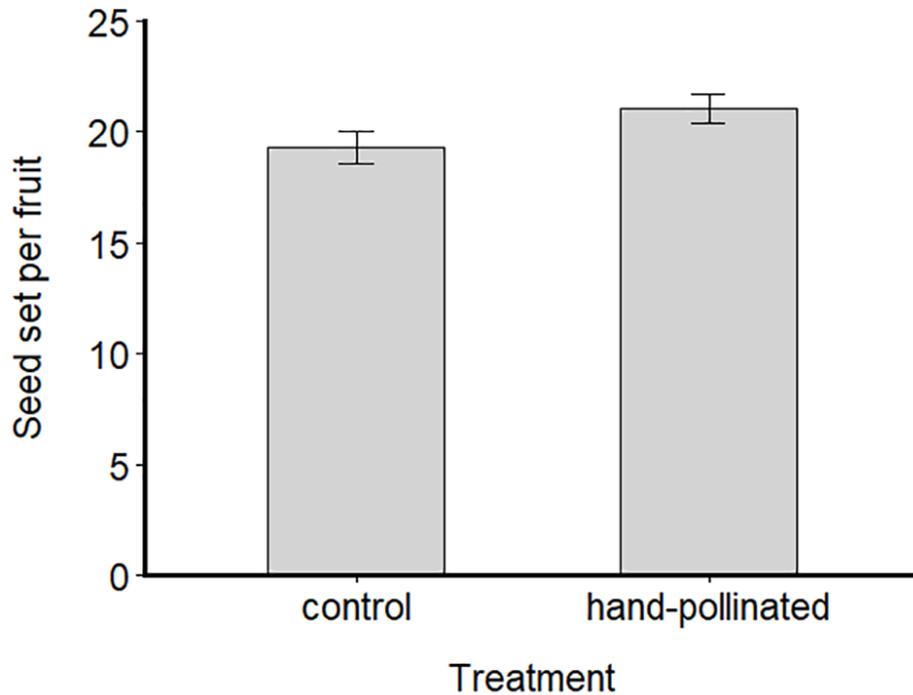


Figure 1.4. Seed set per fruit (mean \pm SE) was significantly higher in hand-pollinated *Dionaea muscipula* flowers compared to open-pollinated control flowers across all sites.

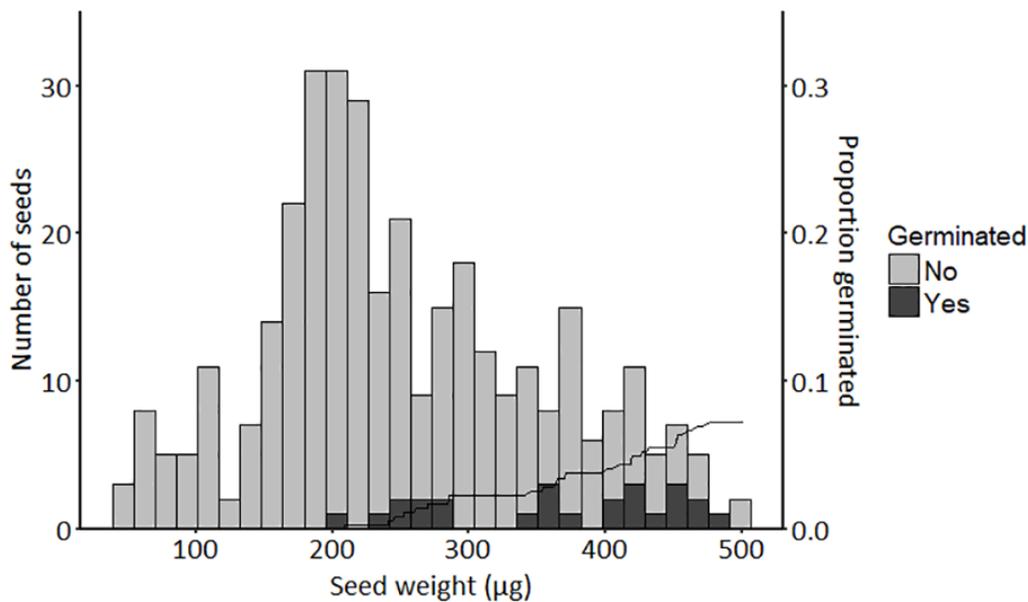


Figure 1.5. Histogram of individual seed weights and germination success (left y-axis). Overlaid on the histogram is a line showing the proportion of seeds that germinated as a function of seed weight (right y-axis). The likelihood of successful germination increased significantly with increasing seed weight.

CHAPTER 2: Local plant density modifies seed set but not pollen limitation or prey capture in the carnivorous *Dionaea muscipula* (Droseraceae)

Laura E. Hamon, Elsa Youngsteadt, Clyde E. Sorenson, and Rebecca E. Irwin

ABSTRACT

Plant reproduction is strongly influenced by conspecific density, both locally and across landscapes. To our knowledge, the relationship between conspecific density and reproductive success has not been quantified in carnivorous plants, which attract insects as both pollinators and prey. In this study, we quantified pollen limitation, floral visitor abundance, pollinator visitation rates, prey capture, and plant reproduction across sites of widely varying density of Venus flytrap (*Dionaea muscipula* J. Ellis). We observed widespread pollen limitation across our sites, with pollen-supplemented flowers producing approximately 27% more seeds per fruit compared to control flowers. However, the degree of pollen limitation did not vary significantly by conspecific density. When we quantified conspecific density at the site level, we observed no significant relationship between conspecific density and seed set per fruit. However, we observed a positive relationship between seed set per fruit and conspecific density local to each experimental plant. Floral visitor abundance, pollinator visitation rates, and prey capture success did not vary significantly with conspecific plant density. Therefore, the relationship between seed set and conspecific density in *D. muscipula* is likely strongly shaped by other measures of resource availability to plants, such as light, water, or previous prey capture success. To inform effective conservation management, future studies should focus on identifying variation in the abiotic factors that limit both density and reproduction in *D. muscipula*.

Keywords: pollination, pollen limitation, carnivorous plants, conspecific density, longleaf pine savanna

INTRODUCTION

Density can have powerful effects on the survival, growth, and reproduction of organisms. These density-dependent effects can be mediated through mechanisms such as intraspecific competition or facilitation for resources or mates. For example, one factor that plays an important role in shaping plant reproduction is conspecific density, with a rich body of literature focusing on the relationship between conspecific flowering plant density and pollination (Thomson 1981, Kunin 1997). Plants with low conspecific flowering densities may be less attractive to pollinators (Sih and Baltus 1987, van Treuren et al. 1993), resulting in lower pollinator visitation rates (e.g., Kunin 1993, Hegland and Boeke 2006, Feldman 2008), lower plant reproductive success (e.g., Kunin 1992, Roll et al. 1997, Lázaro et al. 2013), and greater pollen limitation (e.g., Sih and Baltus 1987, Moody-Weis and Heywood 2001, Waites and Ågren 2004). Plants growing in low conspecific flowering densities may also have a smaller pool of potential mates, resulting in inbreeding depression (Alexandersson and Ågren 1996). As conspecific flowering density increases in low-density populations, facilitation between neighbors may occur, attracting pollinators to dense clumps of flowers between which they can forage efficiently (Dreisig 1995, Field et al. 2005). However, at high conspecific flowering densities plants may compete for pollinator visits, resulting in lower pollinator visitation rates per flower (Steven et al. 2003, Bergamo et al. 2020). The relationship between pollinator visitation rate and flowering plant density for plant reproduction may also depend on pollinator identity (Schmitt 1983, Sih and Baltus 1987), flower sex ratios present at a given time (Elliott and Irwin

1997, Molano-Flores and Hendrix 1999), and interspecific competition between plant species. Consequently, the relationship between conspecific density and reproduction is highly context dependent, and may differ across species, populations, and time.

The relationship between conspecific density and reproductive success is of particular concern for species with restricted or scattered ranges, such as those exhibited by many rare plant species (Karron 1987, Kunin 1997, Lennartsson 2002). Small plant populations tend to have lower conspecific densities, potentially leading to reduced pollinator visitation in instances where density is positively associated with seed set (Kunin 1997, Roll et al. 1997, Bosch and Waser 2001). This pattern, in combination with small and isolated populations, may result in especially low pollinator visitation rates to rare plant species (Lamont et al. 1993, Ågren 1996, Kearns et al. 1998, Steffan-Dewenter and Tschardtke 1999), leading to reduced reproduction and a higher likelihood of extirpation (Groom 1998). Nonetheless, positive correlations between conspecific density and reproductive success may also point to the importance of local environmental factors that are independent of pollinator visitation, such as variation in light, water, or nutrients, that may be driving plant reproduction (Bosch and Waser 2001). To inform conservation management, it is essential to understand how patterns of conspecific density, pollinator availability, and resource availability affect the reproductive success of rare plants.

One group of plants that has received relatively little research on the patterns among conspecific density, pollinator and resource availability, and reproduction is carnivorous plants. In the wild, carnivorous plants face threats from habitat loss, over-collection, and pollution, leading many carnivorous plants to become increasingly rare (Jennings and Rohr 2011). The pollination ecology of many carnivorous plants is not well studied, but prey limitation affects seed-set in some carnivorous species (Stewart and Nilsen 1992, Thorén et al. 1996). Carnivorous

plant leaves have myriad adaptations to attract insect prey, including scents and visual cues (Joel et al. 1985, Kreuzwieser et al. 2014). The effect of conspecific plant density on prey capture in carnivorous plants remains unclear (Savage and Miller 2018), but there is some evidence for interspecific facilitation (Lam et al. 2018). Therefore, carnivorous plants present a unique opportunity to disentangle the links between conspecific density, resource acquisition, and pollinator visitation rates. In addition, carnivorous plants sometimes exhibit an overlap between pollinators and prey (Zamora et al. 1999, Murza et al. 2006), which may cause pollen losses and impose a selective pressure for minimizing capture of pollinators (Jürgens et al. 2012). Some carnivorous plants exhibit temporal separation between traps and flowers (Juniper 1989), but whether this is an adaptation to minimize pollinator-prey overlap is not clear. Insights into whether trap production changes during flowering may provide further insight into the potential for pollinator-prey conflict.

Here we focus on the role of conspecific density of individuals and flowers in the reproduction of Venus flytrap (*Dionaea muscipula* J. Ellis), a rare carnivorous plant that is endemic only to southeastern North Carolina and northeastern South Carolina. This species is currently under consideration for listing under the Endangered Species Act of 1973 (Waller et al. 2016, Hamon et al. 2021). *Dionaea muscipula* was found to be pollen-limited in a study on plants from a single managed area (Hamon et al. 2019). However, the degree to which seed set and pollen limitation vary across its range is unknown. The goal of this study was to address the role of conspecific plant density on the floral visitor abundance, pollinator visitation, pollen limitation, prey capture rate, and seed set of *D. muscipula*, and explore possible causes for variation that could inform conservation management. Assuming as a rare species that *D. muscipula* benefits from conspecifics via facilitation of species interactions that benefit plant

reproduction, we predicted that low density patches of *D. muscipula* would have 1) higher pollen limitation, 2) lower pollinator abundance and visitation rate, 3) a lower proportion of traps with prey, and 4) lower seed set per fruit compared to high density patches. In addition, we explored whether investment in prey capture changes during the flowering period to investigate possible adaptations to minimize pollinator-prey conflict. By determining the relationship between seed set and density, the results of this study will inform priority areas for conservation management of *D. muscipula* and other carnivorous plant species.

METHODS

Study System and Sites

Dionaea muscipula J. Ellis (Droseraceae) is a long-lived perennial with a rosette growth form and a unique terrestrial snap-trap mechanism for capturing prey (Weakley 2020). Each plant has 4-12 trapping leaves (Bailey and McPherson 2012). Following prey capture, traps remain closed for 4 – 7 days before re-opening (Bailey and McPherson 2012). After several bouts of prey capture (about 2 – 3), the trap ceases the ability to close and functions purely as a photosynthetic organ until senescence (Jaffe 1973). Plants reach a mature, flowering stage within about three years. In late May to mid-June, each reproductive individual produces one (rarely more than one) flowering stalk. The single inflorescence is comprised of 8-12 white, actinomorphic flowers with a pentamerous perianth merosity (Smith 1929). This species is protandrous, with the approx. 15 anthers dehiscing roughly 24 hours before the stigma becomes receptive (Smith 1929, Roberts and Oosting 1958, Williams and Scholl 2021). The ovary is superior and unilocular, and the stigma is papillose (Smith 1929, Williams and Scholl 2021).

Dionaea muscipula is self-compatible but requires a mobile pollen vector to transport pollen from anther to stigma (Hamon et al. unpubl., see Chapter 3). The flowers are visited by a generalist suite of pollinators, with sweat bees (chiefly *Augochlorella gratiosa* Smith) and beetles (chiefly *Typocerus sinuatus* Newman and *Trichodes apivorus* Germar) identified as likely primary pollinators (Youngsteadt et al. 2018). Fruits begin to dehisce approximately four weeks after pollination.

Like many plant species in longleaf pine savanna ecosystems (Frost 1993), *D. muscipula* is highly dependent on frequent low-intensity fires to limit the growth of taller light competitors. This species will rapidly decline within several years following a burn (Roberts and Oosting 1958, Gray et al. 2003, Luken 2005a). Burning also promotes flowering in *D. muscipula*, and plants in recently burned sites will exhibit a higher rate of flowering (Luken 2005a) and a greater area of flowering (Hamon et al. 2019) compared to plants in unburned areas.

We studied *D. muscipula* at three sites in each of four managed areas of longleaf pine savanna, for a total of 12 sites scattered throughout the plant's native range of NC and SC, USA. Managed areas were separated from one another by 67.8 – 75.5 km, and were in Horry County (SC), Brunswick County (NC), Pender County (NC), and Carteret County (NC). Within a given managed area, sites were separated from each other by 1.7 – 12.9 km. Due to the poaching risk for *D. muscipula*, we refer to managed areas using the first initial of the corresponding county (H, B, P, and C, respectively) followed by an arbitrary site number (Table 1). All managed areas used controlled burns as part of a land management regimen. Sites included in this study varied widely in the amount of time since the last controlled burn, with the most recent burn occurring two months prior to the study, and the least recent at least three years prior. We selected sites based on a visual assessment of *D. muscipula* density relative to other sites in each managed

area, with one relatively low-, medium-, and high-density site per managed area (Table 1).

Although many studies experimentally manipulate plant or floral density and measure subsequent pollination and plant reproduction (e.g., Kunin 1997, Elliott and Irwin 2009), because we were studying a plant species of conservation concern, we instead relied on natural variation in plant density.

Field Methods

Site Variables

We measured the density of both conspecific and heterospecific flowers at each site on the same day as hand-pollination (described next). To do so, we measured a 25 x 1 m transect at each site that occurred on or next to experimental plants and approximated their extent. At sites where the extent of the *D. muscipula* population was less than 25 m in length on the longest axis, we measured multiple 1 m wide transects with a combined length of 25 m. Within each transect, we counted the number of conspecific and heterospecific plants flowering at the time of hand-pollination and identified them to species using standard references (e.g., Thornhill et al. 2014). On up to 10 plants per species per transect, including *D. muscipula*, we counted the number of flowers on each plant. For taxa with highly compact inflorescences, such as *Polygala* spp. (Polygalaceae), *Lachnocaulon* spp. (Eriocaulaceae) and species in the Asteraceae, we counted inflorescences instead of individual flowers, which we hereafter refer to as ‘flowers’. We also recorded the number of reproductive (i.e., any phenological stage including budding and fruiting) and vegetative (no reproductive stem) *D. muscipula* plants in each transect. To calculate the density of heterospecific and conspecific flowers, we multiplied the mean number of flowers per individual by the total number of individuals within the transect and divided this value by 25. For

sites with multiple transects, we calculated the mean number of flowers per individual across all transects. The total mean number of heterospecific flowers per site was calculated by adding the mean number of flowers per individual across species.

To determine whether the scale of *D. muscipula* density may influence reproduction, we recorded three proxies of *D. muscipula* density at the transect (site) level: the average number of *D. muscipula* flowers per m² (as described above), the average number of reproductive stems (of any phenological stage) per m², and the average number of all *D. muscipula* plants (reproductive and non-reproductive) per m². In addition to estimating the density of flowering *D. muscipula* within each transect, we also measured the density at the focal plant level by recording the number of flowering *D. muscipula* within 1 m² of each experimental plant (experimental plants described in the next section). To do this, we measured a 1 m² square plot around each plant, with the focal flytrap at the center of each plot. We then counted all flowering *D. muscipula* within each square plot. In summary, we recorded three site-level proxies for *D. muscipula* density (N = 12 sites) and one plant-level proxy for *D. muscipula* density (N = 120 plants total, 10 plants per site).

Pollen limitation

To assess pollen limitation, we hand-pollinated plants on eleven dates between late May and mid-June in 2021. Hand-pollinations were conducted between 0930 and 1230 h to ensure both an adequate supply of pollen (new anthers dehisce at approximately 0900 h and steadily lose pollen throughout the day) and the presence of receptive stigmas (stigmas flare between approximately 0930 h and 1230 h). At each of the 12 sites, we haphazardly selected 10 reproductive plants (N = 120 plants total). Each experimental plant was labelled with a piece of

blue laboratory tape (Fisherbrand™ labeling tape, Waltham, MA) placed around the scape. From each inflorescence, we selected two flowers at a similar stage of anthesis, wherein the anthers were dehisced and the stigma was flared. Each flower was haphazardly assigned to one of two treatments: hand-pollinated or open-pollinated control. Flowers in the hand-pollinated treatment were saturated with supplemental pollen by rubbing the dehiscing anthers from another individual (growing at least 5 m away) against the stigma. At least one different pollen donor was used for each experimental flower. Flowers in the control group were handled but not given supplemental pollen. Both treatments were open to natural pollinator visitation. Flower treatments (hand-pollinated or control) were designated by tying a piece of green or black embroidery thread around the pedicel. The color associated with each treatment (hand-pollinated or control) was alternated between sites to avoid any potential color bias to pollinators.

We collected fruits 19-22 days after pollination. At the time of fruit collection, we counted the number of successful and aborted fruits on each plant. Fruits were collected before dehiscence to ensure no seeds were lost. We then counted seeds and recorded the bulk weight of seeds in each fruit to the nearest 0.1 µg. To calculate the average seed weight for each fruit, we divided the bulk weight of seeds by the number of seeds in each fruit. Seeds were returned to their sites of origin after counting and weighing.

Floral visitors

We sampled for flower visitors for at least 60 minutes on the same day as hand-pollination at each site. To do so, we searched the site and collected any arthropods observed touching or resting on *D. muscipula* flower parts. We did not collect arthropods from focal plants in the hand-pollination experiment. Arthropods were captured either using a hand net or by

collecting directly into a vial. With the exception of sites C053 and C107 (Table 1), which were intermittently overcast, arthropod collection occurred on days with fair weather between 1230 and 1652 hours. In total across all sites, we collected for 851 person-minutes. Collected visitors that could be readily identified to species in the field were recorded and released after each hand-netting bout. Butterflies were identified on-the-wing and were counted as a single individual unless another individual of the same species was visible within the same field of view. For these instances, individuals were identified on the wing to family and included in subsequent analyses. Arthropods that were captured but could not be positively identified to species at the time of collection were sacrificed and stored at -30°C until processing. We identified insect species to the lowest possible taxonomic level using standard references (e.g., Gibbs 2011, Evans 2014, and references therein) and comparison to previous collections in the NC State University Insect Museum, Raleigh, NC, USA. Sacrificed specimens were pinned, and will be labelled and archived in the collections at the NC State University Insect Museum.

In addition to hand-netting, we set up a time lapse camera (Brinno Inc© TLC200 Pro) in each site following the arthropod sampling, excluding site B028. The camera was aimed toward at least one *D. muscipula* inflorescence not included in the hand-pollination study and programmed to take a picture every two seconds from dawn to dusk. The number of flowering conspecific individuals within 1 m^2 of the focal inflorescence (as described above) was recorded for nine of the eleven sites where we obtained footage. Cameras were deployed between 1357 and 1922 hrs and retrieved after at least 24 hours. We then watched the footage and recorded any visits to *D. muscipula* flowers. Periods of rain were recorded and excluded from analysis. Visits where the animal touched either the anthers or stigma were labelled as being pollinator visits. During each visit, we recorded the number of stems and open flowers at the time of visit. We

also noted the number of open flowers at the start of each day. Each visitor was then identified to the lowest possible taxonomic resolution (typically order). In total, we analyzed footage representing 11 – 67 hrs of daylight observations at each of 11 sites where a camera was deployed. Our prior research suggested that *D. muscipula* does not receive nighttime floral visitation (Hamon et al. 2019), so cameras were programmed to cease recording at night.

Trapping rate

For each of the 120 focal *D. muscipula* included in the hand-pollination experiment, we recorded the number of developing, open, closed, and partially open (defined as when the marginal teeth were overlapping but the traps were ajar, and no prey items were present) traps. The number of traps of each type were recorded at the time of the hand-pollination treatments (flowering) and again at the time of fruit collection (flowering complete).

Statistical analyses

Statistical analyses were performed using R v.4.1.1 within R studio v.1.4.1717 (R Core Team 2021, R Studio Team 2021). To determine whether the four proxies for *D. muscipula* density were correlated, we calculated pairwise correlation coefficients for all conspecific density proxies, including the three site-level proxies (average number of *D. muscipula* flowers per m², average number of reproductive stems per m², and average number of all *D. muscipula* plants per m²), and the single plant-level density proxy (number of flowering stems within 1 m² of each experimental individual, which was averaged within sites for this analysis) using the package ‘Hmisc’ (v.4.6.0, Harrell 2017). All density proxies were significantly correlated with one another ($r > 0.61$, $P < 0.01$ in all cases), except for the average number of *D. muscipula* flowering stems within 1 m² of each experimental individual ($r = 0.49$, $P > 0.1$). Consequently, we elected to use two uncorrelated proxies of density: average number of all *D. muscipula* plants

per m² in the whole transect and number of flowering *D. muscipula* stems within 1 m² of each experimental individual. These proxies are hereafter referred to as site density and local density, respectively.

Pollen limitation: To determine whether the site density and local density of *D. muscipula* influenced pollen limitation, we constructed a linear mixed-effects model in which seed set per fruit was the response, and hand-pollination treatment (hand-pollinated vs. control), site density of *D. muscipula*, local density of *D. muscipula*, and their interactions were included as predictors. In a separate model examining the effect of heterospecific flower density on pollen limitation, we also constructed a linear mixed-effects model in which total heterospecific density (the sum of average number of flowers per m² across all species observed at each site) and its interaction with pollination treatment were the explanatory variables. Managed area, site nested within managed area, and individual nested within both site and managed area were included in these models as random intercepts. To assess whether pollen supplementation affected investment in seed size in this study, we constructed a similar linear mixed-effects model in which average seed weight was the response and treatment, site density, local density, and their interactions were the predictor variables. All linear mixed effects models were conducted using the package ‘lme4’ (v.1.1.27.1, Bates et al. 2017) and tested using the ‘Anova’ function within the package ‘car’ (v.3.0.11, Fox and Weisberg 2011, summarized in Table 2).

Floral visitors: To determine if there was a difference in floral visitor abundance between sites with differing densities of *D. muscipula*, we calculated the number of flower visitors observed per hour of arthropod searches in the field. We then constructed a linear mixed effects model with number of floral visitors captured per hour as the response variable and site density as the predictor variable. To determine if there was a difference in pollinator visitation rates

between sites with differing densities of *D. muscipula* based on time-lapse camera footage, we added the total number of pollinator visits during each unique combination of number of open flowers and date at each site. We then divided the total number of pollinator visits by the number of open flowers and the number of hours of analyzed footage, resulting in an estimate of number of visits per flower per hour. To obtain the average visitation rate for each site, we calculated the average number of visits per flower per hour, weighted by the number of hours of footage. We then constructed a linear mixed effects model with weighted average of visits per flower per hour as the response variable and site density, local density of the focal filmed inflorescence, and their interactions as predictor variables. To test whether heterospecific flowering density affected floral visitor abundance and pollinator visitation rate to *D. muscipula*, we constructed two additional models in which floral visitor abundance or pollinator visitation rate were the response variables, and total heterospecific density was the explanatory variable. In all models examining arthropod responses, managed area was included as a random variable (Table 2).

Trapping rate: To determine whether the trapping rate differed between sites of different densities, we divided the number traps which were closed at hand-pollination by the total number of traps to obtain a proportion of closed traps per plant. Using a linear mixed effects model, we examined whether the proportion of closed traps differed with site density, local density, and their interactions. Managed area and site nested within managed area were included in this model as random effects. Site H018 was excluded from this analysis because, at the time of hand-pollination, traps were dormant due to drought conditions. In addition, we calculated the proportion of successful fruits per plant by dividing the number of fruits that set seed by the total number of fruits. We constructed a linear mixed effects model wherein proportion of successful fruits was the response variable, proportion of closed traps at pollination was the explanatory

variable, and managed area and site nested within managed area were included as random effects. To determine whether proportion of closed traps influenced seed set and pollen limitation, we constructed a linear mixed-effects model in which seed set per fruit was the response, and hand-pollination treatment (hand-pollinated vs. control), proportion of closed traps at pollination, and their interactions were included as predictors. Managed area, site nested within managed area, and individual nested within both site and managed area were included in this model as random effects. To explore trends that were potentially obscured by comparing proportions, we also ran these models with number of closed traps as the variable of interest. However, we also ran a simple linear regression model to test whether total number of traps per plant had a significant positive relationship with number of closed traps, which would suggest an underlying effect of plant size for which we did not control in our study.

Changes across flowering period: We compared the number of traps between the time of hand-pollination (peak-flowering) and the time of fruit collection (post-flowering) to examine how investment in prey capture changes over the flowering period. To do so, we constructed another linear mixed effects model in which total number of traps or number of developing traps was the response variable, and reproductive phenology (flowering vs. fruiting) was the explanatory variable. Managed area, site nested within managed area, and individual nested within site and managed area were included in the model as random effects.

RESULTS

Pollen limitation

Hand-pollinated flowers produced on average 27% more seeds per fruit compared to open-pollinated flowers ($F_{1,100} = 22.35$, $P < 0.0001$, Fig. 2. See Table 2.2 for all linear mixed-effects

model summaries). Site density varied between 0.52 – 12.55 *D. muscipula* flowers per m², while average local density varied between 1.90 – 21.40 flowering stems within one m² of each experimental plant. Seed set per fruit increased with local density of *D. muscipula* ($F_{1,91} = 5.43$, $P = 0.02$, Fig. 2.). However, seed set per fruit did not vary significantly with site density of *D. muscipula* ($F_{1,10} = 0.15$, $P = 0.71$). Moreover, there were no significant interactions between site density or local density and pollination treatment on seed set per fruit, indicating that degree of pollen limitation did not differ across sites of varying conspecific density ($F_{1,98} = 0.0001$, $P = 0.99$; $F_{1,98} = 0.68$, $P = 0.41$, respectively). There was also no significant interaction between heterospecific flower density and pollination treatment on seed set per fruit, indicating that degree of pollen limitation did not differ between sites of varying heterospecific flower density ($F_{1,84} = 0.79$, $P = 0.38$).

Average seed weight ranged from 0.03 – 0.49 mg. Average seed weight did not depend on hand-pollination treatment ($F_{1,82} = 2.63$, $P = 0.11$), local density ($F_{1,32} = 0.97$, $P = 0.33$), site density ($F_{1,10} = 0.0011$, $P = 0.97$), or their interactions ($F_{1,81} = 0.13$, $P = 0.72$; $F_{1,82} = 2.59$, $P = 0.11$ respectively).

Floral visitors

In field observations, we observed 105 floral visitors to *D. muscipula*, with a range of 0-17.14 floral visitors observed per hour. Of these 105 visitors, 51 (49%) were beetles, 33 (31%) were bees, 13 (12%) were butterflies or moths, 5 (5%) were flies, 2 (2%) were true bugs, and 1 (1%) was an Orthopteran (Supplemental table 1). There was no significant relationship between number of floral visitors observed per hour and site density ($F_{1,9} = 1.50$, $P = 0.25$).

From the time-lapse camera footage, we documented 741 visits wherein the visitor touched either anthers or stigmas, with a range of 0.07 – 1.22 visits per open flower per hour. There was no significant relationship between visits per open flower per hour and site density ($F_{1,3} = 0.02$, $P = 0.90$), camera local density ($F_{1,2} = 0.72$, $P = 0.48$), or their interactions ($F_{1,4} = 2.14$, $P = 0.22$).

Co-occurring flowering species included *Amianthium muscitoxicum* (Walter) A. Gray (Melanthiaceae), *Hypericum tenuifolium* Pursh (Hypericaceae), *Ilex glabra* (L.) A. Gray (Aquifoliaceae), *Lyonia ligustrina* (L.) DC. (Ericaceae), *Polygala lutea* L. (Polygalaceae), and *Utricularia subulata* (L.) (Lentibulareaceae). The average heterospecific flower density ranged between 0.00 – 19.83 flowers per m². We observed a significant positive relationship between pollinator visitation rate and average number of heterospecific flowers per m² ($F_{1,7} = 8.23$, $P = 0.02$). However, we observed no significant relationship between floral visitor abundance and average number of heterospecific flowers per m² ($F_{1,9} = 0.04$, $P = 0.84$).

Trapping rate

Of the total traps per experimental plant, there were an average of 0 – 35% of closed traps at each site. The average proportion of closed traps per site did not depend on either site density ($F_{1,9} = 0.02$, $P = 0.90$), local density ($F_{1,37} = 0.04$, $P = 0.85$), or their interactions ($F_{1,23} = 0.27$, $P = 0.61$). The total number of closed traps per plant also did not differ by site density ($F_{1,9} = 0.29$, $P = 0.61$), local density ($F_{1,36} = 0.44$, $P = 0.51$), or their interactions ($F_{1,23} = 0.01$, $P = 0.90$).

There was no significant relationship between the proportion of successful fruits and the proportion of closed traps at time of hand pollination ($F_{1,80} = 3.48$, $P = 0.07$, Fig. 2a). We observed a positive relationship between the proportion of successful fruits and the number of

closed traps at time of hand pollination ($F_{1,87} = 6.84$, $P = 0.01$, Fig. 2b). However, we also observed a significant positive relationship between total number of traps and number of closed traps ($F_{1,98} = 16.73$, $P < 0.001$), pointing to overall plant size as a possible correlate of reproductive success.

We found no significant relationship between seed set per fruit and the proportion of closed traps at pollination ($F_{1,87} = 0.37$, $P = 0.54$, Fig. 3a) or the interaction between closed traps at pollination and hand-pollination treatment ($F_{1,88} = 0.56$, $P = 0.46$). When examining the number of closed traps instead, there was a significant positive relationship between seed set per fruit and the number of closed traps ($F_{1,89} = 6.23$, $P = 0.01$, Fig. 3b), but no significant effect of the interaction between hand pollination treatment and number of closed traps ($F_{1,90} = 0.59$, $P = 0.44$).

Changes across flowering period

There was a significant effect of reproductive phenology (flowering vs. fruiting) on the total number of traps per individual ($F_{1,115} = 163.45$, $P < 0.0001$, Fig. 4a), with fruiting plants having an average of 2.57 more traps per plant than flowering individuals. There was also a significant effect of reproductive phenology on the number of developing traps per individual ($F_{1,116} = 246.58$, $P < 0.0001$, Fig. 4b), with fruiting plants having an average of 2.2 more developing traps per plant than flowering individuals.

DISCUSSION

The relationship between reproductive success and conspecific density can depend on both biotic and abiotic context (Ye et al. 2014), and understanding this relationship is of particular concern

for rare plants. The goal of this study was to address the role of conspecific plant density on seed set in a rare carnivorous plant with a restricted range. Although *D. muscipula* exhibited widespread pollen-limitation, the degree of pollen limitation, floral visitor abundance, pollinator visitation rate, and prey capture rate did not differ in sites of differing density. These findings contradict our predictions and point to the importance of other patterns of resource availability and density that were not addressed in our study.

When examining localized patterns of density around experimental plants, *D. muscipula* reflects the prediction that density is frequently positively correlated with reproductive success. However, this pattern was not reflected by an increase in floral visitor abundance, pollinator visitation rate, or prey capture success. One possible explanation is that conspecific density may reflect habitat quality, resulting in a correlation between seed set and density via other types of resource limitation (e.g., Ghazoul 2005, Elliott and Irwin 2009). For example, *D. muscipula* requires consistently moist soil (Roberts and Oosting 1958). Roberts and Oosting (1958) observed that *D. muscipula* occurring in moist depressions tend to grow more vigorously, likely reflecting this trend. Both seed production and population density may therefore be most strongly associated with water availability, and by extension, water table depth and soil porosity. In addition, the presence of *Sphagnum* spp., which require a similar moisture regime to *D. muscipula*, is associated with higher seedling establishment in this species (Luken 2005b), which further connects moisture and density in *D. muscipula*.

Local patterns of resource availability to *D. muscipula* may also be shaped by the heterogeneous nature of fire. Understory fuel load and composition influences the intensity of fire at variable scales (Wenk et al. 2011, Wiggers et al. 2013), resulting in variable availability of nutrients such as calcium and nitrogen (Rice 1993, Kennard and Gholz 2001). Though *D.*

muscipula depends largely on insect prey for nutrients, some nutrients are soil derived (Gao et al. 2015), which may shape seed set and density. Fire also influences flowering in *D. muscipula*, with a higher percentage of flowering following a burn (Roberts and Oosting 1958). Therefore, the relationship between seed set and density may also reflect the patchiness of fire and its intensity.

Although pollen limitation did not vary with density, hand-pollination significantly improved seed set per fruit compared to open pollinated controls, reinforcing a previous assessment that *D. muscipula* is pollen-limited (Hamon et al. 2019). If *D. muscipula* is indeed strongly resource-limited and we observed no differences in floral visitor abundance or visitation with conspecific density, it is puzzling that pollen limitation did not differ between sites. In order to observe consistent levels of pollen limitation across a resource gradient, we would expect a parallel increase in the levels of pollination. It is notable that we only had seven data points for comparing pollinator visitation rate by camera local density. Future studies should place increased emphasis on measuring pollinator visitation rate across varying conspecific density. In addition, more proximate metrics for pollination – such as number of pollen grains per stigma – may sidestep the shortcomings of pollinator observations in favor of a more direct measurement of pollen receipt. Nonetheless, the consistent levels of pollen limitation observed in this study point to the troubling possibility that current levels of conspecific pollen deposition may be universally low throughout the range of this species. Pollen limitation occurs when pollen deposition to the stigma is insufficient in either quality (from heterospecific or non-compatible pollen) or quantity (Ashman et al. 2004). Since *D. muscipula* is self-compatible and we observed no correlation between heterospecific flower density and pollen limitation, it is likely that low pollen quantity is driving this pattern in this system (Ashman et al. 2004, Knight et al. 2005).

Species with narrowly endemic ranges may be particularly susceptible to pollen limitation, since they often exhibit high habitat specificity, high fragmentation, and smaller population sizes, thereby disrupting plant-pollinator interactions (Lavergne et al. 2004, Alonso et al. 2010). In a study on the self-compatible, endangered herb *Erysimum popovii* Rothm. (Brassicaceae), which is endemic to southeastern Spain, Fernández et al. (2012) observed that pollen limitation was widespread and posited that this was largely driven by both environmental factors and the relative abundance of certain pollinator taxa. Widespread pollen limitation has also been observed in other narrow endemic plant species, including the South African endemic *Brunsvigia litoralis* R.A. Dyer (Amaryllidaceae) and the Chinese endemic *Sinocalycanthus chinensis* W.C. Cheng & S.Y. Chang (Calycanthaceae) (Li et al. 2020), for which fragmentation was posited as one likely driving factor. In carnivorous plants specifically, pollen limitation has been observed in the endemic *Pinguicula vallisneriifolia* Webb (Lentibulareaceae) (Zamora 1999).

We were surprised to find that neither floral visitor abundance nor pollinator visitation rate were correlated with conspecific density, both at the site level and local level. This suggests that additional factors besides conspecific density shape the foraging behaviors of *D. muscipula* pollinators. *Dionaea muscipula* is visited by a broad suite of pollinators, with sweat bees and beetles forming the bulk of pollen-carrying visitors (Youngsteadt. et al. 2019). It is possible that there is a threshold population size, population area, or density needed to initially attract pollinators (Schmitt 1980), which was met by all sites included in the present study. It is worth noting that our sites, though differing widely in density and size, are all considered part of relatively large populations for *D. muscipula* (Hamon et al. 2021). Once pollinators are present in a population, visitation rates may be shaped largely by interactions with other pollinators or with other plant species (Hegland and Boeke 2006, Perfectti et al. 2009). Though we observed no

significant relationship between heterospecific flower density and pollen limitation, the positive correlation between visitation rate and heterospecific flower density points to the potential role of other plant species in maintaining some degree of pollination to *D. muscipula*.

Though *D. muscipula* employs a scent for prey attraction (Kreuzwieser et al. 2014), we found no evidence that high densities of flytraps amplify the attraction of prey. In addition, they do not appear to compete for prey at higher densities. The number of closed traps – but not proportion of closed traps – was positively associated with both fruit success rate and seed set per fruit. Neither trap measurement was different between local- or site-level conspecific densities. We did observe that plants with more closed traps had more traps overall, and given that leaves are a source of both mineral nutrients and photosynthates, it is not surprising that there is an association between trap number and reproductive success. Increased nutrient supplementation is associated with increased growth in *D. muscipula* (Kruse et al. 2014), and nutrients acquired from prey capture are quickly mobilized to flower tissue (Schulze et al. 2001). We did observe a stark difference between number of traps at the time of flowering and at the time of fruiting. While it is possible that this is partly a byproduct of the advancement of the growing season, it is interesting that there were significantly more developing traps immediately post-flowering. Other carnivorous plants exhibit decreased prey capture during flowering, including members of the closely related *Drosera* L. (Zamora 1999 and Murza et al. 2006). This has been proposed as a possible adaptation for minimizing pollinator-prey conflict (Juniper et al. 1989, El-Sayed et al. 2016). It is unclear whether lower trap development during flowering in *D. muscipula* is due to a shift in resource allocation to reproduction, or an adaptation to minimize prey capture. Given that *D. muscipula* apparently shifts investment during flowering, trapping rates at the time of flowering are not necessarily reflective of trapping rates at other times of year. A future topic for

research may be investigating the temporal variation in prey trapping rates in *D. muscipula*, and whether there is facilitation or competition between conspecifics during peak capture times.

An important caveat of this study is that we relied on natural variation in floral density and did not manipulate density experimentally, which could have helped to disentangle the relative influence of environmental variation on seed set in *D. muscipula*. Multiple other studies have controlled for environmental variation by experimentally manipulating plant density in other systems, resulting in a more nuanced understanding of the role of both biotic and abiotic factors in density-dependent seed-set (Bosch and Waser 2001, Elliott and Irwin 2009). Because of the rare status of *D. muscipula*, it was obviously not ethical to conduct density manipulations with wild-growing plants. To disentangle the effects of environmental variation on seed set of *D. muscipula*, future studies could manipulate the density of potted individuals within its native habitat, but care should be taken to avoid altering the genetic structure of neighboring wild-growing populations via pollen and gene flow. In addition, it is essential to quantify variation in abiotic factors in the wild, such as moisture and nutrient availability, and how they affect reproduction in *D. muscipula*.

In conclusion, we observed significant pollen limitation of *D. muscipula* across the four managed areas included in this study. Pollen limitation can lead to reduced plant abundance, which makes this consistent pattern of pollen limitation an area of conservation concern, assuming a link between seed production and recruitment. Community-wide studies of pollen limitation in longleaf pine ecosystems may shed more light on whether pollen limitation is widespread in this system, and where land management for pollinators may have the highest impact. Our results also suggest that local patterns of *D. muscipula* density are associated with higher seed set per fruit, though the factors that drive this pattern are unclear. This species

continues to be threatened by fire suppression, development, and habitat conversion to timber plantations, all of which can impact local hydrology and nutrient availability (Rice 1993, Kennard and Gholz 2001). To identify areas of conservation priority, it is essential to identify factors limiting the reproduction of *D. muscipula*.

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Table 2.1. A summary of sites and the corresponding density of *D. muscipula* measured in transects at each site, floral visitor abundance, and pollinator visitation rate.

Managed area ID	Site ID	average number of flowers per m²	average number of reproductive stems per m²	average number of all plants per m²	Number of flower visitors observed per hr	Weighted average of number of pollinator visits per monitored flower per hr
H	006	12.55	11.48	15.60	12.95	0.04
H	008	10.07	4.20	5.76	4.48	0.12
H	066	6.60	6.12	9.92	2.90	0.14
B	028	2.40	1.52	1.56	0.98	n/a
B	043	1.14	1.04	1.08	2.86	0.09
B	210	8.28	5.40	12.76	17.14	0.11
P	018	1.45	5.44	7.16	0.00	0.15
P	019	2.35	2.08	1.72	4.62	0.03
P	059	2.54	2.28	2.52	10.77	0.11
C	053	3.02	1.48	1.56	5.54	0.19
C	107	2.7	1.96	4.16	9.87	1.22
C	109	0.52	0.20	0.28	10.13	0.48

Table 2.2. Summary of linear mixed effects models and their results. All linear mixed effects models were conducted using the package ‘lme4’ (v.1.1.27.1, Bates et al. 2017) and summarized using the ‘Anova’ function within the package ‘car’ (v.3.0.11, Fox and Weisberg 2011).

Predictors	Random effects	F value	P value	df regression	df residual
<i>seed set per fruit</i>	managed area, managed area site, managed area site plant				
treatment		22.35	<0.0001	1	100.01
site density		0.15	0.71	1	9.83
local density		5.43	0.02	1	90.97
treatment × site density		<0.001	0.99	1	97.94
treatment × local density		0.68	0.41	1	97.57
site density × local density		1.14	0.29	1	71.90
treatment × site density × local density		0.47	0.49	1	97.52
<i>seed set per fruit</i>	managed area, managed area site, managed area site plant				
treatment		2.58	0.11	1	83.98
heterospecific flower density		0.00	0.95	1	8.25
treatment × heterospecific flower density		0.79	0.38	1	84.21
<i>average seed weight</i>	managed area, managed area site, managed area site plant				
treatment		2.63	0.11	1	81.92
site density		0.00	0.97	1	9.90
local density		0.97	0.33	1	31.65
treatment × site density		2.59	0.11	1	81.57
treatment × local density		0.13	0.72	1	81.31
site density × local density		0.57	0.46	1	23.82
treatment × site density × local density		1.37	0.24	1	81.24
<i>floral visitor abundance</i>	managed area				
site density		1.50	0.25	1	8.59

Table 2.2. (continued)

<i>visitation rate</i>	managed area				
site density		0.02	0.90	1	2.53
camera local density		0.72	0.48	1	2.11
site density × camera local density		2.14	0.22	1	3.96
<i>floral visitor abundance</i>	managed area				
heterospecific flower density		0.04	0.84	1	8.61
<i>visitation rate</i>	managed area				
heterospecific flower density		8.23	0.02	1	7.10
<i>proportion closed traps</i>	managed area, managed area site				
site density		0.02	0.90	1	9.01
local density		0.04	0.85	1	37.49
site density × local density		0.27	0.61	1	23.43
<i>Number of closed traps</i>	managed area, managed area site				
site density		0.29	0.61	1	8.91
local density		0.44	0.51	1	35.64
site density × local density		0.01	0.90	1	22.94
<i>proportion successful fruits</i>	managed area, managed area site				
proportion traps closed		3.48	0.07	1	79.93
<i>proportion successful fruits</i>	managed area, managed area site				
number of closed traps		6.84	0.01	1	86.56
<i>seed set per fruit</i>	managed area, managed area site, managed area site plant				
treatment		21.76	<0.0001	1	87.41
proportion traps closed		0.37	0.54	1	87.28

Table 2.2. (continued)

	treatment × proportion traps closed	0.56	0.46	1	88.37
<i>seed set per fruit</i>	managed area, managed area site, managed area site plant				
	treatment	23.36	<0.0001	1	92.59
	number traps closed	6.23	0.01	1	88.93
	treatment × number traps closed	0.59	0.44	1	90.39
<i>total number of traps</i>	managed area, managed area site, managed area site plant				
	time (pollination vs. collection)	163.45	<0.0001	1	115.29
<i>number of developing traps</i>	managed area, managed area site, managed area site plant				
	time (pollination vs. collection)	246.58	<0.0001	1	116.19

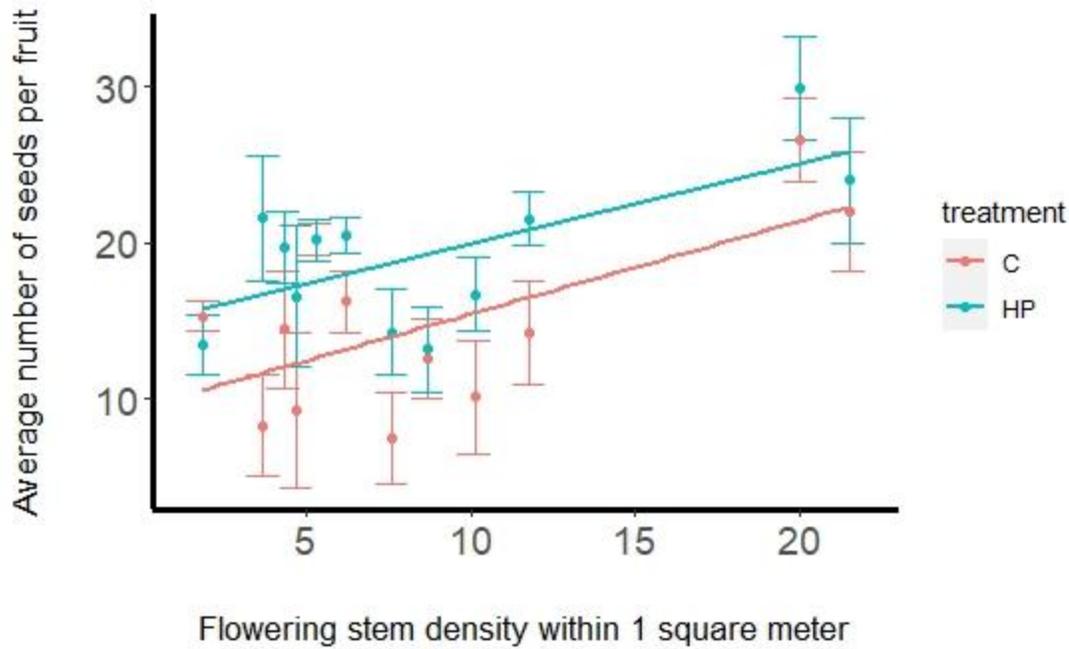


Figure 2.1. There was a significant positive linear relationship between *D. muscipula* flowering stem density within 1 m² of each experimental plant and average seed set per fruit (mean \pm se), with hand-pollinated flowers producing more seeds per fruit on average compared to open-pollinated flowers. Each point represents a site mean for either control (red points) or hand-pollinated (blue points) flowers.

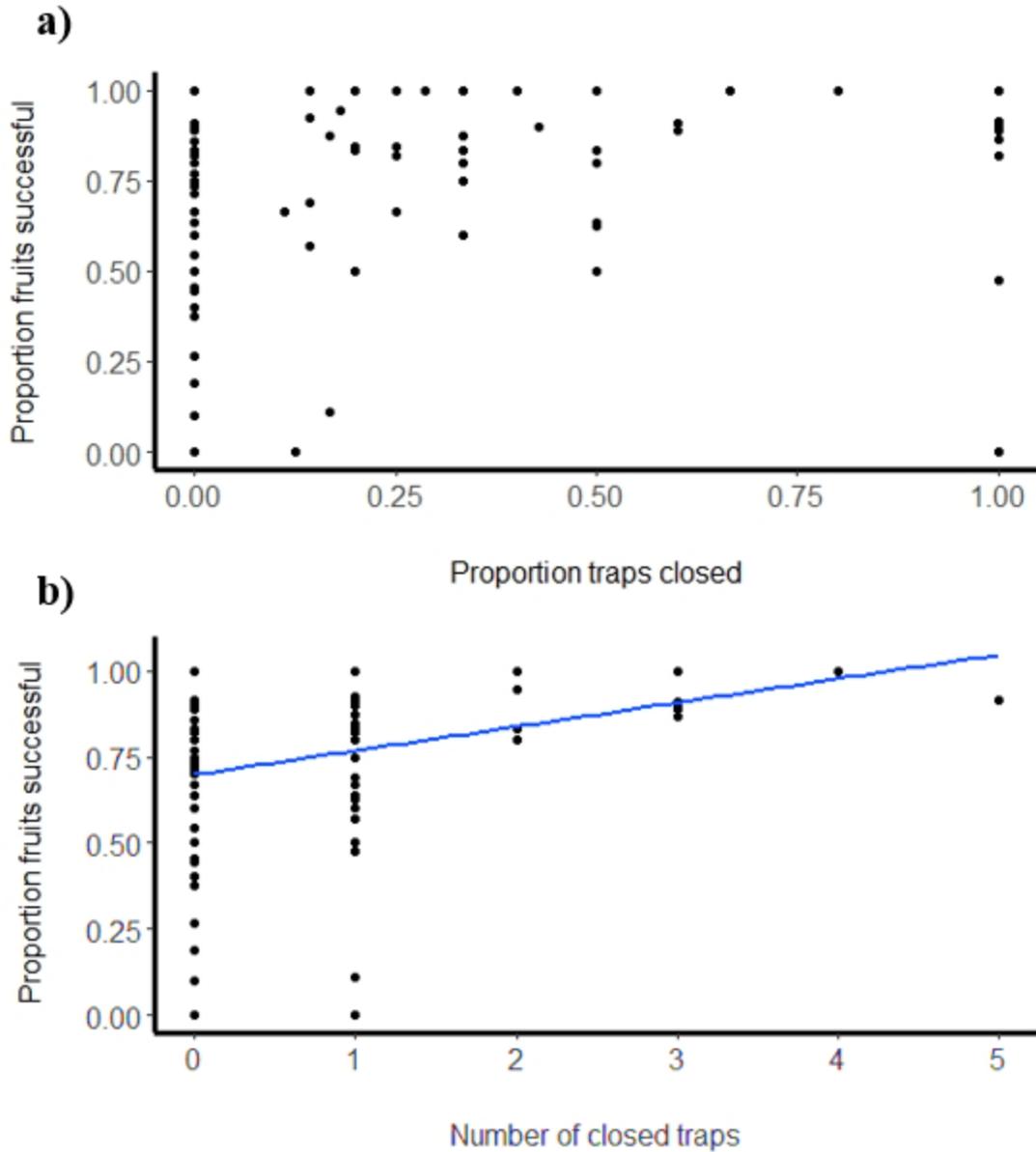


Figure 2.2. There was a) no significant relationship between the proportion of fruits that were successful and the proportion of traps that were closed at the time of hand-pollination and b) a significant positive relationship between the proportion of fruits that were successful and the total number of closed traps. Each point represents an individual plant.

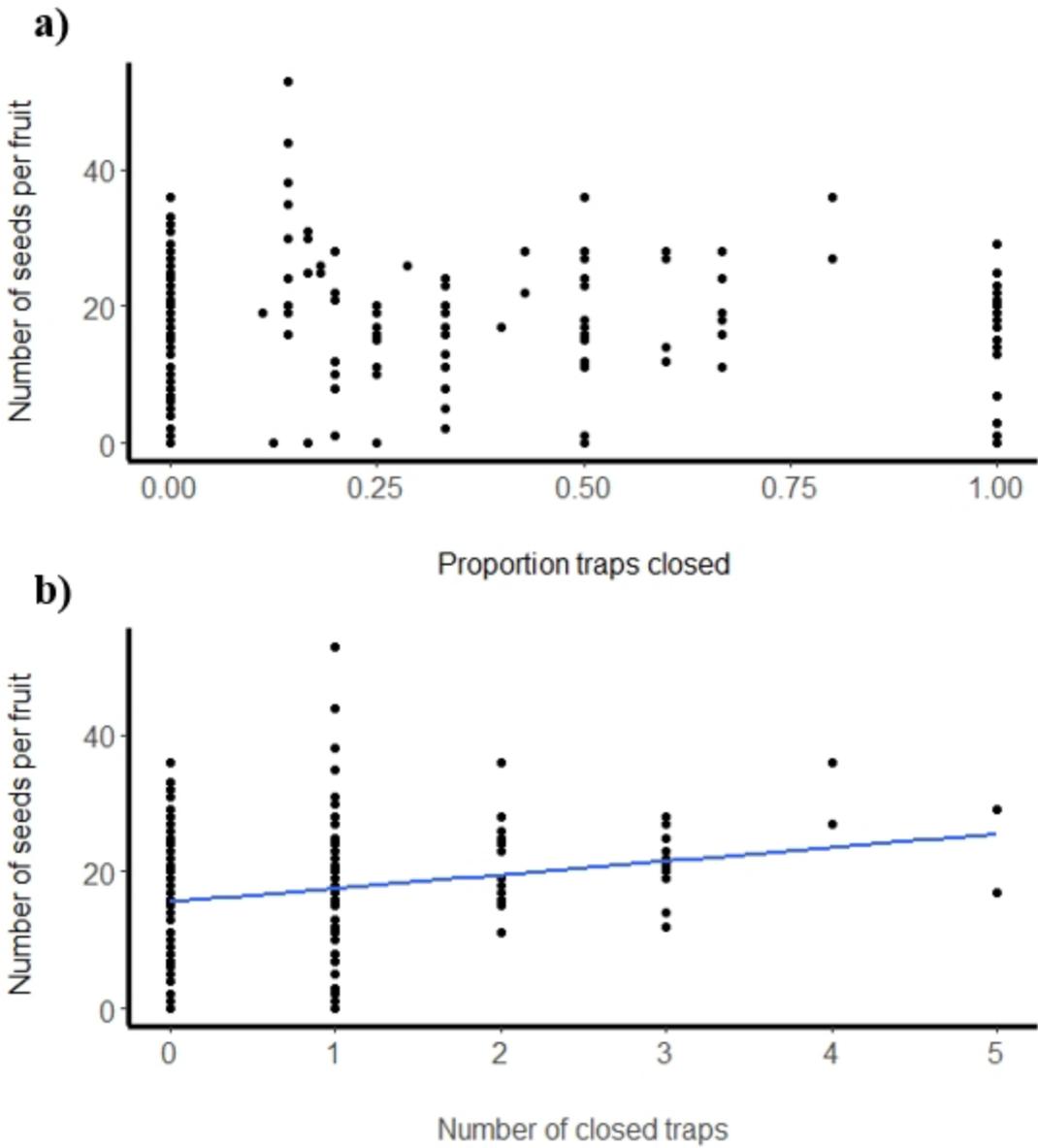


Figure 2.3. There was a) no significant relationship between seed set per fruit and the proportion of traps that were closed at the time of hand-pollination and b) a significant positive relationship between seed set per fruit and the total number of closed traps. Each point represents an individual plant.

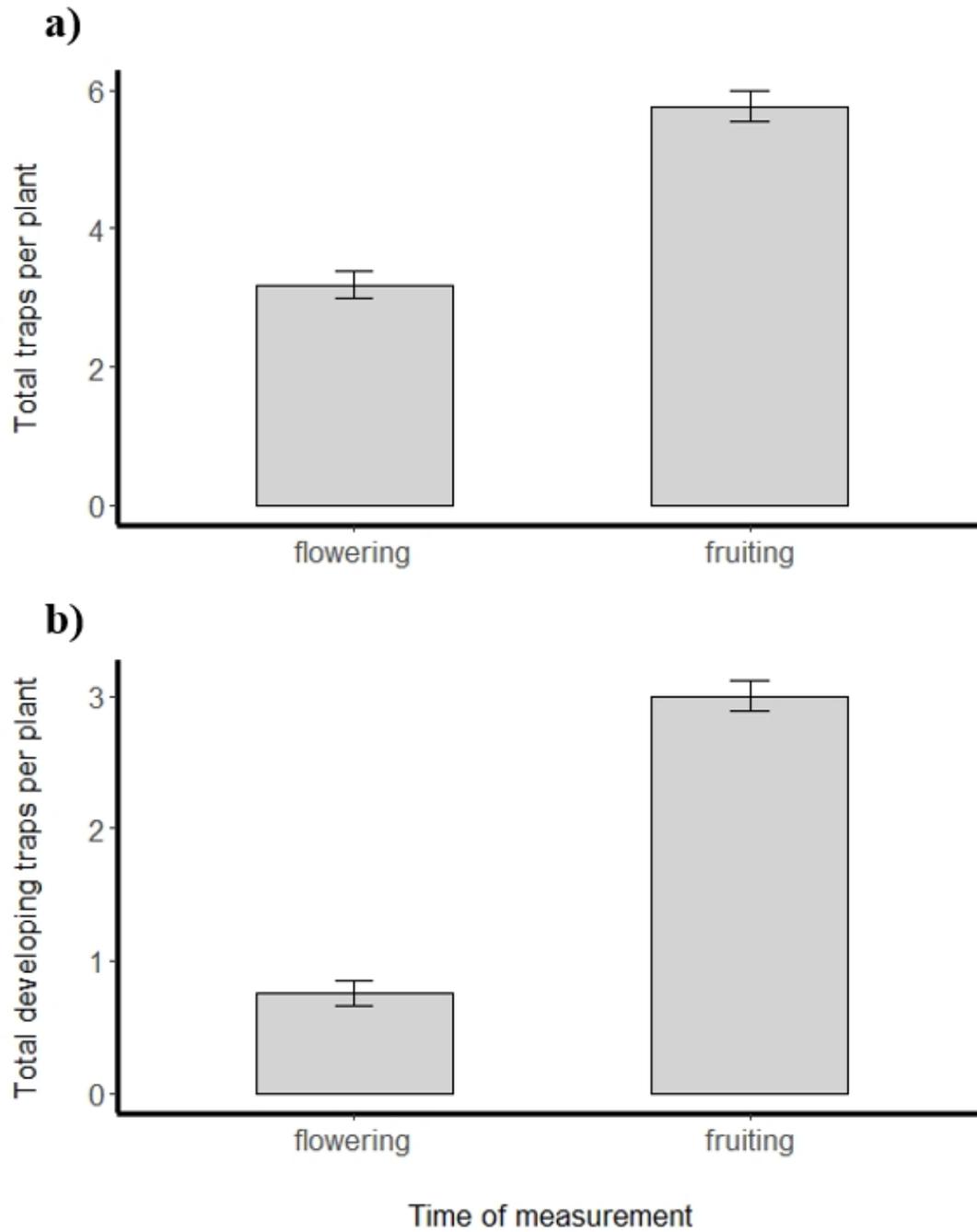


Figure 2.4. Total number of a) traps (mean \pm se) and b) developing traps (mean \pm se) was significantly higher in fruiting plants compared to flowering plants across all sites.

CHAPTER 3: Pollen and prey supplementation in Venus flytrap (*Dionaea muscipula*): implications for flower production and plant reproduction

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ABSTRACT

Carnivorous plants rely on insects as both pollinators and nutrient sources, providing a unique system for studying the effects of both pollen and resource limitation on plant reproduction. In both a 2019 and 2020 trial, we conducted a field experiment using wild-growing Venus flytrap (*Dionaea muscipula*) in which we manipulated prey and pollen in a factorial design and measured estimates of plant reproduction. Because understanding reproduction requires knowledge of a plant species' reproductive and pollination biology, we also examined *D. muscipula*'s pollination system, per-visit pollinator effectiveness, and pollen-ovule (P/O) ratio. We found no difference in seed set per fruit or seed weight between pollen or prey supplementation treatments or their interaction. However, plants that were supplemented with prey just prior to the start of flowering produced significantly more flowers per plant during that same growing season. Hand-pollinations revealed that *D. muscipula* is self-compatible, but pollinators are needed to move pollen from anthers to stigmas. This reliance on pollinator visitation is further supported by a P/O ratio of 332.5, a high ratio compared to other members of Droseraceae. Honeybees deposited more pollen grains per visit than sweat bees, potentially pointing to the role of visitor size and behavior in pollination effectiveness to this species. Taken together, although experimentation revealed no prey or pollen limitation for seed set per fruit, this study reinforces the reliance of *D. muscipula* on adequate prey receipt for flower production and a mobile pollen vector for reproduction.

INTRODUCTION

An essential component of plant ecology is to understand the factors that limit plant reproduction. In particular, many studies have examined how reproduction can be limited by both pollen and resource availability (e.g., Haig and Westoby 1988, Zimmerman and Aide 1989, Burd 2008, Cunningham et al. 2020). These two factors are not mutually exclusive (Campbell and Halama 1993), and resource limitation can also indirectly influence pollen receipt via increased flower or reward production (Zimmerman and Pyke 1988, Campbell and Halama 1993, Carroll et al. 2001). Despite an increasingly nuanced understanding of how resource and pollen limitation affect plant reproduction, these factors have infrequently been studied simultaneously in rare plants (Mattila and Kuitunen 2003, Shi et al. 2010). This is concerning in instances where reduced reproduction can have repercussions for population viability (Wilcock and Neiland 2002, Law et al. 2010). The goal of this study was to understand the degree to which reproduction in a rare carnivorous plant is limited by pollen and resources.

Carnivorous plants get most of their mineral nutrients – chiefly nitrogen (N) and phosphorous (P) – from animal prey (Ellison and Gotelli 2001, Ellison 2006). Low prey capture has been shown to limit reproductive success in several carnivorous plant taxa, including *Pinguicula* L. (Lentibulareaceae) (Thorén and Karlsson 1998) and *Sarracenia* L. (Sarraceniaceae) (Ne'eman et al. 2006). To our knowledge, only one study has investigated the simultaneous influence of pollen and resource limitation on a carnivorous plant (Ne'eman et al. 2006), finding that resource limitation from photosynthates and prey-derived nitrogen had a more significant effect on reproductive output compared to pollen limitation in *S. purpurea* L. The overall importance of pollen limitation to carnivorous plant reproduction remains poorly known.

Since carnivorous plants depend on insects both as pollinators and prey (Juniper et al. 1989), they run the risk of capturing their own pollinators, a hypothetical dilemma known as pollinator-prey conflict (PPC) (Jürgens et al. 2012, El-Sayed et al. 2016). Evidence for PPC is scant (Cross et al. 2018) and has been shown in just a handful of instances (Zamora 1999, Murza et al. 2006). Several scenarios are predicted to minimize PPC in carnivorous plants (Jürgens et al. 2012, Cross et al. 2018). Although captured pollinators are a potential source of pollen loss, they are also a source of nutrients, and PPC may be minimal if reproduction is more resource limited than pollen limited (Cross et al. 2018). Carnivorous plants that can self-pollinate may also have reduced PPC (Jürgens et al. 2012) since a mobile pollen vector would be unnecessary for seed set, with the caveat that this pattern may depend on the level of inbreeding depression (Charlesworth and Charlesworth 1987). Finally, if there is any overlap between pollinator and prey species, PPC may be reduced if the suite of captured insects are relatively ineffective pollinators. To better understand PPC in carnivorous plants, more studies are needed to understand the relative influence of pollen and resource limitation on reproduction, as well as basic aspects of carnivorous plant pollination systems and most effective pollinator taxa.

Though several studies have demonstrated resource limitation to growth and reproduction in carnivorous plants, the patterns and timing of resource allocation in carnivorous plants remains unclear. In the single study where both pollen and prey limitation were simultaneously examined in *Sarracenia*, resource manipulation was performed within a single growing season after bud initiation (Ne'eman et al. 2006). Work with other carnivorous plants has shown that nutrient capture in previous seasons can contribute to trap, flower, or fruit production in the subsequent growing season (e.g., Thum 1988, Thorén and Karlsson 1998, Krafft and Handel 1991).

Therefore, in order to effectively study the role of resource limitation in carnivorous plant reproduction, it is necessary to capture the time window where resources are most limiting.

In this study, we investigated the simultaneous influence of pollen and prey limitation on the reproduction of Venus flytrap (*Dionaea muscipula* J. Ellis, Droseraceae), a rare carnivorous plant. *Dionaea muscipula* can be pollen limited, a pre-condition for PPC (Hamon et al. 2019). However, overlap between pollinator and prey species in *D. muscipula* is minimal, and it is unclear to what degree this is a consequence of adaptation to minimize PPC (Youngsteadt et al. 2018). We used a two-way factorial experiment in which we manipulated pollination and prey to ask: To what degree are flower and seed production pollen and prey limited in *D. muscipula*? In other words, is an insect equally useful as prey or pollinator? We predicted that seed production, estimated as set per fruit, would be highest in plants provided both supplemental pollen and prey. Plants were given supplemental prey either in the previous (summer 2019) or current (spring 2020) growing season of the pollen supplementation experiment in order to compare the importance of prey supplementation timing to resource limitation in this species. We also clarified the pollination system and elaborate the per-visit efficiency of pollinator taxa to better understand factors that limit reproduction in *D. muscipula*.

METHODS

Study system and site

Dionaea muscipula Ellis (Droseraceae) is a carnivorous perennial endemic to pocosin and longleaf pine savanna ecosystems in North and South Carolina. It is the only terrestrial carnivorous plant with a snap-trap mechanism for capturing prey (Cameron et al. 2002). Each

plant has approximately 4 – 12 trapping leaves arranged in a rosette growth form around a short stem (Bailey and McPherson 2012). The entire digestion process takes 4 – 12 days. After several bouts of prey capture, the trap will no longer close, and will remain purely photosynthetic until senescence (Jaffe 1973). Prey capture is generalized, with ground-crawling arthropods (mainly spiders, ants, and beetles) comprising the bulk of captured prey (Hutchens and Luken 2009, Youngsteadt et al. 2018).

Dionaea muscipula reach a reach reproductive maturity three years after germination (Smith 1929). Reproductive stems in *D. muscipula* become visible in early April (Smith 1929, Roberts and Oosting 1958), but it is unclear when the floral primordia begin to develop in this species. When wild individuals are transplanted from the field to a greenhouse setting before April, flowers can begin to develop, indicating that flower development may initiate earlier (Roberts and Oosting 1958). Flowering occurs in late May to mid-June. Reproductive individuals typically produce a single inflorescence, comprising 8 – 12 flowers arranged in an umbelliform cyme. The inflorescence is suspended above the trapping leaves by a 15 – 30 cm scape. The white flowers are perfect and protandrous, with anthers dehiscing about 24 hrs before the stigma flares (Smith 1929, Williams and Scholl 2021). Youngsteadt et al. (2018) found that *D. muscipula* is visited by myriad insect taxa, with sweat bees and beetles identified as the likely primary pollinators. Though originally reported to be self-incompatible (Roberts and Oosting 1958), Juniper (1989) later indicated that *D. muscipula* may be self-compatible, which has also been reported by hobbyist and commercial growers of the plant (FlytrapCare.com). The lifespan of *D. muscipula* in the wild is unknown, but captive individuals can live for up to twenty years (Bailey and McPherson 2012).

We studied a large population of *D. muscipula* at a managed area in Pender Co., North Carolina, USA. Due to the poaching risk for this plant, specific site locations have been omitted. Instead, we assign an arbitrary number to sites (Supp. Table 1). Sites were separated from one another by 0.70 – 5.8 km.

Prey and pollen supplementation

Prey and pollen supplementation to wild plants was conducted as a two-way factorial design with two trials: one starting summer 2019 and a second starting in spring 2020. These two trials allowed for a qualitative comparison of how the timing of feeding affected the degree of prey limitation on plant reproduction. On 9 August 2019, we haphazardly selected 80 plants of a similar size and assigned them to a prey supplementation treatment (hand-fed or control) and a pollen supplementation treatment (hand-pollinated or control) (N = 20 plants per treatment combination). Plants were labelled using metal tags staked into the nearby soil. The site had been burned in May 2019, and plants with the persistent remains of burnt flowering stems were preferentially chosen. However, since flowering stems can sometimes persist from the previous year, it is not certain which plants in this trial had flowered earlier that year.

Prey supplementation. In the 2019 trial (hereafter referred to as 2019 plants), we hand-fed all plants in the prey supplementation treatment on five dates (9 August, 23 August, 15 September, 29 September, 25 October). These dates were selected because the site had been burned in May, and by late summer plants were an ideal size for hand feeding. By November, daytime temperatures were too low to consistently support arthropod prey activity and trapping ability (Bailey and McPherson 2012). Prey supplementation was conducted at least 14 days apart to allow for complete digestion. To supplement prey, we used frozen house crickets (*Achetus*

domesticus) (Premium Crickets, Winder, GA). We chose crickets because Orthopterans are among the natural prey items for *D. muscipula* (Hutchens and Luken 2009, Youngsteadt et al. 2018). Upon receiving each shipment of crickets, we immediately froze crickets for at least 24 hours. We then weighed a subsample of 30 crickets from each shipment to generate an approximate weight per cricket. In 2019, we fed plants with either 0.635 cm or 0.476 cm crickets, using smaller crickets only for traps that were too small to effectively digest large crickets. To hand-feed plants, we placed a frozen cricket in each open trap using a pair of forceps and stimulated the trigger hairs on the trap surface. Following trap closure, we gently squeezed the trap over a duration of roughly 1 minute to continue stimulating the trigger hairs, mimicking struggling prey. During each hand-feeding session, we recorded the number of naturally closed traps at the time of feeding, open traps, and developing traps on plants in both supplemented and non-supplemented treatments. We also recorded the number of 0.635 cm or 0.476 cm crickets that were fed to each plant. Plants in the non-supplemented treatment were handled to manipulate leaves for accurate trap counts. The day following feeding events, we confirmed that a subsample of hand-fed traps proceeded to digestion, indicated by tightly appressed margins.

For the 2020 trial, on 22 May we selected an additional 80 plants of similar size (hereafter referred to as 2020 plants). All selected plants had reproductive stems with buds and had not begun flowering. Plants in this trial in the prey supplementation treatment were fed on two dates, 22 May and 7 June, using similar methods as in 2019. Again, prey supplementation was conducted at least 14 days apart to allow for complete digestion, allowing for two hand-feeding bouts before pollen supplementation was conducted. While we realize that it would have been ideal to have a similar number of feeding events in the 2019 and 2020 trials, feeding supplementation in the 2020 trial was delayed due to the COVID-19 pandemic.

Pollen supplementation. Plants in the 2019 trial that successfully flowered were hand-pollinated on four dates in 2020: 28 May, 31 May, 2 June, and 3 June. On each date, flowers from plants in the hand-pollination treatment with receptive stigmas were pollinated to saturation by rubbing the stigmas with dehiscing anthers collected from one or more individuals at least 5 m away. The first and last flowers to develop within an inflorescence were not included in this experiment. Hand-pollinated flowers were marked using a piece of green embroidery thread tied around the pedicel and were left open for additional open pollination. Simultaneously, we haphazardly selected 1 – 2 flowers with receptive stigmas on plants in the control pollination treatments to serve as open-pollinated controls. In total, each flowering plant from the 2019 trial had 1 – 2 flowers marked for subsequent seed counts, representing 1 – 2 days of hand-pollination bouts for each plant. At the time of each hand-pollination, we recorded the number of open, closed, and developing traps, as well as the number of buds, flowers and fruits on pollen-supplemented and open-pollinated controls. We also recorded the flowering scape height by measuring the distance in cm from the ground to the bottom of the lowest pedicel. Plants in the 2020 trial were hand-pollinated over three dates in 2020: 28 May, 1 June, and 3 June. On plants in the supplemental pollination treatment, we hand-pollinated 1 – 2 flowers on each plant, representing 1 – 2 hand-pollination bouts. Simultaneously, on flowers in the open-pollinated control treatment, we designated 1 – 2 flowers as open-pollinated controls. Again, trap number, flower number, and scape height were recorded during the earliest hand-pollination bout for each plant.

We collected seeds at least 14 days after hand-pollination. Seeds were counted and weighed to the nearest 0.001 mg in the lab. We calculated the average seed weight per fruit by

dividing the total seed weight by the number of seeds in each fruit. Seeds were then returned to the site to replicate natural seed rain.

Statistical analyses. Statistical analyses were performed using R v.4.1.1 within R studio v.1.4.1717 (R Core Team 2021, R Studio Team 2021). All linear mixed effects models in this study were performed using the package ‘lme4’ (v.1.1.27.1, Bates et al. 2017) and summarized using the ‘Anova’ function within the package ‘car’, using a Type II test. If the interaction was significant after running a Type II test, we instead relied on a Type III test, which is more powerful for interpreting significant interactions (v.3.0.11, Fox and Weisberg 2011). For post-hoc comparison between treatments, we conducted a Tukey test using the package ‘emmeans’ (v.1.7.2, Lenth 2022). To determine whether prey supplementation in 2019 plants affected survivorship and flowering probability in 2020, we conducted a Pearson’s chi-squared test comparing the proportion of surviving and flowering plants between fed and control plants. To compare seed set per fruit and average seed weight per fruit between treatments, we fit a linear mixed effects model wherein pollen supplementation treatment, prey supplementation treatment, and their interaction were included as explanatory variables, and plant identity was included as a random variable. We also conducted a t-test to compare scape height, total number of traps at hand-pollination, and total number of reproductive structures at time of hand-pollination (buds, flowers, and fruits) between prey supplementation treatments. Plants that did not flower or had missing inflorescences were excluded from analyses of inflorescence traits. Analyses were performed separately for the 2019 and 2020 trials.

Plant reproductive biology

To test whether *D. muscipula* is self-compatible, we conducted hand-pollinations over six days in late May and early June 2020. At a single site, we selected thirty blocks of four plants each

that had inflorescences with a similar number of total flowers (N = 120 plants total). We selected one flower from each plant to be haphazardly assigned to one of four pollination treatments: 1) self-pollinated with autogamous pollen, 2) self-pollinated with geitonogamous pollen, 3) pollinated with outcrossed pollen from an individual at least 5 m away, and 4) unpollinated control. The focal flower was identified with a piece of green thread tied around the pedicel. Each inflorescence was then enclosed in a drawstring organza bag (pollinator exclusion bag hereafter) before anther dehiscence and about 24 hrs before the stigma became receptive. The day after inflorescence bagging, we conducted hand pollinations by rubbing dehiscing anthers from the relevant treatment against the receptive stigma of the focal flower using forceps. Pollinator exclusion bags were removed following anthesis of the experimental flower, approx. 2-4 days after hand-pollination, when the petals on the focal flower had started to wither.

We collected fruits 18 – 21 days after hand pollination. Seeds were then counted and weighed in the lab and returned to their sites of origin. To compare seed set per fruit and average seed weight among treatments, we conducted a linear mixed effects model in which number of seeds per fruit or average seed weight was the response variable, treatment was the explanatory variable, and block was included as a random effect.

Pollen-ovule ratio

In May 2018, we selected one flower from 28 – 30 inflorescences from each of three sites. We counted the anthers in each flower and then collected all anthers per flower prior to dehiscence (N = 88 flowers total). All anthers within a given flower were collected in the same vial. Anthers were stored in open vials in a desiccator for two weeks after collection, then in closed vials in a desiccator for 4 – 7 mo until they could be counted. In the lab, we used a pestle to lightly grind the anthers until all the anthers were powdery in appearance. We then added 750

μL of 70% ethanol to each vial. Pollen solutions were sonicated for five minutes, followed by five seconds of vortexing. To count pollen grains, we removed 10 μL of the pollen suspension and placed it under the cover slip of one half of a hemocytometer, which was then viewed under a dissecting microscope at 20x magnification. We counted 12 – 16 subsamples from each sample. Samples were vortexed for an additional five seconds between each count (adapted from Kearns and Inouye 1993). To estimate the total number of pollen grains per flower, we calculated the average number of pollen tetrads counted across the subsamples per flower. We then extrapolated the total number of pollen tetrads in the 750 μL solution by multiplying the average value by 75. Like other members of Droseraceae, the pollen grains of *D. muscipula* are combined into permanent tetrads (Halbritter et al. 2012). Therefore, to obtain the total number of pollen grains per flower, we multiplied the total number of tetrads per flower by four (Murza and Davis 2003). We henceforth use the term tetrad only to refer to the aggregated unit of four grains (compare to Youngsteadt et al. 2018 and Hamon et al. 2019, where the term “grain” refers to tetrads).

From each of the same sites where we collected anthers, we also collected the ovaries of 30 – 32 flowers (N = 93 ovaries total). Plants from which we collected ovaries were not the same plants from which we collected anthers, and each ovary we collected came from a different plant. Ovaries were stored in 70% ethanol. Our ovary clearing protocol was adapted from the methods outlined by Kearns and Inouye (1993). Five weeks after collection, we added one-part glacial acetic acid to the samples for every three parts 70% ethanol. The samples were then gently agitated to combine the solution. Ovules were held in this mixture at room temperature for 6 – 16 d. We then rinsed the ovaries two times in distilled water, after which we covered them with 80% lactic acid for 19 – 22 hours. The ovaries were again rinsed twice in distilled water and

returned to a 70% ethanol solution. We dissected the cleared ovules out of the ovaries and counted them under a dissecting scope at 10X magnification.

We tested for a site effect on number of anthers, ovules, and estimated pollen tetrads per flower by constructing an ANOVA and comparing results pairwise between sites using a Tukey test. We then calculated the average pollen-ovule (P/O) ratio per site according to Cruden (1977):

$$\frac{P}{O} \text{ ratio} = \frac{\text{pollen count per flower}}{\text{number of ovules per flower}}$$

Per-visit pollinator effectiveness

We observed pollinators over the course of two flowering seasons – 2018 and 2020. In 2018, we conducted pollinator observations on May 14, May 25, June 1 – 8, and June 14 – 16 for a total of 20.2 person-hours. In 2020, we conducted pollinator observations on eight dates between 31 May and 11 June for a total of 12.6 person-hours. To compare single-visit pollen deposition to the stigma by different floral visitors, we placed pollinator exclusion bags over groups of inflorescences still in bud. During each observation session, the observer removed the exclusion bags from up to 11 inflorescences and watched all flowers that had undergone anthesis while in the exclusion bags. We observed from a distance of at least 0.2 m. When an arthropod visited a flower with a flared stigma, we attempted to identify the visitor down to the lowest possible taxonomic level, noted the time of the visit, recorded the amount of time spent at the flower, and recorded the reward being sought (pollen or nectar) to the best of our abilities. When the visitor stopped visiting flowers within the field of view of the observer, we attempted to photograph or capture the floral visitor for more precise identification, with only 3% of floral visitors (one visitor) successfully captured, and 3% of visitors (again, one visitor) successfully photographed.

Floral visitors were allowed to visit as many flowers as were within the field of view before we attempted capture, regardless of whether the flowers had been bagged or not. We noted order of visitation to observed stigmas, and whether the arthropod visited non-experimental *D. muscipula* inflorescences during the bout. Flowers that were open and exposed to visitation prior to the addition of exclusion bags were marked by cutting a small notch in one petal so that they would be excluded from this study. We then collected all visited stigmas into individual vials and labelled vials with a unique visitor number and stigma number. We collected five unvisited stigmas in 2018 and 10 unvisited stigmas in 2020 to serve as unvisited controls. Stigmas were stored in a cooler with ice until they could be returned to the lab. Stigmas were subsequently stored at 0°C until analysis.

To count pollen, we stained the pollen on the stigmas using basic fuchsin dye (Kearns and Inouye 1993) and counted the total number of conspecific pollen grains (i.e., number of tetrads multiplied by four) deposited on the stigma using a compound microscope at 20x. Since only one visitor was captured and one additional visitor was photographed, we relied on on-the-wing observations to identify visitors to family. To compare pollen deposition per visit between taxa, we fit a linear mixed effects model wherein number of pollen grains per stigma was the response variable and taxon (to family) was included as the explanatory variable. Individual insect number was included in the model as a random effect. We observed a large number of visits from honeybees (*Apis mellifera*), which is a non-native floral visitor to *D. muscipula*. Therefore, to compare per-visit effectiveness of native and non-native visitors, we treated *A. mellifera* separately from other members of the family Apidae. Visits from individuals that could not be positively identified to family were excluded from analysis. This model was visualized using the package ‘sjPlot’ (v.2.8.10, Lüdecke 2021).

RESULTS

Pollen and prey supplementation

Out of the 80 plants that were tagged in the 2019 trial, 68 survived until the next flowering season, and 59 flowered. There was no significant effect of prey supplementation on either survivorship ($X^2 = 1.57$, $P = 0.21$) or proportion flowering ($X^2 = 2.30$, $P = 0.13$) in 2019 plants. For plants that did flower, we found no significant effect of the prey treatment, pollination treatment, or their interaction on seed set per fruit or average seed weight ($F < 1.45$, $P \geq 0.24$ in all cases, Fig. 1a-b). Similarly, in the 2020 trial, we found no significant effect of the prey treatment, pollination treatment, or their interaction on average seed set per fruit or average seed weight ($F_{1,75} < 2.21$, $P \geq 0.14$, Fig. 1c-d).

When examining the effect of prey supplementation on plant traits, in the 2019 trial, there was no significant effect of prey supplementation on the number of reproductive structures (buds, flowers, and fruits; $t_{59} = -1.37$, $P = 0.17$, Fig. 2a). However, in the 2020 trial, we found a significant effect of prey supplementation on total number of reproductive structures, with hand-fed plants having an average of 1.9 more flowers per individual compared to control plants ($t_{66} = -2.37$, $P = 0.02$, Fig. 2b). We observed no significant effect of prey supplementation on scape height or number of traps at the time of hand-pollination for either the 2019 trial (scape: $t_{55} = 0.14$, $P = 0.89$, Fig. 2c; traps: $t_{72} = -1.44$, $P = 0.15$; Fig. 2e) or 2020 trial (scape: $t_{76} = -1.12$, $P = 0.26$, Fig. 2d.; traps: $t_{73} = -1.60$, $P = 0.11$; Fig. 2f).

Plant reproductive biology and pollen-ovule ratio

There was a significant effect of pollination treatment on seed set per fruit ($F_{3,87} = 57.37$, $P < 0.001$, Fig. 3a). Both outcrossed ($t_{87} = -3.52$, $P = 0.0038$) and geitonogamously pollinated flowers ($t_{87} = 11.56$, $P < 0.0001$) had significantly higher seed set per fruit than unpollinated

flowers. Only one out of thirty unpollinated control flowers set seed, likely due to a leaky pollinator exclusion bag. This single unpollinated flower produced four seeds, compared to 21.1, on average, in outcrossed fruits and 20.3 in geitonogamously pollinated fruits. In addition, outcrossed ($t_{87} = -3.52$, $P = 0.0038$) and geitonogamously ($t_{87} = -3.09$, $P = 0.01$) pollinated flowers had significantly higher seed set per fruit compared to autogamously pollinated flowers, producing an average of 32.5% and 28.5% more seeds per fruit, respectively. However, there was no significant difference in seed set per fruit between outcrossed and geitonogamously pollinated flowers ($t_{87} = -0.42$, $P = 0.97$).

There was also a significant effect of pollination treatment on average seed weight ($F_{3,64} = 3.38$, $P = 0.023$, Fig. 3b). However, this significant result was driven by the single control flower that set seed, which produced unusually heavy seeds (29.4% heavier than seeds in all other treatments, on average). When we excluded the control treatment from analysis, there was no significant effect of pollination treatment on average seed weight ($F_{2,56} = 2.11$, $P = 0.13$).

Across all three sites where we collected anthers and ovaries in 2018, we observed an average of 13.96 ± 0.29 anthers per flower, 26.40 ± 0.79 ovules per ovary, and 8776.72 ± 460.23 pollen grains per flower (Supp. Table 2). There was a significant effect of site on number of anthers, ovules, and pollen grains per flower, with S14 displaying an average of 11% fewer anthers, 30% fewer ovules, and 48% fewer pollen grains per flower compared to S3 and S10 (anthers: $F_{2,84} = 3.70$, $P = 0.02$; ovules: $F_{2,90} = 21.43$, $P < 0.0001$; pollen grains: $F_{2,85} = 17.21$, $P < 0.0001$; Supp. Table 2). For each of sites S3, S10, and S14, we estimated a P/O ratio of 350.8, 355.3, and 267.3 pollen grains per ovule, respectively, with an average P/O ratio of 332.5 (Supp. Table 2).

Per-visit pollinator effectiveness

In 2018 and 2020, we identified 33 flower visitors to family representing 98 flower visits (Table 1). Out of the flower visitors identified to family, we further identified those that we could to genus (number of individuals in parentheses): *Apis mellifera* (5), *Bombus* spp. (2), *Augochlorella gratiosa* (1), and *Junonia coenia* (1). Out of 70 flower visits where we recorded the reward being sought, 100% (70) were observed foraging for nectar, while 2.85% (2) were observed foraging for both pollen and nectar. Excluding unvisited control flowers, we counted a mean of 149.24 ± 15.12 grains per stigma. There was a significant effect of visitor taxon on number of pollen grains per stigma ($F_{7,18} = 2.82$, $P = 0.03$, Fig. 4), with flowers visited by *A. mellifera* receiving an average of 198.64 ± 50.00 more grains compared to flowers visited by Halictid bees. However, unvisited control stigmas had an average of 28.52 ± 7.44 grains, with no significant difference between control stigmas and visited stigmas of any taxa ($-0.95 < t < 0.19$, $P \geq 0.19$ in all cases, Fig. 4).

DISCUSSION

Pollination and nutrient resources, individually and in combination, can affect plant reproductive success (Campbell and Halama 1993, Mattila and Kuitunen 2003). For carnivorous plants, their interactions with insects can mediate both pollination and resource acquisition, creating the potential for pollinator-prey conflict if the same insect taxa are involved in both processes. Here we found that neither pollen nor prey supplementation had a significant effect on seed set per fruit in *Dionaea muscipula* in the years of study, but that plants given supplemental prey immediately prior to flowering produced more flowers. Our exploration into the pollination system of *D. muscipula* revealed that insects are required for successful pollination, with both

geitonogamously pollinated and outcrossed fruits resulting in more seed set per fruit compared to unpollinated flowers. Plants are self-compatible, but seed set per fruit is also significantly higher from within-plant compared to within-flower pollen movement. Floral visitors do vary in their per-visit pollinator effectiveness at depositing pollen, with honeybees outperforming native bees. Taken together, this work suggests that *D. muscipula* relies on insects for reproduction, and nutrients from captured insects are quickly mobilized into flower production, potentially decreasing the potential for PPC in this species.

Pollen and prey supplementation

We found no effect of pollen supplementation on reproduction. This contradicts pollen supplementation studies conducted in 2017 and 2020, wherein pollen supplementation increased seed set by approximately 8% and 27% respectively (Hamon et al. 2019, Hamon et al. unpubl, see Chapter 2). Variation in pollen limitation within species is not unusual (Burd 1994, Burd et al. 2009, Ashman et al. 2004), and can vary across years and populations, depending on environmental factors, presence of co-flowering species, fluctuations in pollinator abundance, population size, and other factors (Knight 2005). For example, significant interannual variation in pollen limitation was observed in the rare plant *Polemonium caeruleum* (L.) (Polemoniaceae), possibly in part due to variation in pollinator visitation frequency and temperature (Ryniewicz et al. 2021). Our results compared to prior studies suggest that there is also interannual variation in pollen limitation in *D. muscipula*.

In neither trial did we observe a significant effect of supplemental prey on seed set per fruit. Since prey capture and digestion can invoke a photosynthetic cost in *D. muscipula* (Pavlovič et al. 2010, Kruse et al. 2014), it is possible that the benefits of excess mineral nutrients were partly counteracted by reduced photosynthates. Though nutrient supplementation can also increase

photosynthesis in *D. muscipula* leaves via increased growth, (Kruse et al. 2014), the rate of prey supplementation employed in this study may have outpaced this benefit. In a laboratory study, Kruse et al. (2014) found that moderate prey supplementation to *D. muscipula* resulted in the largest growth rate compared to large prey supplementation, further supporting the notion that the relationship between *D. muscipula* function and excess nutrients may be non-linear, or that *D. muscipula* does not have the plasticity to produce more ovules and seeds when supplemented with prey.

Though prey supplementation did not significantly boost seed set per fruit, we did observe that fed plants produced more flowers in the 2020 trial, representing a likely boost to reproduction at the whole-plant level. This was somewhat surprising, given that the budding stalks in this trial were relatively well-developed at the time of hand feeding. This suggests that *D. muscipula* is able to quickly mobilize nutrients into more buds. Other studies have found that current season prey acquisition can boost investment in flowering, a relatively cheap investment compared to seeds and fruit (Hanslin and Karlsson 1996, Krowiak et al. 2017). For example, Krowiak et al. (2017) found that individuals of *Pinguicula vulgaris* L. that were given supplemental prey had a flowering probability roughly four times greater than plants with natural prey capture. Flowering is likely a major sink of prey-derived nitrogen in *D. muscipula*, with upwards of 76% of N in floral tissues sourced from current-season insect prey (Schulze et al. 2001).

Two caveats are important to consider in the interpretation of our results. First, our study took place over a single year and does not account for the effect of supplemental prey or pollen on growth and reproduction in subsequent years. Reproduction in a given year may impose a cost or change in resource allocation in subsequent years (Thorén et al. 1996, Thorén and Karlsson

1998). In addition, the previous fruiting and flowering success of our experimental plants was not known. This is relevant because previous season fruit production may have influenced success at the time of this study (Zimmerman and Aide 1989). Second, our experiments were designed to assess if excess pollen or prey would provide additional benefits to plant reproduction. However, we do not know how a reduction in pollination or prey capture would affect plant reproduction. Reducing pollen and prey is more challenging to experimentally impose, but would be relevant to consider for *D. muscipula* in future research given its rarity and the potential for habitat degradation to impose negative effects on important ecological processes that affect plant reproduction.

Plant reproductive biology and pollen-ovule ratio

Plants that can self-pollinate are predicted to have less pollen limitation (Larson and Barrett 2000) and reduced PPC (Jürgens et al. 2012). We observed no significant difference in seed set between outcrossed and geitonogamously-pollinated fruits, confirming that *D. muscipula* is self-compatible. However, only one out of thirty unpollinated flowers set seed. We suspect this was due to leakiness in the pollinator-exclusion bag and is not a result of apomixis or facultative self-pollination. Though we did not emasculate unpollinated flowers, anthers flex downwards and away from the stigma during anthesis, minimizing the possibility of self-pollination within a flower in the absence of a visitor (Williams and Scholl 2021). Therefore, *D. muscipula* still relies on the presence of a mobile pollen vector for reproduction.

The P/O ratio provides additional clues about the reliance of *D. muscipula* on pollinators. Plants that can self-pollinate are generally predicted to have low P/O ratios (Cruden 1977). Across all three sites, we observed an average P/O ratio of 332.5. In comparison, *Aldrovanda vesiculosa* L. (Droseraceae) – the closest relative to *D. muscipula* – has a P/O ratio of 28.5

(Okada 2008). Lower P/O ratios have also been observed in other members of Droseraceae, including 9.1 in *Drosera anglica* Huds., 18.7 in *D. linearis* Goldie, 9.0 in *D. rotundifolia* L. (Murza and Davis 2003), and 23.9 in *D. tracyi* (Diels) Macfarl. (Wilson 1995), a pattern which is supported by the prevalence of autogamy and cleistogamy in these taxa. If P/O ratios are indeed an overall indicator of the likelihood that a given pollen grain will help fertilize an ovule, the relatively high P/O ratio exhibited by *D. muscipula* supports outcrossing as a predominant reproductive strategy for this species. Variation in P/O ratios observed between sites may point to variation in local selective pressures imposed by differences in pollinator communities and the environment (Erbar and Langlotz 2005).

Per-visit pollinator effectiveness

When comparing single-visit pollen grain deposition, *A. mellifera* outperformed Halictid bees. Several apiaries are maintained at the managed area where we measured per-visit pollinator effectiveness, resulting in a high number of floral visits from this non-native pollinator. Despite this result, observations of pollinator behavior made during this study may provide clues about the most effective native pollinators of *D. muscipula*. Halictid bees were observed foraging for both nectar and pollen, but both of these resources are presented away from the stigma. Consequently, the small sweat bees were rarely observed touching the stigma. By comparison, larger, heavier bees, such as *A. mellifera* and Megachilid bees, tended to touch the stigma while foraging. These observations point to the possibility that larger visitors may play an outsize role in pollinating *D. muscipula*.

Unfortunately, despite the prevalence of beetle visitors to flowers of *D. muscipula* in prior research (Youngsteadt et al. 2018, Hamon et al. 2019), we observed no beetle visits to our experimental flowers in 2018 or 2020. Beetles were observed visiting adjacent, non-experimental

flowers, so it is unlikely that the presence of the observer prevented these visits. During this study and previous research (Youngsteadt et al. 2018, Hamon et al. 2019), beetles were intermittently observed resting in flowers for extended periods of time while feeding on pollen, which seemingly aligns with the notion that beetles tend to be poor pollen dispersers and voracious floral predators (Grimaldi 1999, discussed further in Willmer 2011). However, Youngsteadt et al. (2018) found that beetles – chiefly the longhorned beetle *Typocerus sinuatus* and the checkered beetle *Trichodes apivorus* – frequently carried *D. muscipula* pollen on their bodies, supporting their potential importance as pollinators. Indeed, the per-visit effectiveness of beetle pollinators across plant taxa is poorly known and has been examined in just a few studies (e.g., Englund 1993, Matsuki et al. 2008). More observation is necessary to determine the per-visit effectiveness of beetle visitors to *D. muscipula* and other plant taxa with a generalist pollination system.

Conclusions

Though we observed no significant effect of prey or pollen limitation on seed set per fruit in the present study, the increase in number of flowers in fed plants reinforces the reliance of *D. muscipula* on insect prey. Moreover, we confirmed that *D. muscipula* requires pollinators for the movement of pollen within or among plants for successful reproduction. It remains unclear whether *D. muscipula* exhibits adaptations to minimize PPC, but our results suggest that occasional pollinator capture may grant an outsize benefit in fitness due to a boost in flower numbers. In order to better understand how pollinator and prey access affects reproductive success in this species, future studies should focus on the multi-year effects of pollen and prey limitation on individual fitness and population demographics.

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Table 3.1. Summary of flower visitors observed in 2018 and 2020 and their average per-visit pollination efficiency (number of pollen grains deposited in a single visit)

Order	Family	No. individuals	No. stigmas	Mean pollen grains (\pm SE)
Hymenoptera	Apidae	5	36	175.00 \pm 24.54
Hymenoptera	Apidae (<i>Apis mellifera</i>)	5	25	285.76 \pm 37.30
Hymenoptera	Halictidae	16	23	67.13 \pm 16.23
Hymenoptera	Megachilidae	4	10	114.00 \pm 37.65
Hymenoptera	Vespidae	1	2	146.00 \pm 46.00
Diptera	Syrphidae	1	1	4.00
Lepidoptera	Nymphalidae	1	1	12.00
Unvisited control	n/a	n/a	15	28.53 \pm 7.43

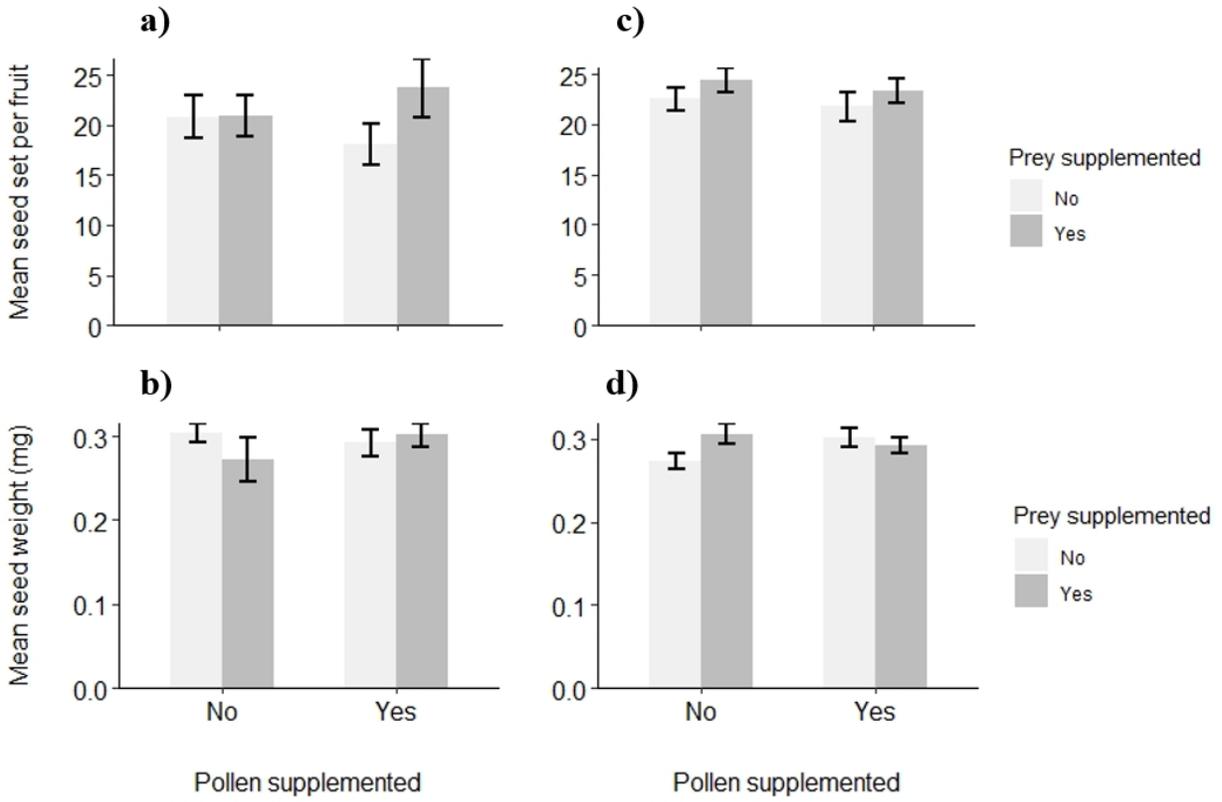


Figure 3.1. Seed set per fruit (a,b) and mean seed weight (mg) (c,d) in the 2019 trial (left column) and 2020 trial (right column). Bars are mean \pm SE.

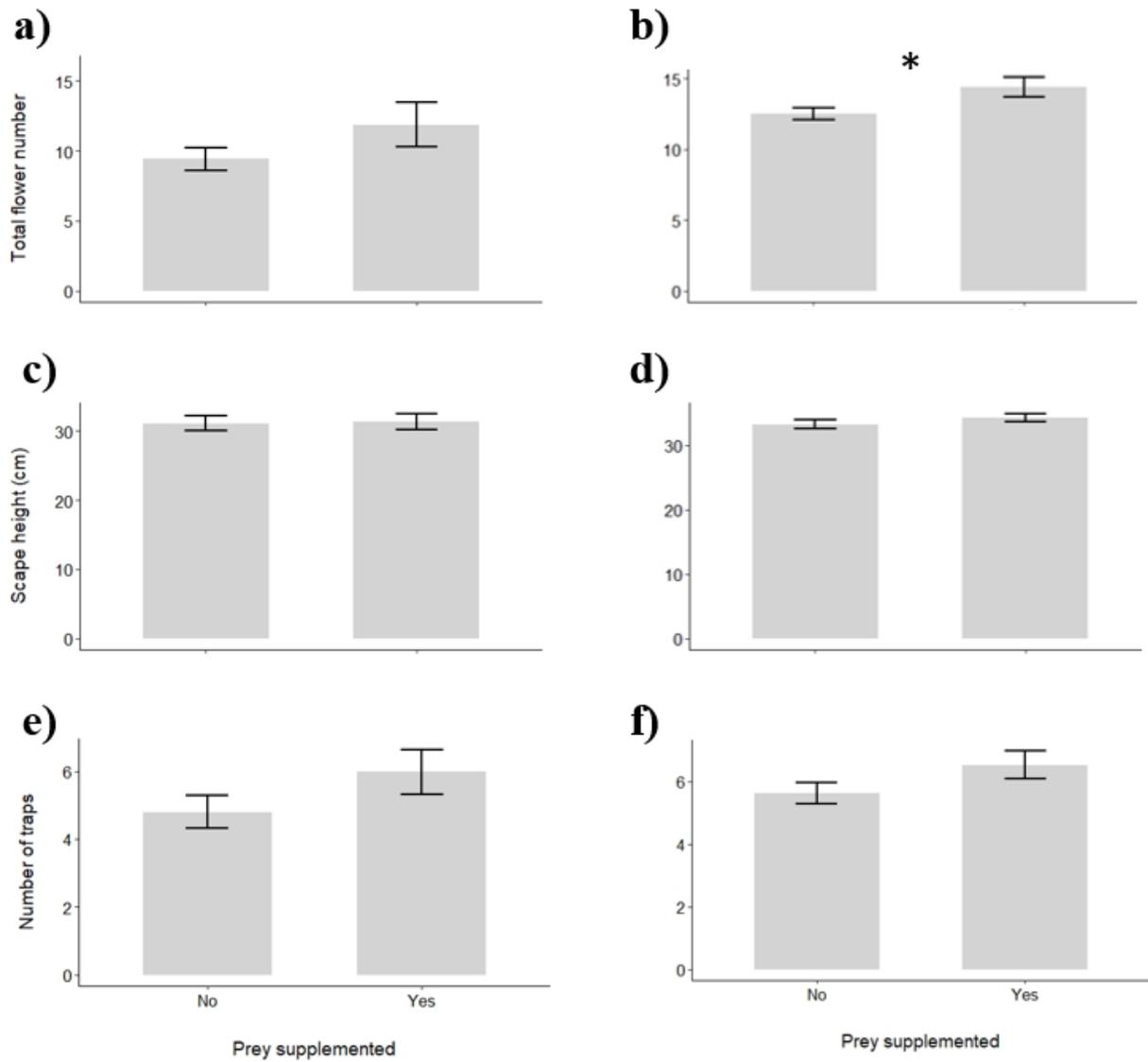


Figure 3.2. Number of reproductive structures (a,b), scape height (cm) (c,d), and number of traps (e,f) by prey supplementation treatment in the 2019 trial (left column) and 2020 trial (right column). Bars and error bars represent means \pm SE. Significant differences ($P < 0.05$) in traits between prey supplementation treatments are marked with an asterisk.

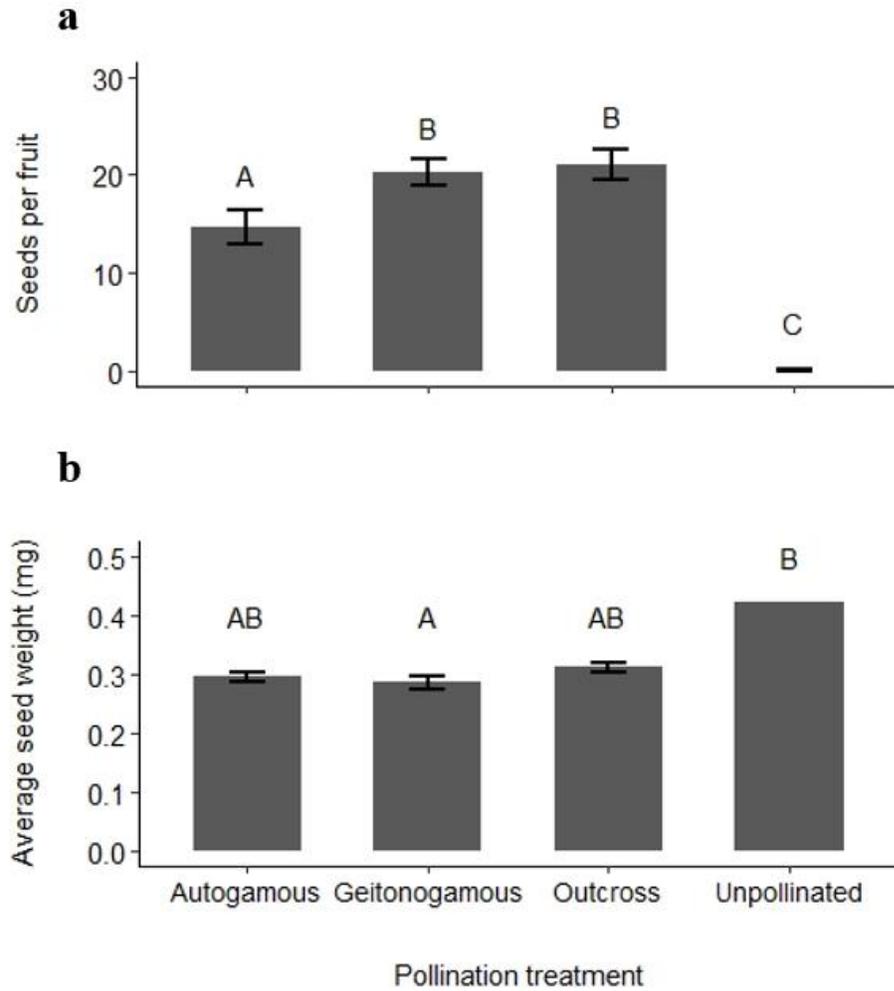


Figure 3.3. Seed set per fruit (a) and mean seed weight (mg) (b) by hand-pollination treatment. Bars are means \pm SE. Different uppercase letters above the bars indicate significant differences in response variables from a Tukey HSD test at $P < 0.05$. Average seed weight for unpollinated fruits is based on a single flower that set seed. Otherwise, unpollinated flowers did not set seed.

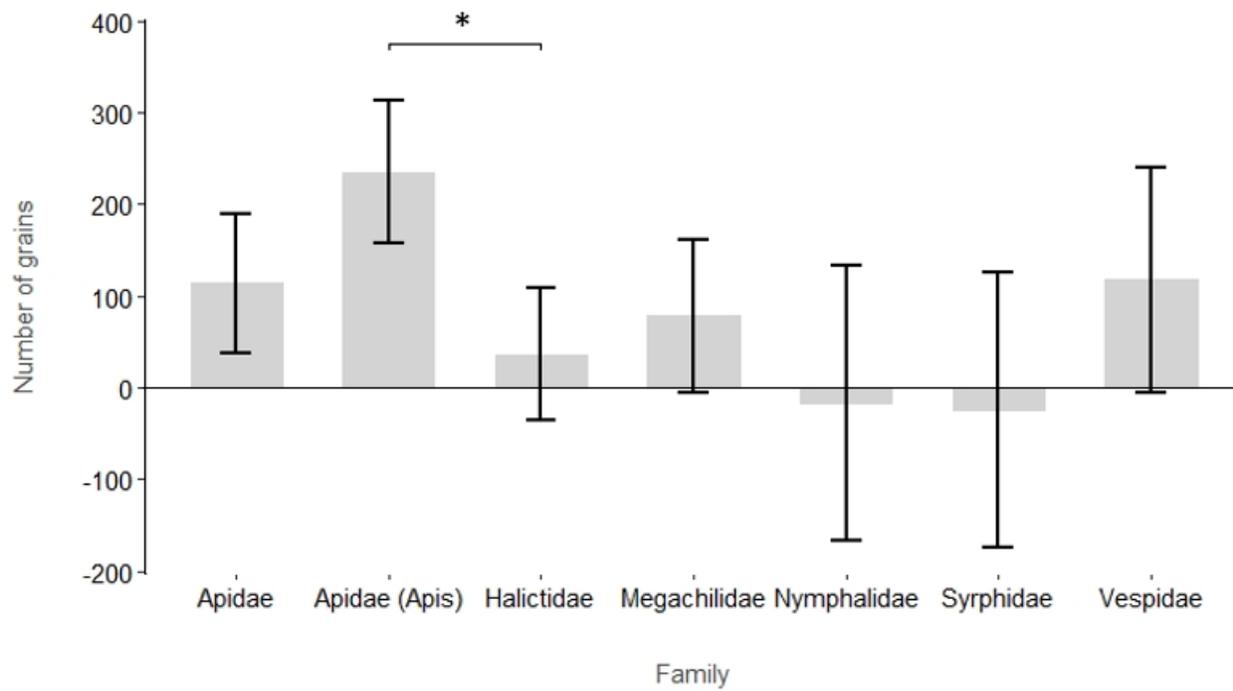


Figure 3.4. Summary of fixed effect estimate of number of pollen grains deposited per visit by insect family. Error bars indicate standard error.

**CHAPTER 4: A range wide status survey of Venus flytrap *Dionaea muscipula*
(Droseraceae)**

Laura Hamon, Daniel Hannon, Suzanne Mason, Stephanie Horton, and Misty Buchanan. 2021. A range wide status survey of Venus flytrap *Dionaea muscipula* (Droseraceae). Unpubl. report to US Fish and Wildlife Service Raleigh Ecological Services Office, Raleigh, NC.

ABSTRACT

Venus flytrap (*Dionaea muscipula* J. Ellis) inspires scientific and public fascination, thanks to its carnivorous habit and charismatic snap-trap strategy for capturing prey. Venus flytrap is native only to a small region of North and South Carolina, where it is impacted by fire suppression and land use change. The last concerted range-wide effort to survey Venus flytrap was conducted in 2002, and development and land management changes in the last two decades necessitate a reassessment of populations. An updated survey to assess extant Venus flytrap populations was conducted from 2019 to 2021. Surveyors visited as many populations as feasible and used a combination of absolute counts and estimative efforts to quantify population sizes. In addition, surveyors recorded qualitative information about each population, including habitat type and extant threats. Populations on both public and private lands were included. In total, 28 distinct populations were visited by the core North Carolina Natural Heritage Program (NCNHP) survey team and other collaborating surveyors from 2019 to 2021, with a total count of 811,980. Of this number, 402,934 individuals were counted directly, and 409,046 individuals were estimated via a subsampling method. The total estimate for populations not visited during this survey is 67,187, for a total minimum estimated population size of 879,167. This far outstrips the estimated population size of Venus flytrap in 2018 of approximately 73,000 – 158,000 individuals. However, this population size is still far smaller than the historic estimated population size of Venus flytrap.

Evidence of impact from development, changes in hydrology, fire suppression, and illegal collection were observed during this survey. Following survey completion, populations were ranked according to their size.

This survey represents the most thorough recent effort to assess the population size of wild Venus flytrap. Out of the populations previously ranked “Fair” (at least 500 individuals) or better, 68% were included in this survey, with 36% of all extant populations visited. Since the previous survey, 8 populations were assigned an improved rank and 16 populations were downgraded. One population was newly confirmed as extirpated, and 117 previously unknown patches were recorded.

Due to the risk of illegal collection, references to specific locations have been omitted from this report. Instead, Element Occurrence numbers are used. Element Occurrences are units used by the NatureServe network to delineate populations and are numbered arbitrarily. Element Occurrences in North Carolina are prefixed with “NC”, while Element Occurrences in South Carolina are prefixed with “SC”.

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Multiple other survey teams provided data between 2019 and 2021. Justin Bashaw (U.S. Army Corps of Engineers), Keith Bradley (South Carolina Department of Natural Resources), Keleigh Cox (U.S. Army Corps of Engineers), Laurel Davis (U.S. Army Corps of Engineers), James “Trapper” Fowler (South Carolina Department of Natural Resources), Alicia Jackson (Dr. J.H. Carter III & Associates. Inc.), Janice Johnson (U.S. Army Corps of Engineers), Teresa Russell (U.S. Army Corps of Engineers), Dale Shew (University of North Carolina-Wilmington, The Nature Conservancy), Roger Shew (UNC-Wilmington, The Nature Conservancy), and Eric Ungberg (University of North Carolina-Chapel Hill Herbarium, NCNHP) all contributed records and maps based on their field observations.

Hunter Cox (Student Conservation Association intern), Holly Ferreira (U.S. Marine Corps), Maggie Hughes (Student Conservation Association intern), Nathan Shepard (NCNHP), Craig Ten

Brink (U.S. Marine Corps), Sudie Thomas (Natural Resources Conservation Service), Cason Verhine (N.C. Parks & Recreation), and Andy Walker (U.S. Forest Service) provided additional assistance in the field. Nathan Burmester (The Nature Conservancy) provided information about burning practices at relevant properties.

NCNHP staff Suzanne Mason, Brenda Wichmann, Meredith Wojcik, Stephanie Horton, and David Siripoonsup entered updated survey data into Biotics, revised ranks, and provided the tables necessary for this report. Rodney Butler helped with equipment access and recruitment of field assistants. Linda Rudd assisted with the writing and editing of this report and other interim reports. Michael Schafale provided information on potential survey locations.

Stakeholder meetings were held on January 24th, 2019, and January 28th, 2020, to review known research, present preliminary data, and solicit feedback on survey protocols. Many people from myriad agencies and organizations attended these meetings, and we would like to thank the following for their feedback and expertise: Jame Amoroso (NCNHP), Keith Bradley (South Carolina Department of Natural Resources), Angie Carl (The Nature Conservancy), Ed Corey (N.C. State Parks), Carla Edwards (N.C. State Parks), James “Trapper” Fowler (South Carolina Department of Natural Resources), Jeff Glitzenstein (consulting biologist), Stephen Hall (consulting biologist), Stacy Huskins (U.S. Army Civilian), Rebecca Irwin (N.C. State University), Alicia Jackson (Dr. J.H. Carter III and Associates, Inc.), Natalie Kerr (Duke University), Cheryl Knepp (N.C. Department of Transportation), Michael Kunz (N.C. Botanical Garden), Allison Louthan (Duke University), James Luken (Coastal Carolina University), Michelle Ly (The Nature Conservancy), Hervey McIver (The Nature Conservancy), Melissa Miller (N.C. Department of Transportation), Julie Moore (consulting biologist), William Morris (Duke University), Milo Pyne (consulting biologist), Dale Shew, Roger Shew (University of North Carolina-Wilmington), Clyde

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PART I. GENERAL BIOLOGY AND ECOLOGY

INTRODUCTION

This report details the results of a status survey of native Venus flytrap (*Dionaea muscipula* J. Ellis) populations conducted from 2019 to 2021 by the North Carolina Natural Heritage Program (NCNHP) and other collaborating agencies. The goal of this survey was to assess as many populations as feasible, update the total known population size of this species, and record extant threats.

Venus flytrap is a carnivorous herb with a rosette growth form and sensitive, hinged trapping leaves. It is the sole member of its genus and the only terrestrial carnivorous plant that employs a snap-trap mechanism to capture prey (Weakley 2020). This species is native solely to the Coastal Plain region of N.C. and S.C., where it is generally found in Wet pine savannas, Flatwoods, Savanna/pocosin ecotones, and Sandhill seeps (NatureServe 2021). Like many co-occurring species in these communities, Venus flytrap is dependent on intermittent fire to limit light competition from the overgrowth of taller understory plants (Frost 1993). The southeastern Coastal Plain is noteworthy for its high species diversity and endemism (LeBlond 2001, Noss et al. 2014). Following European colonization, much of this region was exploited for agriculture, timber, and the naval stores industry. In addition, widespread fire suppression became increasingly common (Frost 1993). Consequently, only a fraction of original viable habitat remains, and fire suppression and land conversion continue to be the chief threats to Venus flytrap viability.

Multiple survey efforts have focused on Venus flytrap within the last several decades. Prior to 2019, the most recent, concerted, range-wide survey effort for Venus flytrap was conducted in 2002 by Roger and Dale Shew in a survey for the North Carolina Plant Conservation Program (NCPCP). This survey was unprecedented in its scope and scale, but ongoing development pressures and land management changes necessitate a reassessment of this species.

In 2016, Donald Waller (University of Wisconsin-Madison) petitioned the U.S. Fish and Wildlife Service (USFWS) to consider Venus flytrap for listing under the Endangered Species Act of 1973. The petition was cosigned by 25 individuals and submitted to USFWS on 18 October 2016. At the time of the petition, Venus flytrap was listed as a Federal Species of Concern, an informal term designating species which appear to be in decline but require additional information to merit listing.

The USFWS awarded a Coastal Program grant to NCNHP in 2018 for the completion of a survey to assess extant Venus flytrap populations. The findings from this survey would provide the latest information on the known population of this species, which is essential for the listing decision.

The primary goal of this survey was to assess the number of individuals at as many populations as possible to generate an accurate total population estimate. In addition, this survey sought to:

- 1) Survey and map as many natural populations as possible.
- 2) Record extant threats to wild populations.
- 3) Update geographical records with current range and distribution.
- 4) Describe habitat type and quality at each population.
- 5) Search for the Venus flytrap cutworm moth (*Hemipachnobia subporphyrea*), a specialist herbivore of Venus flytrap.

This survey was conducted in spring and summer of 2019 and 2020 in N.C. and S.C., with some additional survey work in spring 2021. Surveyors visited 28 populations and reported a

minimum estimate of 811,980 plants. When accounting for the most conservative recent counts for populations that were not visited during this survey, there are an estimated 879,167 individuals in the wild. Of the 69 extant populations of Venus flytrap, 25 are ranked as “Fair” or better (500 or more individuals), while 44 are ranked as “Poor” (fewer than 500 individuals). Only 17 populations are considered “Good” to “Excellent” (more than 1,000 individuals). The current population count suggests a far more promising outlook than previous estimates, though many populations have been reduced or extirpated since the earliest surveys of this species.

Many historical and low-quality sites were not visited during this survey. In addition, efforts to contact and access private sites were truncated by the COVID-19 pandemic. However, the current survey represents the most thorough range-wide survey of this species.

Taxonomy and taxonomic history

Venus flytrap is in the family Droseraceae, which also includes the carnivorous genera sundews (*Drosera* spp.) and waterwheel (*Aldrovanda vesiculosa*). This family is characterized by leaves that are adaxially circinate and that usually occur in a spiral or rosette (Wood 1960). The pollen grains are distinctive, forming a permanent endoaperturate and echinate tetrad (Halbritter et al. 2012, Takahashi and Sohma 1982). The flowers in this family are racemose and perfect (Wood 1960, Schnell 2002).

Dionaea is a monotypic genus believed to be sister to *Aldrovandra* (Cameron et al. 2002). Both genera employ a snap-trap mechanism for capturing prey. Genetic and fossil evidence suggests that this snap-trap trait evolved once from a sundew-like common ancestor of *Dionaea* and *Aldrovanda* (Cameron et al. 2002, Heubl et al. 2006, Renner and Specht 2011). The trigger hairs and marginal teeth of the leaf traps are likely homologous to the sticky trichomes of *Drosera* (Heubl et al. 2006). A detailed draft genome of Venus flytrap was released in 2016 (Hackl 2016).

Though it was certainly already familiar to Indigenous tribes in the area, Venus flytrap was first introduced to European botanists in 1759 by N.C. governor Arthur Dobbs (Roberts and Oosting 1958). Naturalist John Ellis formally described the species in *The St. James's Chronicle*, an English newspaper, in 1768. The generic name *Dionaea* refers to the Greek goddess of love, Aphrodite (daughter of Dione) and was coined by Swedish botanist Daniel Solander. It is unclear whether this name derives from Solander's concurrent studies of the planet Venus (named after the Roman goddess of love), or whether it refers to the appearance of the traps, which early European scientists found suggestive of human anatomy (Bailey and McPherson 2012). The specific epithet means "mouse trap". Though Ellis uses the common name "Venus's Flytrap" in the original description, various spellings are often used, and this report employs "Venus flytrap,"

the spelling favored by USFWS and NC Department of Agriculture and Consumer Services (NCDA&CS). Other seldom-used common names include Meadow clam, Tippitiwitchet, Fly trap sensitive, and Catch fly sensitive (Bailey and McPherson 2012, Weakley 2020).

Characteristics

Venus flytrap is a long-lived perennial herb with approximately 4-12 leaves arranged in a rosette. The photosynthetic, leaf-like petioles are loosely triangular to heart-shaped, with fleshy, persistent bases that overlap around a short underground rhizome (Bailey and McPherson 2012). Leaves are hinged at a stiff midrib, and each lobe is bordered by 14-21 stiff marginal teeth that interlock when the trap is closed (Juniper et al. 1989, Bailey and McPherson 2012). The adaxial surface of the leaf contains numerous digestive glands and possesses 3-6 touch-sensitive trichomes on each lobe. Due to the presence of anthocyanins, the digestive surface may vary from yellow green to deep red (Di Gregorio and DiPalma. 1966).

The allometry and orientation of the leaves and petioles may vary according to genetics and environmental conditions. During the summer, individuals tend to have long, thin, somewhat erect petioles with relatively large leaves. Conversely, plants in the autumn and winter tend to have broad petioles, relatively small leaves, and an overall squat growth form (Bailey and McPherson 2012). Venus flytrap individuals that receive supplemental nitrogen develop a higher ratio of petiole to trap area, indicating that this species may allocate growth according to the relative availability of light and prey (Gao et al. 2015)

During flowering, an individual produces a single (occasionally more than one) flowering stem. The inflorescence is an umbelliform cyme that is suspended approximately 15-30 cm above the traps by a slender scape (Smith 1931, Roberts and Oosting 1958). Each inflorescence has an average of 8-12 flowers, with as few as two and as many as 40 flowers possible. The flowers are

actinomorphic and perfect, with 5 sepals and 5 white petals. The ovary is superior and unilocular, with basal placentation and 5 fused carpels. The stigma is bifurcated and papillose. Each successful fruit produces an average of 25-40 black, obovoid seeds. There are an average 15 stamens in each flower (Diels 1906, Smith 1931, Wood 1960).

Prey capture

Plant carnivory has independently evolved at least six times and is represented by over 600 species (Ellison and Gotelli 2009). Many – but not all – carnivorous plants are found in environments where the soil is poor in nutrients but where light is abundant and the soil is moist, such as the conditions found in longleaf pine savannas (Givnish et al. 1984). Trapping leaves are generally less efficient at capturing light, and Givnish et al. reason that carnivory is still a competitive strategy where light and moisture are not limiting resources (1984). Venus flytrap co-occurs with multiple carnivorous species, including North American pitcher plants (*Sarracenia* spp.), *Drosera* spp., bladderworts (*Utricularia* spp.), and butterworts (*Pinguicula* spp.). However, Venus flytrap is the only terrestrial plant in the world that uses a snap-trap to capture prey. Charles Darwin was notably fascinated by Venus flytrap, and his experiments with sundews (*Drosera* spp.) provided evidence that this species derives nutrients from insect prey (Darwin 1875, 1878). The unique trapping mechanism of Venus flytrap continues to be an area of active research, with numerous studies devoted to understanding how this species captures and digests prey.

The entire capture, digestion, and reopening process takes approximately 4-12 days. When fully open, the trapping lobes of Venus flytrap are oriented at an 80-degree angle to one other (Poppinga et al. 2016). There are 3-6 modified trichomes, or trigger hairs, on the surface each lobe. These trigger hairs are stiff, but flexible at the point of attachment (Buchen et al. 1983). When displaced, the trigger hair bends at the base, stimulating a concentric row of mechanosensory cells

at the basal portion of the trichome (Buchen et al. 1983, Juniper et al. 1989). This triggers the opening of ion channels, depolarizing the cell membranes and generating an action potential that propagates across the trap surface (Juniper et al. 1989, Hodick and Sievers 1988, Sibaoka 1991, Krol et al. 2006, Volkov et al. 2008, Escalante-Perez et al. 2011, Volkov et al. 2014). Very little force is required to deflect a trigger hair and stimulate an action potential, as indicated by observations of ants moving over a trap surface (Scherzer et al. 2019).

The number of action potentials required to trigger trap closure depends on environmental conditions. At room temperature, two action potentials within 20-30 seconds are required to stimulate trap closure (Williams and Bennett 1982, Hodick and Sievers 1988). At higher temperatures, a single touch may be necessary to close the trap (Buchen et al. 1983, Juniper et al. 1989). Drought-stressed plants may require additional stimuli to close (Escalante-Perez et al. 2011). Once enough action potentials occur and the required electrochemical threshold is reached, the trap snaps shut in approximately 0.1 s, one of the fastest known movements by a plant (Forterre et al. 2005, Volkov et al. 2008, Volkov et al. 2011, Cross 2012, Poppinga et al. 2016).

Several mechanisms may contribute to the rapid trap closure. The movement may be due in part to hydraulic action, such as through the growth of cells in the outer surface via acid-induced wall-loosening (Williams and Bennet 1982) or through the opening of water channels (Markin et al. 2008, Escalante-Perez et al. 2014). Elastic forces, via a difference in hydrostatic pressure between the inner and outer trap surface (Hodick and Sievers 1989, Markin et al. 2008), or by a geometric instability in the trap curvature (Forterre et al. 2005) may also account for the very rapid closure. A combination of mechanisms is likely responsible for trap closure, and the precise process for trap closure remains an area of ongoing investigation.

Immediately after the trap closes, gaps remain between the marginal teeth. Darwin proposed that this allows small arthropods to escape, favoring large prey items (Darwin 1875) and there is mixed support for this hypothesis (Gibson 1991, Hutchens and Luken 2009, Davis et al. 2019). The struggling prey continues to stimulate trigger hairs, creating further action potentials and initiating complete trap closure. As the prey struggles, additional action potentials, chemical cues from the prey, and hormonal signaling by the plant induce full closure and digestion (Lichtner and Williams 1977, Escalante-Perez et al. 2011, Libiakova et al. 2014). When fully closed, the trap margins form a hermetic seal, and the marginal hairs appear erect. If the trap closes and there are no additional action potentials, the trap will reopen after approximately 1-2 days. This likely serves to prevent the trap from wasting energy on nonnutritive stimuli, such as falling rain or debris.

The trap surface is lined with digestive glands that produce hydrolases for digesting prey (Schulze et al. 2001). Once digestion is complete, the trap reopens, and the chitinous, nondigested parts remain. Trap reopening occurs either through cell growth or hydraulic pressure changes (Markin et al. 2008). A single leaf can complete the trapping and digestion process several times before dying or becoming inactive.

The degree to which Venus flytrap relies on attractants is unclear. The trapping zone of Venus flytrap reflects ultraviolet light, a possible attractant (Joel et al. 1985, Kurup et al. 2013). Analysis of the volatile headspace of Venus flytrap reveals that traps produce an odor similar to the profile of flowers and fruits, another potential attractant (Kreuzweiser et al. 2014). Some have proposed that red trap coloration plays an attractive role, but the attractive qualities of anthocyanins in Venus flytrap and other carnivorous plants has been debated in multiple studies (Ichiishi et al. 1999, Briscoe and Chittka 2001, Foot et al. 2014, Jürgens et al. 2015, Potts and Krupa 2016).

The common name “flytrap” is somewhat of a misnomer, as flies make up a small fraction of natural prey. In the wild, Venus flytrap chiefly captures ground-crawling arthropods that inadvertently crawl into the traps (Williams 1980, Hutchens and Lukens 2009, Youngsteadt et al. 2018). In a study that identified trapped prey in wild, Youngsteadt et al. recorded four invertebrate classes and 11 orders, with ants, beetles, and spiders composing the bulk of the prey community (Youngsteadt et al. 2018).

Reproductive biology

Flowering occurs in the early summer from approximately mid-May to mid-June (Figure 1). The inflorescence is visited by a generalist suite of pollinators, which chiefly features sweat bees and beetles. Frequent floral visitors that carry Venus flytrap pollen include the sweat bee *Augochlorella gratiosa*, the longhorned beetle *Typocerus sinuatus*, and the checkered beetle *Trichodes apivorus*. There is virtually no overlap between the arthropod taxa captured in the traps and visiting the flowers (Youngsteadt et al. 2018).

Venus flytrap is self-compatible, but dependent on a motile pollinator to move pollen from anther to stigma (Hamon et al., unpublished). Moreover, Venus flytrap is protandrous, and the anthers dehisce one day before the stigma becomes receptive (Williams and Scholl 2021). Venus flytrap can be pollen limited, such that seed set may be partly limited by the amount of pollen delivered to the stigma (Hamon et al. 2019).

Life history

Venus flytrap is perennial, with hobbyists reporting a relatively long lifespan of 15-25 years in cultivated plants (Bailey and McPherson 2012). The lifespan of wild plants is unclear but believed to be long-lived.

Fruits dehisce in June-July. Germination generally occurs within 2-6 weeks and individuals achieve maturity within several years (Smith 1931, Schnell 2002, Bailey and McPherson 2012). It is believed that seeds exhibit some conditional dormancy (Schnell 2002) and efforts to store and germinate seeds over a long period have been somewhat successful (Pietropaulo and Pietropaulo 1986, Schell 2002). It is unclear how long Venus flytrap seeds can persist in the seed bank, but this information would shed light as to whether populations could return after a period of fire suppression (Luken 2012).

Seedlings possess functional traps, though they close slowly and tend to capture small, slower prey such as springtails (Poppinga et al. 2016). Ex-situ studies suggest prey capture may be important for growth even in the seedling stage (Hatcher and Hart 2014).

Venus flytrap does not trap insects or produce new leaves in low temperatures, but individuals frequently remain green and retain their leaves through the winter. In very cold, waterlogged, or drought conditions, this species may enter a dormant state, existing as an underground rhizome (Bailey and McPherson 2012).

Habitat

Venus flytrap occurs in the Fall-Line Sandhills and Outer Coastal Plain of the Carolinas. In the latter region, this species is chiefly found in the ecotonal areas between pocosins and adjacent Pine Savannas or Wet Pine Flatwoods (Schafale and Weakley 1990). In the drier Sandhills, Venus flytrap most frequently occurs in the ecotone between moist Streamhead Pocosins or Sandhill Seeps and upland longleaf pine/scrub oak savannas (NatureServe 2021). This species can also be found between bay rims and adjacent communities. Venus flytrap can sometimes occur in depressions and ditches and may be found densely clustered in powerlines and

roadsides. Where conditions are favorable and fire management is practiced, Venus flytrap can be abundant, comprising a major component of the herbaceous understory.

Venus flytrap habitat has a humid subtropical climate somewhat mediated by its proximity to the coast. Daytime summer temperatures reach upwards of 38 °C and winter temperatures occasionally dip below freezing (Roberts and Oosting 1958). The habitats where Venus flytrap thrives are fire dependent, and many plant species in these habitats require frequent fires to prevent overgrowth of shrubs and deciduous trees, release nutrients, and promote seed germination (Frost 2000, Luken 2005, Evans et al. 2012).

Venus flytrap is typically found growing in acidic, moist, nutrient-poor soil (Roberts and Oosting 1958). It is frequently — but not always — found growing in association with *Sphagnum* moss. Associated soil types include Baymeade (Arenic Hapludults), Blaney (Arenic Hapludults), Foreston (Aquic Paleudults), Grifton (Typic Orchaqualfs), Johnston (Cumulic Humaquepts), Kureb (Spodic Quartzipsamments), Leon (Aeric Haplaquods), Murville (Typic Haplaquods), Onslow (Spodic Paleudults), Pactolus (Aquic Quartzipsamments), and Woodington (Typic Paleaquults) (Hudson 1984, Barnhill 1986, NCNHP 1993, NatureServe2021).

High plant diversity is typical in longleaf pine savannas and adjacent habitats, and Venus flytrap may be found growing alongside numerous other species. *Pinus palustris* frequently dominates the canopy, with *Pinus serotina* and *Taxodium ascendens* also possible. Grasses include *Aristida stricta*, *Ctenium aromaticum*, and *Muhlenbergia expansa*. Other herbs include *Aletris* spp., *Calopogon* spp., *Polygala* spp., *Rhexia alifanus*, *Spiranthes* spp., *Xyris caroliniana*, and *Zigadenus* spp. Low-growing shrubs commonly associated with *Venus flytrap* include *Gaylussacia frondosa*, *Ilex glabra*, *Kalmia caroliniana*, *Lyonia mariana*, *Morella cerifera*, and *Vaccinium*

crassifolium. Other carnivorous plants are commonly found co-occurring with Venus flytrap, including *Drosera* spp. and *Sarracenia* spp. (NatureServe 2021).

Lysimachia asperulifolia – a state and federally listed species – can co-occur with Venus flytrap. Other rare plants that may co-occur with Venus flytrap include *Rhynchospora pallida* (S3G3), *R. stenophylla* (S3G4), and *Solidago pulchra* (S3G3) (NatureServe 2021).

Range

Venus flytrap is endemic only to southeastern N.C. and northeastern S.C. It has also been introduced outside of its native range to Yancey County in N.C., the Apalachicola region of the Florida panhandle, and southern New Jersey (Weakley 2020).

The ability of Venus flytrap to survive outside its native range points to clues about the evolutionary history of the Atlantic Coast Plain. Though Venus flytrap is narrowly endemic to N.C. and S.C., the restriction does not seem to be due to narrow environmental requirements. Rather, it reflects the geographic history of the region, which has experienced several bouts of inundation with fluctuating sea levels, accompanied by speciation on isolated upland regions (Sorrie and Weakley 2001, Noss et al. 2014). The Carolina Coastal Plain is consequently a region of high endemism, with at least 20 other species similarly restricted in range. The evolutionary history of Venus flytrap as it relates to distribution remains an area of active inquiry.

Venus flytrap has historically been reported from 18 N.C. counties (Beaufort, Bladen, Brunswick, Carteret, Columbus, Craven, Cumberland, Duplin, Hoke, Jones, Lenoir, Moore, New Hanover, Onslow, Pamlico, Pender, Robeson, and Sampson) and three S.C. counties (Charleston, Georgetown, and Horry) (NatureServe 2021). Currently, Venus flytrap is known to be extant in 11

N.C. counties (Bladen, Brunswick, Carteret, Columbus, Craven, Cumberland, Hoke, New Hanover, Onslow, Pender, and Sampson) and one S.C. county (Horry).

Hemipachnobia subporphyrea

The management and conservation of Venus flytrap has implicit repercussions for other species. One notable instance of this is the Venus flytrap cutworm moth (*Hemipachnobia subporphyrea* Walker), a moth in the Noctuidae whose range is ostensibly restricted to N.C. and S.C. Adults of this species have purple to reddish-brown forewings, with paler grey hindwings. Adult individuals are approximately 17 mm in width. Genital morphology is apparently the most certain way to distinguish *H. subporphyrea* from a nearby related species, *H. monochromatea* (Forbes 1954). Larvae have a prominent pale stripe running mid-dorsally. They appear green in early instars, turning a dark brown before pupation. This species is univoltine. The late-stage caterpillar overwinters and pupates in early March. Following eclosion, the adult is present for only a short period between late March and early May. Most observations of adults occur in mid- to late-April (Hall and Sullivan 2000, Hall and Sullivan 2005).

Venus flytrap appears to be the primary larval host plant of *H. subporphyrea*, and this species has been found almost exclusively in Venus flytrap populations. However, in a 2004 feeding trial, one individual was observed successfully feeding solely on *Drosera brevifolia* and *D. intermedia*. There is some evidence to suggest that later instars may switch host species, and captive individuals have been observed feeding successfully on the Ericaceous taxa *Gaylussacia* spp. and *Vaccinium crassifolium* (Hall and Sullivan 2005).

Nonetheless, this species appears to be chiefly dependent on Venus flytrap. Therefore, the factors that threaten Venus flytrap are likely also threats to *H. subporphyrea*, including development and fire suppression. However, while Venus flytrap can flourish under a fire regime

that prioritizes burning large areas in a single bout, it is likely that *H. subporphyrea* requires a patchier burn strategy for individuals to persist in unburned refugia and recolonize burned areas (Hall and Schweitzer 1993). This may explain why searches for *H. subporphyrea* have been relatively unsuccessful in some of the largest – and most frequently burned – Venus flytrap populations. Other threats to this species include fire ants and improper use of insecticides (Hall and Sullivan 2000).

Hemipachnobia subporphyrea is listed as “Significantly Rare” in N.C. and direct sightings are limited (NCNHP 2020). After it was first collected by European scientists in the late 18th or early 19th century, it was not recorded again until 1974, when it was found in Carteret County. Between 1986-1996, sporadic individuals were recorded. The first status survey for this species was conducted in 1998 and 1999 by Stephen Hall and Bo Sullivan. To sample for adults, Sullivan and Hall used UV light traps and sheet sampling. They also performed direct plant searches for larvae. Hall and Sullivan sampled for adults at 25 locations across 4 sites in N.C. throughout April 1998. In addition, larval sampling occurred in June, July, and October 1999 at 5 sites. Hall and Sullivan confirmed extant populations only at 3 sites, in Carteret, Pender, and Onslow Counties (Hall and Sullivan 2000).

In 2004, Hall and Sullivan collected gravid females at a limited number of sites and performed a rearing study. Though genital morphology is a key difference between potentially co-occurring *Hemipachnobia* species, *H. subporphyrea* is reportedly identifiable from high-quality photos and Hall and Sullivan identified individuals nonlethally during this survey (Hall and Sullivan 2005). A thorough status survey based on larval presence and feeding signs was conducted in 2005. A total of 16 sites were surveyed and potential diagnostic herbivory damage was found at 12 Venus flytrap populations.

No individuals of this species were found during a later 2016 survey. Based on NCNHP records, only five individuals have been sighted since 2009, including two larvae that were observed by our survey team at a new location for the species in 2019. The profound rarity of this species makes it a high priority for further study, especially while its host plant is under review for listing. A planned survey of this species in 2020 was delayed due to the COVID-19 pandemic. A survey for adults of this species was conducted in spring and summer of 2021, with report pending.

Threats

The Carolina Coastal Plain has undergone significant changes since European colonization, including the rapid decline of longleaf pine savannas due to the naval stores industry, damage from livestock, and widespread fire suppression (Outcalt and Sheffield 1996, Frost 2000, Frost 2006). Consequently, a fraction of viable habitat remains. Venus flytrap is threatened chiefly by ongoing trends of fire suppression, development, and hydrological changes in this area. To a lesser but non-negligible degree, this species is also vulnerable to illegal collection.

Fire suppression

Like many species in the longleaf pine savanna and associated habitats, Venus flytrap depends on frequent fire to limit taller competitors that impede access to light (Schulze et al. 2001, Luken 2005). Fire also promotes seedling establishment and flowering in this species (Roberts and Oosting 1958, Luken 2005, Hamon et al. 2019). After approximately 4 years following a fire, Venus flytrap populations quickly decline.

Historically, lightning strikes ensured that fires were a frequent natural disturbance in the Carolina Coastal Plain. Fire was also used to manage open game land by Indigenous communities. Indigenous groups whose ancestral land contains Venus flytrap habitat include — but are not limited to — the Coharie, Chicoran, Coree, Lumbee, Tuscarora, and Waccamaw Siouan peoples

(Native Land Digital 2021). Many tribes on the east coast of N.C. were decimated by European diseases, hostility from colonists, and forced displacement, which denied Indigenous sovereignty and eroded traditional land management (Coharie Tribe, Lumbee Tribe of North Carolina, Tuscarora Nation of North Carolina, Waccamaw Siouan Tribe). Although European farmers and later game hunters continued to burn some areas, attitudes toward fire changed, and fire prevention was widespread in the first half of the twentieth century (Frost 2006).

It is now well-understood that fire is an essential component of the longleaf pine ecosystem and associated habitats. Multiple landowning agencies incorporate prescribed fire as a management tool. Many of the largest and densest Venus flytrap populations occur on land that is intermittently burned. However, controlled burns are difficult for populations on private land or near residential areas. Though mowing can simulate the benefits of fire by increasing access to light (Luken 2005), fire suppression remains an ongoing threat to populations where burns are difficult or not practiced.

Habitat conversion

Another primary threat to Venus flytrap is habitat change, including conversion to timber plantations, agriculture, golf courses, and residential areas. The southeastern U.S. is undergoing rapid urbanization (Terando et al. 2014). Surrounding developments can also make planning for controlled burns difficult or limited. Therefore, the effects of development can also exacerbate the effects of fire suppression (Evans et al. 2012).

Illegal collection

The charismatic appearance of Venus flytrap has made it popular in the horticultural trade. Though this species is amenable to propagation via tissue culture or cuttings, wild plants are vulnerable to poaching.

Historically, it was legal to collect flytraps from the wild for sale, and wild plants may have been exported in the millions (Sutter et al. 1982). Prior to the start of widespread tissue culture in the trade by the early 1990s, wild-collected plants likely constituted the bulk of the flytrap trade (Bailey and McPherson 2012). In the 1960s, it became illegal to collect plants from N.C. state lands without a permit. It would become a Class 2 misdemeanor in N.C. to take Venus flytrap without permission, punishable by a fine of up to \$50. Later, offenders could receive a fine of \$1,000 and up to 60 days in jail, with a possible penalty of up to \$2,000 for repeat offenders (NC GS § 106-202.19). In S.C., it is currently a misdemeanor to cut or damage plants on private or public property without a permit. The penalty is a fine of up to \$200 and up to 30 days in jail (SC ST SEC 16-11-590).

In December 2014, N.C. legislature made it a Class H felony to remove Venus flytrap plants or seeds from public or private land without permission, punishable by a maximum of 25 months in prison (NC GS § 14-129.3). Each individual plant taken counts as a separate offense. This, coupled with laws that impose greater penalties for repeated felonies, means that an individual could potentially incur a sizeable prison sentence for a single bout of illegal collection. Since that law was implemented in 2014, at least seven arrests have been made, with most of these resulting in conviction. The most recent arrest at the time of this report was made in 2019, and some evidence of illegal collection was reported by surveyors in July 2020.

Multiple questions surround flytrap poaching. Namely, it is currently unclear where the demand for wild plants comes from. Online collectors, informal local vendors, and medicinal companies have all been proposed as sources of demand for wild plants (McManus 2014). Poverty almost certainly plays a role in driving the supply side of illegal collection (Outland 2018), pointing to potential systemic drivers. A single plant reportedly earns up to \$0.10, but since thousands of

plants can be harvested a time, illegal collection may still be a reliable source of funds for those living with economic desperation.

Changes in hydrology

Venus flytrap thrives in moist soil and is sensitive to changes in hydrology. Large swaths of the Carolina Coastal Plain have been drained and converted to agriculture and timber (Carter 1975), particularly wetter Venus flytrap habitats like pocosins and Carolina bays (Richardson 1983). Drainage ditches can also cut through habitat along roadsides. In one particularly dramatic example of hydrologic change, a lake in Brunswick County drained in 2018 after a hurricane damaged its dam. Multiple patches of Venus flytrap occur near this lake, and the effect of this incident on these populations remains to be seen.

Right-of-way management

Powerline corridors and roadsides are susceptible to herbicide use and the effects of roadside maintenance, potentially impacting Venus flytrap populations (Figure 2).

Existing protections

Venus flytrap was previously considered for listing under the Endangered Species Act in 1993 but was determined to have insufficient data for consideration (Federal Register 1993) In N.C., Venus flytrap is listed as a Threatened by the N.C. Plant Conservation Program (NCPCP) under the Plant Protection and Conservation Act. This denotes a species “which is likely to become an endangered species within the foreseeable future” (GS19B-106:202.12.). Under this act, Venus flytrap cannot be collected without a written permit from the owner or agency of the land where collection will occur. Collectors must also carry the permit with them while collecting.

Venus flytrap is ranked by NatureServe as S2 in N.C., S1 in S.C., and G2 globally (Wichmann 2021). These ranks designate a species that is considered imperiled N.C., critically imperiled in S.C., and imperiled globally. Conservation ranks are based on a number of factors, including abundance, range, threats, trends, and distribution.

Under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), Venus flytrap is listed as an Appendix II species. In addition, Venus flytrap is listed as “Threatened” by the International Union for the Conservation of Nature (IUCN) Red List (Schnell et al. 2000).

PART II. STATUS SURVEY

METHODS

Protocol development

Stakeholder meetings were held on January 24th, 2019, and January 28th, 2020, to review known research on Venus flytrap, present preliminary data, and solicit feedback on survey protocols. These meetings were attended by land managers, researchers, and other collaborators.

Delineating populations

This report uses three different terms to refer to groupings of Venus flytrap: population, subpopulation, and patch. A generic distance of 2 km was used to denote separate populations, or Principal Element Occurrences (or parent EOs), of Venus flytrap. Data analysts buffered the element occurrence records by 2 km and denoted a single population as occurring where the buffer overlapped. When occurrences within 2 km were disjunct from one another for more than 1 km due to unsuitable habitat, the data team used a 1 km separation distance rule to separate populations. A population of Venus flytrap may vary widely in distribution, ranging from a single cluster of individuals, to thousands of individuals distributed over several kilometers.

A subpopulation or sub-EO refers to geographically distinct groups of individuals within a population. The subpopulation designation is somewhat subjective but is broadly a method of distinguishing parts of a population for the purposes of conservation planning (NatureServe 2021). While population ranks were used to identify key areas of focus, subpopulations were used as the primary unit for planning the survey schedule.

In this report, patch refers to a group of individuals represented by a single source feature or polygon. A subpopulation may be composed of multiple patches. Surveyors generally used patch as a distinct survey unit, with separate observations and a new line of data recorded for each

patch. In instances where patches were so close that the habitat quality and type were indistinguishable, a single line of data was sometimes used.

Site priorities

The goal of this survey was to visit as many Venus flytrap populations as possible. However, given time restrictions, subpopulations were assigned priority levels to guide the survey schedule. Since the overarching goal of this survey was to update the known population count of this species, higher priority was given to subpopulations where a recent survey had not occurred, but that were reported to have abundant individuals. Within each managed area, subpopulations were prioritized according to the following criteria:

High priority

- a) Subpopulation is ranked as B (“Good”) to A (“Excellent”) (roughly $\geq 1,000$ individuals) and has not been surveyed in three or more years.
- b) Site is ranked as B (“Good”) to A (“Excellent”), has been surveyed within the last three years, but estimates — not absolute counts — were used in the most recent survey.

Medium priority

- a) Population is ranked as B (“Good”) to A (“Excellent”) (roughly $\geq 1,000$ individuals) and exact counts were performed in the most recent survey.
- b) Population has approximately 200-999 individuals according to the most recent survey but has not been surveyed in three or more years.
- c) Population has approximately 200-999 individuals, has been surveyed within the last three years, but exact counts were not performed.

- c) Populations with unclear or contested status. This generally denotes populations with brief recent surveys that reported few plants but have a history of abundant individuals and whose status may be affected by recent management practices.

Low priority

- a) Population has approximately 200-999 plants according to the most recent survey, has been surveyed within the last 3 years, and exact counts were performed.
- b) Population is ranked as “Historical.”
- c) Population has fewer than 200 plants according to the most recent survey.
- d) Extirpated populations. Habitat is recorded as destroyed or projected for certain development.

Absolute counts

In both years, counts were made through one of two primary methods. For most patches, surveyors performed counts by walking consecutive parallel transects approximately 5 meters wide and counting all individuals seen within 2.5 meters of the surveyor. This was typically done in teams of two individual surveyors, with one surveyor leading the survey direction and a second surveyor counting plants.

To ensure the transects were parallel and nonoverlapping, surveyors used a combination of visual landmarks, compasses, and pathway-mapping software via the GIS Pro mobile application (Garafa LLC 2019). At a minimum, the survey team repeated the transect method across the extent of the entire patch polygon as recorded in previous surveys. If plants or suitable habitat extended outside of the polygon border, the survey team would extend the transect to capture the most

comprehensive distribution of the patch. At either end of each transect, the survey leader recorded the present GPS location to map an updated polygon delineating the current patch extent.

To count plants, the surveyor carried two counters – one to count reproductive individuals, and a second to count purely vegetative individuals. All rosettes arising from a discrete point were counted as a single individual. Surveyors also generally assumed that a single flowering stem correlated with a single individual, since individuals infrequently produce more than one inflorescence.

This method was referred to as an “absolute” count, since it prioritized making a comprehensive, nonestimative assessment of population size. This allowed the survey team to survey large populations with thousands of individuals with relative efficiency. However, surveyors are visually biased to reproductive individuals, likely leading to a systematic underestimate of total population size and an overestimate of the fraction of reproductive individuals in each population. In addition, mapping software revealed the tendency of the survey team to “cut wide” in order to prevent overlapping transects, often resulting in up to 3 m between adjacent transects. This reinforces that the absolute counts are likely still underestimates.

Estimated counts

In instances where time was limited and sites were high priority, the core survey team used an alternate estimative method to assess population counts. In this method, surveyors delineated the outline of the population using GIS Pro software. The resultant polygon was then used to calculate the total area of the population. Surveyors then virtually marked regular transects through the population, such that the total area of the transects (5 m x the length of the transect) represented approximately 10% of the population area. The number of individuals counted in this subset of transects was then used to extrapolate total area. This method was used sparingly, but its influence

on the results is significant because it was used at two sites with exceedingly large populations. Limited comparisons between the estimative method and the absolute method suggest that the estimative method may lead to a slight overestimate.

Site variables

At each site, the core survey team recorded additional data related to location and site conditions (Appendix 1). This included GPS coordinates, survey date, surveyor names, and access location. As a proxy for phenology, surveyors also recorded a visual estimate of the percentage of individuals that were vegetative, budding, flowering, or fruiting. Vigor was recorded as the general impression of the health of the plants. Prevailing habitat type was noted as either savanna, flatwood, bay rim, pocosin, right-of-way, or a mosaic of habitat types. Habitat type was elaborated in a description, and generally a photo of the site was taken as a record of habitat composition. Surveyors also noted co-occurring plant species at each population.

At each site, surveyors made qualitative notes of the extant threats, focusing on development, fire suppression and illegal collection. For this survey, the core NCNHP survey team used “development” as a blanket term to include habitat change from construction, road maintenance, and foot traffic. To record fire suppression, surveyors recorded the date of the last burn wherever possible and noted whether the site was overgrown. The scale of these threats was then described qualitatively in terms of severity, scope, and immediacy, which are defined further below:

Severity

- High: Loss of species population (all individuals) or destruction of species habitat in area affected, irreversible or requiring long-term recovery (>100 yr.).

- Moderate: Major reduction of species population or long-term degradation or reduction of habitat in area affected, requiring 50–100 yr. for recovery.
- Low: Low but nontrivial reduction of species population or reversible degradation or reduction of habitat in area affected, with recovery expected in 10–50 yr.
- Insignificant: Essentially no reduction of population or degradation of habitat due to threats, or populations or habitats able to recover quickly (within 10 yr.) from minor temporary loss. Note that effects of locally sustainable levels of hunting, fishing, logging, collecting, or other harvest from wild populations are generally considered Insignificant as defined here.

Scope

- High: >60% of total population, occurrences, or area affected.
- Moderate: 20–60% of total population, occurrences, or area affected.
- Low: 5–20% of total population, occurrences, or area affected.
- Insignificant: <5% of total population or area affected.

Immediacy

- High: Threat is operational (happening now) or imminent (within a year).
- Moderate: Threat is likely to be operational within 2–5 yr.
- Low: Threat is likely to be operational within 5–20 yr.
- Insignificant: Threat not likely to be operational within 20 yr.

Other survey methods

In addition to the core NCNHP survey team, several other survey teams focused on *Venus flytrap* populations from 2019 to 2021. Most significantly, many plants were reported from two populations counted by two separate survey teams in Brunswick County, N.C. Because these results are summarized in this report, their survey methods are outlined here.

The Nature Conservancy property

At a property owned by The Nature Conservancy (TNC) in Brunswick County, surveyors use a combination of direct counts and estimative methods to monitor the size of a Venus flytrap population. To do this, surveyors record the number of plants and flowering rate in 14 1 x 3 m plots and four 10 x 2.5 m transects. For the purposes of the present survey, the surveyors incorporated additional methodology. In two subpopulations, surveyors counted the total number of flowering plants. They then selected several 1 x 3 m plots representing variable habitat conditions. In each of these plots, the surveyors counted the percent of individuals that were flowering. They then used this percentage to extrapolate the total number of individuals in these two subpopulations. In an additional subpopulation, the surveyors counted the number of plants in two 10 x 2.5 m transects and one 1 x 3 m plot. They then used this value to extrapolate the total number of individuals in one acre.

In addition to population sizes, surveyors recorded burn times and signs of illegal collection, as well as qualitative descriptions of plant sizes and understory thickness.

Department of Defense property

At a Department of Defense-owned property in Brunswick County, a team of surveyors estimated Venus flytrap populations while surveying for the federally listed species *Lysimachia asperulifolia* in 2019. Additional surveys were conducted by a consulting botanist in 2020.

To assess populations in 2019, surveyors focused on Venus flytrap patches that overlapped with or were adjacent to *L. asperulifolia* sites. For each site or patch, surveyors conducted an absolute count (U.S. Army Corps of Engineers 2019). In 2020, comprehensive absolute counts were conducted at this property, most of which did not overlap with counts from the previous year, and many of which represented new occurrences for the species (Dr. J.H. Carter III and Associates, Inc. 2021.).

Search for *Hemipachnobia subporphyrea*

While surveying, surveyors from NCNHP conducted a haphazard search for *Hemipachnobia subporphyrea* larvae or feeding damage. Multiple invertebrates may leave feeding damage on Venus flytrap, including myriad Orthopterans and Lepidopterans. Surveyors focused their attention for feeding damage described by Stephen Hall as potentially diagnostic for *H. subporphyrea* (Hall and Sullivan 2003). This damage is characterized by numerous small holes in the leaves and petioles, which may be accompanied by frass. It is possible that the larvae, like many other Lepidopterans, feed during the night and remain inconspicuous during the day. For this reason, if suspected feeding damage was spotted, the surveyor inspected the base of the plants near the soil. When suspected feeding damage was found, the surveyor recorded the GPS coordinates of the finding, usually accompanied by a photo. Suspected photos were sent to entomologists Stephen Hall and Bo Sullivan for assessment.

Timeline

In 2019, surveys focused on public lands and lands owned by The Nature Conservancy. Surveys began on 5 May 2019 and were completed 1 August 2019. The core NCNHP survey team visited 18 populations across six N.C. counties and one S.C. county. Populations were distributed across counties as follows; Brunswick (6), Carteret (1), Columbus (2), Horry (2), New Hanover (1), Onslow (3), Pender (2), and one population overlapping Onslow and Pender. In total, the core NCNHP survey team visited 14 public managed areas, 2 private sites, 7 TNC-owned preserves, and 7 roadside sites. An interim report was submitted to USFWS on 19 September 2019.

During the summer of 2020, surveys focused on private lands and remaining populations in public lands. Surveys began on 19 May 2020 and were completed 22 July 2020. The core NCNHP survey team visited 18 populations across 6 N.C. counties. Populations were distributed across counties as follows; Brunswick (5), Bladen (1), Carteret (1), New Hanover (1), Onslow (5), Pender (3), and two populations overlapping Onslow and Pender. In total, the core NCNHP survey team visited 7 public managed areas, 28 private sites, 1 TNC-owned preserve, and 30 right-of-way sites. An interim report was submitted to USFWS on 15 September 2020.

In May 2021, Venus flytraps were observed at one new S.C. population and one S.C. population that was previously listed as Historical (James Fowler and Keith Bradley, S.C. Department of Natural Resources, pers. comm.). These sites were again visited by an NCNHP surveyor for inclusion in the present survey.

Data entry and rank assessment

In Fall of 2019 and 2020, a data team at NCNHP entered survey data into Biotics, a data management system used by the NatureServe network. Population designations were then reconfigured according to the updated survey data.

To assess rank designations, data analysts summarized the total estimate of plants in each population. Ranks were then assigned according to generic Biology and Conservation Database (BCD) rank specifications, which consider only population size. In more modern rank specifications, other factors including conditions and habitat quality are also incorporated. Though modern rank specifications are a goal for this species, BCD rank specifications are used to allow for comparison with previous rank assessments. For Venus flytrap, ranks are assigned as follows:

A rank (“Excellent”) – $\geq 2,000$ plants.

B rank (“Good”) – 1,000-2,000 plants.

C rank (“Fair”) – 500-1,000 plants.

D rank (“Poor”) - <500 plants.

E rank – Extant.

F rank – Failed to Find.

H rank – Historical.

X rank – Extirpated.

i = Introduced.

r = Reintroduced.

Venus flytrap data from S.C. were also newly assessed according to BCD rank specifications.

Data Analysis

To generate the total number of wild Venus flytrap in each population, the number of individuals counted or estimated in the most recent survey of each subpopulation was added. For

the total wild population count for this report, the latest conservative survey estimate was incorporated from populations that were not visited in 2019. When addressing threats, the proportion of patches and individuals assigned each threat level was calculated.

Since the bulk of wild Venus flytraps are concentrated in just a small number of populations, this report focuses on discussing A-ranked populations. For each A-ranked population, the updated rank assessment and habitat quality are described. Where the percentage of reproductive individuals is included, only patches where reproductive individuals were counted were included in the calculation. In addition, previous survey estimates are discussed. To summarize threats, the general prevalence of each primary factor is discussed, with a description of specific threats.

RESULTS

Total counts

From 2019 to 2021, the core NCNHP survey team visited 26 distinct populations (Table 1). Other survey teams and contributors visited two additional populations, with a total of 28 populations visited during the scope of this survey (Table 1). There are 65 extant populations in N.C. and four extant populations in S.C., with a total native global distribution comprising 69 populations (Table 2).

Though only 28 of the possible 69 populations were visited, this survey reflects the focus on populations with a high abundance of individuals. Surveyors visited 13 out of 17 populations with an estimate of 1,000 or more individuals, and 17 out of 52 populations with fewer than 1,000 individuals. From 2019 to 2021 surveys alone, the core NCNHP survey team counted 361,055

individuals via absolute counts and estimated 151,976 individuals via estimative methods for a total of 513,031 individuals. The survey team at a property in Brunswick County estimated an additional minimum of 256,070 individuals in Population 263. Surveyors at a federal property counted at least 41,157 individuals. Other collaborating surveyors counted 1,722 plants. When combining all agency survey results from 2019 to 2021, a total of 811,980 were counted or estimated. When accounting for the most recent conservative estimate from extant patches and populations that were not included in the present survey (41 populations), we estimate 879,167 individuals of wild Venus flytrap in N.C. and S.C.

Distribution

From 2019 to 2021, surveyors visited populations in seven N.C. counties (numbers of populations visited in each county listed in parentheses): Bladen (2), Brunswick (8), Carteret (1), Columbus (2), New Hanover (1), Onslow (8), and Pender (4). Surveyors also visited two populations in Horry County, S.C.

As of 2021, Venus flytrap is extant in 11 counties and is considered extirpated, historical, or failed-to-find in nine counties (Figure 3).

Surveyors mapped survey polygons covering 3.8 km² and recorded over 100 new patches.

Threats

The core NCNHP survey team recorded threats at 373 distinct patches from 2019 to 2021. In terms of severity, development was noted as a high threat at 8% of patches comprising over 17,600 individuals, fire suppression was noted as a high threat at 13% of patches comprising 4,100 individuals, and illegal collection was recorded as a high threat at one patch comprising 27 individuals (Figure 4, Figure 5). Other threats — generally hydrology issues — were recorded as

high threat in terms of severity at 8% of patches comprising 9,241 individuals. It is worth noting that this measurement applies to patches where damage has already occurred. Fire suppression was frequently noted as a prevalent factor in patches where Venus flytrap were once found but were not found from 2019 to 2021.

When assessing the immediacy of threats, development was noted as a high threat at 9% of patches comprising 17,742 individuals, fire suppression was noted as a high threat at 14% of patches comprising 4,308 individuals, and illegal collection was recorded as a high threat at 7% of patches comprising 18,911 individuals. Other threats were noted as an immediate, high threat at 9% of patches comprising 14,607 individuals. These values represent individuals under imminent danger of damage from these factors (Figure 4, Figure 5).

Rank evaluations

Out of the 27 N.C. populations that received updated counts from 2019 to 2021, five populations received an improved rank designation and four populations received a worse rank designation. The ranks of 17 other N.C. populations were also reassessed, and in total three populations received an improved rank designation and 10 populations received a worse rank designation compared to their previous assessment.

After assessing S.C. records according to BCD rank specifications, we identified 11 distinct populations. Of these, one population is “A” ranked, three populations are “D” ranked, and the remaining populations are considered historical or failed-to-find.

Moth observations

Suspected feeding damage was noted at 16 patches of Venus flytrap in 2019 and two patches in 2020. None of the records where feeding damage alone was recorded were positively

identified as evidence of *Hemipachnobia subporphyrea*. However, two larvae were noted in Population 256 in May 2019, and were positively identified as *H. subporphyrea* by Stephen Hall. These larvae represent a new known population for the species. Distinctive feeding damage and frass were noted on the Venus flytrap individuals where the larvae were found, and one larva was observed feeding on Venus flytrap tissues.

Limitations

As previously noted, the absolute count method likely resulted in an underestimate due to the visual bias towards nearby, reproductive individuals. In addition, surveyors identified individuals as each discrete rosette arising from a single point. However, since Venus flytrap can divide asexually and form dense mats, this method was likely inefficient at distinguishing separate individuals that were growing very close together. Drought conditions in the summer of 2019 (National Drought Mitigation Center; U.S. Department of Agriculture; National Oceanic and Atmospheric Administration 2019) likely further contributed to an underestimate, particularly in June and July. Many plants entered dormancy, often forcing the surveyors to rely on the dried fruits and flowers as a visual cue. The dormant leaves appeared black and shriveled, making it difficult to count vegetative individuals at sites where plants had entered dormancy (Figure 6).

Limited comparisons between absolute counts and estimative methods suggest that the subsampling method may result in a slight overestimate. Though the estimative method was only employed at a few populations, the superabundance of these populations makes this potential source of error important.

Major Population Centers

NC263

Surveyors: Roger Shew, Dale Shew

Summary

This population occurs in Brunswick County, N.C., and is estimated to have at least 257,951 plants, with a maximum estimate of over 500,000 plants. Therefore, this population represents at least 30% of the total wild population of Venus flytrap, making it the most major population center for the species. Based on estimates from subplots and transects, the average percentage of reproductive individuals was 13.8%. The entirety of this population occurs within land owned by a private land conservancy and is managed expressly for conservation.

This survey was not performed by the core NCNHP team. A concerted effort to monitor this population has been conducted for over six years by Roger and Dale Shew, and this report focuses on the most recent survey of this population performed in 2019.

Rank evaluation

This Principal EO is comprised of seven sub EOs, all of which occur on land owned by a private land conservancy. Of these seven sub EOs, six were last surveyed in 2019 and one was last surveyed in 2017. The final sub EO was last surveyed in 2009, when it was simultaneously created via the introduction of 400 poached plants. Counts from this population were estimated primarily using transect and subplot monitoring. When counts from sub EOs are combined, a minimum of 257,951 plants are estimated, handily giving this Principal EO an A-rank according to the BCD rank specs.

Habitat description

This population co-occurs with numerous plant species of conservation concern, including 14 species of carnivorous plant, 16 species of orchid, and the federally listed Rough-leaf loosestrife (*Lysimachia asperulifolia*) (The Nature Conservancy 2021). The habitat is burned on a fire return interval of 2-3 years, allowing for a rich understory and an amenable habitat for Venus flytrap.

Within the preserve, almost all Venus flytrap individuals are found in a string of roughly interconnected longleaf pine savanna openings that are each surrounded by a matrix of dense pocosin. The largest of these savannas, containing one subpopulation, is over 40 acres, with most individuals occurring in the wetter regions of the savanna. At the time of this survey, this savanna was last burned in February 2017. The surveyors noted that the grass had become thick in the nearly two years since the previous burn, so the rate of flowering was reduced, at only 4.9%. In a partial survey of two 1 x 3m plots and two 10 x 2.5 m transects, surveyors counted 2,043 plants, and estimated that Subpopulation 011 contains the most individuals out all the savannas in this population. However, a full count was not conducted in 2019 because the low flowering rate would likely lead to an inaccurate approximation under the protocol used at this site.

A second, smaller, wet savanna (approx. 7 acres) lies immediately adjacent to the largest savanna and contains a second subpopulation. At the time of survey, this savanna was last burned August 2017, and surveyors again noted that the rate of flowering was very low compared to previous years. Surveyors recorded 2,592 individuals during a partial survey of two 1 x 3 m plots and two 10 x 2.5 m transects, with a total estimate of 179,790 – 359,580 individuals in this savanna alone. Surveyors also noted an average flowering rate of 16.2% within the plots, and 9.6% within the transects.

A third subpopulation occurs in a savanna where individuals are chiefly found in old fire line ditches. Here, conditions are wet and amenable to flytraps. This savanna is small (<3 acres) and contains few wet areas outside of the fire lines. At the time of this survey, this area was last burned in January 2016, but individuals persisted well in the fire lines where thick grasses tend to encroach less. From five 1 x 3 m plots, surveyors noted an average flowering rate of 16.3 %.

The fourth and fifth subpopulations occur over roughly three disjunct savanna openings. One of these savanna openings covers approximately 20 acres. Here, most individuals occur in the ecotone between pocosin and pine savanna, particularly in the wetter areas. This region also supports many other carnivorous plants. Surveyors recorded 16,645 total flowering plants in this savanna and noted a flowering rate of 40.6% in selected plots, resulting in an overall estimate of 39,741 individuals for this subpopulation. The second savanna covers only 1.4 acres. Here, surveyors have conducted full counts since 2014 to monitor the impact of controlled burns on Venus flytrap. This subpopulation was last burned August 2018, and the recent burn contributed to a relatively high flowering percentage. Surveyors counted 10,706 total flowering plants in this savanna and noted a flowering rate of 29.3% in selected plots. Therefore, they estimated a total of 36,539 individuals in this savanna. Parts of this savanna were missed by the most recent burn – or burned incompletely – leading to a flowering rate that, while high, was lower than some adjacent subpopulations that were burned at a similar time. In a final string of savannas covering approximately 31 acres, flytraps were noted largely in the ecotonal and wetter regions of the savanna. At the time of this survey, the most recent burn in this area occurred in June 2018. In two subplots from this area, the average flowering percentage was 19.5%.

The two remaining subpopulations were last surveyed in 2009 and 2017, where they were estimated to have approximately 400 and 100 individuals, respectively.

Previous population estimates

The overall rank of this population remains unchanged since the previous rank designation. This population has undergone regular monitoring using subplot and transect methods since 2014. Surveyors have consistently reported estimates reflecting an overall population of hundreds of thousands of individuals based on the protocols used at this site. Prior to 2014, this population had intermittent incomplete surveys dating to the late 1980s.

Threats and Protections

Fire suppression

The land managers burn the savanna habitat on a fire return interval of 2-4 years. However, burning the pocosin areas is difficult, and feasibility for burning these areas may be an ongoing challenge.

Development

This population occurs entirely on land owned by a private land conservancy designated as a Dedicated Nature Preserve. This refers to land that is owned by state agencies, local governments, or nonprofit conservation groups, and which is managed for conservation via a legally binding agreement with the N.C. Department of Natural and Cultural Resources (DNCR). Consequently, land development is only an indirect threat to this population.

Illegal collection

The site where this population occurs is locally known as a destination where the public can view carnivorous plants in the wild. This region therefore has high value as an educational site, where visitors can learn about the longleaf pine savanna and its conservation. Unfortunately,

popularity can also increase the risk of illegal collection of rare plants, including Venus flytrap. Surveyors at this site have recorded intermittent signs of collection in their research plots since 2014, and other surveys have noted signs of poaching in prior years. However, signs of poaching were low in 2018 and 2019. Repeated poaching over time can increase the severity of impacts that may seem low in any given year. Given the rate of collection in a population of this size, illegal collection could be considered a low-to-moderate threat in terms of scope and severity, but a high threat in terms of immediacy.

Other

Historically, significant parts of the larger region where this population occurs was dominated by cedar and gum. In the first half of the twentieth century much of the swamp forests in the area were logged. In the 1960s, further portions of this region were converted to pine plantation, and it is likely that this included some Venus flytrap habitat. However, this population likely persists because its current extent includes swaths of habitat that were unsuitable for logging and agriculture (Michael Schafale, NCNHP, pers. comm.).

NC286

Surveyors: Laura Hamon, Daniel Hannon, Summer Lauder, Ryan Martin, John McLaughlin, Libby Seay, Eric Ungberg, Andy Wood

Survey Dates: 2019 – 14 May, 16 May, 21 May, 23 May, 4-5 June, 14 June, 17-18 June, 24-26 June, 28 June, 1-3 July, 8-15 July, 17 July, 19 July. 2020 – 19 May, 26 May, 30 June, 10 July, 22 July.

Summary

This population occurs in Pender County, N.C., and is estimated to have at least 221,611 individuals. This total count comes entirely from surveys from 2019 to 2021. Of this total estimate, 217,789 were counted via absolute counts and 3,822 were estimated using a transect method. Most of these individuals (95%) were recorded on state-owned land managed for wildlife. The remaining individuals were recorded on land owned by a private land conservancy (4%) or private property (0.2%). Much of this population had experienced a burn within the last 1-2 years, and over 70% of the counted individuals were reproductive. Surveys from 2019 to 2021 recorded 39 previously unmapped patches in this population.

In both 2019 and 2020, surveys were conducted from mid-May to mid-July. In both years, land managers conducted extensive burns during May. In burned habitat, surveys were prioritized for later in the growing season, when new flytrap growth was readily visible in the burned soil.

Rank evaluation

This population is comprised of five sub EOs, four of which are almost entirely on state property, and one of which occurs largely within a private land preserve. A small portion of one source feature overlaps with private property. With over 200,000 plants, this population easily surpasses the requirements for an A-rank according to the BCD rank specifications.

Habitat description

Of the five sub EOs, four occur almost entirely on public land comprising over 208,000 individuals. Here, nearly 134,000 individuals occur in wet longleaf savanna with a diverse herbaceous understory. An additional 54,000 individuals are found occurring on bay rims, or in the narrow ecotone between bay rims and adjacent habitat. Nearly 3,500 individuals were recorded

in powerline rights-of-way. Fewer individuals are patchily distributed in low, wetter patches in an otherwise relatively dry longleaf pine savanna. Savanna in this area is burned on a fire return interval of 2-3 years. At the time of survey in 2019, over 129,000 individuals occurred in habitat that had been burned within the previous three months (Figure 7). Co-occurring species here include *Acer rubrum*, *Andropogon* spp., *Aristida stricta*, *Arundinaria gigantea*, *Asclepias* sp., *Calopogon pallidus*, *Cyperophorus* sp., *Ctenium aromaticum*, *Cyrilla racemiflora*, *Drosera capillaris*, *Eriocaulon* spp., *Gaylussacia* sp., *Gordonia lasianthus*, *Hypericum* spp., *Ilex glabra*, *Kalmia angustifolia*, *Lachnanthes caroliniana*, *Lyonia mariana*, *Magnolia virginiana*, *Morella cerifera*, *Pinus elliotii*, *Pinus palustris*, *P. serotina*, *Polygala* spp., *Pteridium pseudocaudatum*, *Pterocaulon pycnostachyum*, *Rhexia alifanus*, *Sphagnum* spp., *Spiranthes* spp., *Vaccinium* sp., and *Xyris* sp. Additionally, this population occurs in the vicinity of approximately 12 other carnivorous plant species, including *Drosera* spp., *Sarracenia* spp., and *Utricularia* spp.

A patch of approximately 546 individuals, 28% of which were reproductive, were recorded on private property. In this area, pine flatwoods grade into pocosin. Co-occurring species include *Aristida stricta*, *Ilex* sp., *Morella cerifera*, *Pinus palustris*, *P. serotina*, *Polygala lutea*, *Pteridium pseudocaudatum*, *Rhexia* sp., and *Sphagnum* sp.

One subpopulation in this population occurs on land owned by a private land conservancy and comprises nearly 10,000 individuals. Of these individuals, approximately 99% were recorded in damp, seldom-used roadbeds, alongside *Drosera* sp. and *Sphagnum* sp. The borders of these roadbeds grade into diverse savanna and meadow habitat that includes other carnivorous plants, Ericaceous shrubs, *Rhynchospora* spp, and *Sabatia difformis*. Scattered individuals in this sub-EO occur in a meadow habitat with occasional *Pinus serotina*. Grass in this area is dense, and it is

possible that some individuals were not readily visible during the survey. An additional survey following a burn could be informative.

Previous population estimates

The current survey represents the most intensive survey of this population, but the rank remains unchanged from the last rank assessment. Previous surveys of the two largest subpopulations reflect the superabundance of this population, with surveyors frequently describing patches using qualitative descriptors such as “abundant” or “numerous” (Sorrie 1995). The rank of one subpopulation – which occurs in drier habitat compared to other parts of this population – was upgraded from BC to A-rank. The previous rank designation was assigned according to data from 1995, which broadly described this population as “uncommon” or “scattered” (Sorrie 1995).

Threats and Protections

Fire suppression

The bulk of this population occurred on land that is frequently burned to promote wildlife, including the federally listed Red-cockaded woodpecker (*Leuconotopicus borealis*). The largest and densest populations were observed in wet savanna that had been burned recently. Therefore, fire suppression is likely not a high-priority threat to this population. However, fire suppression was noted as a likely factor preventing the growth of individuals in one patch that had contained flytraps in previous surveys. In the subpopulation occurring in a private land preserve, surveyors noted dense grass in several patches that previously contained more individuals, likely resulting in an underestimate in those areas. Though controlled burns occur at this habitat, several years had elapsed since the previous burn, and the need for a new burn was apparent. Mild fire suppression was also noted in the small part of this population that overlaps with private property.

Development

No portions of this population are slated for imminent development. However, patches occurring in roadbeds are at increased risk of trampling, whether by vehicles or pedestrians. Development is overall a low-priority threat.

Illegal Collection

Much of this population is visually apparent from the road, which may increase the risk of illegal collection. In 2016, nearly 1,000 plants were stolen during a single poaching event, though the poaching rate in more recent years is unclear, and most of this population is regularly monitored for signs of poaching.

NC264

Surveyors: Laura Hamon, Gary Haught, Daniel Hannon, Summer Lauder, Ryan Martin, Libby Seay, Craig Ten Brink

Survey Dates: 2019 – 28-29 May. 2020 – 25-26 June 2020.

Summary

This population occurs in Onslow County, N.C., and is estimated to have more than 105,100 individuals. A total of 84,875 were counted from 2019 to 2021. Of these, 78,251 individuals were estimated using the transect method, while 6,624 were counted using absolute counts. During this status assessment survey, approximately 60% of individuals were reproductive. This population occurs entirely on federal land, which is managed in part to promote federally listed species, including *Leuconotopicus borealis* and *Lysimachia asperulifolia*. In some areas of

this habitat, fire is frequently ignited by military exercises, which is beneficial for many fire-dependent species.

Due to limited time at this population from 2019 to 2021, surveyors relied largely on estimative methods to conduct counts. In two patches, surveyors were not able to survey at least 10% of the total patch area, and instead extrapolated the total count from a smaller survey area.

Rank evaluation

This population is composed of 18 subpopulations, all of which occur on federal property. Of these subpopulations, six were visited from 2019 to 2021. An additional eight subpopulations were surveyed in 2016 (Walker 2016). The remaining four subpopulations were last surveyed in 1995. If a partial survey occurred in 2020 that overlapped with surveys from 2016 or 2019, the older surveys were prioritized for determining total counts, as these were more complete. When only surveys from 2016, 2019, and 2020 are considered, the total population is estimated to include over 105,100 individuals, surpassing the minimum of 2,000 individuals required for an A-rank.

Habitat description

Of the 84,475 individuals counted from 2019 to 2021, 45,746 (54%) are from a single patch, characterized by longleaf pine savanna with an understory dominated by *Aristida stricta*, with most individuals clustered in the ecotonal areas between the savanna and an inset patch of pocosin habitat. An additional 26,086 (31%) occur in a mosaic of pocosin and other habitats. Scattered individuals occur in shallow furrows dominated by *Sphagnum* in a matrix of Ericaceous shrubs. Parts of the surveyed area consist of a dry longleaf pine savanna, with exposed sand and shrub oaks. No individuals were found in this habitat, but were instead scattered in the lower, wetter edges of this area.

Co-occurring species with this population include *Aristida stricta*, *Asclepias pedicellata*, *Cyrilla racemiflora*, *Gordonia lasianthus*, *Hypericum* sp., *Lysimachia asperulifolia*, *Osmundastrum cinnamomeum*, *Polygala lutea*, *Pinus palustris*, *P. serotina*, *Pteridium pseudocaudatum*, *Rhexia* spp., *Sphagnum* sp., and *Vaccinium* sp.

Previous population estimates

In 1995, one subpopulation EO was purported to have more than 100,000 individuals. A complete survey of this subpopulation has not been performed since this time. In 2016, an estimate of 10,000 individuals was reported from this subpopulation. In 2019, only 7,091 individuals were estimated from this subpopulation using transect methods. However, none of these three surveys overlapped geographically. This subpopulation is therefore high priority for additional survey. In 2016, botanist Andy Walker visited 12 subpopulations of this EO and visually estimated Venus flytrap population size, totaling 74,200 individuals. The A-rank designation of this population therefore remains unchanged compared to the previous rank assessment.

Threats and Protections

Fire suppression

Regular fires occur at the largest and densest patch surveyed from this population. In another large patch, explosives frequently ignite fires, such that fire may occur at an unusually short return interval. In some locations, surveyors noted that the grass was dense and several years had elapsed since the previous burn. From 2019 to 2021, mild fire suppression was recorded at patches comprising a total of 9,353 plants. Fire suppression is therefore a moderate threat at this population.

Development

This population occurs on land that is protected and managed partially for wildlife and partially for military training exercises. Some populations may experience occasional trampling by vehicles or individuals. Overall, disturbances from development are a low to insignificant threat at this population.

Illegal collection

Access to this population is extremely restricted, and illegal collection is therefore an insignificant threat.

NC262

Surveyors: Hunter Cox, Holly Ferreira, Laura Hamon, Gary Haught, Daniel Hannon, Maggie Hughes, Summer Lauder, Ryan Martin, Libby Seay, Craig Ten Brink

Survey Dates: 2019 – 28-29 May. 2020 – 25-26 June 2020.

Summary

This population occurs in Onslow County, N.C., and comprises an estimated 95,150 individuals. A total of 81,569 individuals were counted from 2019 to 2021 alone. Of this total, 74,945 were estimated using the transect method, and the remaining 6,624 were counted using an absolute count. Of the individuals counted in the present status survey, over 71% were reproductive. This population is comprised of 15 subpopulations, including 12 on federal land, one on state land, one on land overlapping state and private property, and one entirely on private

property. However, over 99% of individuals recorded in this status survey occurred entirely on federal land. Surveyors recorded five previously unknown patches from 2019 to 2021.

Rank evaluation

This population includes a large geographic area, comprising 15 subpopulations occurring over a length of 19 km. There are additional EOs just outside the 2.0 km separation distance from this population with suitable habitat between. Consequently, this population has the potential to be expanded if additional plants are found within the separation distance that would warrant merging with nearby EOs. Of the 15 subpopulations in this population, four have not been surveyed since 2002 or earlier. No plants were found at another subpopulation during its last survey, and another subpopulation is presumed partially extirpated due to development. Approximately 720 plants are found on state land, while only 3 are recorded on private land. The remaining individuals from this population occur entirely on federal land. A single subpopulation on federal land accounts for over 81,200 (85%) of the total count, largely comprising newly recorded patches for this population. Consequently, the EO-rank was upgraded to A in 2020, up from an AB-rank as of 2018.

Habitat description

In the current status survey, the largest subpopulation occurs in a largely open, meadow-like habitat with very few scattered *Pinus palustris* and *P. serotina*. Individuals are densest in the ecotone between this meadow and adjacent pocosin habitat, but Venus flytrap is a prevalent component of the understory throughout this area, with a total of 75,945 estimated in one continuous meadow. Co-occurring species include *Andropogon spp.*, *Aristida stricta*, *Lysimachia asperulifolia*, *Polygala lutea*, and *Rhexia alifanus*. In other parts of this subpopulation, numerous additional individuals occur in a mosaic of pocosin and flatwood. Scattered individuals were

recorded in *Sphagnum*-filled furrows between thick Ericaceous shrubs and *Smilax* sp. in an area that was formerly used for timber.

In 2020, 28 individuals were recorded in a powerline right-of-way intersecting with federal land. Co-occurring species here included *Aristida stricta*, *Hypericum* spp., *Ilex* sp., *Lysimachia asperulifolia*, *Osmundastrum cinnamomeum*, *Polygala lutea*, *Rhexia* sp., and *Xyris* sp.

In one subpopulation on state land, surveyors recorded 264 individuals, many of which occur in *Sphagnum* depressions. Most of these are found in openings amidst a dense shrub understory or bordering trails. Some individuals are found growing in a pine forest with a relatively closed canopy, albeit in an area that had experienced a recent burn. Co-occurring species in this subpopulation include *Drosera* spp., *Pinus taeda*, *Pteridium pseudocaudatum*, *Sarracenia rubra*, *S. purpurea*, *Smilax* sp., and *Vaccinium* spp.

Previous population estimates

Prior to 2018, previous estimates placed this population as having fewer than 5,000 individuals. The present survey visited only four out of the 15 subpopulations in this population, making direct comparisons difficult. Nonetheless, with the addition of approximately 81,200 from a previously unrecorded patch, the current estimate of this population is far greater than previously assumed from Element Occurrence data.

Threats and Protections

Fire suppression

Fire suppression is a moderate threat to this population. Dense shrubs indicating a relatively long amount of time since the previous burn were noted in five patches of this population

comprising approximately 3,800 individuals. Considerable fire suppression was noted as a likely factor impacting one particular patch comprising 197 individuals. A previous survey of this patch by land managers reflected a far more extensive number of plants.

Development

It is assumed that two patches of one subpopulation have recently been lost to development. An additional subpopulation occurs entirely on private land and has not been surveyed since 1998, when three individuals were observed. Patches in powerlines may be vulnerable to the effects of powerline maintenance. However, most of this population is located on federal land that is managed in part for conservation and military use. Development is therefore a low-priority threat to this population.

Illegal Collection

No evidence of illegal collection was observed during this survey. Most of this population occurs on land with extremely limited access, making illegal collection unlikely.

NC256

Surveyors: Justin Bashaw, Hunter Cox, Keleigh Cox, Laurel Davis, Laura Hamon, Daniel Hannon, Alicia Jackson, Janice Johnson, Summer Lauder, Ryan Martin, John McLaughlin, Jack Nolan, Teresa Russell, Libby Seay

Survey Dates: 2019 – 4 May, 6 May, 15 May, 20 May, 12 June, 20 June, 26 June, 16 July, 1 August; 2020 – 20 March, 14 May, 20-21 May, 26-29 May, 1-2 June, 4-5 June, 7-11 June, 16-19

June, 22 June, 14 July, 17 July, 28 July, 31 July, 18-19 August, 22 September, 24-27 September, 1-2 October.

Summary

This population occurs in Brunswick County, N.C., and comprises an estimated 75,106 individuals. From 2019 to 2021, surveyors counted 75,106 individuals, 72,106 of which were counted via absolute methods. This total includes data from June-October 2020 surveys (Dr. J.H. Carter III and Associates, Inc. 2021.), which included the addition of three newly recorded subpopulations (Mason 2021). Of the patches where reproductive rate was recorded, approximately 64% were reproductive. This population is distributed across many different roadsides, managed areas, and private parcels. Of the individuals counted from 2019 to 2021, 46% were on federal land, 32% were in rights-of-way, 9% were on private parcels, 8% were on state land, and 5% were on land owned by a private land conservancy. From 2019 to 2021, more than 50 previously unmapped patches were recorded. Because of the diverse management contexts present, monitoring this population is complex and faces unique challenges.

Rank evaluation

With 48 sub EOs, this population is composed of more subpopulations than any other Principal EO for this species. Out of these subpopulations, 14 are on private property, 11 are on state property, 14 are on federal property, two are on municipal property, and one is on a land trust easement. The remaining subpopulations refer to areas that overlap, including three that overlap state and private property, two that overlap land trust easement and private property, and one that overlaps a private land preserve and private property.

Out of the 14 subpopulations on private land, nine were visited in the present survey, with 15,482 plants located at six subpopulations and no plants found at the remaining three subpopulations. If both the current and previous surveys are considered, then an additional 130-310 plus “many plants” were found on private property at three subpopulations, and no plants were found at an additional two subpopulations. In total, four of the 14 subpopulations had no plants found in the most recent survey, with one additional subpopulation listed as a historical record.

Of the 11 subpopulations entirely on state land, eight were visited from 2019 to 2021, with a total of 3,996 plants recorded. If both the current and previous data are considered then an additional 170 plants have been recorded, totaling 4,166 plants. If subpopulations that overlap state and private property are added, an additional 8,336 brings this total to 12,332 plants. Of the 14 subpopulations on federal land, nine were surveyed between 2016 and 2019 by separate survey teams, with at least 40,000 recorded. Both subpopulations on municipal property were last reported in 2009, with one reported only as present, and the second comprising 400 planted individuals. The subpopulation on a land trust easement was last visited in 1994, with only 10 plants reported compared to thousands of plants in 1987. The subpopulation largely occurring on a private land preserve comprises over 3,100 individuals counted from 2019 to 2021. With over 75,000 individuals, this population handily surpasses the 2,000 individuals required for an A-rank according to BCD rank specifications (Mason 2021).

Habitat Description

Populations on roadsides and rights-of-way make up a large proportion of this population, comprising over 20,000 individuals. Frequently, these patches are adjacent to unpaved roads and grade into longleaf pine or pond pine flatwoods. Co-occurring species are variable, and include *Aletris* spp., *Andropogon* sp., *Aristida stricta*, *Calopogon tuberosus*, *Cleistosiopsis* spp., *Drosera*

sp., *Ilex glabra*, *Leiophyllum buxifolium*, *Lyonia* sp., *Morella cerifera*, *Osmundastrum cinnamomeum*, *Persea borbonia*, *Pinus palustris*, *Pogonia ophioglossoides*, *Polygala lutea*, *Rhynchospora* sp., *Schizachyrium* sp., *Sphagnum* sp., *Spiranthes* sp., and *Utricularia* sp. Roadside patches adjacent to paved roads are often narrow and frequently characterized by moist, shallow ditches co-occurring with *Sphagnum* or mowed, lawn-like conditions.

For the individuals on private land preserve property, plants are generally found in the wetter portions of pine flatwoods, which sometimes grade to sand ridges or thick shrubs. Co-occurring species include *Aristida stricta*, *Gaylussacia* sp., *Hypericum* sp., *Ilex* spp., *Pinus palustris*, *P. serotina*, *Polygala* sp., *Rhexia* sp., *Vaccinium* sp., and *Xyris* sp.

For plants occurring largely on state property, many individuals occur in longleaf or pond pine savanna with *Aristida stricta*, *Andropogon* spp., and low shrubs (Figure 9). Some areas had been recently burned, while others were densely shrubby, making surveying difficult. Where conditions are dry or shrubby, individuals are often found persisting in moist, *Sphagnum*-filled depressions. Other associated species include *Aristida stricta*, *Eriocaulon* sp., *Lachnanthes caroliniana*, *Magnolia virginiana*, *Rhexia alifanus*, *Sarracenia flava*, and *Vaccinium* sp.

Within this population, most plants counted on federal land had received a burn within the last 1-3 years. However, one partially surveyed patch had not been burned since 2005 and the shrub layer was dense in areas.

Patches solely on private lands are found in variable habitats. Some of these patches occur in savanna-like habitat, with wet sandy soil, low shrubs, and co-occurring species including *Aristida stricta*, *Ilex glabra*, *Persea palustris*, *Pinus palustris*, *Polygala lutea*, *Pteridium pseudocaudatum*, *Rhexia* sp., and *Sarracenia* spp. Nearly 3,500 individuals are found in lawn-like

environments in backyards or near golf courses. Over 250 individuals were counted in moist ditches.

Previous Population Estimates

The overall rank of this population remains the same since the previous rank assessment. However, based on the data in the present survey, five subpopulations were downgraded in rank and ten were upgraded in rank. Combining the five subpopulations that were downgraded, at least 900 fewer plants were counted in comparison to previous assessments. This includes one subpopulation that was not found in 2020, whereas a survey in 1992 counted 25 individuals. When examining the subpopulations that were upgraded, at least 46,000 more plants were counted in the present status survey compared to previous surveys. Much of this represents new patches recorded by Alicia Jackson on federal land, necessitating four new subpopulation designations.

Threats

Fire suppression

Fire management is practiced for the parts of this population that are on state, federal, and land-conservancy-owned land. However, fire suppression was noted as a moderate threat at 35 patches comprising 19,363 individuals, and as a high-priority threat at 24 patches comprising 2,532 individuals. Overgrown conditions were often noted as an imminent threat at private properties, where fire management is difficult.

Development

Parts of this population occur on roadsides and private property, making development is a significant threat. Development was noted as a high-priority threat at 28 patches comprising 17,517

individuals. Much of this refers to patches on land that is slated for development and is therefore likely to be extirpated. Development was also noted as a moderate threat at approximately 20 patches comprising nearly 4,000 individuals. Road maintenance poses an additional threat to roadside patches in this population. Conversely, at least 4,000 individuals occur on land that is protected as a Dedicated Nature Preserve. Most of the population surveyed on federal land occurs on land that is designated as a Registered Heritage Area. This refers to a voluntary agreement between DNCR and the landowner that signifies the landowner's commitment to protecting the natural heritage values of the property.

Illegal collection

Poaching is frequently a moderate- to high-priority threat in this population. Evidence of illegal collection was recorded at two patches, with an estimated loss of more than 100 individuals. Over 13,000 individuals were noted as being areas with high apparency, such as roadsides, which may increase the risk of illegal collection. At three large, roadside sites, surveyors were stopped by locals or law enforcement who suspected illegal collection.

Other

Changes in hydrology are noted as a potential issue at several patches, with some sites being too dry to support Venus flytrap. This may be due in part to drought conditions in the southeastern U.S. in 2019 (National Drought Mitigation Center; U.S. Department of Agriculture; National Oceanic and Atmospheric Administration 2019). Other patches are adjacent to a lake that has had dramatic changes in water level in the past several years, potentially altering hydrology in the region.

The use of herbicide is also noted as a potential threat for patches near powerlines, golf courses, and residential sites. Herbicide use is noted at nine patches comprising 5,723 individuals. Mowing during the reproductive period may also impact seed set. Reproductive season mowing was recorded at five patches comprising over 500 individuals.

NC237

Surveyors: Misty Buchanan, Laura Hamon, Daniel Hannon, Summer Lauder, Ryan Martin, John McLaughlin, Libby Seay, Nathan Shepard, Andy Walker

Survey Date: 2019 – 13 May, 22 May, 6-7 June, 13 June, 17 June. 2020 – 3 June, 9 June, 20-21 July.

Summary

This population occurs in Carteret County, N.C., and includes an estimated 55,264 individuals based solely on 2019 and 2020 data, with 970 plants occurring on land owned by a land trust, 865 plants occurring on state land, and the remaining plants occurring on federal land. Of the individuals counted in this status survey, over 74% were reproductive. Surveyors recorded 12 patches that were previously unmapped. When accounting for older data in patches not covered by this survey, an additional 3,000 individuals are estimated, with a total of over 55,000 individuals. Almost the entirety of this population occurs on land managed in part for recreation, biodiversity, and timber products.

Rank Evaluation

This population is comprised of 26 subpopulations. Of these subpopulations, 20 occur on federal land, one occurs on state land, two occur on private property, one occurs on both municipal and private property, one occurs on both federal and private property, and one occurs on both federal and land trust property. If only recent data are considered, this population has over 48,300 individuals, easily exceeding the minimum count required for an A-ranking according to the BCD rank criteria.

Habitat Description

Much of this population is found densely clustered in the zones between shrubby bay rims and flatwood or savanna, particularly for those subpopulations federal land. Over 21,000 individuals occur in expressly bay rim habitat. Thousands of additional individuals occur in savanna or flatwood habitat, with a mosaic of predominant herb or shrub layer. Co-occurring species include *Aristida stricta*, *Arundinaria gigantea*, *Ilex spp.*, *Morella cerifera*, *Pinus palustris*, *P. serotina*, *Pteridium pseudocaudatum*, *Sabatia difformis*, *Sarracenia flava*, and *Spiranthes eatonii*. In multiple patches, relatively dry, scrubby flatwoods with few Venus flytrap grade into wetter, high quality habitat.

Moist, *Sphagnum*-filled furrows or ditches within savanna or shrubs is another prevalent habitat type. Here, individuals are often densely clustered. Over 12,500 individuals occur in habitat where these ditches are a major component, including all 970 individuals counted on land trust property.

Previous Population Estimates

The overall rank of this population remains the same since the previous rank assessment. However, based on the data in the current survey, four subpopulations were downgraded in rank and three subpopulations were upgraded in rank since their previous assessment. Of the four subpopulations that were downgraded, over 4,400 fewer plants were counted than in previous surveys, likely in part due to drought conditions and mild fire suppression. Of the three subpopulations that were upgraded, surveyors counted an additional 5,137 compared to previous assessments, likely due to the thoroughness of the present survey.

Threats

Fire suppression

Much of this population is intermittently burned. Controlled burning could not occur on federal property in 2019, and therefore some areas were becoming somewhat overgrown at the time of this survey. Moderate fire suppression was noted at 12 patches comprising nearly 8,700 individuals. Shrubs are particularly dense in the parts of the population on state property.

Development

In three patches, surveyors noted that plants may be vulnerable to trail traffic or maintenance, comprising over 3,200 individuals. Overall, development is a low risk to this population.

Illegal Collection

Some parts of this population are close to trails or roads, potentially increasing the risk of illegal collection.

Other

Illegal vehicle off-roading has been noted as a threat to habitat quality at this site. Tire tracks from All Terrain Vehicles were observed running directly through several populations, and trash dumping was observed near some major patches. Bedding for forestry was also noted as a possible threat in some patches.

NC272

Surveyors: Laura Hamon, Summer Lauder, John McLaughlin, Libby Seay, Eric Ungberg, Cason Verhine

Survey Date: 2019 – 14 May, 12 June, 15 June.

Summary

This population occurs in Pender and Onslow Counties in N.C., and includes an estimated 20,800 individuals, of which 18,869 individuals were counted in 2019. Most of these individuals – over 17,400 – were counted on property owned by a private land conservancy. The remaining individuals were counted on state property. This population largely occurs on land managed for biodiversity conservation.

Rank Evaluation

Overall, this population is comprised of 17 subpopulations, with six on state property, three on a private land preserve, three overlapping state and land conservancy property, and five entirely on private property. There are additional EOs just outside the separation distance from this population, with suitable habitat between. Consequently, this population could potentially be

expanded if additional plants are found. Of the 17 subpopulations, ten have been visited in recent years, since 2017. No plants were found at four of these subpopulations. The remaining subpopulations have not been visited since 2002. A single subpopulation accounts for over 17,400 of the individuals in this population. Other individuals were found largely on subpar habitat in powerline corridors, roadsides, and fire-suppressed savannas, with approximately 1,000 found on state property and over 2,000 on private land.

Habitat Description

The largest and densest patch in this population occurs in a diverse meadow with sparse pines, dense grasses, and *Sarracenia* spp. Other parts of this subpopulation are observed in wet, recently burned longleaf and pond pine savanna. Scattered individuals occur in fire-suppressed habitat, in a matrix of dense grasses and thick ericaceous shrubs. Associated species include *Calopogon* spp., *Hypericum* spp., *Osmunda regalis*, *Osmundastrum cinnamomeum*, *Pogonia* spp., and *Pteridium pseudocaudatum*.

For subpopulations on state property, plants occur in *Sphagnum* depressions in a powerline, in pine savanna with sparse tree cover, and in an open meadow with a very dense herb layer. Co-occurring species include *Andropogon* spp., *Arundinaria gigantea*, *Aristida stricta*, *Cyrilla racemiflora*, *Hypericum* spp., *Pinus serotina*, *Rhexia alifanus*, *Sabatia difformis*, and *Sarracenia* spp.

Previous Surveys

The overall rank of this population remains the same since the previous rank assessment. One subpopulation was upranked from CD to B, since approximately 700 more plants were counted in 2019. Another population was downranked from BC to D based on the present survey. At one subpopulation, no plants were found compared to the previous 2007 survey, when 18 plants

were counted. Another subpopulation was reranked as “Historical” from “Failed-to-Find” based on the number of times no plants have been found at this subpopulation.

Threats

Fire suppression

Though most of this population represents a subpopulation that is burned on an interval of 2-4 years, significant fire suppression was noted in at least three patches comprising just four individuals. Some of these patches were previously noted to contain many more individuals.

Development

Plants on or near roadsides may be vulnerable to the effects of foot or vehicle traffic. Over 5,800 plants were noted as occurring near roads or trails. The present survey did not include subpopulations on private property, and more survey work is needed to assess the development pressure to these sites.

Illegal Collection

High traffic areas may be more vulnerable to illegal collection. Significant amounts of trash were noted at one site. Another site occurred close to residential area. No evidence of poaching was observed, however. Overall, illegal collection appears to be a low-priority threat to this population.

Other

Plants in powerlines may be vulnerable to powerline maintenance. There were 284 individuals counted in powerline rights-of-way.

NC266

Surveyors: Daniel Hannon, Ryan Martin

Survey Date: 2019 – 23 May, 24 June. 2020 – 13 July.

Summary

This population occurs in Pender County, N.C., and includes an estimated 18,700 individuals. From 2019 to 2021, 14,152 individuals were estimated using the transect method and 4,610 were counted. Approximately 52% of individuals were reproductive during this survey. Surveyors mapped two previously unrecorded patches. This population occurs entirely on state property that is managed for wildlife and recreation.

Rank Evaluation

This population is comprised of six subpopulations, all of which are located entirely on state property. Out of these subpopulations, three were visited from 2019 to 2021 and three were last visited in 1995, 2002, or 2017. Even when combining older data, almost 19,000 plants have been recorded in this population. One subpopulation contains over 15,300 plants.

Habitat Description

The largest patch in this population, containing an estimated 14,152 individuals, occurs in a powerline right-of-way grading into savanna with interspersed flatwood and pocosin habitat. Co-occurring individuals in this patch include *Aristida stricta*, *Asclepias* sp., *Carphephorus* sp., *Osmundastrum* sp., *Pinus palustris*, *P. serotina*, *Polygala brevifolia*, *P. lutea*, *Pteridium aquilinum*, *Rhexia* spp., *Sarracenia purpurea*, and *Sarracenia flava*. An additional 1,191 individuals were also found in other right-of-way areas.

Savanna habitat is the predominant habitat type observed in three patches containing 2,973 individuals. Most of these individuals occur in very dense mats alongside *Drosera* spp., *Rhynchospora* spp., and *Sarracenia flava*.

Previous Surveys

The overall rank of this population remains the same since the previous rank assessment. Notably, one subpopulation was upgraded from D to A, when over 15,300 individuals were counted in a subpopulation previously reported to have fewer than 100 individuals. If subpopulations that were not visited in the present survey are considered, only 276 individuals were not considered from 2019 to 2021.

Threats

Fire Suppression

Dense grasses are observed in two patches containing 446 individuals, suggesting a need for fire.

Development

One small patch is adjacent to a road and may be vulnerable to any road maintenance or traffic.

Illegal Collection

The largest patch in this population is accessible and visually apparent, which may increase the risk of illegal collection, making this a low- to moderate-priority threat.

SC042

Surveyors: Laura Hamon, Summer Lauder, John McLaughlin, Libby Seay

Survey Date: 2019 – 9 May, 17 May, 25 May, 27 June.

Summary

This population occurs in Horry County, S.C., and includes at least 5,622 individuals, all of which were counted using absolute methods. Nearly all of this population occurs on state-owned property that is managed for biodiversity and recreation. Some patches occur outside state property, but none of these were surveyed from 2019 to 2021.

Rank Evaluation

With over 2,000 plants occurring within 2km of each other, this easily qualifies as an A-ranked population under BCD rank specifications employed by NCNHP.

Habitat Description

Bay rims are a prevalent component of this population. Thousands of individuals occur in extremely dense clusters in moist furrows with *Sphagnum* moss within savanna or flatwood habitat. Over 1,700 individuals grow in an old roadbed. Co-occurring species include *Magnolia virginiana*, *Pinus palustris*, and *Pteridium pseudocaudatum*.

Previous Surveys

The present survey presents a more optimistic total population estimate compared to a previous recent survey, which estimated over 2,000 individuals (James Luken, Coastal Carolina University, pers. comm.).

Threats

Fire Suppression

Overgrown conditions are observed at seven historical patches. Individuals are unlikely in these areas. However, this population experiences regular prescribed fire, and most extant patches had been recently burned. Fire suppression is a low-priority threat to this population.

Development

Ongoing residential development is prevalent outside of this state property. Consequently, if this population includes patches outside on private land, development poses a moderate to high risk. Populations adjacent to trails and roads may also be vulnerable to road maintenance.

Illegal Collection

At least one of the patches in this population is locally known as a destination to view Venus flytrap, which potentially increases the risk of illegal collection. In addition, thousands of individuals are highly visually apparent from the road. To deter this risk, site managers have installed game cameras. Illegal collection is therefore a moderate threat to this population.

Other

At five historical patches, conditions appeared too dry to support Venus flytrap.

NC200

Surveyors: Daniel Hannon, Gary Haught, Ryan Martin

Survey Date: 2020 – 26 June.

Summary

This population occurs in Onslow County, N.C. and has an estimate of at least 3,000 individuals all of which are on federal land that is managed for wildlife and military training exercises. In 2020, surveyors counted 338 individuals, approximately 48% of which were reproductive.

Rank Evaluation

The A-rank of this population is based on a more complete survey from 2016, when 3,000 individuals were counted.

Habitat Description

In 2020, surveyors described this population as resembling sandhill-like habitat grading into pocosin. Co-occurring species include *Andropogon* sp., *Aristida stricta*, *Cyrilla racemiflora*, *Ilex* spp., *Kalmia* sp., *Pinus palustris*, and *Rhexia* sp.

Previous Surveys

This population has been surveyed only three times, with the most complete survey occurring in 2016. The first survey of this population estimated only 50 individuals.

Threats

Fire Suppression

Moderate fire suppression was noted at this population, pointing to the need for prescribed fire.

Development

This habit is not at risk of development.

Illegal Collection

Access to this population is very restricted, making illegal collection unlikely.

NC287

Surveyors: Cheryl Knepp, Laura Hamon, Daniel Hannon, Ryan Martin, Melissa Miller, Andy Wood

Survey Dates: 2019 - 30 July, 2020 - 1 July.

Summary

This population occurs in Pender and New Hanover Counties, N.C., and has an estimated total of 2,593 individuals. From 2019 to 2021, 1,507 of these individuals were counted via an absolute count, with approximately 51% of these individuals reproductive. In the present survey, 94% of individuals were counted on private property and 6% were counted on state property. Surveyors recorded five previously unmapped patches in this population.

Rank Evaluation

This Principal EO includes seven sub EOs, five of which are on private property, one of which is on state property, and one of which partially overlaps state property and private property. The A-rank of this population is largely driven by two subpopulations that had 1,409 and 1,000 plants in 2019 and 2016, respectively, and were both located on private land. Two additional subpopulations have been extirpated due to development. Only 184 plants have been recently reported from the patches on state land. Remaining three patches on private land have not been surveyed since 1996, and two of these patches have likely been impacted by development.

Therefore, although this population meets the minimum number of individuals required for an A-rank, that status is not entirely secure.

Habitat Description

In 2019, only 84 individuals were counted on state property, in a small opening in a young loblolly forest. The *Aristida stricta* layer was thicker than in 2014, when this patch was last surveyed. Other co-occurring species include *Sarracenia flava* and *Vaccinium* sp. Surveyors unsuccessfully searched for individuals in a relatively dry patch dominated by *Arundinaria gigantea*, which previously was reported to have individuals.

In 2020, surveyors counted 1,423 individuals on private property, 711 of which occur in a gas line right-of-way. Co-occurring species in this patch include *Ilex* spp., *Magnolia virginiana*, *Pinus palustris*, *P. serotina*, *Polygala* spp., *Rhexia* sp., and *Sphagnum* sp. An additional 581 individuals occur in the ecotone between pine savanna habitat and adjacent pocosin. Finally, 38 individuals occur in a lawn-like habitat.

Previous Surveys

The rank of this population increased compared to the previous rank assessment, largely due to the 1,423 individuals counted on private property. Notably, the current survey excludes a private population last reported to have at least 1,000 individuals in 2016. A reassessment should be considered when all private patches have been surveyed.

Threats

Fire Suppression

Lack of fire was noted in seven patches surveyed from 2019 to 2021. However, four of these patches experience intermittent mowing, which may mimic some of the benefits of fire. Fire may be particularly difficult on private lands. Fire suppression is therefore a moderate to high threat to this population.

Development

In 2016, a large patch on private property occurred adjacent to land under development. Several other patches appear to have been extirpated due to development within the last 20 years. Therefore, development is a high-priority threat to this population. In the patch observed on state land in 2019, areas were compacted due to recent foot traffic.

Illegal Collection

Trash and informal trails were observed at the patch on state property in 2019. These plants may be vulnerable to illegal collection by passersby, though this appears to be a low-to-moderate threat overall.

Population 298

Summary

This population occurs in Brunswick County, N.C., and was not surveyed under the scope of this survey. Rather, this site was last visited in 2018 (Kunz) and is estimated to have approximately 2,000 plants based on surveys from both 2018 and 2015. The bulk of these

individuals occur in a relatively broad strip of habitat adjacent to a state highway, with additional plants occurring on private land between a field or meadow and denser pocosin.

Rank Evaluation

This population has approximately 2,000 individuals, meeting the minimum requirement necessary for an A rank under BCD rank specs.

Habitat description

The largest patch of this population occurs in an open roadside depression (Figure 9)

Previous population estimates

Only one survey of this population occurred prior to 2015. In 2002, surveyors estimated 1,000-2,000 plants in this population, which was confirmed in 2015.

Threats and Protections

Fire suppression

It is unlikely that prescribed fire is a prospective management tool at this population. Instead, mowing or lack of grass encroachment in wet, ditch-like conditions may serve as a proxy for the effects of fire. Without additional information, it is likely that fire suppression is a threat at this site.

Development

The effects of road maintenance and pollution may pose a threat to this population. Heavy machinery can trample plants, while runoff and flooding can degrade habitat quality. Development and other forms of habitat conversion may therefore pose a threat. Whether these factors are a

major threat in this specific case is unclear, but this population regardless should be a high priority for ongoing monitoring.

Illegal collection

Roadside populations have high visibility to passersby, particularly during flowering. Therefore, these populations may have an elevated risk of illegal collection, making this a moderate threat at this population.

CONCLUSION

The present survey represents the most comprehensive survey of Venus flytrap populations in nearly 20 years. Prior to this survey, the most recent population estimate for wild Venus flytrap by NCNHP was 73,000-158,000 individuals across 11 N.C. counties and one S.C. county (NatureServe 2021). Survey efforts from 2019 and 2020 reveal an estimate of over 800,000 individuals in the wild.

Although our population estimate provides a more optimistic outlook for the species compared to previous estimates, it is worth noting that a small number of very large populations comprise the bulk of this count. Of the 69 extant populations, only 25 rank as “Fair” or better (500 or more individuals). The remaining populations have fewer than 500 individuals and are at risk of stochastic change. Upon reassessment, 14 populations were downgraded in rank due a smaller population estimate, representing a potential loss of approximately 1160 individuals based on previous estimates. During the duration of this survey, surveyors also noted evidence of fire suppression, active development, illegal collection, and hydrological change. Individuals near residential areas, in rights-of-way, or in unburned habitat were frequently at higher risk of extirpation.

Future efforts should focus on the viability of small and disjunct Venus flytrap populations. In addition, the effects of management for Venus flytrap on the extremely rare moth *Hemipachnobia subporphyrea* should be investigated.

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Table 4.1. Summary of results from 2019 and 2020 survey.

Principa l EO	County	State	Absolute Count	Estimate	Total	Reproductive (%)	Federal (%)	State (%)	Private conservancy (%)	Private (%)	ROW (%)
NC263	Brunswick	N.C.	0	256,070	256,070	NA	0	0	100	0	0
NC286	Pender	N.C.	217,789	3,822	221,611	36	0	95	4	0	0
NC262	Onslow	N.C.	6,624	78,251	84,875	71	100	0	0	0	0
NC264	Onslow	N.C.	19,667	52,837	72,504	71	100	0	0	0	0
NC256	Brunswick	N.C.	60,287	3,000	63,287	64	46	8	5	9	32
NC237	Carteret	N.C.	52,044	3,220	55,264	74	96	2	2	0	0
NC272	Pender, Onslow	N.C.	18,869	0	18,869	76	0	7	93	0	0
NC266	Pender	N.C.	4,610	14,152	18,762	52	0	100	0	0	0
SC042	Horry	S.C.	5,622	0	5,622	65	0	100	0	0	0
NC287	New Hanover	N.C.	1,507	0	1,507	51	0	6	0	94	0
NC027	Brunswick	N.C.	11,89	0	1,189	46	0	100	0	0	0
NC326	Brunswick	N.C.	817	0	817	69	0	0	0	0	100
NC291	Brunswick	N.C.	645	0	645	0	0	0	100	0	0
NC251	Onslow	N.C.	525	0	525	24	82	0	18	0	0
NC242	Brunswick	N.C.	508	0	508	67	0	0	0	100	0
NC200	Onslow	N.C.	338	0	338	48	100	0	0	0	0

Table 4.1. (continued)

NC265	Pender	N.C.	270	0	270	26	0	0	100	0	0
NC275	Brunswick	N.C.	219	0	219	35	0	100	0	0	0
NC028	Brunswick	N.C.	116	0	116	45	0	0	0	0	100
NC274	Columbus	N.C.	39	0	39	0	0	100	0	0	0
NC172	Duplin	N.C.	18	0	18	33	0	0	100	0	0
NC46	Pender	N.C.	3	0	3	100	0	0	100	0	0
SC080	Horry	S.C.	3	0	3	NA	0	100	0	0	0
NC219	Columbus	N.C.	2	0	2	100	0	100	0	0	0
NC362	Duplin	N.C.	1	0	1	100	0	0	0	0	100
NC206	Onslow	N.C.	0	0	0	0	0	0	0	0	0

Table 4.2. Summary of population estimates for all populations in North Carolina and South Carolina, including data from previous NCNHP records.

A= “Excellent,” B= “Good,” C= “Fair,” D= “Poor,” E = “Extant,” F = “Failed to Find,” H = “Historical,” i = “Introduced,” r = “R”

EO Number	County	State	Most recent survey year	Rank 2018	Rank 2021	Minimum population estimate	Land Protection Status
NC263	Brunswick	N.C.	2019	A	A	257951	dedicated nature preserve, private land conservancy
NC286	Pender	N.C.	2020	A	A	221611	dedicated nature preserve, easement, game land, private land conservancy
NC264	Onslow	N.C.	2020	A	A	105100	military installation
NC262	Onslow	N.C.	2020	AB	A	95150	easement, game land, military installation, powerline easement
NC256	Brunswick	N.C.	2020	A	A	75106	dedicated nature preserve, easement, local government, military installation, powerline easement, private individual, private land conservancy, registered heritage area, state
NC237	Carteret	N.C.	2020	A	A	55264	dedicated nature preserve, easement, game land, registered heritage area, state forest, U.S. forest
NC272	Onslow, Pender	N.C.	2020	A	A	20800	dedicated nature preserve, easement, other protection, powerline, private land conservancy, state

Table 4.2. (continued)

NC266	Pender	N.C.	2020	A	A	19000	dedicated nature preserve, easement, game land, powerline
SC042	Horry	S.C.	2019	?	A	5622	heritage preserve
NC200	Onslow	N.C.	2020	A	A	3000	military installation
NC287	New Hanover, Pender	N.C.	2020	BC	A	2593	mitigation site, private individual, state
NC298	Brunswick	N.C.	2018	A	A	2040	private individual, roadside
NC192	New Hanover	N.C.	2002	B	B	1800	private individual
NC265	Pender	N.C.	2019	B?	B?	1270	private individual, private land conservancy
NC167	Hoke	N.C.	1994	B	B	1100	dedicated nature preserve, game land, private individual
NC027	Brunswick	N.C.	2020	D?	B	1100	military installation
NC033	New Hanover	N.C.	2018	D	B	1012	registered heritage area, state park
NC291	Brunswick	N.C.	2020	C	C	885	dedicated nature preserve, game land, private land conservancy
NC326	Brunswick	N.C.	2020	D	C	817	private individual roadside
NC295	Hoke	N.C.	2018	B	C	770	military installation
NC242	Brunswick	N.C.	2020	BC	BC	762	private individual, roadside
NC251	Onslow, Pender	N.C.	2020	D	C	734	easement, military installation, private land conservancy
NC119	Hoke	N.C.	1992	C	C	600	military installation
NC029	Brunswick	N.C.	2018	BC	C	500	roadside
NC135	Hoke	N.C.	2009	CD	CD	500	military installation
NC288	New Hanover	N.C.	2014	D	Dr	400	easement, other protection
NC190	Onslow	N.C.	2016	D	D	334	military installation
NC275	Brunswick	N.C.	2019	C	CD	319	dedicated nature preserve, game land, private land conservancy
NC056	Sampson	N.C.	2017	CD	D	300	private land conservancy, state

Table 4.2. (continued)

NC166	Hoke	N.C.	1994	D	D	265	military installation
NC132	Cumberland	N.C.	1992	D	D	200	military installation
NC104	Pender	N.C.	1993	F	D	200	military installation, game land
NC301	Onslow	N.C.	2008	D	D	200	private individual
NC189	Onslow	N.C.	2016	D	D	200	military installation
SC081	Horry	S.C.	2021	NA	D	197	roadside
SC029	Horry	S.C.	2021	H	D	146	conservation easement
NC171	Bladen	N.C.	2018	D	D	130	private individual
NC028	Brunswick	N.C.	2020	D	D	116	roadside
NC165	Cumberland, Hoke	N.C.	2010	D	D	112	military installation
NC290	Columbus	N.C.	2002	D	CD	110	private individual
NC218	Brunswick	N.C.	2002	D	D	100	local government, private individual
NC255	Brunswick	N.C.	2018	D	D	100	powerline corridor
NC038	Carteret	N.C.	2014	D	D	99	U.S. forest
NC363	Onslow	N.C.	2020	?	D	75	private individual
NC243	Brunswick	N.C.	2004	D	D	50	private individual
NC213	Brunswick	N.C.	2018	CD	D	50	powerline easement, private individual, registered heritage area
NC245	Bladen	N.C.	2015	CD	D	45	powerline easement, private individual
NC365	Pender	N.C.	2019	?	D	40	private individual
NC274	Columbus	N.C.	2019	BC	D	39	private individual, private land conservancy, registered heritage area
NC219	Columbus	N.C.	2021	C?	D	27	state park
NC279	Brunswick	N.C.	1995	D	D	25	private individual, registered heritage area
NC224	Pender	N.C.	2002	D	D	25	powerline corridor, private individual
NC315	Jones, Craven	N.C.	2017	D	D	25	game land, registered heritage area, U.S. forest

Table 4.2. (continued)

NC206	Onslow	N.C.	2020	C	D	24	local government, powerline corridor, private individual, roadside
NC246	Cumberland	N.C.	1996	D	D	20	military installation
NC289	Brunswick	N.C.	2002	D	D	20	private individual, transmission line corridor
NC220	Brunswick	N.C.	2002	D	D	19	powerline corridor
NC172	Bladen	N.C.	2020	D	D	18	private individual
NC285	Onslow	N.C.	2002	D	D	10	private individual, transmission line corridor
NC014	Bladen	N.C.	2017	D	D	10	registered heritage area, state park
NC136	Hoke	N.C.	2014	D	D	8	military installation
NC292	Brunswick	N.C.	2002	D	D?	8	private individual
NC232	Bladen	N.C.	2017	D	D	3	state forest
NC046	Pender	N.C.	2019	D	D	3	dedicated nature preserve, private land conservancy
SC080	Horry	S.C.	2019	?	D	3	heritage preserve
NC332	New Hanover	N.C.	2017	D	D	2	local government
NC116	New Hanover	N.C.	2002	D	D	1	public university
NC115	Brunswick	N.C.	2002	D	D	1	private individual
NC362	Bladen	N.C.	2020	?	Dr?	1	private individual
NC134	Hoke	N.C.	2014	D	F	0	military installation
NC055	Bladen	N.C.	1992	F	F	0	private individual, roadside
NC048	Onslow	N.C.	2002	F	F	0	roadside
NC062	Beaufort	N.C.	1992	F	F	0	private individual
NC018	Brunswick	N.C.	2002	F	F	0	private individual
NC080	Bladen	N.C.	2002	F	F	0	dedicated nature preserve, easement, game land
NC096	Pender	N.C.	1992	F	F	0	private individual
NC084	Bladen	N.C.	2002	F	F	0	state forest
NC098	Pender	N.C.	2002	F	F	0	federal, registered heritage area
NC194	Bladen	N.C.	2001	F	F	0	private individual

Table 4.2. (continued)

NC006	Columbus	N.C.	2002	F	F	0	private individual, registered heritage area
NC284	Beaufort, Pamlico	N.C.	1992	F	F	0	private individual, roadside
NC185	Columbus	N.C.	2002	F	F	0	private individual
NC005	Craven	N.C.	2004	F	F	0	powerline easement, registered heritage area, U.S. forest
NC162	Pender	N.C.	2015	F	F	0	powerline corridor
NC181	Brunswick	N.C.	2002	F	F	0	private individual
NC193	New Hanover	N.C.	2002	F	F	0	private individual
NC293	Bladen	N.C.	2002	F	F	0	roadside
NC294	Cumberland	N.C.	2002	F	F	0	dedicated nature preserve
NC083	Brunswick	N.C.	2002	F	F	0	dedicated nature preserve, easement, private individual
NC205	Duplin	N.C.	2002	F	F	0	private individual
NC163	Sampson	N.C.	2006	F	F	0	easement, private individual
NC244	Brunswick	N.C.	2018	D	F	0	private individual
NC258	Sampson	N.C.	2015	F	F	0	private individual, roadside
NC299	Brunswick	N.C.	2019	D	F	0	dedicated nature preserve, easement, private individual
NC310	Cumberland	N.C.	2014	D	F	0	military installation
SC002	Charleston	S.C.	1938	?	F	0	private individual
SC030	Horry	S.C.	1992	?	F	0	powerline corridor, private individual
NC019	Bladen	N.C.	2002	F	Fi	0	dedicated nature preserve, state park
NC024	Bladen	N.C.	1991	H	H	0	private individual
NC036	Lenoir	N.C.	1939	H	H	0	private individual
NC032	Brunswick	N.C.	1941	H	H	0	private individual
NC120	Craven	N.C.	1949	H	H	0	private individual, roadside
NC071	Robeson	N.C.	1957	H	H	0	private individual
NC313	Jones	N.C.	1958	H	H	0	private individual
NC068	New Hanover	N.C.	1992	H	H	0	private individual

Table 4.2. (continued)

NC001	Cumberland	N.C.	1974	H	H	0	easement, private individual
NC043	Columbus	N.C.	1981	H	H	0	private individual
NC045	New Hanover	N.C.	2002	H	H	0	private individual, roadside
SC003	Charleston	S.C.	1901	?	H	0	U.S. forest
SC007	Horry	S.C.	1941	?	H	0	private individual
SC008	Horry	S.C.	1974	?	H	0	private individual
SC011	Horry	S.C.	1976	?	H	0	private individual
SC021	Georgetown	S.C.	1941	?	H	0	private individual, private land conservancy
NC035	Onslow	N.C.	2002	H?	H?	0	private individual
NC085	New Hanover	N.C.	1963	H	Hr	0	private individual
NC031	Onslow	N.C.	1981	X	X	0	private individual
NC042	Columbus	N.C.	1991	X	X	0	private individual, roadside
NC073	Pender	N.C.	1981	X	X	0	private individual
NC066	Moore	N.C.	1991	X	X	0	private individual
NC074	Pender	N.C.	1997	X	X	0	private individual
NC054	Pender	N.C.	1992	X	X	0	private individual, roadside
NC058	Duplin	N.C.	2006	X	X	0	private individual
NC060	Duplin	N.C.	2006	X	X	0	private individual
NC072	Bladen	N.C.	1992	X	X	0	private individual, roadside
NC067	Onslow	N.C.	1992	X	X	0	private individual
NC016	Bladen	N.C.	1992	X	X	0	roadside
NC059	Duplin	N.C.	2006	X	X	0	private individual, roadside
NC039	Columbus	N.C.	1991	X	X	0	private individual
NC069	New Hanover	N.C.	1981	X	X	0	private individual
NC077	Brunswick	N.C.	1991	X	X	0	private individual
NC041	Columbus	N.C.	1991	X	X	0	private individual, roadside
NC009	Brunswick	N.C.	2002	X	X	0	private individual

Table 4.2. (continued)

NC131	Brunswick	N.C.	2002	X?	X	0	private individual
NC174	Columbus	N.C.	2002	F	X	0	private individual, roadside
NC020	Bladen	N.C.	1992	X	X	0	private individual, roadside



Figure 4.1. A reproductive individual. A reproductive individual demonstrating the vertical separation between flowers and traps.

Photo by Clyde Sorenson.



Figure 4.2. Roadside effects. Tire tracks mark where vehicles impacted a roadside population in Brunswick County. This population had 116 individuals in 2019, compared to a 2018 estimate of approximately 1,000 individuals. It is unclear whether the damage was accidental.

Photo by Laura Hamon.

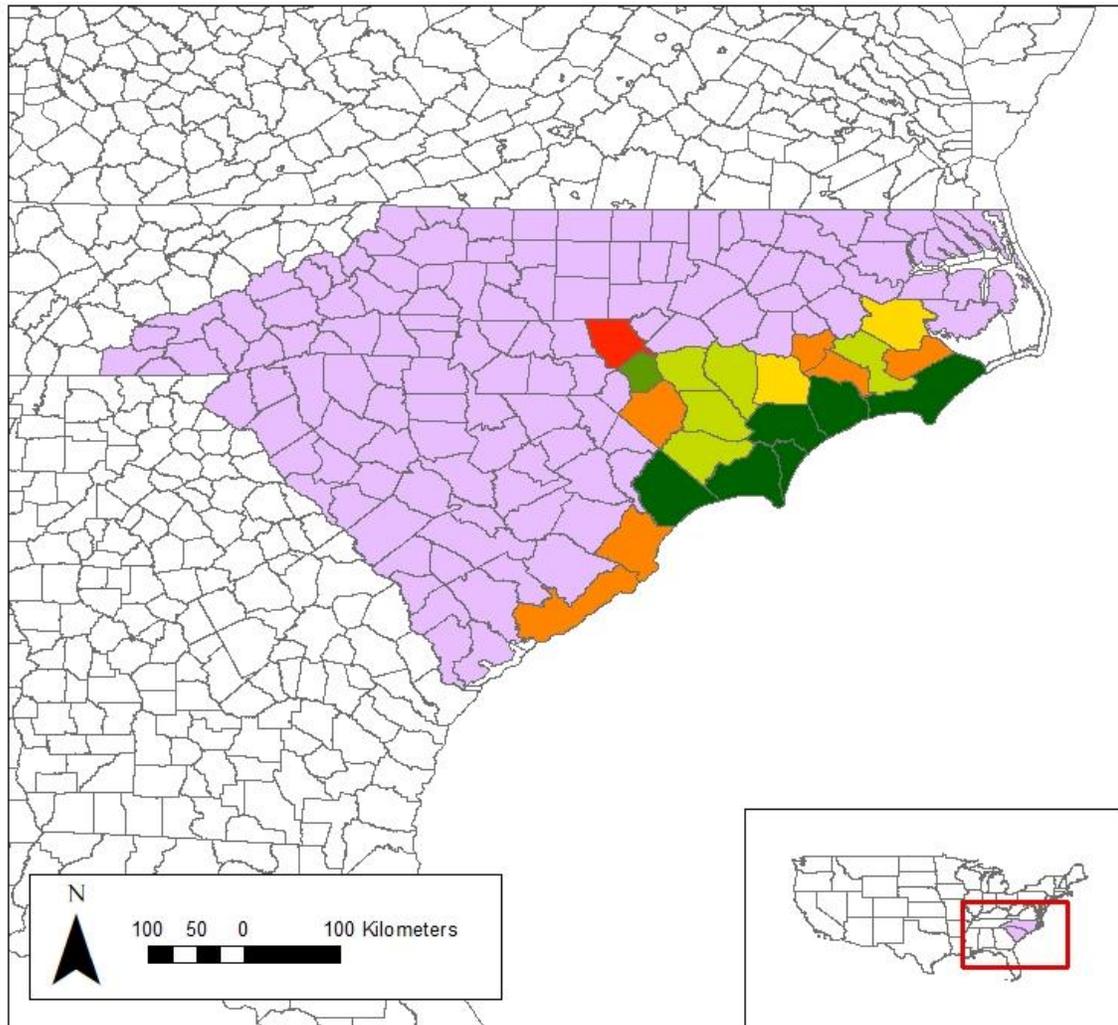


Figure 4.3. A map of *Dionea muscipula* county occurrences in North Carolina and South Carolina. States where Venus flytrap natively occurs are purple. Counties are colored by the highest-ranking population present in each as follows; A (Excellent) = dark green, B (Good) = green, C (Fair) = light green, F (Failed to Find) = yellow, H (Historic) = orange, X (Extirpated) = red. Map compiled using ArcGIS Version 10.6.1. County shapefile obtained from the US Census Bureau.

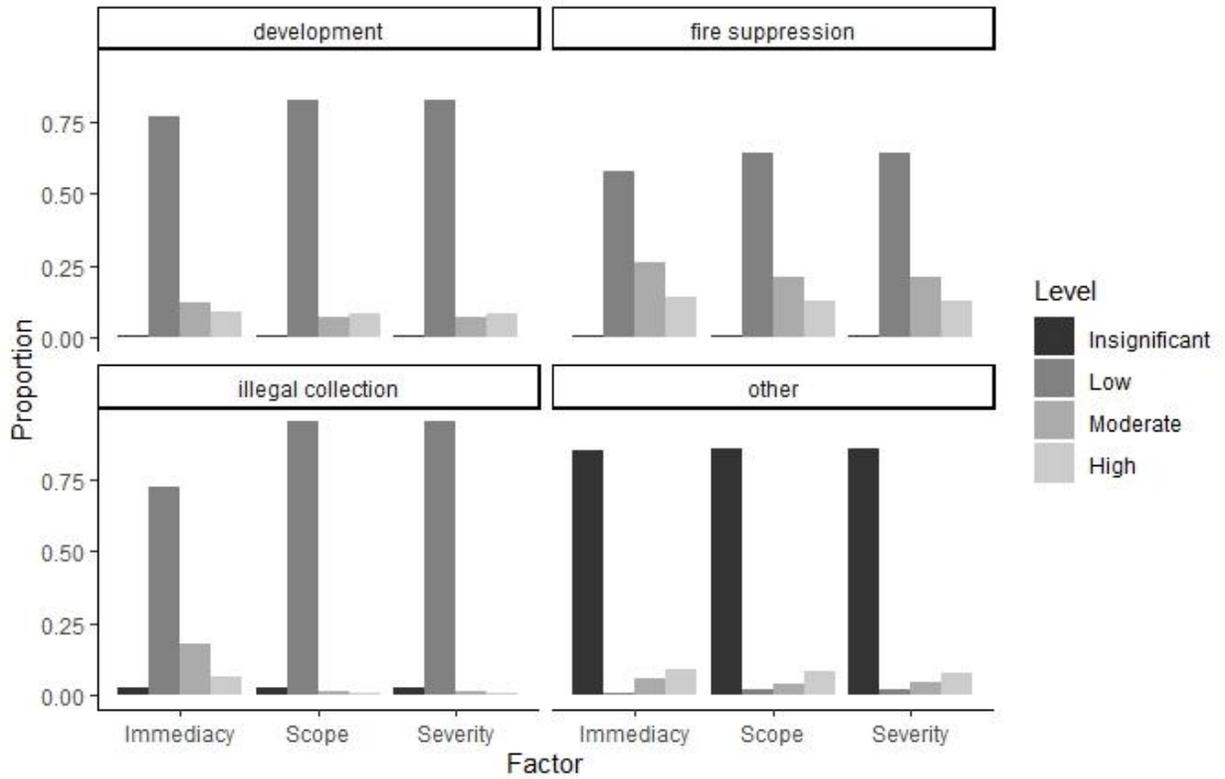


Figure 4.4. Proportion of patches of Venus flytrap surveyed by core NCNHP survey team ranked by the severity, scope, and immediacy of threats.

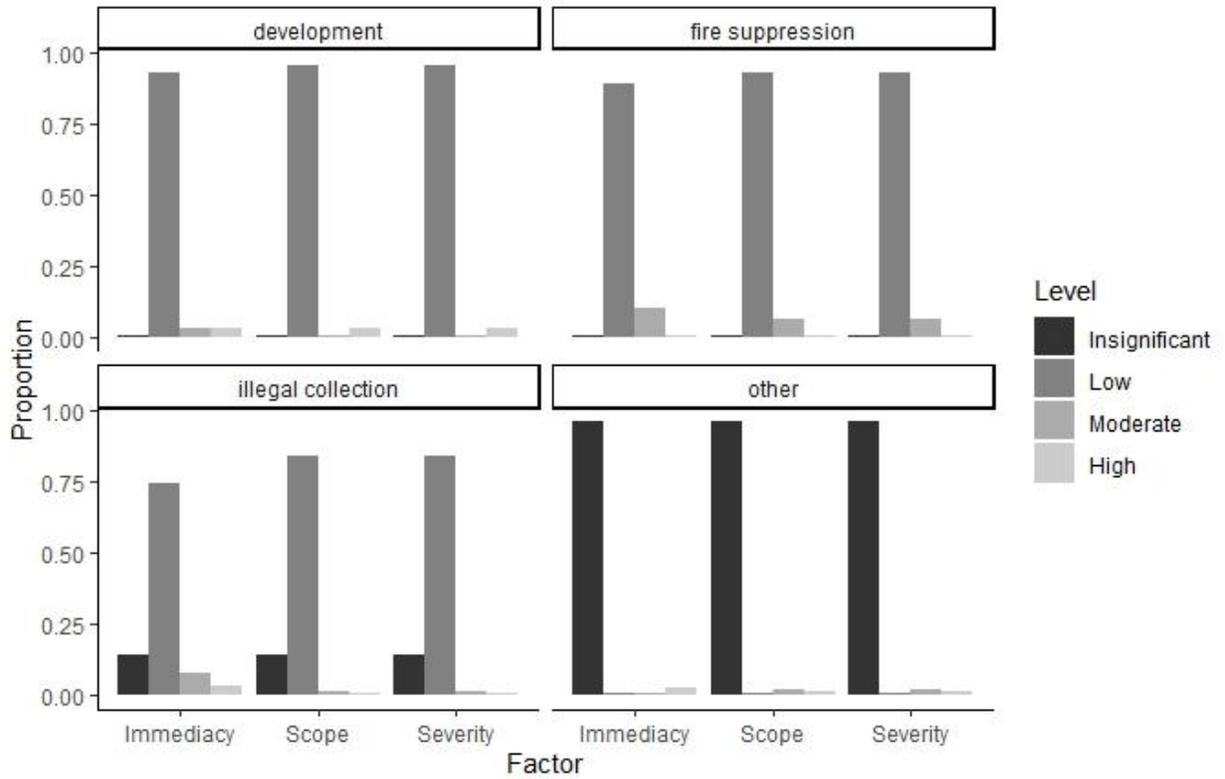


Figure 4.5. Proportion of individuals of Venus flytrap surveyed by core NCNHP survey team ranked by the severity, scope, and immediacy of threats.



Figure 4.6. Drought conditions in 2019. Dried *Sphagnum* moss in pocosin vegetation in Brunswick County demonstrating dry conditions.

Photo by Laura Hamon.



Figure 4.7. Habitat in Population 286. Wet pine savanna habitat in Pender County that was burned on 16 May 2019, approximately one month prior to this photograph.

Photo by Laura Hamon.



Figure 4.8. Habit in Population 256. Example of pond pine/longleaf pine savanna habitat with a shrubby understory from Population 256 in Brunswick County.

Photo by Laura Hamon.



Figure 4.9. Habitat in Population 298. A roadside patch of Population 298 in Brunswick County in 2018.

Photo by Johnny Randall.

APPENDICES

APPENDIX A: Supplementary figures and tables

Table S1.1. Pearson correlation coefficients among morphological traits, seed set per fruit, and average seed weight. Plant sample sizes are in parentheses. Bolded values and asterisks indicate significant correlations ($*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$). In each sub-table, correlations for two sites are depicted, with one site above the diagonal and another site below the diagonal, as follows: (a) S1 below and S2 above, (b) S3 below and S4 above, (c) S5 below and S6 above, and (d) S7 below and S8 above the diagonal.

		Scape height	No. traps	No. flowers	No. seeds	Average seed weight
(a)	Scape height	—	0.51 (20)*	0.28 (20)	-0.01 (20)	0.30 (20)
	Number of traps	0.07 (20)	—	0.34 (20)	0.45 (20)*	0.20 (20)
	Number of flowers	0.61 (20)**	-0.09 (20)	—	0.08 (20)	0.52 (20)*
	Number of seeds	0.10 (20)	-0.20 (20)	-0.23 (20)	—	0.03 (20)
	Average seed weight	0.36 (20)	0.20 (20)	0.04 (20)	-0.17 (20)	—
(b)	Scape height	—	0.51 (20)*	0.19 (20)	0.00 (20)	0.40 (20)
	Number of traps	0.44 (20)	—	0.41 (20)	0.16 (20)	0.07 (20)
	Number of flowers	0.32 (20)	0.28 (20)	—	0.14 (20)	-0.18 (20)
	Number of seeds	0.36 (20)	0.46 (20)*	-0.15 (20)	—	0.32 (20)
	Average seed weight	0.40 (17)	0.57 (17)*	0.19 (17)	0.61 (17)**	—
(c)	Scape height	—	-0.27 (19)	-0.03 (19)	-0.46 (19)*	0.28 (18)
	Number of traps	0.04 (20)	—	0.44 (19)	-0.02 (19)	0.06 (18)
	Number of flowers	0.37 (20)	0.29 (20)	—	-0.10 (19)	0.36 (18)
	Number of seeds	0.13 (20)	0.07 (20)	0.24 (20)	—	-0.56 (18)*
	Average seed weight	0.47 (20)*	0.05 (20)	0.2 (20)	-0.23 (20)	—
(d)	Scape height	—	0.54 (20)*	0.07 (20)	-0.04 (20)	0.52 (19)*
	Number of traps	-0.06 (18)	—	0.13 (20)	-0.13 (20)	0.44 (19)
	Number of flowers	0.42 (18)*	0.31 (18)	—	0.30 (20)	-0.20 (19)
	Number of seeds	0.35 (18)	-0.05 (18)	0.00 (20)	—	0.08 (19)

Table S1.1. (continued)

Average seed weight	0.08 (18)	0.04 (18)	-0.14 (20)	-0.20 (0.20)	—
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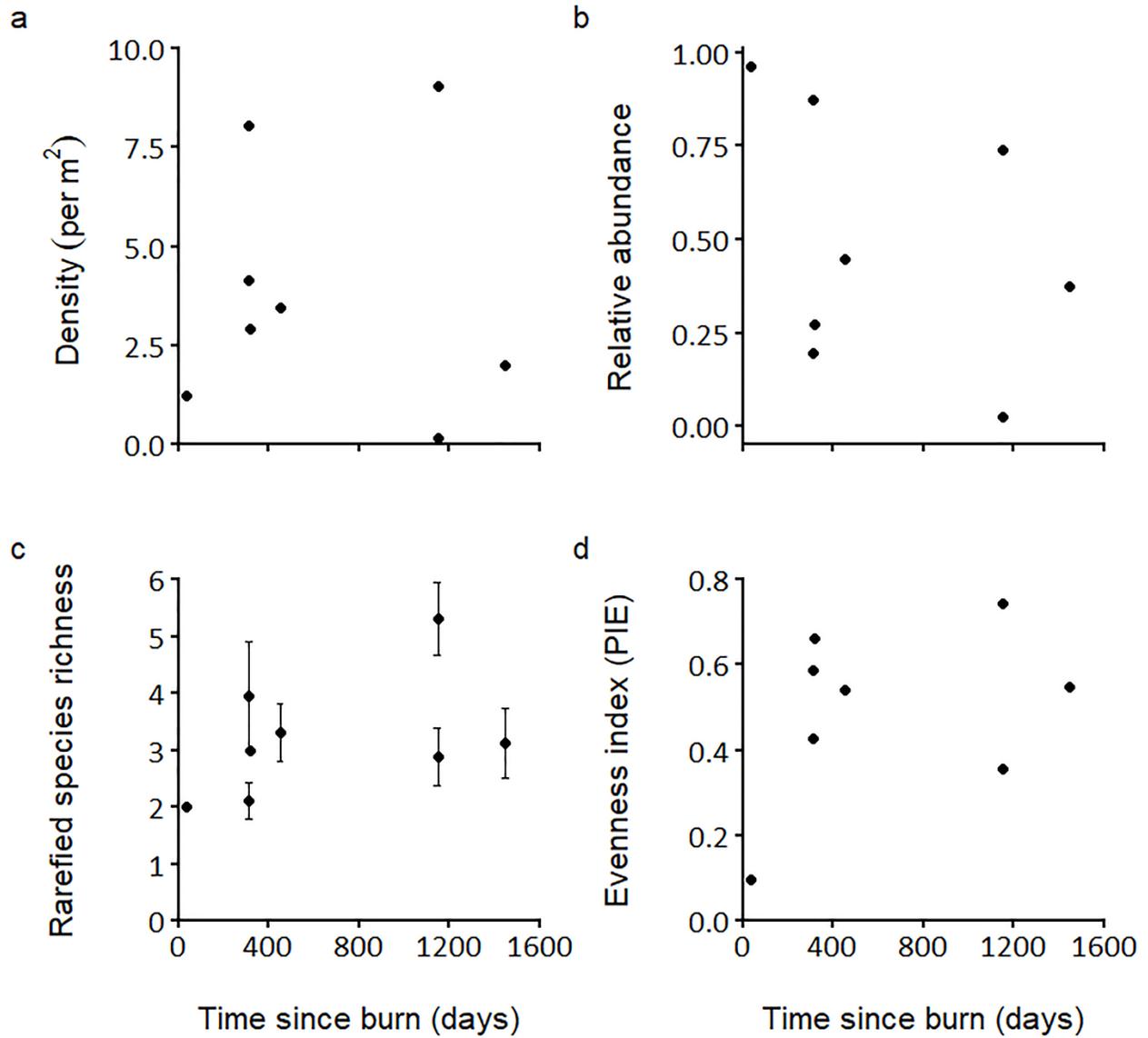


Figure S1.1. There were no significant relationships between (a) *Dionaea muscipula* flower density, (b) relative abundance, (c) rarefied richness (\pm SE) of flowering plant species, and (d) evenness of flowering plant species and time since burn. Each point represents a site.

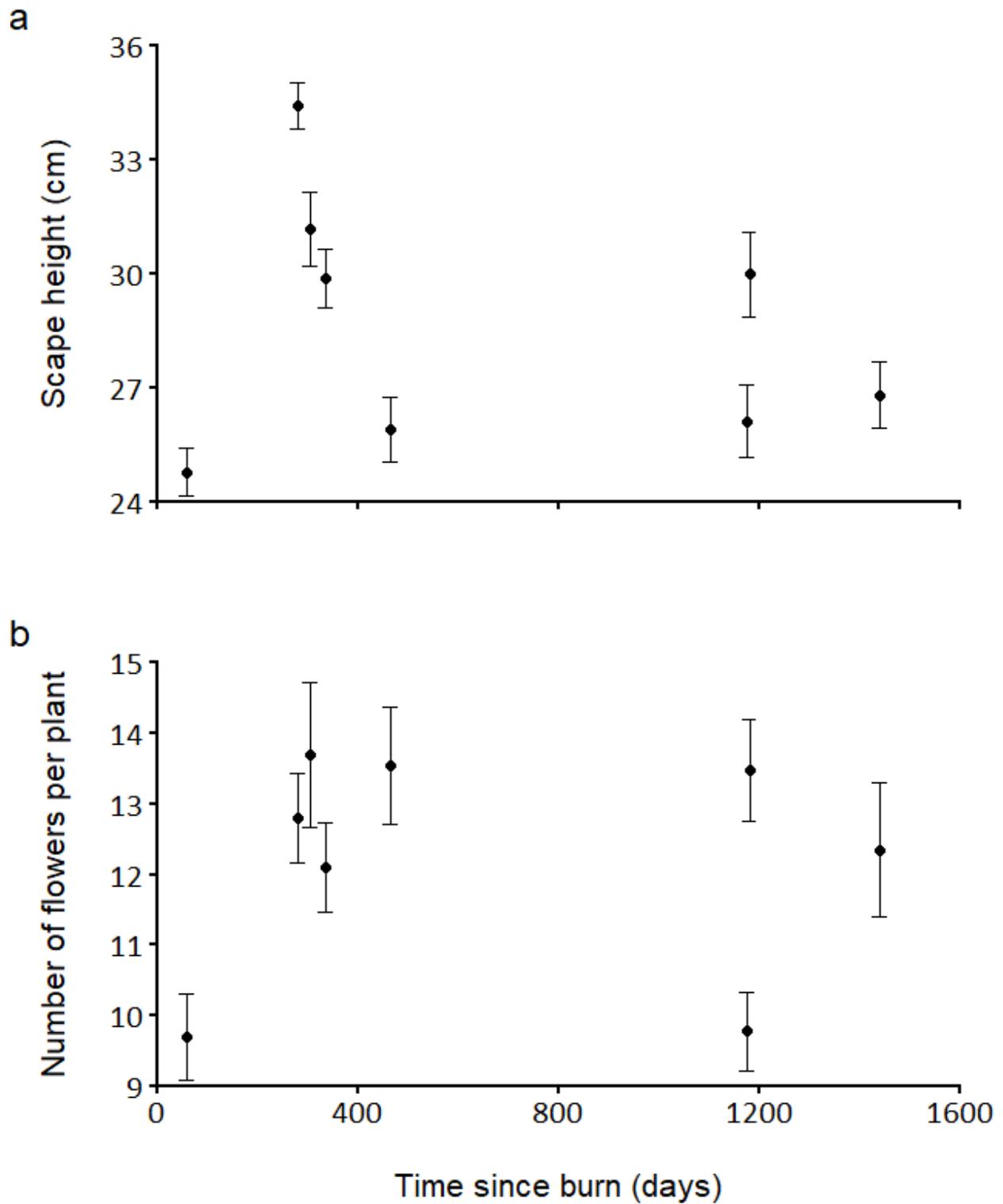


Figure S1.2. There were no significant relationships between (a) mean scape height and (b) mean number of flowers per plant and time since burn. Points represent site means \pm SE. Points are jittered for clarity.

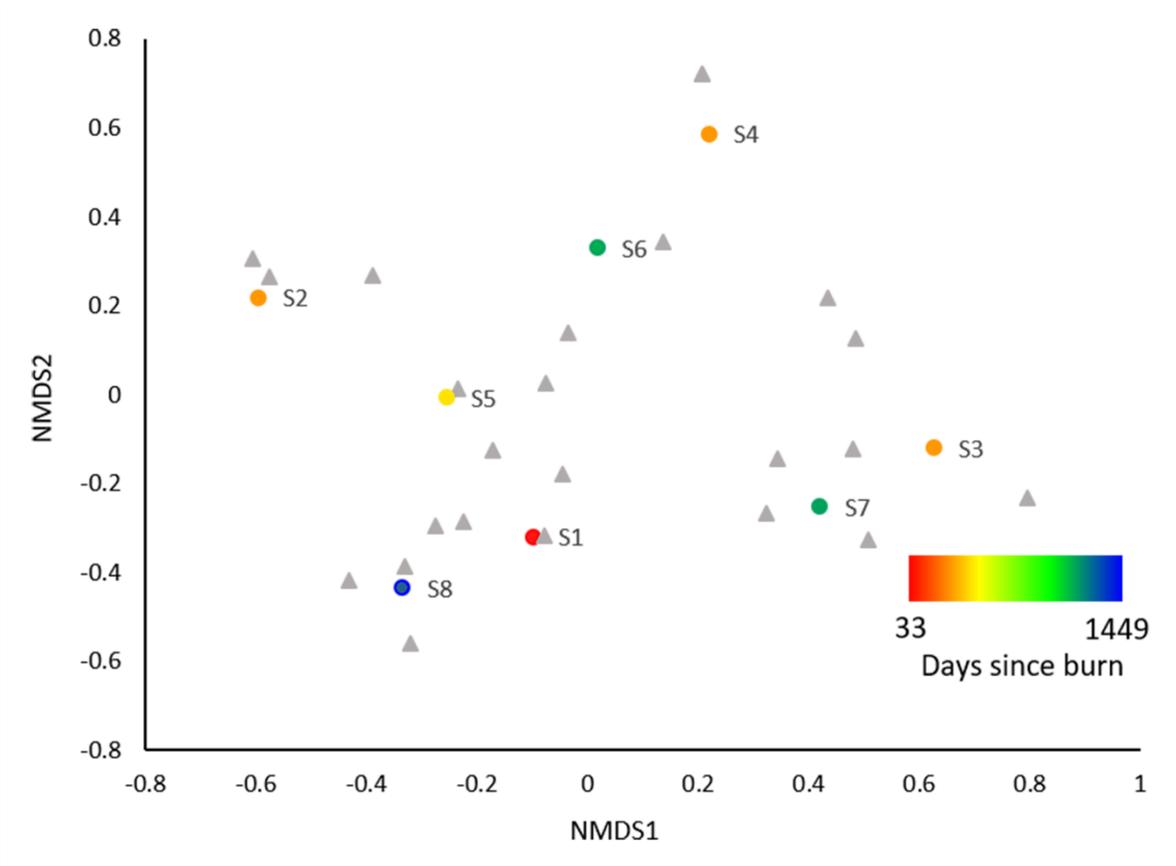


Figure S1.3. Ordination plot of sites and floral visitors. Triangles represent different species of floral visitors and circles represent sites. Site ID numbers correspond to increasing time since burn. There was no clustering of the sites as a function of time since burn.

Table S2.1. List of arthropod visitors to *D. muscipula* identified from hand-netting, including individuals identified in the field and post-collection.

Taxon	Order	Family	No. of individuals
<i>Mordella atrata</i>	Coleoptera	Mordellidae	23
<i>Lasioglossum</i> sp.	Hymenoptera	Halictidae	18
<i>Typocerus sinuatus</i>	Coleoptera	Cerambycidae	15
<i>Cryptocephalus</i> <i>venustus</i> group	Coleoptera	Chrysomelidae	7
micromoth	Lepidoptera	unknown	6
<i>Junonia coenia</i>	Lepidoptera	Nymphalidae	6
<i>Bombus</i> sp.	Hymenoptera	Apidae	4
<i>Trichiotinus piger</i>	Coleoptera	Scarabaeidae	3
<i>Oedancala</i> <i>crassimana</i>	Hemiptera	Pachygronthidae	2
<i>Apis mellifera</i>	Hymenoptera	Apidae	2
<i>Augochlorella</i> <i>gratiosa</i>	Hymenoptera	Halictidae	2
<i>Heriades</i> sp.	Hymenoptera	Megachilidae	2
<i>Trichodes apivorus</i>	Coleoptera	Cleridae	1
<i>Odontocorynus</i> <i>salebrosus</i>	Coleoptera	Curculionidae	1
<i>Trigonopeltastes</i> <i>delta</i>	Coleoptera	Scarabaeidae	1
<i>Anthrax irroratus</i>	Diptera	Bombyliidae	1
<i>Poecilognathus</i> <i>unimaculatus</i>	Diptera	Bombyliidae	1
Bombyliid	Diptera	Bombyliidae	1
<i>Agopostemon</i> <i>sericeus</i>	Hymenoptera	Halictidae	1
Unknown Halictid	Hymenoptera	Halictidae	1
<i>Coelioxys mitchellii</i>	Hymenoptera	Megachilidae	1
<i>Megachile</i> sp.	Hymenoptera	Megachilidae	1
Unknown Megachilid	Hymenoptera	Megachilidae	1
<i>Panoquina ocola</i>	Lepidoptera	Hesperiidae	1
Acridid (nymph)	Orthoptera	Acrididae	1

Table S2.2. List of insect visitors observed visiting flowers during time-lapse footage.

Taxon	Number of pollinator visits
Hymenoptera	346
Coleoptera	204
Lepidoptera	109
Identity unclear	67
Orthoptera	6
Hemiptera	5
Diptera	4

Table S3.1. Summary of experiments by site and year conducted.

Study name	Site	Year
Prey and pollen supplementation	S12	2019, 2020
Pollination system	S12	2020
Per-visit pollinator effectiveness	S3, S10, S12, S14	2018, 2018
Pollen-ovule ratio	S3, S10, S14	2018

Table S3.2. Average number of reproductive structures per flower between and across sites. Values represent means \pm SE, with sample size in parentheses.

Site	Number of anthers	Number of pollen grains	Number of ovules	P/O ratio
S3	14.31 \pm 0.33 (30)	10,602.20 \pm 690.13 (30)	30.22 \pm 1.06 (32)	350.8
S10	14.67 \pm 0.61 (30)	10,086.54 \pm 780.72 (30)	28.39 \pm 1.41 (31)	355.3
S14	12.86 \pm 0.49 (28)	5,417.48 \pm 526.52 (28)	20.27 \pm 1.06 (30)	267.3
Average	13.96 \pm 0.29 (88)	8,776.72 \pm 460.23 (88)	26.40 \pm 0.79 (93)	332.5

APPENDIX B: Data form and landowner report templates

NCNHP *Dionaea muscipula* Survey Data Sheet (template)

EO num:

Parent EO:

New Site?

Survey Date:

Surveyors:

Site Location:

Coordinates:

Owner/site manager:

State / County:

Location/access/parking Notes:

Phenology Notes: % Vegetative: % Bud: % Flower: % Fruit:

Vigor Notes: very feeble feeble normal vigorous exceptionally vigorous

Number of individuals (rosettes) counted: **Of these, number flowering/fruiting:**

Number of individuals estimated (if population is larger than 2,000 rosettes): **Estimated number of flowering/fruiting:**

Prevailing habitat type: savanna flatwoods bay rim pocosin right-of-way mosaic

General habitat description:

Summary of co-occurring plant species, in order of dominance:

Most recent burn date:

Threats (Indicate high, moderate, low, or insignificant for each):

Threat	Severity	Scope	Immediacy	Notes
Development				
Fire Suppression				
Poaching				
Other (indicate)				

Evidence of *Hemipachnobia subporphyrea* (feeding damage, larvae)?

Other notes:

Photo taken?	Yes	No	Photo ID:
Full extent mapped?	Yes	No	

Occupied area (based on mapped polygon):

Hours spent surveying:

Person-hours spent surveying:

VENUS FLYTRAP SITE SURVEY REPORT (template)

SITE NAME:

DATES VISITED:

INVESTIGATORS:

REPORT AUTHOR:

DATE OF REPORT:

BACKGROUND INFORMATION/PURPOSE OF VISIT:

The site was surveyed as part of the range-wide status survey of Venus Flytrap (*Dionaea muscipula*) by the Natural Heritage Program (NCNHP). The main objective was to determine the current population viability of Venus Flytrap, to document current site conditions and threats, and to record the range-wide population status of the species.

OWNER CONTACT:

COUNTY: QUAD:

LOCATION/ACCESS:

GENERAL DESCRIPTION:

SIZE:

FLYTRAP POPULATION:

SITE CONDITION AND THREATS:

MANAGEMENT OPTIONS:

NCNHP staff members plan to update flytrap population numbers and site information in the Biotics database as soon as possible. Contact NCNHP for more details.