

Evidence of Protocarnivory in Triggerplants (*Stylidium* spp.; Stylidiaceae)

D. W. Darnowski¹, D. M. Carroll², B. Płachno³, E. Kabanoff⁴, and E. Cinnamon¹

¹ Department of Biology, Indiana University Southeast, 4201 Grant Line Road, New Albany, IN 47150, USA

² Department of Immunology and Microbiology, Rush University Medical Center, 600 South Paulina St. Suite 440, Chicago, IL 60612, USA

³ Department of Plant Cytology and Embryology, Jagiellonian University, 52 ul. Grodzka, 31-044 Krakow, Poland

⁴ Microscopy and Image Analysis Centre for Horticulture and Plant Sciences (CHAPS), University of Western Sydney, Hawkesbury Campus, Bldg S8, Locked Bag 1797, Penrith South DC, NSW 1797, Australia

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Abstract: Australian triggerplants (*Stylidium* spp.; Stylidiaceae) trap small insects using mucilage-secreting glandular hairs held at various points on their inflorescence stems and flower parts. Triggerplants are generally found in habitats also containing genera of plants already accepted as carnivorous, two of which (*Drosera*, *Byblis*) use the same basic mechanism as *Stylidium* to trap their prey. In the herbarium, sheets of triggerplants and of accepted groups of carnivorous plants held similar numbers of trapped insects, and in the field, trapping of small prey per unit of glandular surface area was the same at a given site for triggerplants and for nearby carnivorous plants at three sites in northern Australia. Even more important, protease activity was produced by glandular regions of both triggerplants and *Drosera* after induction with yeast extract. A panel of negative and positive controls, including use 1) of plants grown in tissue culture, which therefore lack surface microorganisms, and 2) of protease inhibitors, shows that this activity 1) is generated by the glandular regions of the triggerplant itself, not by organisms that might reside on the surface of the plants, and 2) is due to proteases. All of this evidence taken together provides strong evidence of protocarnivory in *Stylidium*, something not previously suggested in the scientific literature, though the insect trapping has been noted informally. Experiments remain to be done to determine nutrient uptake, so triggerplants may well be fully carnivorous.

Key words: *Stylidium*, triggerplant, carnivorous, protocarnivorous, Australia, insectivorous, *Drosera*, protease activity.

Introduction

Carnivory in plants involves the trapping and digestion of prey, ranging from unicellular organisms to small mammals, though by far the most common prey are insects (Juniper et al., 1989). In order to be carnivorous, a plant must attract, trap, and digest prey, followed by nutrient absorption. This standard was most clearly laid out by Lloyd (1976), who also created a list that is often used as a canon of accepted genera of carnivorous plants

and was longer than that used by Darwin (1893) for his seminal work. Plants which trap prey but depend on assistance for digestion are given names such as “subcarnivores” or “protocarnivores” (e.g., Spomer, 1999; Heinrich et al., 2002). This paper presents evidence that Australian triggerplants (*Stylidium* spp.; Stylidiaceae), not previously suspected of being carnivorous plants, are at least protocarnivorous. They display these abilities for part of their life cycle, and some authors classify them with *Triphyophyllum peltatum* as “part-time” carnivorous or subcarnivorous plants. For this paper, “insectivorous” and “carnivorous” are used synonymously.

Trapping and digestion are the most crucial stages for demonstrating carnivory, since attraction must have occurred for plants to have trapped insects, and many plant surfaces will absorb nutrients placed on them. A variety of active and passive mechanisms exist for trapping prey. A well-known example of an active mechanism is that of the Venus flytrap, *Dionaea muscipula* (Droseraceae), which possesses modified leaves, the lobes of which close on prey when trigger hairs are touched. A passive example is found in the New World pitcher-plants (*Sarracenia* spp., *Heliophora* spp., and *Darlingtonia californica*; Sarraceniaceae), which present pitfall traps filled with water or digestive fluid.

Sundews (*Drosera* spp.; Droseraceae) use traps which are at least partly passive in their action, consisting of leaves covered with glandular hairs that secrete mucilage. When prey land on the mucilage, they become trapped. Leaves are induced by the presence of trapped insects to secrete enzymes for digestion of the prey. In the laboratory, various substances can substitute for prey, including aqueous solutions of yeast extract. Other genera of carnivorous and subcarnivorous plants that use the same basic mechanism of trapping include *Byblis* (Byblidaceae), *Pinguicula* (Lentibulariaceae), *Roridula* (Roridulaceae), *Drosophyllum* (Droseraceae), and *Triphyophyllum* (Dioncophyllaceae; Lloyd, 1976; Bringmann et al., 2001, 2002).

Similar passive traps are found in a number of other genera, some of which are considered to be non-carnivorous. These include some wild members of the Solanaceae, as well as *Probo-scidea* (Martyniaceae). However, these non-carnivorous plants are often, as in the case of members of the Solanaceae, only able to secrete resin rather than mucilage. Resin is unable to support the digestive activity found in mucilage-secreting carnivorous plants (Juniper et al., 1989).

Triggerplants have not previously been examined for carnivory. Triggerplants are found primarily in Australia, with a few species occurring to the north of Australia. *Stylidium* spp. range widely in their habit, from minute species only a few cm tall to moderately tall plants like the tree triggerplant, *S. laricifolium*, that can grow to 1.5 m in height. Triggerplants reside in areas with alpine, tropical, and Mediterranean climates (Erickson, 1981; Gibson, 1990). In general, they grow on the same nutrient-poor, seasonally, or permanently wet soils, as do accepted genera of carnivorous plants.

At the centre of the *Stylidium* flower runs a column formed from the fused filament and style, which can be *triggered* into seismonastic motion, thus giving the genus its common name. Stimulation occurs when insects seeking nectar rub their proboscises against the posterior side of the column, or otherwise jostle this general region, thereby initiating the trigger-like mechanism. This mechanism is solely for pollination (Findlay and Findlay, 1975; Erbar, 1991) and is uninvolved in carnivory.

The stalked, mucilage-secreting glands of triggerplants are present on peduncles, pedicels, sepals, and/or other parts, depending on the species, and triggerplants share habitats with several genera accepted to be carnivorous such as *Drosera* and *Utricularia* (bladderworts; Lentibulariaceae). Since nutrient-poor soils, particularly wet soils lacking nitrogen, are often associated with the presence of carnivorous plants, the presence of a carnivorous assemblage near triggerplants on such soils in the wild serves as a first indicator that they might be carnivorous.

Although *Stylidium* spp. have been known to western science for several centuries (Morren, 1838), little, if any, research has been done on the mucilage-secreting glands and their function. To assess whether they are indeed carnivorous, a biochemical analysis of the glandular regions of these plants, including their secretions, was performed. Proteases found on the surface of insectivorous plants are needed for the digestion of animal protein and thus N (Heslop-Harrison and Knox, 1970). The secretion of this class of enzymes is a good indicator of carnivory and, as a result, biochemical proof of carnivory usually relies on studies of proteases, although other digestive enzymes are known to be secreted by carnivorous plants (Juniper et al., 1989). Studies utilizing the substrate film method to localize proteases have resulted in the confirmation of carnivory in genera such as *Pinguicula* (Heslop-Harrison and Knox, 1970). This method qualitatively determines the presence of enzymatic activity using the fact that exposed, processed photographic film is comprised of pigmented, proteinaceous gelatin layers and is therefore subject to digestion by proteases. In the presence of proteases, at high humidity incubation, the film is digested, revealing clear holes that appear as the exposed and developed photographic emulsion is removed by enzymatic activity. Coarser grained, higher ASA film yields clearer results than lower speed films (Fratello, 1968; Hartmeyer, 1997).

Materials and Methods

Species used

Due to the restricted flowering time for many *Stylidium* species, it was necessary to use different species in different parts of this work. When it was not possible to use the same species, species from the same sections of the genus *Stylidium*, one tropical/northern section (*Debilis*; Lowrie and Kenneally, 1996) and one temperate/southeastern section (Lineares; Mildbraed, 1908), were used.

Determination of mucilage

To confirm that *Stylidium* species secrete mucilage, not resin, and are therefore capable of secreted protease activity, two species were tested using the periodic acid/Schiff test (Sigma, St. Louis, MO, USA; #3951), which gives a dark purple colour when mucilage is present and pink-purple colour when reducing sugars are present. For a negative control, water only was used, while for positive controls, sucrose, *Drosera adelae* and *Pinguicula ehlersae* glandular hairs were used. The last two are mucilage-secreting carnivorous plants.

SEM

In order to illustrate the structure of the glandular hairs, the tropical *S. fimbriatum* and temperate *S. graminifolium* were examined by SEM. Live floral specimens were frozen in liquid nitrogen, critical point dried, coated with gold, and examined in the SEM.

Trapping of prey

Based on initial observation of gnats and other small insects trapped on inflorescences of cultivated triggerplants, principally on tropical *S. fimbriatum*, further observations were made in the field and the herbarium. The southeastern species *S. lineare* and *S. productum* were examined in the field in the Blue Mountains of New South Wales, near Leura, for the presence of trapped insects. Specimens were similarly examined in the herbarium at the Royal Botanical Gardens in Sydney. For the herbarium specimens, plants known to be carnivorous and using the same passive trapping mechanism as *Drosera* and *Stylidium* were examined, together with non-carnivorous plants with similar hairs and non-carnivorous, non-glandular species. Trapping was estimated as abundant (+++) through to sparse (+) or non-existent (-), and as variable from plant to plant (+/-), occurring on some plants for a herbarium sheet but not on others. In addition, different groups of Australian sundews were included, since carnivorous Australian pygmy, tuberous and *petiolaris* type sundews trap fewer insects than annual species of Australian sundews.

For more quantitative determination of trapping, three sites over approximately 1000 km in northern Australia were examined which contain both triggerplants and at least one genus of carnivorous plants that uses the same trapping mechanism. The number of insects trapped was counted for a number of plants, and the surface area of the glandular region was estimated by treating *Drosera* leaves as circular, *Byblis* leaves as rectangular, and *Stylidium* inflorescence stems as tubular. Measurements of radius, length, and/or width were made as

appropriate and the number of prey per m² was calculated based on the modelled geometric shapes.

Digestion of protein

Tropical *S. fimbriatum* and southeastern *S. laricifolium* were the two species used to test for digestion of trapped insects. *S. fimbriatum* has a long, slender inflorescence topped by several serially-opening flowers. Its dark pedicels are densely glandular nearer to the flower. The flowers have bright pink petals with a few glandular hairs on their abaxial surfaces (Fig. 1). *S. laricifolium*, on the other hand, forms a small bush, with scapes lacking glandular hairs. Glands are present most densely at the base of the pedicel but more sparsely on the sepals, petals, and labellum (Erickson, 1981). The respective glandular regions on each of these plants were tested for proteolytic activity. *S. laricifolium* was raised from seed and cuttings, while *S. fimbriatum* was micropropagated on 0.2 × MS/B5 medium (Sigma, St. Louis, MO, USA; M0404), pH 5.8, 3% sucrose, 0.8% Bactoagar, based in part on McComb (1985). Some plants were left in aseptic culture for testing while others were placed on a mixture of 3:3:3:1 sphagnum peat:long fibre sphagnum:silica sand:commercial peat-based soil-free potting mix (brands of components varied) in a terrarium.

Enzymatic secretion was induced by priming the leaves of the plant with a solution of Difco yeast extract (Becton Dickinson, Sparks, MD, USA), using methods similar to those described by Hartmeyer (1997). Serial dilutions of a saturated solution of yeast extract were painted onto leaves of *D. capensis* to determine the concentration that induced optimal digestion, and 2% (aq, w/v), was used for all other experiments. It is important to note that tests of the yeast extract showed that it lacked proteolytic activity under the assay conditions used (data not shown).

The approximate pH of the surfaces of induced leaves was determined using pH paper in order to choose the buffer for protease assays. For the substrate film test, pieces of exposed and developed Kodak (Rochester, NY, USA) Max 400 ASA colour film were pre-moistened with 0.15 M KH₂PO₄ buffer at pH 7.

For each treatment, plant material was induced for 24 h after being painted with a 2% yeast extract. Then, induced parts were excised and mounted in humidified dishes for 24 h on separate pieces of the pre-moistened processed film. Each treatment was conducted in a separate dish. Negative controls included non-induced and induced leaves of *Citrus* sp. (non-carnivorous) and non-induced leaves of *Drosera capensis*. Positive controls included McCormick™ Meat tenderizer (McCormick & Co., Hunt Valley, MD, USA), induced leaves of *D. capensis*, and frozen-thawed pieces of papaya. Both frozen-thawed papaya and McCormick™ Meat tenderizer contain abundant papain, a chymotrypsin-like protease found in papaya (Stryer, 1988). No induction was required for those controls.

The pieces of mounted film were gently removed from plant material, rinsed with 25°C water, and air-dried. Great care was taken to avoid tearing or otherwise damaging the moistened emulsion, which would have caused false positive readings. For reproduction, the pieces of film were scanned using a flatbed scanner. Since digestion was usually complete or nearly complete, visualization of different colours from the



Fig. 1 Flower of *Stylidium fimbriatum*, showing various parts and regions including the location of glandular hairs. (G) Area where glandular hairs are especially common in this species. Flower is approximately 1 cm wide.

colour negatives was not crucial. For simplicity, images were converted to grey-scale, in which complete digestion appears as a white region, nearly completely digested are pale grey, with other regions appearing darker.

To determine whether any of the observed proteolytic activity was due to microbes on the surfaces of the soil-grown plants used in most experiments, aseptically-cultured plants of *S. fimbriatum* were also tested for carnivory. To further validate any removal of the film gelatin layers from digestion by proteases, in some experiments a general cocktail of protease inhibitors (Sigma; P2714) was applied in excess to the film after it was dampened with buffer but prior to mounting plant material. The inhibitor cocktail contained 2.0 mM AEBBSF (inhibits serine proteases), 1.0 mM EDTA (inhibits metalloproteases), 130 µM Bestatin (inhibits aminopeptidases), 1.4 µM E-64 (inhibits cysteine and thiol proteases), 1.0 µM Leupeptin (inhibits serine and thiol proteases), and 0.3 µM Aprotinin (inhibits serine proteases).

Results

Mucilage

Stylidium glandular hairs produced abundant mucilage, which gave a dark purple colour very similar to that from *Drosera* and *Pinguicula* (Table 1). The hairs are most numerous on the inflorescence stem and back of sepals (Fig. 1), are not as large as those of *Drosera*, but are of similar size and complexity to those of *Pinguicula* (Fig. 2).

Table 1 Chemical tests of mucilage using the periodic acid/Schiff (PAS) test. Various materials were tested using the PAS test for the presence of mucilage. Water only was used as a negative control, and both *Drosera* and *Pinguicula* were used as the carnivorous plants which secrete mucilage. Sucrose should also react positively due to the presence of appropriate chemical groups. *Stylidium debile* and *S. candelabrum* are in the same sections of the genus as *S. laricifolium* and *S. fimbriatum* that were used for protease tests

Material	PAS result
Water only	negative
Sucrose	positive
<i>Drosera adelae</i> F. Muell. exudate	positive
<i>Pinguicula ehlersae</i> Speta and Fuchs exudate	positive
<i>Stylidium debile</i> F. Muell. exudate	positive
<i>Stylidium candelabrum</i> Lowrie and Kenneally exudate	positive

Trapping of prey

Plants of *S. lineare* and *S. productum* in the field trapped numerous small insects, with > 6 on some scapes. These insects were generally much smaller (estimated at 0.1 × the size) than those which visited flowers and triggered pollen transfer. Trapping varied widely from population to population, with some populations capturing no prey and others catching the higher numbers previously mentioned. It should also be noted that the area examined had experienced dry conditions for some time, which could have affected mucilage properties.

Herbarium specimens of many species from the Royal Botanical Gardens, Sydney retained trapped insects (Table 2). This was particularly true for *S. hispidium* and *S. musicola*, which trapped not abundantly but measurably, similar to some annual sundew specimens such as *Drosera burmanii* and *D. glanduligera*, not to mention pygmy, tuberous and *petiolaris* type sundews. These last types usually trap fewer prey in the field than annual members of their genus, for reasons unknown, as also found in herbarium sheets. The trapped insects were generally much smaller than those that pollinate triggerplants. Table 2 shows the results from the examination of these and other plants, including three major types of sundews found in Australia (annual, pygmy, tuberous).

Trapping in the field in northern Australia again showed strong similarity among *Stylidium* spp., various types of *Drosera*, and other carnivorous plants (Table 3) on the basis of the surface area of their glandular regions.

Digestion of prey

Soil-grown *S. laricifolium* and *S. fimbriatum* only digested film when induced. The same was true for leaves of *D. capensis*. Citrus leaves, induced or not, failed to digest the emulsion, but papaya and meat tenderizer completely digested the layers of the film (Fig. 3 and additional data not shown). These results were replicated several times.

In all trials, soil-grown *Stylidium*, *S. laricifolium*, yielded the most complete digestion, but *S. fimbriatum* grown *in vitro* showed the most complete digestion (Fig. 3 and additional data

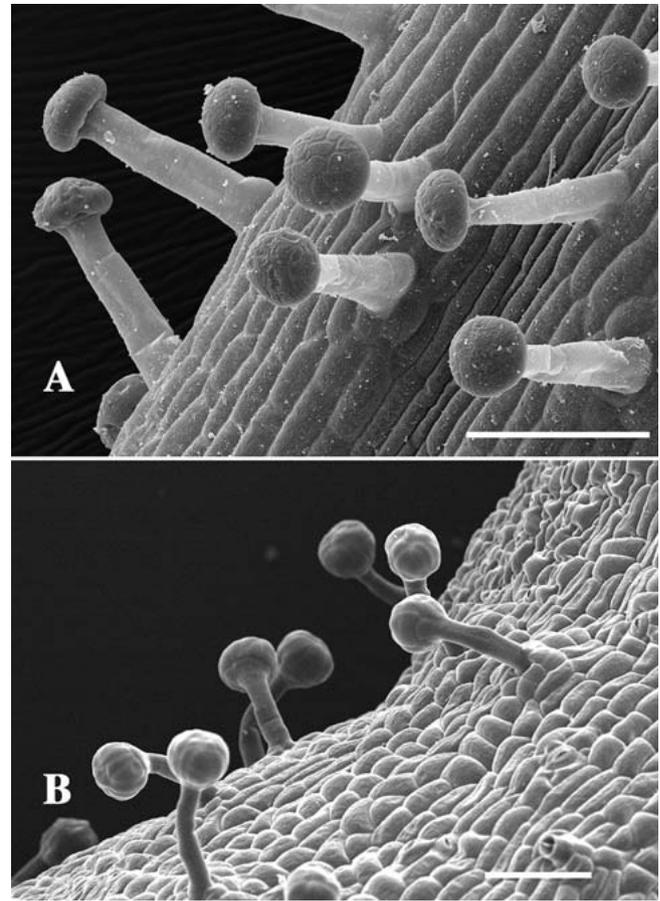


Fig. 2 Glandular hairs of two species of triggerplants. The glandular hairs of triggerplants share a general structure, consisting of a stalk made by two parallel rows of cells leading up from the epidermis and a glandular head of 10–20 cells which varies in roundness/flatness. (A) *Stylidium fimbriatum*. (B) *S. graminifolium*, which comes from the same section of the genus as *S. laricifolium*, which was used for protease assays. Bars = 100 µm.

not shown). In all the trials involving glandular plant material, digestion was concentrated in areas possessing glands.

Discussion

Mucilage

Since *Stylidium* spp. produce mucilage (Table 1), not resin, they are capable of being carnivorous plants. Resins are unable to support the activity of digestive enzymes, while mucilage is able to do so (Juniper et al., 1989).

Trapping

Trapping by most triggerplants was not as vigorous as in Australian annual sundews, though some herbarium specimens had trapped insect numbers similar to samples from annual sundews. Qualitatively, trapping was highly similar to that of carnivorous pygmy, tuberous and *petiolaris* type and sundews from Australia in the carnivorous genus *Drosera*. This leads to the interesting question of the relative ecological importance of carnivory for triggerplants. Perhaps extra nutrients

Table 2 Insects trapped on leaves of various plants. Specimens were examined in the Herbarium of the Royal Botanical Gardens, Sydney, New South Wales, Australia, and classified according to 1) known carnivory and 2) presence of glandular hairs. The number of insects trapped per preserved plant was determined by visual inspection. Insects were trapped: – not at all; + one or two per plant preserved; ++ 3–6 per plant; +++ numerous. The number of different sheets examined for each species is indicated in parentheses. Since many sheets were typically available for any one species, one or more sheets were selected at random. n.a. = not applicable

Species	Type of sundew	Carnivorous, glandular hairs	Trapping
<i>Dolichandrone heterophylla</i> (R. Br.) F. Muell. (1)	n.a.	no, no	–
<i>Proboscidea louisianica</i> (Mill.) Thell. (1)	n.a.	no, yes	+++
<i>Drosera burmannii</i> Vahl (2)	annual	yes, yes	+ / ++
<i>D. glanduligera</i> Lehm. (1)	annual	yes, yes	+++
<i>D. platystigma</i> Lehm. (1)	pygmy	yes, yes	+ / –
<i>D. pygmaea</i> DC. (1)	pygmy	yes, yes	+ / –
<i>D. gigantea</i> Lindl. (1)	tuberous	yes, yes	+ / –
<i>D. macrantha</i> ssp. <i>planchonii</i> N. Marchant (1)	tuberous	yes, yes	+ / –
<i>Stylidium laricifolium</i> Rich. (3)	n.a.	?, yes	– / +++
<i>S. brunonianum</i> Benth. (3)	n.a.	?, yes	–
<i>S. hispidum</i> Lindl. (1)	n.a.	?, yes	+
<i>S. roseo-alatum</i> Erickson and Wills (1)	n.a.	?, yes	–
<i>S. calcaratum</i> R. Br. (3)	n.a.	?, yes	+ / –
<i>S. graminifolium</i> Swartz (1)	n.a.	?, yes	+ / –
<i>S. musicola</i> F. Muell. (1)	n.a.	?, yes	++
<i>S. affine</i> Sond. (1)	n.a.	?, yes	+

Table 3 Trapping by known carnivorous plants and triggerplants at three sites across Northern Australia. One site was in the Northern Territory (Darwin) while the other two were in Western Australia. For normalization, the lowest number trapped per unit area was set to 1 and other data normalized to it. Given the current state of uncertainty in names for tropical *Stylidium* spp. due to many new names being recognized, up to three different species were found at each site with similarities to *S. leptorrhizum*. These were counted separately but are not given different specific epithets. The same is true for species of *Drosera* from the *petiolaris* complex. Annual species of sundews are indicated

Site	Species	Number of plants	Mean 10 ³ (prey) (m ⁻²)	SD (as % of mean)	Normalized by site
Darwin	<i>Drosera</i> aff. <i>petiolaris</i>	2	170	77	1.0
	<i>Stylidium</i> aff. <i>leptorrhizum</i>	20	170	70	1.0
Kununurra	<i>Drosera burmannii</i> Vahl	3	1.9	84	1.0
	<i>Drosera</i> aff. <i>petiolaris</i>	4	7.3	47	4.0
	<i>Byblis</i> sp.	3	4.1	38	2.2
	<i>Stylidium</i> aff. <i>leptorrhizum</i> 1	10	4.6	58	2.5
	<i>Stylidium</i> aff. <i>leptorrhizum</i> 2	10	4.9	56	2.7
Broome	<i>Stylidium</i> aff. <i>leptorrhizum</i> 3	11	11	77	6.0
	<i>Drosera indica</i> L.	5	7.4	67	2.2
	<i>Drosera</i> aff. <i>petiolaris</i>	6	19	18	6.0
	<i>Byblis</i> sp.	6	12	55	3.6
	<i>Stylidium</i> aff. <i>leptorrhizum</i>	9	3.3	86	1.0

are shuttled to developing seeds, thus the restriction of glandular hairs to inflorescences in most species, and/or nutrients, are shared with symbiotic mycorrhizae. In general, tropical triggerplants seem to trap more prey than temperate species (Table 2), and this deserves further study. Expression of carnivory during only part of the life cycle is also known in *Triphyophyllum peltatum* (Dioncophyllaceae), a liana from tropical West Africa (Green et al., 1979; Bringmann et al., 2001, 2002).

Trapping of insects by triggerplants in the field varied widely, both from population to population near Leura, New South Wales (Table 2), and from plant to plant in the populations described in Table 3. The reason for variation in New South Wales may have to do with weather, as long-term drought had occurred in that area. Triggerplant mucilage may have lacked sufficient moisture to provide the appropriate viscosity for trapping in some populations, while others may have been in more humid locations that provided the mucilage with sufficient moisture for normal function. Indeed, the best trapping

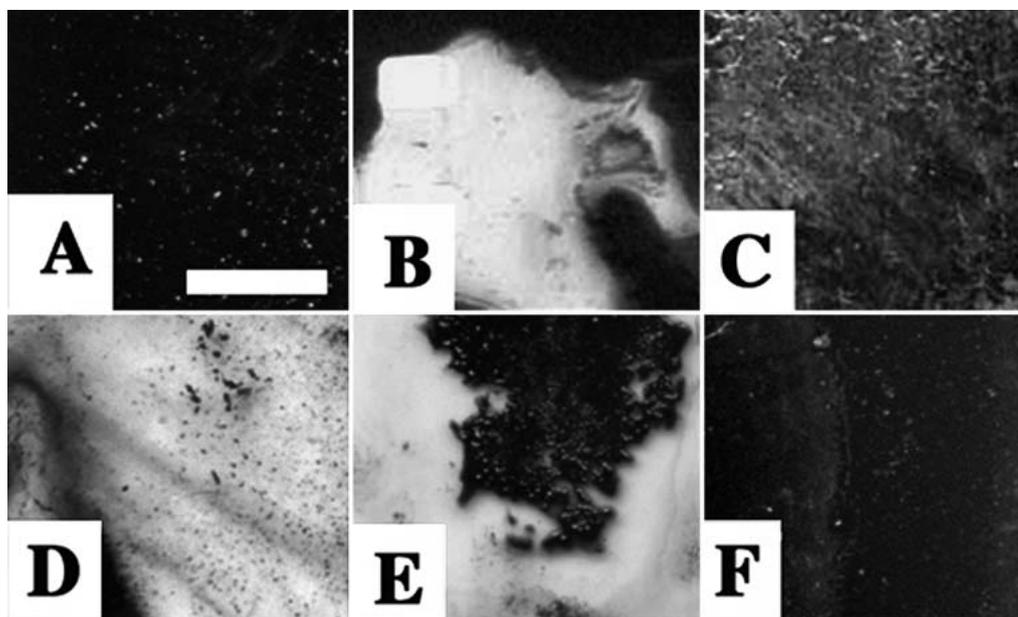


Fig. 3 Digestion of photographic film emulsion treated with various plant materials. As described in the results, 400 ASA colour negative film was pre-moistened and incubated with various materials. Shown are parts of treated film, scanned and converted to grey scale for simplicity since digestion was usually complete or nearly so. As the leaves, flowers or other materials applied did not cover the entire strip of film, the portion shown is part of the area which was covered by plant material plus part of the adjacent uncovered area for comparison. Where digestion occurred, as evidenced by a clearing to a white or grey colour, part of the edge of the limit of digestion is shown, so that the appearance of undigested film is also seen. Some panels show light spots

in a dark area. These are generally produced by physical contact, even light physical contact, between the moistened film and plant material. Areas which were well digested by positive controls showed areas of significant clearing, as in **B** with papaya. (**A**) Yeast extract-primed citrus leaf, negative control. (**B**) Papaya, positive control. (**C**) Papaya, treated with protease inhibitors. (**D**) Yeast extract-primed leaf of sundew, positive control. (**E**) Yeast extract-primed pedicel and sepal of *Styloidium fimbriatum* grown *in vitro*. (**F**) Yeast extract-primed pedicel and sepal of *Styloidium fimbriatum* grown *in vitro*, treated with protease inhibitors. Bar = 0.5 cm.

was seen in plants growing either in shaded areas, where humidity might remain higher during the day, or moist gullies.

The variation in trapping from plant to plant within the three sites can be seen from the high standard deviations in Table 3. This might be due to differences in exposure of the inflorescences to illumination or some other natural factor which affects the attraction mechanism involved. What this mechanism of attraction is – whether light shining from mucilage drops, UV markings, or scent – still needs investigation, particularly in the field.

Trapping also varied from site to site in northern Australia, but all populations examined there had trapped some insects. This variation might have been due to the same atmospheric factors described above, as well as to variation in the availability of insects across such a wide area. The critical fact is that trapping was highly similar for triggerplants and for other accepted carnivorous and subcarnivorous genera within each site, where the same populations of insects would have been available to the plants.

In general, whether in the field or the herbarium, by far the most prey trapped were small and delicate insects, most probably Australian members of the Bibionomorpha (gnats) or Culicoida (midges; Brunet, 2000). This makes some sense as the glandular hairs of *Styloidium* are relatively short, perhaps best adapted to trapping physically delicate prey as opposed to

more robust insects such as bees, flies, or beetles. Taken together, these results demonstrate that several species of triggerplants used the glandular, mucilage-secreting hairs on different parts of their inflorescences to trap small animal prey, mostly insects, and that this trapping was very similar to that performed by accepted genera of carnivorous plants.

Field and laboratory studies are needed to address this issue by following the uptake of labelled material by triggerplants, for example by following labelled amino acids through autoradiography, fluorescence microscopy, or metabolomic analysis using ^{13}C -labelled compounds. These studies are all in the planning stages or already underway. Also needed and planned are studies of the enzymology of the digestive secretions of *Styloidium* to identify which classes of proteases are secreted; whether other digestive enzymes, such as nucleases, are secreted; and examination of possible carnivory in the glandular sister genus *Levenhookia*.

Digestion

More importantly for the demonstration of carnivory in triggerplants, two very different triggerplants, the temperate *S. laricifolium* and the tropical *S. fimbriatum*, from two very different sections of the genus (Mildbraed, 1908; Lowrie and Kenneally, 1996) digested protein after induction. This behaviour was identical to that of the carnivorous sundew, *D. capensis*, and digestion was not due to the presence of yeast extract

since primed leaves of *Citrus* sp., having yeast extract on their surfaces, did not digest the film.

The positive results obtained from *S. fimbriatum* grown in culture rule out the possibility of digestion having been performed by microorganisms that could reside on the plant surface in natural conditions or when plants are grown on soil in terraria or the greenhouse. No microbial growth could be observed in the cultures that were only opened during the time of the repetition of these experiments. Also, results were consistent from the first to the last, which would not have been the case if microbial contamination had occurred. The *Citrus* leaves were handled similarly, including mock induction using yeast extract, and still did not display any digestion of the emulsion.

It might also be argued that digestion was due simply to acid hydrolysis. However, the cessation or significant reduction in proteolysis when a mixture of protease inhibitors was applied strongly indicates that proteases were secreted by the triggerplants.

From the observation that digestion was more appreciable on areas of the film where glands were present, it can be inferred that digestion originated from these stalked glands in both species of *Stylidium* tested. Digestion in adjacent areas probably occurred due to wicking of the digestive fluid for short distances along the photographic film. Both undigested and digested areas are shown in Fig. 3, showing, for positive tests, the edge of the area digested and the edge of the place where plant material contacted the film. Pedicels of fully developed flowers exhibited more enzymatic activity than those of undeveloped flower buds. This suggests that the enzymatic activity of the glands increases with the maturity of the flower or inflorescence, but this intriguing point deserves further study, as does the contrast in the size of prey and the size of pollinators. Taken together, this indicates that the two most crucial parts of carnivory in triggerplants, trapping and induced digestion, are highly similar to *Drosera* spp. and other carnivorous/subcarnivorous plants. Added to the consistent occurrence of triggerplants and carnivorous plants together in nature, this strongly suggests that triggerplants are carnivorous.

The genus *Stylidium* has grown rapidly in recent years, moving from 100–150 named species a few decades ago to over 250 species (Wege, 2005) named or in the process of being named. This makes the genus one of the largest in Australia, and the addition of *Stylidium* to the list of accepted carnivorous genera increases the number of known carnivorous species dramatically, from over 500 to over 800. Given carnivory in *Stylidium*, it is likely that *Levenhookia*, another glandular member of the Stylidiaceae, will also prove to be carnivorous.

Carnivory in the triggerplants is a second example of the type found in *Triphyophyllum*, in which the carnivorous syndrome is not expressed throughout the life cycle but rather only at certain times (Green et al., 1979; Bringmann et al., 2001, 2002). In *Triphyophyllum*, this occurs before flowering and after an initial stage of rosette-type growth. In contrast, triggerplants display carnivory just prior to and during sexual reproduction, the first example of this developmental timing.

While the first assumption is usually that carnivorous plants are carnivorous to obtain N, recent work by Ellison and colleagues (Wakefield et al., 2005; Ellison and Farnsworth, 2005) has shown that, in some cases, P is a more important element for carnivorous plants. Given the high demand for P for DNA synthesis in the new cells forming as flowers open and seeds form, this may be the case for carnivorous *Stylidium* species.

Carnivory depending only on the inflorescence stem may seem unusual, but Cieslack et al. (2005) describe a strongly supported clade of three species within the genus *Pinguicula* (*P. ramosa*, *P. villosa*, *P. variegata*), a genus sharing the same trapping method as *Stylidium*, which catch their prey almost exclusively on inflorescence stems. Again, this supports the identification of the carnivorous syndrome in *Stylidium*.

Triggerplants are also unusual in how closely they place their carnivorous regions to the area visited by pollinators. However, given the small size of the prey compared to pollinators and the relatively small size of triggerplant glandular hairs compared to those of *Drosera*, for example, the carnivorous syndrome in triggerplants may be tuned to discriminate between these two groups of insects. There is precedent for carnivorous plants discriminating carefully between pollinators and prey, as provided by Murza et al. (2005) who studied the sundew *Drosera anglica* in Canada. This plant has the same trapping mechanism as *Stylidium*, with very little overlap between pollinators visiting the top of the tall inflorescence and prey caught at the bottom of that inflorescence on the leaves. This supports the idea that the prey and pollinators of triggerplants may be distinct insect species, even though the trapping and pollinating areas in triggerplants are closer than in the *Drosera* studied by Murza et al. (2005). Many species of *Drosera* also have glandular hairs on their inflorescences, including sepals, and catch a small amount of their prey on the inflorescence.

Question may also arise as to the appropriate term for triggerplants: carnivorous or protocarnivorous/subcarnivorous? In some species that clearly trap insects it has been difficult to show that they secrete proteases and/or other digestive enzymes. These plants usually have symbionts, often microorganisms, on their epidermal surface which aid in digestion. In other cases, insects that avoid capture may play this role, as in the case of *Roridula* spp. (Roridulaceae; Midgley and Stock, 1998). Also, it has recently been suggested that a range of plants with sticky leaves may be protocarnivorous (Spomer, 1999). Since triggerplants clearly secrete digestive enzymes, they are properly called carnivorous, *not* sub- or protocarnivorous.

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D. W. Darnowski

Department of Biology
Indiana University Southeast
4201 Grant Line Road
New Albany, IN 47150
USA

E-mail: ddarnows@ius.edu

Guest Editor: S. Porembski