First observations of UV-induced fluorescence in *Heliamphora* (Sarraceniaceae) and other tepui flora

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Abstract: Seven species of the carnivorous plant genus *Heliamphora* were studied in the wild across four tepuis of the Venezuelan Guyana. All were found to exhibit UV-induced blue fluorescence in their young and developing pitchers, the fluorescence being largely confined to the downward-pointing trichomes of the pitcher interior, with a small contribution from the nectaries. Subsequent work on cultivated plants confirmed the universality of this trait across all known members of the genus. Fluorescence microscopy localized the blue emissions to the surface of the trichomes and unequivocally showed that it represents true fluorescence. The phenomenon was found to be highly transient, generally being seen only in recently opened pitchers. Whether it has a biological function or is an incidental property remains to be determined. Possible roles in the attraction of prey and pitcher inquilines are discussed.

Introduction

Fluorescence, a form of photoluminescence, involves the absorption of electromagnetic radiation at shorter wavelengths and almost immediate emission at longer wavelengths. Once the source of excitatory radiation is extinguished, so too is the fluorescent emission, on a nanosecond timescale. In this it differs from phosphorescence, where the emitted radiation persists much longer (milliseconds to hours) and which has a different underlying mechanism.

Fluorescence in plants has long been the subject of scientific inquiry and the best-known example is undoubtedly that of chlorophyll *a*, which emits in the red to far-red, though blue-green plant fluorescence is also common. No studies on fluorescence in *Heliamphora* have previously been published, but a 2013 study by Kurup *et al*. claimed to show that three other carnivorous plant genera—*Dionaea*, *Nepenthes*, and the closely related *Sarracenia*—employ UV-induced blue fluorescence as a visual lure to attract prey. The results were widely reported, but concerns were soon raised about the study's methodology and conclusions. Clearly, more work is needed to elucidate the basis and functional importance (if any) of this phenomenon.

Methods

Field studies were carried out on seven species and one non-autonymic variety of *Heliamphora* across the summit plateaus of four tepuis: *H. chimantensis* on Apacará-tepui; *H. huberi*, *H. pulchella*, and *H. uncinata* on Amurí-tepui; *H. minor* var. *minor* and *H. minor* var. *pilosa* on Auyán-tepui; and *H. purpurascens* and *H. sarracenioides* on Ptari-tepui. Additionally, *Catopsis berteroniana* was imaged on the lower slopes of Auyán-tepui, and *Xyris* sp. and an undetermined Eriocaulaceae were photographed on the summit plateau of Ptari-tepui. Some species could only be studied in daylight

owing to their inaccessibility and therefore had to be artificially shaded prior to being photographed. A high-power UV hand torch with an emission peak of 365 nm was used in all cases.

All remaining species of *Heliamphora* that could not be studied in the wild were observed as cultivated specimens in the author's live collection. To confirm fluorescence, plants were viewed under the same UV torch as before.

Small pieces of tissue were prepared from a freshly opened *Heliamphora pulchella* pitcher from the author's collection. The resulting slides were viewed with a fluorescence microscope, being imaged under bright-field illumination as well as four different fluorescence filter cubes, with excitatory wavelengths ranging from UV to yellow.

Results

Field observations confirmed the presence of UV-induced blue fluorescence in seven species and one non-autonymic variety of *Heliamphora* (Figs. 1–3). Observations on cultivated plants of all remaining *Heliamphora* species showed that some level of blue fluorescence is exhibited by all members of the genus, though it is highly variable in its intensity, localization, and persistence (Figs. 4–5).

Under fluorescence microscopy, the retentive hairs were the only sampled parts of the trapping surface to show significant blue fluorescence under UV, with the surrounding epidermal tissue emitting no perceptible fluorescence (Fig. 6). This situation was largely reversed when the excitatory light was changed to blue and the emissions filtered to green, with the epidermal tissue showing strong green fluorescence in contrast to the weakly visible trichomes. Similarly, no trichome fluorescence was observed at the longer excitatory wavelengths. These microscopy studies unequivocally showed that the blue emissions represent true UV-induced fluorescence and not merely reflected blue light.

Wild *Heliamphora sarracenioides* pitchers were found to fluoresce internally despite lacking retentive hairs (Fig. 3). Subsequent observations revealed that *Heliamphora* pitcher nectaries also give off blue fluorescence, as does the nectar itself (Fig. 5).

Developing and freshly opened pitchers were found to be the most intensely blue-fluorescent due to subsequent quenching of trichome fluorescence. This quenching appeared to progress

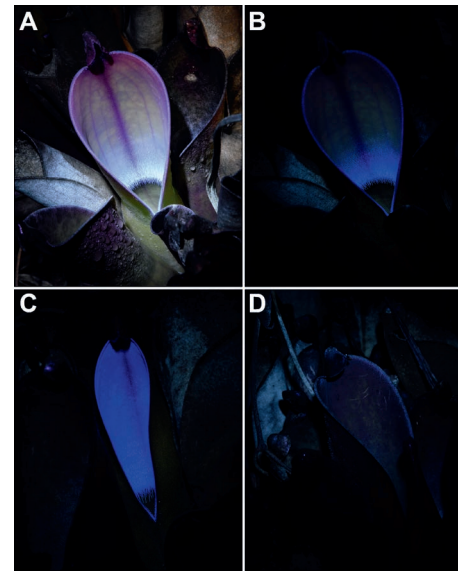

Figure 1: *Heliamphora chimantensis* on Apacará-tepui: (A–B) a freshly opened pitcher under white and UV illumination; here the majority of the inner pitcher surface lacks conspicuous blue fluorescence, the trichomes being confined to the area just above the waterline and to a narrow band lining the pitcher rim; (C) a pitcher in the process of opening; here the entire inner surface is lined with hairs and shows uniformly strong blue fluorescence; (D) an older pitcher from the same patch, showing no obvious UV-induced fluorescence (the pitcher is illuminated only by the small amount of visible light emitted by the UV torch, which necessitated a longer exposure).

Figure 2: *Heliamphora uncinata* at its type locality on Amurí-tepui. Strong blue fluorescence is apparent in the freshly opened pitcher; note that the older adjacent pitcher exhibited no perceptible UV-induced fluorescence whatsoever.

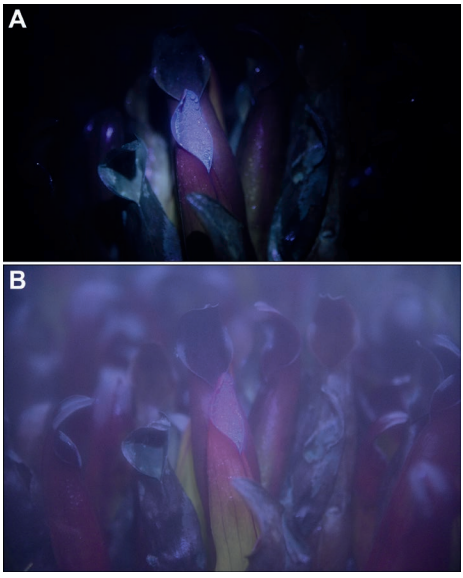

Figure 3: A freshly opened *Heliamphora sarracenioides* pitcher on the summit plateau of Ptari-tepui, (A) showing modest UV-induced fluorescence throughout the hairless interior surface (photo taken at 5:26 am local time). (B) The same clump photographed 14 minutes later (5:40 am local time). At this point, ambient light is already beginning to overwhelm the fluorescent signal (at least as perceived by human eyes).

very rapidly in most species, with the fluorescent signal rendered clearly diminished to non-existent within likely no more than a few weeks of pitcher opening. Of the species observed in the wild, this progression was most clearly seen in *Heliamphora chimantensis*, owing to its vigorous clumping habit. In pitchers of this species in the process of opening, the entire interior fluoresces brightly (Fig. 1C). Fully opened but still very young pitchers also fluoresce, but this is mostly confined to the ring of long hairs just above the fluid level (Fig. 1B). Fully pigmented adult pitchers (otherwise apparently completely functional) generally lack discernible fluorescence under 365 nm (Fig. 1D). Consequently, only a small fraction of functional pitchers fluoresce at any given time on any given plant (generally up to one pitcher per growth point and, in large clumps, as few as one per several dozen pitchers, as was observed in wild *H. chimantensis*). Fluorescence in *H. sarracenioides* was also found to be restricted to freshly opened pitchers, despite this species lacking retentive hairs (Fig. 3).

Preliminary observations revealed UV-induced blue fluorescence in a range of other tepui plants, including the carnivorous bromeliad *Catopsis berteroniana*, an undetermined Eriocaulaceae, and a species of *Xyris*. By chance, a single scorpion was found in association with pitchers of *Heliamphora purpurascens* on Ptari-tepui. It is likely to be the first scorpion ever recorded from the summit plateau of Ptari-tepui and probably represents a previously undocumented species. Scorpions are

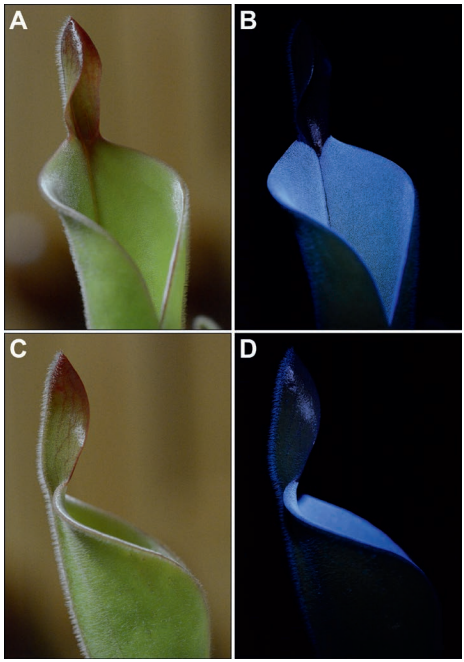

Figure 4: *Heliamphora parva* under white light and under UV in the dark. Note the modest blue fluorescence from the hairs of the exterior pitcher midrib (D).

not uncommon on tepui summits and, owing to their brilliant blue fluorescence, are among the animals most likely to be encountered during night-time fluorescence studies.

Discussion

Retentive hair fluorescence in *Heliamphora*: a biosignal?

The brilliant blue fluorescence of the retentive hairs is instantly captivating. Since retentive hairs are the primary structures involved in prey trapping, the question naturally arises: might they also play a role in prey attraction?

To be involved in biosignalling, the wavelengths of fluoresced light must lie within the sensitivity range of the putative target species. Most insects are trichromats, having visual sensitivity maxima in the UV, blue, and green wavebands. However, when it comes to ants, which make up the bulk of prey caught by *Heliamphora* in the wild, UV–green dichromacy appears to be the norm, with some limited evidence for UV–blue–green trichromacy. This would argue against a role in prey attraction, at least of ants, though in any case ants would seem an unlikely biosignalling target given their terrestrial nature

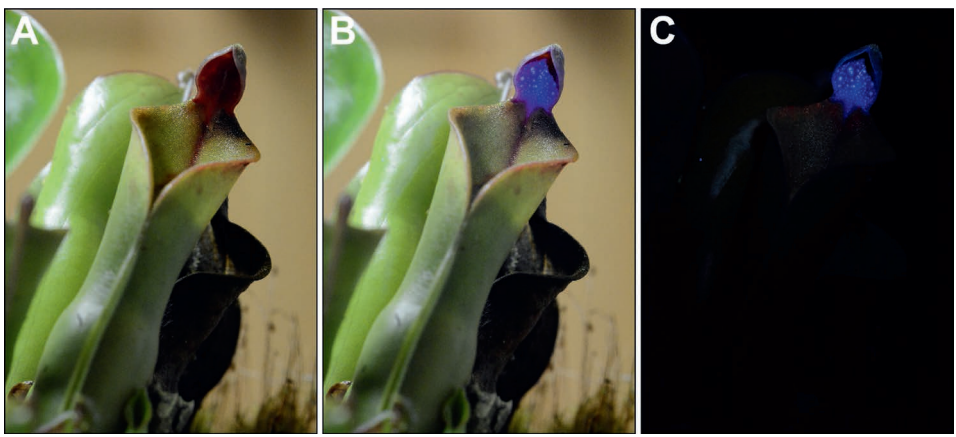

Figure 5: Cultivated plant of *Heliamphora nutans* from Yuruaní-tepui. This old pitcher lacked trichome fluorescence but showed strong nectar-derived fluorescence. The photos were taken under white light (A), a combination of white and UV light (B), and UV light alone (C).

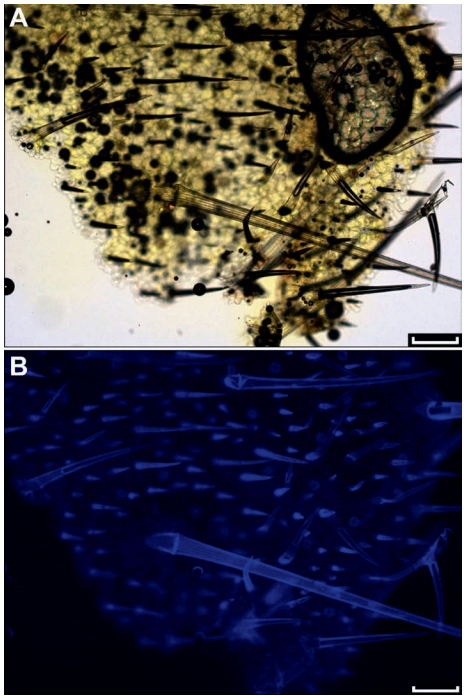

Figure 6: Thin slice of the interior surface of a young *Heliamphora pulchella* pitcher. At least two discrete size classes of trichomes are readily discernible. (A) As viewed under bright-field microscopy. (B) The same piece of tissue viewed under fluorescence microscopy with a DAPI filter cube (specimen excited by UV light and emitted light filtered to blue). It is clear that the trichomes are the only structures exhibiting significant blue fluorescence under this UV excitation range. Scale bars = 250 µm. (Image adjusted for the print article. See online article for original.)

and primary reliance on other sensory modalities. Further casting doubt on this idea is the brief temporal window of fluorescent activity, particularly when compared to the functional lifespan of an individual pitcher (many months to several years). On the other hand, assuming the ants can see the fluoresced wavelengths, early attraction might be sufficient, with subsequent capture facilitated by pheromone-based foraging trails. If the plant–ant interaction is a simple predator–prey relationship, attraction to blue fluorescence should not be selected for (unless a form of aggressive mimicry is involved), but if at the level of the ant colony it represents a mutually beneficial interaction then this would be expected.

Another possible function of retentive hair fluorescence might be early recruitment of pitcher inquilines. *Heliamphora* are known to commonly host mosquito (*Wyeomyia* spp.) and midge larvae, all of which are apparently obligate pitcher inhabitants. These likely benefit the plant by making nutrients from captured prey more readily available as part of a mutualistic relationship. One can see how it might be advantageous for ovipositing insects to be able to identify newly opened pitchers to ensure that their offspring (a) complete the aquatic stage of their life cycle before conditions deteriorate due to pitcher senescence and (b) are able to exploit the higher input of prey at the beginning of a pitcher's functional life. Likewise, the plant would presumably stand to benefit from having larval hatching coincide with the onset of prey acquisition. Indeed, it has been shown that mosquito (but not midge) oviposition overwhelmingly takes place in the very youngest *Heliamphora* leaves, even prior to the commencement of prey capture. A similar pattern has been consistently observed in the North American *Sarracenia purpurea*, which also hosts a *Wyeomyia* species in its pitchers, though in *S. purpurea* the inquiline mosquitoes are apparently attracted by chemical cues. Interestingly, color appears to be a factor in the selection of ovipositional sites by at least some bromeliad-breeding *Wyemoyia*, and multiple studies have demonstrated the same for other mosquitoes, including specific attraction to blue wavelengths.

Fluorescent biosignalling: a caveat

Caution should be exercised whenever attempting to assign ecological importance to fluorescent phenomena, since it is tempting to view them solely through the prism of human visual perception,

which often differs markedly from that of the putative target species. This is especially true when viewing UV-induced visible fluorescence at night under a high-power UV source, rather than in a biologically relevant context. The fluorescent 'glow' may appear impressive, but that is because the powerful excitatory (UV) light is invisible to us and so the comparatively weak fluoresced light is sensitizing eyes accommodated to darkness; when viewed under natural light the fluorescence is likely to become imperceptible (again, to us!).

The biological 'impracticality' of UV-induced visible fluorescence is down to both the inherent inefficiency of the fluorescence conversion process and to the low intensity of UV radiation reaching the Earth's surface as compared to visible light (the photon flux density of the former being around 5% of the latter). It is also important to remember that since UV radiation is visible to most insects, it can be utilized for biosignalling not only through fluorescence but also (much more efficiently) through reflectance. Similarly, a biosignal in the visible spectrum could be created by simply reflecting visible light. Of course, reflection and fluorescent signals need not be mutually exclusive, and it is possible that they act in concert to enhance or broaden the range of emerging (reflected plus fluoresced) light that corresponds to the visual sensitivity maxima of the intended recipients.

Other possible explanations

Fluorescence need not be ecologically relevant to have adaptive value. One possibility is that it is photoprotective, converting damaging UV-A radiation into less energetic blue wavelengths. Alternatively, blue fluorescence might enhance photosynthesis by 'creating' photosynthetically active radiation from otherwise unproductive UV light. There is evidence for both of these processes in corals and it is quite possible that they work in tandem in various green plants and lichens. In *Heliamphora*, however, neither role seems likely given the highly transient nature of the blue fluorescence and the small fraction of the total pitcher surface that fluoresces.

Finally, it is important to remember that many natural compounds and materials autofluoresce as a by-product of their chemical makeup. It is entirely possible that the observed fluorescence in *Heliamphora* is merely an epiphenomenon: an incidental, non-adaptive property of phytochemicals that evolved to serve some unrelated function.

Avenues for future research

To the author's knowledge, this paper constitutes the first published demonstration of fluorescence in *Heliamphora* or indeed any component of the specialized tepui flora. Further studies should be undertaken to confirm the chemical source of the blue fluorescence, characterize the timing and mechanism by which it is quenched, determine its excitation and emission spectra, and quantify the contribution of fluoresced light to total emerging blue light under various irradiance scenarios. The latter could then be related to known or inferred spectral sensitivities of ecologically relevant species (viz. prey and inquilines) to establish whether a biosignalling role is plausible. Ultimately, behavioural studies involving the putative target species would be needed to confirm fluorescent biosignalling. Potential non-ecological functionality, such as in photoprotection or photosynthesis enhancement, should also be investigated.

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https://cpn.carnivorousplants.org/articles/CPNv49n4pe1_31.pdf