of the Purple Pitcher Plant*:* the noctuid moths *Exyra fax* (Grote) (Pitcher Plant Moth) and *Papaipema appassionata* (Harvey) (Pitcher Plant Borer Moth). The more common Pitcher Plant Moth will lay ~5 eggs on the interior of the pitcher lip (Jones 1921). When the eggs hatch to larvae, the larvae will then start consuming the interior plant tissue wall from just above the pitcher fuid to the pitcher opening, which the moth larvae have enclosed either with a silken web or by girdling the plant to protect themselves from predators while feeding (Atwater et al. 2006, Folkerts and Folkerts 1996, Lafontaine and Poole 1991, Rymal and Folkerts 1982). The closed pitcher opening does not allow rainwater to further accumulate in the pitchers or allow prey to enter the system, creating an unsuitable habitat for the Purple Pitcher Plant inhabitants and a decline in nutrients for inhabitants and the plant (Atwater et al. 2006). The Pitcher Plant Moth larvae are capable of consuming numerous pitchers in addition to the pitcher they were initially deposited in as eggs, as well as pitcher fower buds and fowers (Folkerts and Folkerts 1996, Jones 1921). In preparation for pupation, each larva will leave the pitcher it is actively consuming, fnd a mature pristine pitcher, in which it chews a drainage hole at the back base, causing fuid to drain from the pitcher (Fish 1976, Folkerts and Folkerts 1996, Jones 1921, Rymal and Folkerts 1982). After drainage, the larva will enter the empty pitcher, pupate, and then eventually emerge as an adult through the pitcher opening. Moth herbivore damage to the Purple Pitcher Plant in the form of drained pitchers can be found across the eastern seaboard of the United States (L.A. Pett, pers. observ.) .

There is little evidence that the Pitcher Plant Midges are capable of emigration out of unsuitable pitchers to suitable fuid-flled pitchers while in the aquatic larval stage. The only biological rationale as to why emigration is plausible comes from Wiens (1972) in which they noted that midge larvae are incapable of swimming but can crawl up the interior of the pitcher wall. As well, Paterson and Cameron (1982) noted observing late instar midge larvae in newly opened pitchers. Not only would emigration from pitchers be advantageous for midge larvae when faced with unsuitable habitat but also to the plant or surrounding Purple Pitcher Plants, as the midge larvae are one of the most abundant organisms within the plant and play a critical role in processing prey. As mentioned previously, the midge larvae break down prey that enter the system, creating the basis of the pitcher plants' detritus system and simultaneously releasing nutrients and organic matter into the aquatic ecosystem (Cochran-Stafra 1993, Heard 1994, Paterson and Cameron 1982). It is also noteworthy to mention that besides impacting the plant and the aquatic ecosystem, the Pitcher Plant Midge larvae also directly impact other obligate organisms, as the midge larvae will consume other species of mosquito larvae that enter the system, thereby decreasing the competition for the obligate Pitcher Plant Mosquito larvae (Petersen et al. 2000).

Although it is known that the Pitcher Plant Midge larvae can crawl up the interior pitcher wall, it is unknown if the larvae can successfully emigrate out of their pitcher of origin to other surrounding pitchers. The objective of our experiment was to measure the success of midge larvae emigrating to suitable habitat in the form of fuid-flled pitchers when presented with mimicked moth herbivory causing

drainage of their origin pitcher. We hypothesized drained pitchers would induce emigration of midge larvae, as the habitat would not be sustainable for the aquatic midge larvae to survive in. We also hypothesized that initial abundance of midge larvae could impact success of emigration and survival in new pitchers. We predicted that with a high abundance of midge larvae in drained pitchers, the emigration rate would increase due to competition for resources and habitat, in turn pushing midge larvae out into suitable pitchers thereby increasing survival in a new pitcher. This work will provide insight on the commonly used model system *S. purpurea* and whether this system is truly closed in terms of its larval aquatic invertebrates.

Methods

To measure the success of Pitcher Plant Midge larvae emigrating out of drained pitchers of the Purple Pitcher Plant to viable, fuid-flled pitchers, we conducted a greenhouse experiment in the summer of 2021 using 15 plants potted in sphagnum moss in individual plastic pots. We cleaned all plants and flled the pitchers with purifed reverse osmosis water that contained no obligate invertebrates. Pots were separated by ~13 cm from one another within water-holding trays inside insect tents. For each plant, we designated the largest pitcher as the experimental pitcher to be drained. The number of viable, fuid-flled pitchers per plant varied between 1 and 12. The low number of plants in the study and the variable number of pitchers present in each plant were due to monetary constraints as well as pitchers being damaged in the shipment process. If pitchers were not capable of holding water, we deemed them not viable for aquatic invertebrates.

We collected Pitcher Plant Midge larvae from Molly Bog, Stowe, VT. Five plants received high abundance (10 individuals) of midge larvae in the experimental pitcher, 5 plants received medium abundance (5 individuals) of larvae, and 5 plants received low abundance (2 individuals). We collected all larvae at the same time but they may have varied in stage of development, as the most accurate way to determine stage is by examining eye development, which we did not do (Weins 1972). Pitcher Plant Midges are present in larval stages for ~60 days, which exceeded our experiment time. However, we used insect tents in case any midge larvae were late instars that would eclose and emerge as adult midges during the course of the experiment.

We kept the abundance of midge larvae and number of replicates of experimental treatments relatively low as to minimize the quantity of larvae we needed to collect and thereby not impact the natural abundance of midge larvae at Molly Bog, in which the plant population is declining. All midge larvae in experimental pitchers received aquarium fsh fakes on day 1 as a food source. Midge larvae were then allowed to acclimate to experimental pitchers for 4 days.

After 4 days, we drained 4 of the 5 experimental pitchers of high, medium, and low abundance, while 1 of each abundance treatment was left flled with fuid, serving as controls. We used a small proportion of control pitchers because of the historical literature on the system stating that midges are incapable of movement out of the aquatic (control) environment and to have a larger sample size of unsuitable

habitat to hedge against emigration being a rare event. To mimic drainage caused by *E. fax*, we used a sterilized 18-gauge needle to punch a hole in the back of the leaf near the base of the pitcher, in what Lloyd (1954) termed zone 5 of the pitcher. Plants were then exposed to ambient climatic conditions. After 2 weeks, we clipped experimental pitchers at the base of the plant and dissected them to determine the number of midges present, and their status (living or dead). We then repeated this process for every other pitcher in each plant.

Using the statistical software R (version 3.6.2; R Core Team 2019) and pooled data for control and drained pitchers, we employed Fisher's exact test for count data, with Monte Carlo simulation, to test the effect mimicked damage of the Pitcher Plant Moth, in the form of pitcher puncture and drainage, has on (1) the proportion of initial larvae abundance surviving in place, (2) the proportion of the initial larvae abundance dying or going missing, and (3) the proportion of initial larvae abundance emigrating and then surviving in a new, viable, fluid-filled pitcher. We also tested the effect of initial abundance on surviving in place, dying or going missing, or successfully emigrating and then surviving in a new, viable, fluid-filled pitcher by pooling data for each of the 3 levels of initial abundances.

Results

After 2 weeks, 7.4% of the initial larvae abundance survived in the experimental drained pitchers while 94.1% of the initial larvae abundance survived in the control pitchers. Of the larvae in drained pitchers 54.4% died or went missing compared to 5.9% of larvae in control pitchers. Finally, 38.2% of initial larvae abundance,

were unaccounted for at the end of the experiment should be considered when interpreting the results.

The unaccounted-for midge larvae were most likely present in the sphagnum-moss media that each plant was potted in, or a more unlikely scenario could be that midges were unidentifable due to decomposition by protists and bacteria. The latter is less probable as numerous dead midges were found and not decomposed, completely intact. Midge larvae could have also pupated, eclosed, and emerged as adults during the experiment if we initially gathered late-stage larvae. However, the experiment was surrounded by insect tents and no adult midges were found. Future experiments should limit the midge larvae to only instars that would require more time than the experimental period to pupate and eclose. Future experiments should also use only plants that contain the same number of pitchers, as plants with more pitchers may have an increased likelihood of successful emigration.

We assume that no long-distance dispersal of midge larvae occurred between plants. The larvae, which are at best poor swimmers (Wiens 1972), would have to leave experimental drained pitchers, traverse though the sphagnum, down the plastic pots, swim through the tray of water, and crawl back up another plastic pot to reach a new plant. If long-distance dispersal was occurring in this environment,

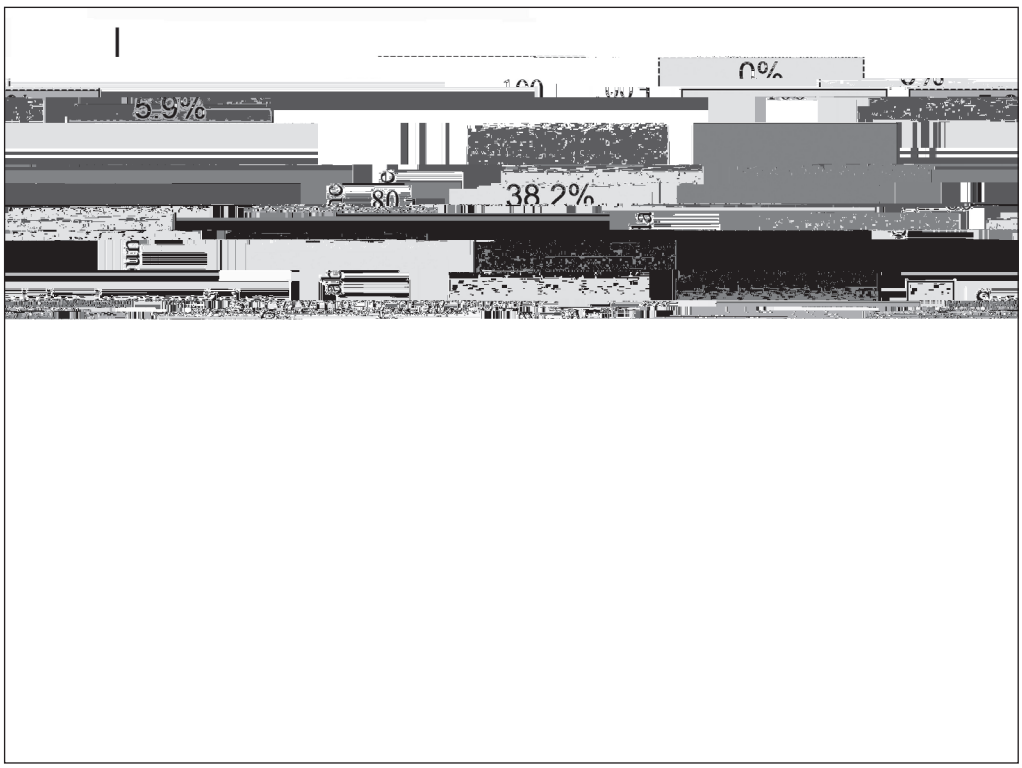

Figure 1. The difference of effect of control (fuid flled) and drained pitchers on the proportion of the initial *M. knabi* (Pitcher Plant Midge) abundance surviving in place (dark grey, bottom), dead or missing (grey, middle), and emigrating to and surviving in a new fuid-flled pitcher (light grey, top).

we would expect to see a net gain at least once in 1 of the plants, which we did not. We did find 3 live larvae within the tray water when it was filtered at the end of the experiment (we coded these larvae as missing in the analysis). Perhaps in a natural environment such as a sphagnum bog, where plants are potentially touching pitchers and are separated only by sphagnum moss and not standing water, long-distance dispersal is plausible.

Future work should compare the morphology of midge larvae that were able to emigrate in comparison to larvae that were not, as we believe larger posterior proleg hooks allow the midge to successfully crawl the pitcher walls. The ability of midge larvae to promote conspecific survival during emigration should also be investigated. In addition, path of emigration for midge larvae should be determined, as this could occur through the pitcher opening (which is not enclosed when the pitcher is drained) or through the drainage hole caused by the Pitcher Mining Moth larvae. Pitcher Plant Midge

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