Uptake of the Amino Acid Alanine by Digestive Leaves: Proof of Carnivory in the Tropical Liana *Triphyophyllum peltatum* (Dioncophyllaceae)[1]

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This paper is dedicated to Prof. Wittko Francke, on the occasion of his 60th birthday.

Summary

The uptake of organic matter by the insect-trapping glandular leaves of the Western tropical African liana *Triphyophyllum peltatum* (Dioncophyllaceae) is demonstrated for the first time. After feeding carbon-13 labelled L-alanine to the trapping leaves, the label is detected in apical shoot parts and normal (non-trapping) leaves within 2 d of application. With this proof of resorption and transport, the carnivorous syndrome of *T. peltatum* is demonstrated to be complete. The prey composition reveals the glandular leaves of *T. peltatum* to be traps for flying insects, predominantly.

Introduction

The glandular leaves produced in juvenile stages of the rare West African liana *Triphyophyllum peltatum* (Dioncophyllaceae, see Figure 1) trap considerable amounts of arthropods (Green *et al.*, 1979). The homology of the stalked and sessile glands on these organs to those of *Drosophyllum lusitanicum* (Drosophyllaceae) was very early recognized by plant anatomists (Metcalfe, 1951). From these data and the detection of endogenous proteolytic enzymes in the secretions of these glands, the hypothesis of carnivory in *T. peltatum* was derived (Marburger, 1979; Green *et al.*, 1979). The resorption of organic matter by the trapping leaves has, however, not been demonstrated.

Recent genetic studies (Fay *et al.*, 1997; Meimberg *et al.*, 2000) indicate a close phylogenetc relationship of the Dioncophyllaceae to the carnivorous families Drosophyllaceae (with sticky leaves), Nepenthaceae (with pitfalls), and Droseraceae (with sticky leaves or spring traps), as well as the non-carnivorous families Ancistrocladaceae, Plumbaginaceae, and Polygonaceae. Phytochemical (Bringmann

[1]Part 144 in the series 'acetogenic isoquinoline alkaloids'. For part 143, see Bringmann, G., Mühlbacher, J., Repges, C., and Fleischhauer, J. 2000. MD-based CD calculations on the naphthylisoquinoline alkaloid dioncophylline A, for the assignment of the absolute axial configuration. *J. Comp. Chem.* (submitted).

& Pokorny, 1995; Bringmann *et al.*, 1998) and anatomical (Schlauer, 1997) similarities support grouping these families together in a caryophyllid clade (a redefined order Nepenthales).

First data on carnivorous properties of the glandular leaves formed at certain developmental stages of *T. peltatum* (Dioncophyllaceae) were obtained earlier (Green *et al.*, 1979; Marburger, 1979). Here we describe experiments that demonstrate the ability of *T. peltatum* to absorb the amino acid alanine applied to the glandular leaves, which completes the knowledge of the carnivorous syndrome in this species. Furthermore, the fauna trapped by these leaves has been analyzed in order to further characterize carnivory in *T. peltatum*.

Materials and Methods

Plant material: 24 specimens of *T. peltatum* (Hutch. & Dalz.) Airy Shaw bearing glandular leaves were fed with 500 mg [2,3-^{13}C]-labelled L-alanine (Promochem, Wesel, Germany) at the Parc de Taï (Ivory Coast) in April 1996, just before the beginning of the rainy season (Figure 2), and harvested after 2 d incubation (Bringmann *et al.*, 1996). The material was air dried at the Centre National de Floristique, Abidjan (Ivory Coast) and stored at 4°C in Würzburg, Germany. The prey animals attached to the traps were removed and determined taxonomically. All work and collection of material for research was performed in accordance with the official permit conditions of Ivory Coast. Voucher specimens of *T. peltatum* are deposited at the Centre National de Floristique, Abidjan (UCJ) and at Herb. Bringmann, Institute of Organic Chemistry, Würzburg.

Extraction and analysis of plant material: Dried *T. peltatum* plants were divided into roots, basal stems, apical stems, normal leaves, and trapping leaves, then washed with distilled water (3-5 times) until alanine was no longer detected in the supernatant. The material was then lyophilized and homogenized. The resulting finely powdered fractions were extracted three times with the tenfold (w/w) amount of distilled water, each with ultrasonification for 2 d at 22°C and subsequent filtration. 5 ml each of the aqueous extracts were filtered through preconditioned RP-18 columns (Waters, Eschborn, Germany) and the columns were washed with 1 ml of distilled water each. The procedure was repeated with preconditioned anion exchange columns. The solvent was removed by lyophilization. The residues were dissolved in 1 ml of distilled water, neutralized with 1N NaOH, and applied on preconditioned cation exchange columns. The columns were washed with 1 ml of distilled water each and vacuum-dried for 1 min. The cations (including alanine) were eluted by 1.5 ml of 1N HCl each, the eluates were dried at 22°C, redissolved in a solution of 0.108 ml (1.5 mmol) thionyl chloride in 1 ml of isopropanol each, refluxed for 1 h and subsequently deprived of the solvent by evaporation. The residues were treated with 50 µl of trifluoroacetic acid anhydride in 400 µl of dichloromethane each and stirred for 1 h at 22°C. The solvent was removed by superfusion with nitrogen, the residues obtained were redissolved in 300 ml of toluene and analyzed by GC-MS.

GC-MS: A gas chromatograph HP 5890 Series II with on-column injector (Hewlett-Packard, Avondale, USA) was coupled directly with a quadrupol mass spectrometer MSD 5971 A (Hewlett-Packard, Avondale, USA). The temperature of the transfer line was 280°C, resulting in an ion source temperature of 180°C. A DB-17 column (J&W Scientific, 10 m × 0.16 mm, film thickness 0.18 µm) with helium as the carrier at a pre-column pressure of 100 kPa was applied. Temperature program: 50°C (4 min), 6°C/min, 80°C, 60°C/min, 210°C (3 min). Alanine was analyzed as its *N*-trifluoroacetyl-L-alanine isopropyl ester. The label (fragment weights increased by two units) was detected by the shift in the ratio between the *m/z* 142

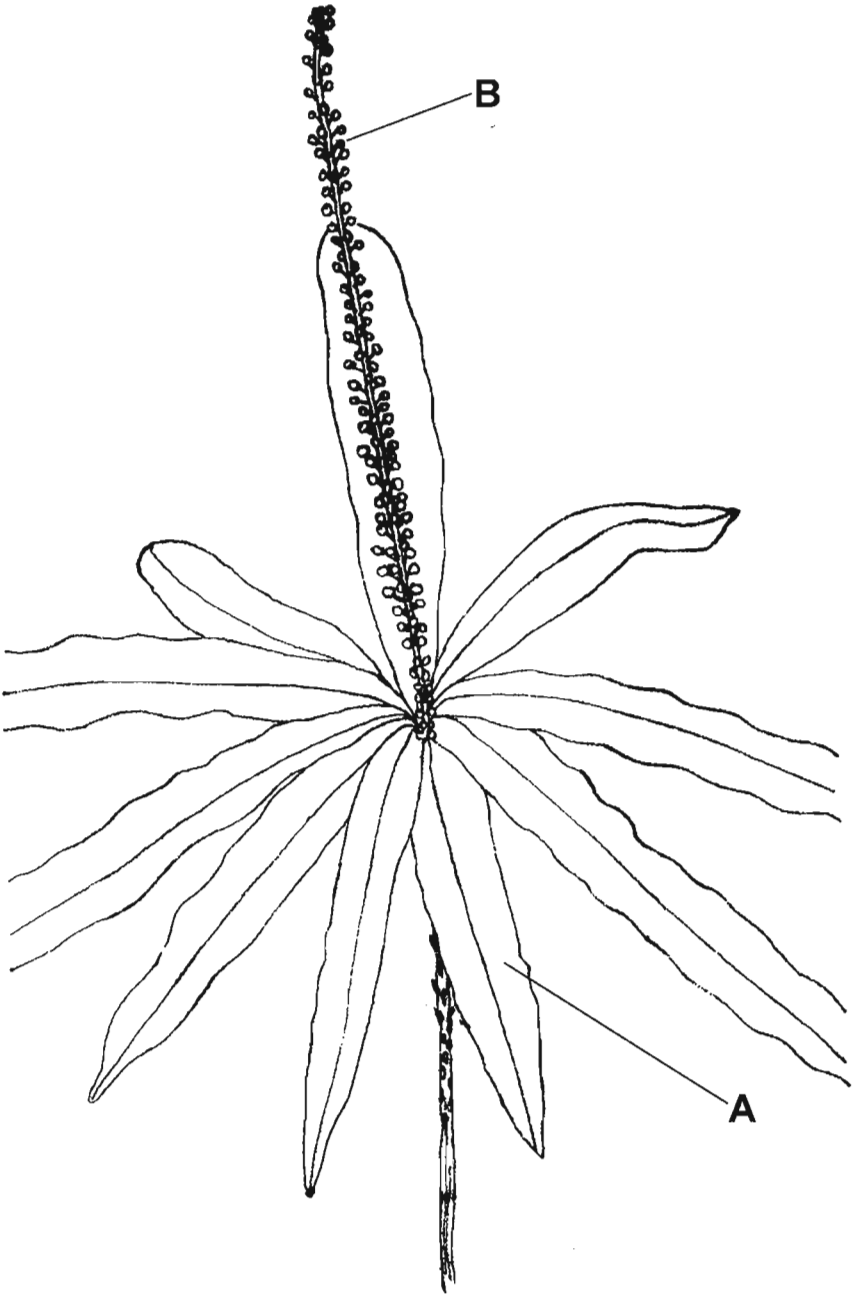

Figure 1: Juvenile plant of *T. peltatum* with rosette of normal leaves (A) and trapping leaf (B). Drawn by J. Schlauer

and 140 [M+- COO*i*Pr] peaks, which correspond to characteristic fragment ions of the respective derivatives. Unlabelled leaf tissue was extracted and derivatized in the same way and analyzed as a control. All values were obtained from triplicate measurements (from three separate chromatograms each).

Results

Alanine uptake and redistribution: After uptake, labelled alanine was found predominantly in the trapping leaves and the adjacent stem by GC-MS analysis. A somewhat smaller content of labelled alanine was detected in the normal (i.e. non-carnivorous) leaves of fed plants of *T. peltatum* (Table 1). No alanine (either labelled or unlabelled) was detected in the roots by the analytic method employed.

	Tissue				
	Control	Roots	Stem	Leaves	Traps
142/140	0.048±0.004	no alanine detected	0.538±0.031	0.101±0.046	1.163±0.188
Label/control	1	-	11.2	2.1	24.2

Table 1: Distribution of labelled alanine after feeding to T. peltatum. Relative abundance (mean, SE calculated from three independent experiments) of characteristic fragment ions of labelled (m/z 142) vs. Unlabelled (m/z 140) alanine in different parts of T. peltatum fed with $^{13}C_2$ labelled alanine and unfed control plants (likewise with glandular leaves) of the same species.

Figure 2: Feeding labelled alanine to a carnivorous plant of *T. peltatum* in the tropical rain forest in the Parc de Taï (Ivory Coast); interestingly this specimen bears two glandular leaves. Photo by H. Bringmann

Fauna trapped by T. peltatum: The invertebrates collected from the glandular leaves of 24 specimens of *T. peltatum* were determined to their order (Table 2). A total of 197 specimens was obtained. The mouthparts of the prey and the presence/absence of wings were investigated in order to estimate relative abundances of different prey types.

Members of following suborders, families, or subfamilies were found:

Diptera:

Brachycera, Nematocera, Sciaridae, Mycetophilidae, Anisopodidae, Phoridae, Ceratopogonidae, Scatopsidae, Macroceridae, Bolitophilidae, Dixidae

Coleoptera:

Chrysomelidae, Lycidae, Scolytidae, Curculionidae, Staphylinidae (predator), Elateridae, Malachiidae

Hymenoptera:

Formicidae, Myrmecinae (winged sexuals and few workers)

Aranea:

Theridiidae, Salticidae, Linyphiidae

Prey Type	no.	% of total	% winged	mouthparts
Insecta				
Isoptera	13	6.5	100	biting
Auchenorryncha	1	0.5		sucking
Heteroptera	2	1.0		sucking
Thysanoptera	1	0.5		sucking
Hymenoptera	43	21.8	93	biting
Coleoptera	41	20.8	100[1]	biting
Diptera	80	40.6	100	licking, licking-sucking
Lepidoptera	2	1.0	50	sucking, biting (one larva)
Arachnida				
Aranea	12	6.0	0	
Acari	2	1.0	0	
Total	197			

Table 2: Prey trapped by T. peltatum. Twenty-four specimens of T. peltatum with glandular leaves were investigated. [1]Wings not always visible through elytra.

Discussion

These results demonstrate that *T. peltatum* takes up and redistributes alanine applied to the digestive glands. The relative concentration of incorporated alanine was highest at the trapping leaves, which is not unexpected since the plants were harvested only a brief time after application of the label (2 d). Since the material had been washed repeatedly, the high signal found was apparently caused by alanine that had really been taken up. A considerable proportion of the label (almost half of the relative concentration found in the trapping leaves) was detected in the stems, which had not been in any external contact with labelled alanine. This region should be the first one to be reached by compounds taken up by the glandular leaves. As the normal (not trapping) leaves showed significant (although lower) label concentrations, while the roots were devoid of label, the leaves might be a sink for nutrients derived from animal prey. This would also accord with previous results obtained for pitcher plants (Schulze *et al.*, 1997), in which non-trapping leaves were

found to be a sink for probably insect-derived nitrogen. The fact that no alanine was detected in the roots could be due to very low steady state concentrations of free (i.e. not protein-bound or otherwise fixed) alanine in these organs.

Previous to this investigation, the resorption of amino acids by the glandular leaves and the distribution of these metabolites to other tissues had been the missing evidence required to show that *T. peltatum* is able to perform the entire series of carnivorous activity—to attract, capture, kill, decompose and absorb animal prey to a nutritional benefit. Our findings complete the "carnivorous syndrome" (Juniper *et al.*, 1989): In the growth phase marked by the formation of glandular leaves, *T. peltatum* is, beyond doubt, a carnivorous plant.

The major groups of animals found attached to the glandular leaves of *T. peltatum* in Ivory Coast differ only slightly from those previously reported to be captured by the same species in Sierra Leone (Green *et al.*, 1979), while the trapping efficiency (197 identified carcasses from 24 plants) was apparently lower than in that study (164 captured arthropods from 8 plants). Like in Sierra Leone, the trapping season in Ivory Coast coincides with periods of peak activity of the prey (many winged, sexual specimens) at the onset of the rainy season. Most of the identified arthropods trapped by *T. peltatum* in this study were winged. Only few other animals were found to be trapped, although they appeared to be abundant everywhere, so that those species seem not to be specific prey of *T. peltatum*. Most specimens caught had biting or licking-sucking mouthparts. Surprisingly few Lepidoptera were captured, which could imply that the traps are not attractive to them.

Acknowledgements

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Writings from the Readership

Refining The Terrarium: Alternative Techniques For The Indoor Gardener

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The quantity of literature pertaining to the indoor cultivation of *Nepenthes* is far from overwhelming. The two essays I have found most useful appeared in the pages of this journal: "A Rainforest In The Basement: *Nepenthes* Cultivation Under Lights" (Butler, 1987), and "The Potted Terrarium" (D' Amato, 1996). Butler's piece described the construction of a basement grow-chamber for his "intermediate" *Nepenthes*. D'Amato's article, which is more important for our purposes, described two styles of terraria which were well-suited for a general carnivorous plant collection. Both of the foregoing works were instrumental in my early attempts to grow carnivorous plants, and especially *Nepenthes*, indoors. Nonetheless, neither article provides an optimal method for growing lowland *Nepenthes* indoors. In this article I will describe and advocate two modifications to D'Amato's "potted greenhouse style terrarium" (henceforth "potted terrarium"). These modifications yield improved growing conditions for lowland *Nepenthes* without any sacrifice in convenience. Furthermore, the "refined" potted terrarium has a few other useful applications. Before getting on to this, I will review D'Amato's article.

At the time of publication D'Amato's article was perhaps the most important discussion of carnivorous plant culture in terraria. I remember thanking him personally at a Bay Area Carnivorous Plant Society meeting for sharing such useful information. (The piece reappeared in his book, much supplemented.) In contrast with the paradigmatic terrarium containing a planted soil bed, D'Amato advocated simply placing potted plants directly on the terrarium floor. As he pointed out, this simple change allowed one to grow plants requiring a variety of different soils and soil moisture levels in the same terrarium. This versatility, I think, is the chief benefit of the potted terrarium; it constitutes a dramatic improvement over the planted tank.

While acknowledging the great utility of the potted terrarium, it proves deficient for the lowland *Nepenthes* grower in two areas. The first is watering. Unlike many carnivorous plants, *Nepenthes* will not tolerate standing in water. Hence, one must remove the plants, water them, let them drain, and then return them to the terrarium. This is incredibly inconvenient. The alternative D'Amato suggested is to sit each potted *Nepenthes* in "a shallow saucer and water overhead as soon as the water in the saucer evaporates" (D'Amato, 1998, p 277). Though he cautioned the