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Testing The Appetites of *Ibicella* And *Drosophyllum*

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Abstract

A simple method of detecting enzymes was used to test thirteen species. As expected, *Drosera*, *Dionaea*, *Pinguicula*, and *Drosophyllum* were all shown to secrete digestive enzymes. The method was tested on noncarnivorous plants and controls. *Ibicella lutea* and *Proboscidea parviflora* are in the *Martynia* family, and are sometimes suggested to be carnivorous. *Ibicella lutea* and *Proboscidea parviflora* showed no enzymatic activity.

Introduction

In 1997, Siegfried Hartmeyer discovered that *Byblis liniflora* does not produce digestive enzymes (Hartmeyer, 1997), so *B. liniflora* is not strictly a carnivorous plant. Hartmeyer established the hypothesis that it requires the aid of arthropods to benefit from its captured prey (i.e., Hartmeyer, 1998). While his results were fascinating, the experimental method he used, first developed by Heslop-Harrison & Knox (1971), was particularly remarkable because it involved a technique of enzyme testing that is so simple, anyone with a pair of scissors, tape, and inexpensive black and white film could perform it!

In summary, this is Hartmeyer's approach. He stimulated the leaves of carnivorous plants into producing digestive enzymes by smearing them with a yeast solution. Then he placed photographic film (right out of the roll with no processing) in contact with the stimulated leaves. The digestive enzymes from the leaves digested the protein layer of the film, so after twelve hours or so parts of the previously opaque film became transparent. Subsequent photoprocessing was optional.

Procedure

I decided to try the enzymatic test. I bought a roll of Ilford HP5 ASA 400 film (as Hartmeyer recommended) and a packet of baker's yeast. I made a 10% solution of yeast by mixing 7 grams of yeast with 70 ml of distilled water. For each leaf tested, I did the following. 1)I smeared a few drops of yeast solution onto a leaf. Instead of waiting several hours as did Hartmeyer, I immediately proceeded with the next step. 2)I taped one edge of a 2-4 cm length of film to a paper backing. 3)I coded the film with holes from a deftly wielded pin and a hole-puncher. 4)I delicately sandwiched the stimulated leaf between the film and the paper, and taped the other edge of the film to the paper backing. 5)If the film and paper sandwich was too heavy for the plant, I affixed the sandwich to an appropriate support pole. 6)I recorded the details of the trial in my notes, referring to the code I made in step two. 7)I returned the plant to its normal location in the greenhouse for 24 hours before removing the film sandwich.

Some important but mundane matters should be mentioned. First, photographic film consists of an inert plastic layer that functions as a support for the emulsion. When preparing the individual tests, I took care that the dull emulsion

side—and not the shiny plastic side—was pressed against the leaf. Even a hungry carnivorous plant cannot digest plastic! Second, I used acid-free archival cardboard for the backing in each test (specifically, the sturdy paper used in mounting herbarium specimens). Third, when making each film-leaf-paper sandwich, I used the tape to make the sandwich snug enough so it would not slip off the leaf, but not so snug that the leaf was crushed. Finally, as an alternative to yeast, I experimented with using a dilute solution of Bovine Serum Albumin (BSA) to stimulate the plants' leaves. The results in all cases were identical to my yeast trials. (As I dripped BSA onto the glistening leaves I enjoyed thinking how, decades after I killed my first Venus Flytraps by giving them hamburger, I was once again feeding cows to my plants, albeit in the guise of high science!)

The first group of tests included those plants I thought would certainly demonstrate enzyme production. Specifically, I tested *Dionaea muscipula*, *Drosera adelae*, *D. binata* var. *multifida* f. *extrema*, *D.* × *californica*, *D. capensis* (red- and wide-leaved clones), *D. spatulata*, *D. venusta*, and a Mexican *Pinguicula* hybrid of unknown parentage (but obviously closely related to *P.* 'Sethos'). *Dionaea* was tested by feeding the traps small pieces of photographic film which were retrieved from the traps when they reopened a week later. A total of twenty-four yeast and BSA trials unanimously shouted these plants were carnivorous. In Figure 1 I show the results of a test using *Drosera capensis* (a red-leafed clone). The positive enzyme secretions are indicated by the clear spots digested into the normally black opaque emulsion.

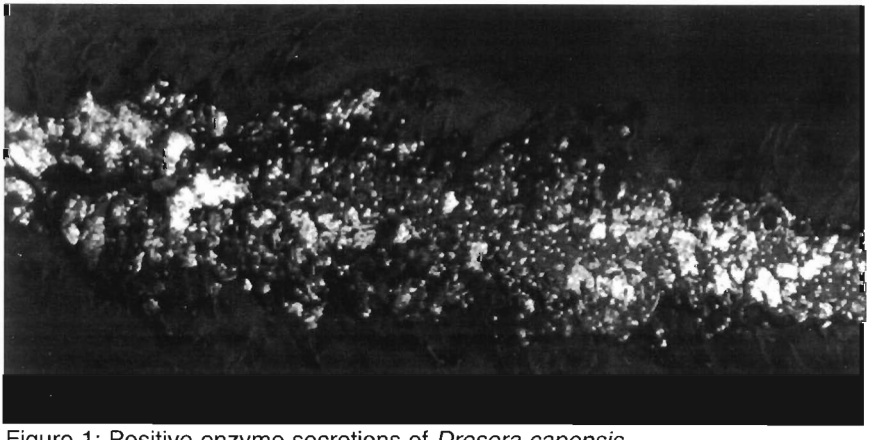

Figure 1: Positive enzyme secretions of *Drosera capensis*.

Six control tests were made upon *Abutilon* × *hybridum* 'Sugar Plum' (a non-carnivorous Malvaceous species), and four control tests were made using no plant at all (yeast solution or BSA was applied directly to the film's emulsion). No relevant emulsion damage was observed in these tests. These control tests demonstrated that a lack of enzymatic activity was properly indicated by the method. As a bonus, these control tests illustrated that when kept wet for 24 hours, film emulsion becomes delicate and is easily damaged. Do not mistake such damage for enzyme activity!

The third test group consisted of three species which particularly interested me: *Drosophyllum lusitanicum*, *Ibicella lutea*, and *Proboscidea parviflora*. In each of its five tests, the *Drosophyllum* digested all the emulsion it contacted (Figure 2) and left only the transparent plastic substrate—the evidence of enzymes was clear! Seven trials were made of *Ibicella* and seven of *Proboscidea*, targeting leaves both very young and mature. At the end of twenty-four hours the films showed numerous tiny clear dots or dashes, unlike any seen in the other tests (Figure 3). However,

Figure 2: Positive enzyme secretions of *Drosophyllum*.

these dots were not the result of enzymes. Instead they were caused by the leaf hairs being driven into the emulsion (although I could not tell if the marks were due to the glandular or the longer, eglandular hairs). The stiff hairs had left their imprints in the emulsion! (As noted above, after a day of exposure to water, the emulsion layer becomes mushy and very susceptible to such mechanical damage.) In some cases I used paper clips to hold the film sandwiches onto the leaves, and the clear marks were often clustered around where the paper clips had been, further indicating the marks were a result of mechanical damage and not enzymatic activity. Soaking the leaf with water so the film detached more easily still resulted in some tearing damage.

Ibicella and *Probiscidea* tests in which film was left on the leaves for another twenty-four hours still resulted in no enzymatic damage to the film.

Discussion

My tests have verified the effectiveness of Hartmeyer's method of enzymatic detection, and extended them to show enzymatic activity in a few new genera. A significant oversight in Hartmeyer's work was a lack of appropriate null tests. I have addressed this by showing his method does not falsely detect enzymes when none are present. While he did test *Roridula* as a noncarnivorous species, this plant has a suspicious history in the annals of plant carnivory, and also damaged the emulsion mechanically. More appropriate controls needed to be tested.

Plants in the Martyniaceae, in particular *Ibicella lutea*, did not show any indication of producing digestive enzymes. Is *Ibicella* just another sticky, but non-carnivorous plant? Probably. But it might be carnivorous in one of three ways and still have slipped past my enzyme tests.

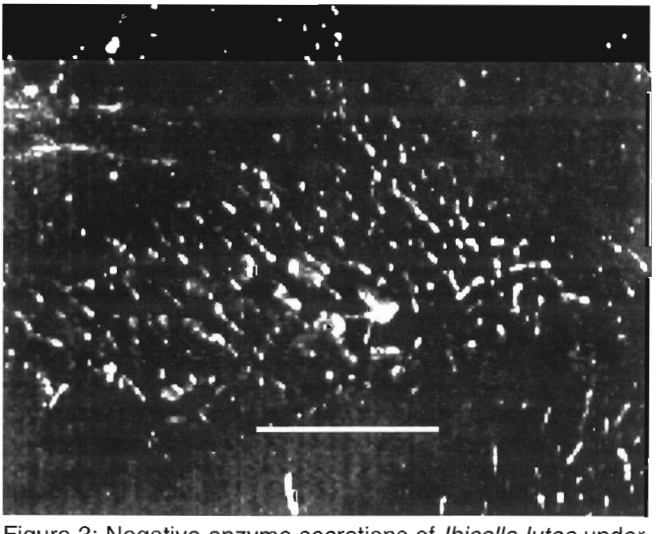

Figure 3: Negative enzyme secretions of *Ibicella lutea* under high power. The horizontal bar indicates 1 mm.

First, it is possible *Ibicella* is not carnivorous its entire life and I may have tested it during the wrong time. While both young and old leaves were tested for enzyme activity, might it be that overall plant age is relevant? For example, *Triphyophyllum peltatum* is usually carnivorous only prior to

Figure 4: A close-up view of a leave of *Ibicella lutea*, with trapped whitefly and fungus gnats.

changing its growth form into a climbing plant. However, unlike *Triphyophyllum*, *Ibicella* has no habit change so clearly marked. Leaves on branches bearing flowers, fruit, or no reproductive organs were all tested. No enzymatic behavior was detected.

Second, it is possible only specific parts of the plant are carnivorous and were overlooked by my tests. Indeed, small insects are trapped on all parts of the plants except the petals (Figure 4), but only the leaves were tested. Nonetheless, the trapped insects on these untested areas do not appear any more digested than the insects on the leaves. If carnivory were a factor, it would be much more likely to occur over the large overall leaf surface area than the relatively small stem, petiole, or inflorescence surface areas.

Finally, it is possible that *Ibicella* requires an arthropod analogous to those observed on *Roridula* (Hartmeyer, 1998). No arthropod candidates were observed on the plants grown outdoors in Davis, California or Tucson, Arizona. It may be that the appropriate arthropods are only found in the plant's native range in South America, but no such fauna has ever been observed on the related *Proboscidea*, which I have grown for many years well within its native range.

In conclusion, I have found no sturdy evidence that *Ibicella* and *Proboscidea* are carnivorous. Personal communication with Jan Schlauer (1998), revealed he had been unable to detect any enzymatic activity when he applied peptone to the leaves of *Ibicella lutea* and *Proboscidea louisianica*. These are interesting plants, but I have no room for them in my carnivorous garden. The seeds I will send to the ICPS seedbank will be my last.

I would like to thank Tim Metcalf and the staff at the University of California at Davis Botanical Conservatory for the use of their facilities for this experiment. A particular apology is due to any of the staff who brushed against the foul-smelling *Ibicella lutea* plants the long year I grew them at the greenhouses.

References:

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