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Observations on prey preference and other associations of *Aldrovanda vesiculosa* in a new culture system

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Abstract. *Aldrovanda vesiculosa*, also known as the waterwheel plant, is an aquatic free-floating carnivorous plant noted for its difficulty of cultivation. A new method of cultivation allows for the rapid propagation of *A. vesiculosa* without using complex additives, and this method is effective with plants from Europe, Asia, and Australia. This involves the creation of a complex periphyton and associated community, including invertebrate animals and protists over a rich soil substrate. To better understand the associations of *A. vesiculosa* with protists and invertebrate animals in this system, two lines of investigation were pursued. The first line of investigation involved examination of whether *A. vesiculosa* is selective of the prey that it traps, and if so, what characteristics the different types of trapped prey share. Several prey items were offered to the plants, and observations were made for 1 wk with amphipods and snails preyed upon the most, perhaps due to their seeking shelter in or on the plants of *A. vesiculosa*. The second line of investigation involved the examination of microscopic animals and protists living with *A. vesiculosa* in this new system of cultivation to determine if the types of organisms associated with the periphyton may indicate any salient features of the new system. Large numbers of rotifers, testate amoebae, and other organisms indicative of mesoeutrophic or eutrophic conditions were observed.

Introduction

Aldrovanda vesiculosa (Droseraceae), also known as the waterwheel plant, is an aquatic free-floating carnivorous plant found growing just below the surface of the water. It is native to Australia, Europe, Africa, and Asia, first being discovered in 1699 by Leonard Plunkenet. Europe's population of *Aldrovanda* has become endangered in recent years due to pollution, and in Japan *Aldrovanda* has gone extinct in the wild (D'Amato 1998).

A simple method of cultivating *A. vesiculosa* has recently been described (Darnowski 2002) which uses rich garden soil and companion plants with abundant roots to create an environment in which *A. vesiculosa* plants from Europe, Asia, and Australia rapidly grow and reproduce. To better understand this method, which includes the formation of a periphyton mat with associated organisms, the nature of the prey trapped by *A. vesiculosa* and the nature of the animals and protists associated with *it* may provide significant clues. That a periphyton mat is produced may not be surprising given the observation that in the wild in the Northern Territory, Australia, *A. vesiculosa* can be seen growing among large floculant particles (Darnowski, unpublished observation), which may represent a periphyton mat along with other organisms.

Waterwheel plants can reach a maximum length of 20 cm and have whorls of leaves perpendicular to its stem. These leaves bear traps that prey on small aquatic organisms such as *Daphnia* and amphipods. Each whorl usually consists of eight leaves with a trap at each leaf's apex. As the plant grows and produces more whorls, the aged whorls at the basal end soften and fall from the plant. Under favorable conditions this plant is able to grow at a rate of 0.4 to 0.9 cm d^{-1} (Breckpot 1997).

Most carnivorous plants, including, *A. vesiculosa*, live in nutrient poor environments, which is thought to be the reason for their carnivory. *A. vesiculosa* inhabits shallow bodies of water such as lakes, bogs, lagoons, and billabongs that are dystrophic with sparse bottom fauna. Filamentous algae and competition from denser stands of plants can lead to the disappearance of this plant in a habitat. Other carnivorous plants that share the same habitat as *A. vesiculosa* include various bladderworts (Lowrie 1998; Adamec 1995).

The trapping mechanism of *A. vesiculosa* is nearly unique among carnivorous plants since only one other plant, the Venus Fly Trap (*Dionaea muscipula*), utilizes this same technique known as the spring or beartrap (Gentle 1996). The spring trap's shape is similar to that of a bivalve, with two semicircular halves usually measuring 4 mm long and 2.5 mm wide. Possible prey items such as *Daphnia* or amphipods are attracted to the plant by four to eight long apical bristles on which they can seek shelter. Although these are important in bringing organisms into contact with the plant, the primary purpose of these bristles is to prevent the traps from capturing floating debris near the surface of the water (Breckpot 1997). The trap is stimulated when an organism touches one of the forty small trigger hairs that the trap possesses on its inner margins (Lowrie 1998). The trap shuts in only 20 msec as a result of an action potential traveling across the excitatory cells in one of the most rapid movements seen in plants (Iijima and Sibaoka 1981).

After the leaf ingests the prey, approximately sixty to eighty tiny teeth inside the trap lock together and excess water is pumped out. Last, the plant releases a secretion to seal the trap shut. Digestion takes place over a few days as the digestive glands on the inside walls of the trap secrete enzymes and acid to dissolve the organism. After the organism is dissolved, its nutrients are absorbed. Predation is not affected by the digestive process, allowing traps to catch more organisms even if there is already prey in a trap (D'Amato 1998). *A. vesiculosa* will consume anything that touches the trigger hairs stimulating the trap to shut even if the organism is larger than the trap. When this scenario occurs, the trap will remain closed and eventually die (Gentle 1996).

Much research has been conducted on *A. vesiculosa*, but one area of study that has not been given much attention is that of prey selection. It is known that the plant eats different organisms but in what proportions is not known. In order to determine if the plant does select which prey it captures when different species of aquatic organisms are available, an experiment was carried out using several prey items that were offered to the plant in a laboratory setting.

Similar studies have been performed on other aquatic carnivorous plants from the genus *Utricularia*. Like *Aldrovanda*, *Utricularia* sits and waits for prey to approach it. Hence, it has been proposed that prey selection probably depends more on the characteristics of the prey, like its activity and swimming patterns, and not on the behavior of the predator (Harms and Johansson 2000). In three sympatric species of *Utricularia*, planktonic prey were preyed upon in smaller amounts than what was available while prey living on a substrate, plant or sediment, were captured in proportion to availability (Harms 1999).

Others have observed that prey selection and carnivory may not be important aspects in one species, *U. purpurea*. A substantial amount of biomass is invested to develop and maintain traps but they only capture a small number of living organisms. Rather than a predator-prey relationship, a mutualistic relationship was observed between mature bladders and algae and zooplankton (Richards 2001). There may be similar connections between occupants of the periphyton and *A. vesiculosa* in culture.

Materials and Methods

Cultivation of *Aldrovanda* *A. vesiculosa* was cultivated in the laboratory to make a stock supply according to Darnowski (2002). 8 l aquaria were used, filled with approximately 5 cm inches of garden soil from Queen Anne's County, Maryland, USA. This soil, clay-based with organic amendments, had added to it double distilled water to a depth of approximately10 cm. One or two water hyacinths (*Eichhornia crassipes*; Pontideriaceae) were added to each tank, and several segments of *A. vesiculosa* were placed on the surface of the water. The large root mass of these plants may help to condition the water for *A. vesiculosa.* The condition of the plants and tanks were observed periodically and more distilled water was added when necessary to maintain the water level.

Selection and description of prey items Based on their size relative to that of the traps, prey items were selected. *Daphnia magna*, copepods, and amphipods were ordered from Ward's Natural Science (Chicago, Illinois, USA) and mosquito larvae, baby pond snails (*Amnicola limnosa*) , and rotifers were purchased from Carolina Biological Supply Company (North Carolina, USA). Some pond snails, already present in tanks used to culture *A. vesiculosa* before experiments were performed, were also included among prey organisms.

These prey can be divided into three size classes, the smallest being the rotifers which were microscopic and which have been found to live in the traps of *U. purpurea* (Richards 2001). The copepods and *Daphnia magna* were slightly larger, approximately 0.5 -1.5 mm long. The largest class of prey ranging from 2-3 mm included the amphipods, baby snails, and mosquito larvae. Prey organisms were fed a 1:1 mix of Brewer's yeast and Spirulina (Debittered Brewer's Yeast, Now Foods, Bloomingdale, IL USA; Spirulina, Earthwise Nutritionals, Petaluma, CA, USA) until used in experiments.

Daphnia magna or water fleas are small crustaceans measuring 0.2-6 mm. These are found mainly in freshwater (Fox 1994). Copepods and amphipods are larger aquatic crustaceans measuring 1-2 mm and 2-50 mm, respectively (www.nmnh.si.edu/iz/copepod / and http://web.odu.edu/sci/biology/jrh/whatis.htm). The larval stage of mosquitoes is the second stage of their metamorphosis, lasting 1-3 wk. During this period they feed on bacteria and algae and brush-like hairs help them swim. Rotifers are microscopic organisms which use beating cilia to swim in ponds and lakes (Carolina Biological Supply Company 1998).

Experiments on selection of prey From the stock supply, robust pieces of *A. vesiculosa* containing six whorls were cut. Three pieces of the plant were used for each experiment. All three pieces were examined with a dissecting microscope prior to the experiment to record any organisms already present in the traps--any such organisms were subtracted from the totals in Tables 1-4. The plants were then placed in a small GladWare® container three quarters full of filtered pond water. The pond water used in these experiments was taken from containers in Washington College's greenhouse that had aquatic plants growing in them. The original water in these pots had aged over time, meaning minerals and nutrients had been taken up by the plants and small animals, resulting in water properties that would support *Aldrovanda* in the wild. A coffee filter was used to trap vegetation and organisms present in the pond water so that only conditioned pond water was used for experiments and so that no additional organisms from the greenhouse were included.

Prey were then added using a disposable plastic pipette. The first experiment involved amphipods, *Daphnia magna*, and copepods with five of each added per container. In the second experiment two additional prey items were used, mosquito larvae and rotifers. The same quantities of prey employed in the first experiment were also used here, except for mosquito larvae of which there was a limited supply, and the rotifers because they were much smaller than the other prey. Three mosquito larvae were added to *Aldrovanda* numbers 4 and 5 and only 2 to *Aldrovanda* number 6. 0.5 mL of rotifers (3.6×10^8 rotifers l^{-1}) were added to these three containers.

After determining which prey were caught in the greatest quantity, a third experiment was conducted to see if the predation pattern would change given the addition of other organisms of similar size and behavior. This last experiment involved only amphipods and baby snails. These two animals are similar in their behavior because they both periodically seek shelter in floating aquatic plants such as *A. vesiculosa.* The baby snails cling to the sides of a container or to plants in the water, and the amphipods actively seek plants to hide from predators. *Aldrovanda* plants 7-9 (Table 3) received two amphipods and two snails while *Aldrovanda* plants10-12 (Table

4) received the same number of snails but three times the number of amphipods. All containers in these experiments were then covered with plastic wrap to prevent any additional prey from entering the containers. The traps were then observed every 2 d under the dissecting microscope for 1 wk. Notes were made of what was in the traps and the condition of the plants.

Observation of associated animals and protists 20 l tanks, prepared according to Darnowski (2002), were prepared and placed in a frost-free greenhouse in Queen Anne's County, Maryland, USA, and allowed to grow for approximately 5-8 months. At the start, these tanks contained tap water, *A. vesiculosa* from a variety of locations (Europe, Girraween Lagoon in Australia's Northern Territory, Japan, Australia's New South Wales, southern Western Australia), water hyacinths, and bladderworts (*U. gibba* and other species). In addition, small volumes of water from active tanks of *A. vesiculosa* including both protists and invertebrate animals were added to promote the formation of the periphyton mat. This addition always included a red-orange cyanobacterial species.

During the late winter and early spring of 2002, samples of water and the periphyton mat were removed from these tanks, but especially the tank containing *A. vesiculosa* from Girraween Lagoon, Northern Territory, Australia and examined from 40-400x total maginfication using bright field microscopy. Images of the organisms present were taken using a 1.5 megapixel RCA digital camera pointed into one eyepiece of the stereo compound microscope. Organsisms were identifed using a variety of guides to protozoa and other organisms commonly associated with them (Patterson 1996).

Results

In the first experiment, only a small number of prey were caught in the traps. *A. vesiculosa* captured only *Daphnia* and amphipods, and mostly examples of the latter organism (Table 1). The second experiment yielded similar results to the first, only amphipods and *Daphnia* were caught (Table 2). All the plants caught prey with *A. vesiculosa* number 6 capturing the most, a total of three amphipods between the third and fourth whorls. Again, amphipods were the most commonly trapped prey. Several plants showed outgrowth of new shoots, a normal response to decapitation of the plants as was performed in preparing the experiments.

The least amount of prey caught was observed in the last experiment. *A. vesiculosa* numbers 8-11 did not catch any prey. *A. vesiculosa* number 7 caught an amphipod and a baby snail in the fourth whorl (Table 3), and *A. vesiculosa* number 12 caught one amphipod in the sixth whorl on the second day as illustrated in Table 4.

Associated animals and protists Many microscopic organisms were found in abundance in the cultures of *A. vesiculosa*. This being a qualitative study rather than a quantitative, the organisms occurring the most obvious numbers were recorded. Most obvious were testate, or shelled, amoebae, rotifers, and cyanobacteria.

Discussion

Selection of prey Based on the data, two conclusions can be drawn: 1) amphipods were preyed upon the most and 2) a majority of the traps did not catch any prey. A possible explanation as to why the amphipods were chosen over the other prey is their swimming behavior which was observed under the dissecting microscope. Frequently, the amphipods would seek shelter in the plant, thus increasing the probability of coming into contact with the traps and being caught. This pattern has previously been observed by Harms (1999) in the genus *Utricularia*, and it may explain the capture of a snail in the third experiment since the snails also come into close contact with the plants. Harms concluded that planktonic prey such as *Ceriodaphnia* and *Bosminia* were at a lower risk of being captured than prey living on the plant like copepods or ostracods. Why more copepods were not caught is unclear and deserves further study, especially since these organisms are abundant in non-experimental cultures of *A. vesiculosa.*

Prey size in relation to trap size is another possible reason the amphipods were caught in greater quantities. Several of the *Daphnia magna* and mosquito larvae appeared to be larger than the traps so they were less likely to be caught, while there was a possibility that the rotifers were too small to even trigger the traps, also indicated by their ability to live in the traps of *U. purpurea* (Richards 2001). Careful observation for leaf absence, resulting from trap death after a prey item larger than the trap was caught, was made so this predation did not go unnoticed. However, this was not observed. Therefore, the size of the trap may determine the nature of the prey caught (Harms 1999).

Many of the traps did not catch any prey, perhaps because there was a limited amount of prey available for experiments. In the first experiment there were only 15 prey items compared to approximately 48 traps. The second experiment had 2 or 3 more prey items depending on the container plus the 0.5 mL of rotifers, yet similar results compared to those from the first experiment were seen. Prey was present in even smaller quantities for the third experiment and this was illustrated in the data.

Compared to the second experiment, the dramatic decrease observed in this experiment might have been because of soil clogging the traps and covering the trigger hairs, or the low availability and condition of the prey which was not optimal--this last experiment occurred later than the others, at a time when many prey cultures were no longer in excellent condition.

The effect of an already low abundance of prey could be enhanced if the prey were to die once in the container. Some prey might die naturally or perhaps because of attack by other prey species used in the same experiment. This activity was observed on one occasion between an amphipod and *Daphnia*, where the *Daphnia* was being eaten by the amphipod. If this process occured regularly than it would greatly decrease the probability of *Daphnia* being caught because a proportion of them would have been killed by the amphipods.

Another condition which might have affected the number of prey caught was the number of traps per whorl. Throughout the course of the experiment, 1 wk, several traps fell off each of the plants. This could be a result of a natural process as the plant grows or shock from being cut and placed in a new medium. Also, the number of leaves per whorl is dependent on how fast the plant develops. Younger plants might have less than eight leaves per whorl and these leaves are much smaller than more mature plants. In future, use of intact plants instead of sets of six whorls is indicated.

The last problem encountered that might have affected the data is difficulty in determining the contents of some traps. Several of the traps, especially in the third experiment, contained soil which made it difficult to see through them and determine if they were occupied prior to the experiment. A temporary drop in the water level brought the plants into close proximity with the soil bottom where they were able to catch soil particles. During the experiment most of this soil was released by traps as they opened to catch prey. In addition, several prey items were very small, such as the rotifers, making it difficult to determine what the trap had caught. These traps could have been dissected to identify the prey, but the plant would have been sacrificed, ruining the experiment.

In summary, this part of the study shows that *A. vesiculosa* does select prey based on characteristics of the prey. Prey behavior leading to increased contact with the plant, either living on the plant or seeking its shelter periodically, will increase the probability of predation for that species. The prey also has to be similar in size to the traps to stimulate the trigger hairs within the lobes.

Associated protists and Invertebrates

The protists and invertebrate animals which were found in successful cultures of *A. vesiculosa* from a variety of sites around the world indicate mesoeutrophic to eutrophic conditions, especially the abundance of cyanobacteria and testate amoebae. This shows the need for precise chemical tests to determine the concentrations of various mineral nutrients in solution in these cultures, especially N, P, K, and B, in the study of this new culture method. In particular the question is raised of the role that these companion species may play in the ecology of *A. vesiculosa*, perhaps related to the non-carnivorous associations proposed for *U. purpurea* (Richards 2001).

General conclusion

The new method for cultivating *A. vesiculosa* (Darnowski 2002) shows great promise not only for producing many plants in a simple manner but also for studying important ecological associations of these plants, both associations with prey and associations with other organisms.

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Table 1. Number and type of prey caught in each whorl in the first experiment.

	Aldrovanda 1				Aldrovanda 2				Aldrovanda 3			
Whorl	Day 2	Day 4	Day 6	Day 7	Day 2	Day 4	Day 6	Day 7	Day 2	Day 4	Day 6	Day 7
1	0	0	0	0	0	Daphnia	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	Amphipod	0	0	0	Amphipod	0	Amphipod

Table 2. Number and type of prey caught in each whorl in the second experiment.

	Aldrovanda 4				Aldrovanda 5				Aldrovanda 6			
Whorl	Day 2	Day 4	Day 6	Day 7	Day 2	Day 4	Day 6	Day 7	Day 2	Day 4	Day 6	Day 7
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	Amphipod	0	0	0	0	0	0	0	Amphipod (2)	Amphipod	0	0
4	Daphnia	0	0	0	0	Amphipod (2)	0	0	0	Amphipod	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0

Table 3. Number and type of prey caught in each whorl of Aldrovanda plants 7-9 in the third experiment.

	Aldrovanda 7				Aldrovanda 8				Aldrovanda 9			
Whorl	Day 2	Day 4	Day 6	Day 7	Day 2	Day 4	Day 6	Day 7	Day 2	Day 4	Day 6	Day 7
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	Amphipod	Snail	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0

Table 4. Number and type of prey caught in each whorl of Aldrovanda plants 10-12 in the third experiment.

	Aldrovanda 10				Aldrovanda 11				Aldrovanda 12			
Whorl	Day 2	Day 4	Day 6	Day 7	Day 2	Day 4	Day 6	Day 7	Day 2	Day 4	Day 6	Day 7
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	Amphipod	0	0	0

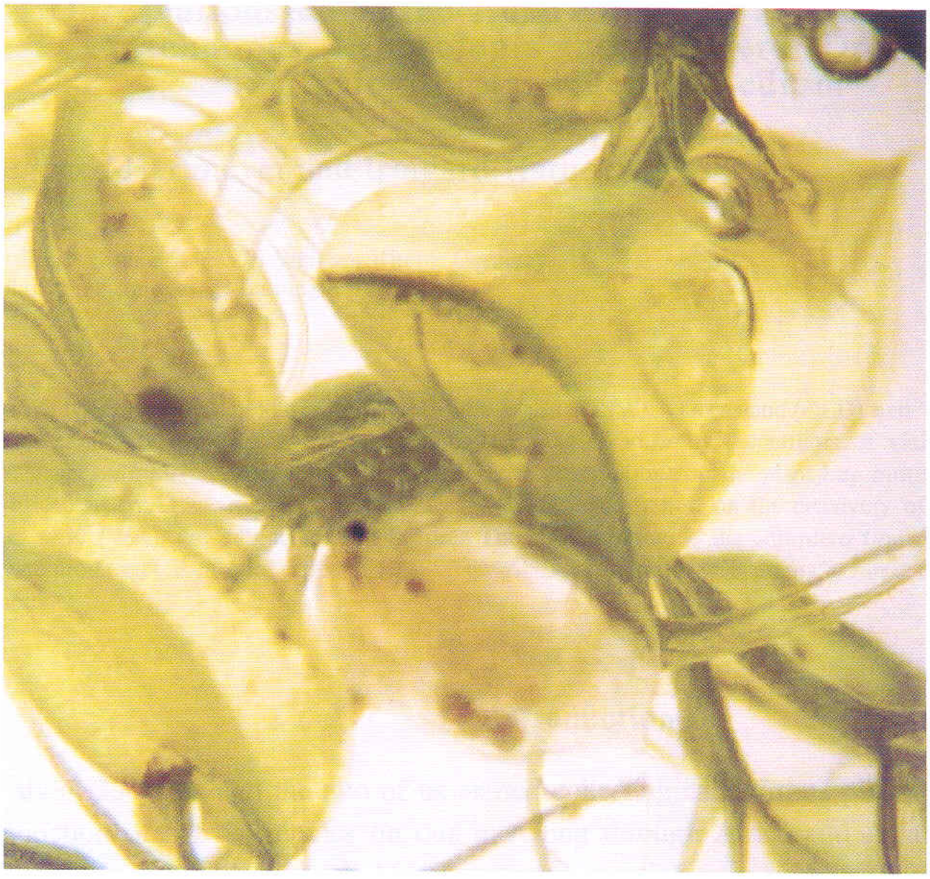

Figure 1. *Daphnia magna* on an *Aldrovanda* leaf (length of trap is approximately 4mm).

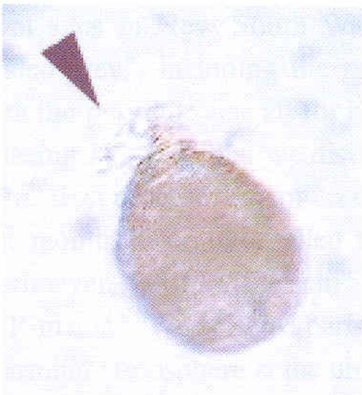

Figure 2. Testate amoeba from a culture of *Aldrovanda vesiculosa* from Girraween Lagoon, Northern Territory, Australia. The amoeba is approximately 20μm long, and the arrowhead indicates the extended pseudopodia.