Further evidence of carnivory in triggerplants (*Stylidium* spp.; Stylidiaceae)

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Introduction

Triggerplants (*Stylidium* Sw.; Stylidiaceae) grow from Southeast Asia through Australia, with well over 100 species in the genus. Almost all species make sticky hairs on parts of their inflorescences. These sticky hairs trap small insects, much smaller than the flowers' normal pollinators, which include arthropods such as bee flies. Digestive enzymes have been detected in this genus in the area of sticky hairs where prey are trapped (Darnowski *et al.* 2006).

Field observations in Australia almost unfailingly show that triggerplants grow alongside known carnivores, such as *Drosera* and *Utricularia* (Darnowski 2002; Darnowski *et al.* 2006). That ecological association is another indicator that triggerplants may be truly carnivorous, using their sticky hairs to obtain extra nutrients while growing on nutrient-poor soils.

To prove that triggerplants are carnivorous plants, it must be demonstrated they uptake nutrients from prey, and that there is a benefit being derived from those nutrients (Płachno *et al*. 2009; Adamec 2013).

This work uses heavy-isotope amino acids to look for nutrient uptake by triggerplants in the same region in which they have their trapping hairs.

Materials and methods

^{13}C-alanine (Cambridge Isotope Laboratories, Tewksbury, Massachusetts, USA) was provided to flowering plants of *Stylidium fimbriatum* and *S. debiles* by soaking a small piece of Whatmann #1 filter paper in a solution of the amino acid so that each plant received 30 mg of ^{13}C-alanine. This heavy amino acid can be traced in the plant by mass spectrometry (MS), since ^{13}C is rare in nature and easy to differentiate by MS from normal ^{12}C.

The filter paper containing the labelled amino acid was applied to the area of sticky hairs, which is the same region in which digestive enzymes have been observed.

Three days later, plants were separated into shoots and roots and then freeze-dried. Because of the small plant size, three plants were pooled per species and the results reported as a whole. ^{13}C was detected in the various samples using MS (UC Davis Stable Isotope Facility, University of California-Davis, Davis, California, USA), and the amount detected was converted to give the percentage of the original sample detected.

Results and discussion

Stylidium debiles showed 20% of the initially-applied sample of ^{13}C-alanine was detected in the shoot of the plant and 45% in the root (Fig. 1). *Stylidium fimbriatum* had 5% in the shoot and 20% in the root.

In both species of triggerplant examined, the plants took up the nutrients provided and translocated them to the roots. In fact, while the initial absorption must have occurred in the shoot, broadly defined to include the inflorescence, most of the label found in the plant three days later was in the root, confirming transport of the nutrients by the plant. This strengthens the idea that triggerplants are carnivorous plants.

The portion of the initially-applied 30 mg of ^{13}C-alanine which was not detected in the plants may have been lost to cellular respiration. This idea is supported that a greater amount was not found in tropical *S. debiles* than in temperate/subtropical *S. fimbriatum* since tropical plants tend to lose more of their carbon to cellular respiration than other plants (Taiz & Zeiger 2010).

Further studies are needed to check uptake and translocation in more species, and ecological studies are needed to show some benefit from the uptake of nutrients from prey, such as increased biomass or the production of more seeds, but it can be said that triggerplants are probably carnivores.

References

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Figure 1: A drawing of *Stylidium debiles*. 20% of the initially-applied sample of ^{13}C-alanine was detected in the shoot and 45% in the root.