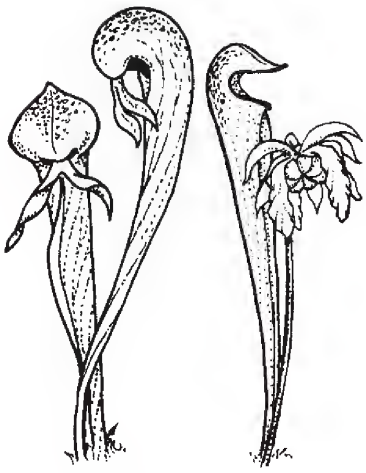
CARNIVOROUS PLANT NEWSLETTER

Journal of the International Carnivorous Plant Society

Volume 41, No. 1 March 2012



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Journal of the International Carnivorous Plant Society
www.carnivorousplants.org

Volume 41, Number 1
March 2012

Front Cover: Dark purple color variant of *Drosera cistiflora* growing in the Kalbaskraal area of South Africa, one of the most stunning and rarest variants from the *D. cistiflora* complex, with flowers up to 3 cm in diameter. Due to habitat loss, only a few remaining populations of this variant are known in areas just north of Cape Town. Photo by Andreas Fleischmann. Article on page 24.

Back Cover: *Sarracenia* 'Zjahnine'. Photo by Julian Brook. Article on page 29.

Carnivorous Plant Newsletter is dedicated to spreading knowledge and news related to carnivorous plants. Reader contributions are essential for this mission to be successful. Do not hesitate to contact the editors with information about your plants, conservation projects, field trips, or noteworthy events. Advertisers should contact the editors. Views expressed in this publication are those of the authors, not the editorial staff.

All correspondence regarding dues, address changes and missing issues should be sent to the Membership Coordinator at the ICPS. Do not send such correspondence to the editors. Checks for subscriptions should be made to the ICPS in US funds. Dues for 2012 are $35 for the first year of membership; renewals are $30 per year.

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Date of effective publication of the December 2011 issue of Carnivorous Plant Newsletter: 1 December 2011.

The ICPS is the International Cultivar Registration Authority (ICRA) for the names of cultivated carnivorous plants according to the International Code of Nomenclature for Cultivated Plants. Send relevant correspondence to the ICPS, Inc.

Carnivorous Plant Newsletter is published quarterly in March, June, September, and December by the ICPS, Inc., 2530 Patra Drive, Richmond, CA 94803, USA. Periodicals postage paid at Richmond, CA and additional mailing offices. Postmaster: Send address changes to ICPS, Inc., PMB 322, 1564-A Fitzgerald Drive, Pinole, CA 94564-2229, USA. Printed by Allen Press, Inc., 810 E. 10th Street, Lawrence, KS 66044. Logo and masthead art: Paul Milauskas.
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2012 ICPS Conference update

Registration is still OPEN for the 2012 ICPS Conference! The conference will be held August 11-13, 2012, at the Johnson and Wales Inn in Seekonk, Massachusetts, USA. There is an "early bird" discount of $50 US for those who register before April 15. If you are planning to attend, and haven't yet registered, there is still a little time to take advantage of this great deal! Please visit our website at necps.org/icps2012 for details on how to register.

A number of hotel rooms at the conference center have been reserved for conference attendees and are available at a discounted rate. When registering by phone, mention that you are part of the New England Carnivorous Plant Society or International Carnivorous Plant Society to reserve one of these rooms. Information on the conference center, and other nearby lodging, can be found at necps.org/icps2012.

An itinerary for the field trips has also been posted on our website. If you are interested in attending the post-conference field trips, please visit necps.org/icps2012 and click on Field Trips. There you will find details about the sites and information on how to register.

Abstracts are currently being accepted for those who are interested in presenting at the conference, either as a speaker or as part of the poster presentation. Also, there will be classes and presentations open to the public, and we invite members of the ICPS community to be a part of this series. This is a great opportunity to share your knowledge and love of carnivorous plants! Please submit abstracts or speaking proposals to emily.troiano@gmail.com by June 1.

Are you interested in being a vendor or sponsor at the conference? There are still spaces open! One exciting aspect of the 2012 ICPS Conference is the public show and plant display that will be running in conjunction with the presentations. The NECPS has had over 600 visitors at our annual Carnivorous Plant Show, and we hope to bring in even larger crowds to this show! This is a unique opportunity, with exposure to both a large public crowd and a group of dedicated carnivorous plant enthusiasts. Contact emily.troiano@gmail.com or johnatthebeach@cox.net for more information.

As the date for the conference draws closer, and we see our plans starting to take shape, we members of the New England Carnivorous Plant Society are growing more and more excited to be hosting the event! I hope you will join us this August for what promises to be an incredible conference!

Errata

September 2011, vol. 40 no. 3, page 85: For some unknown reason, our printer substituted four therefore symbols (∴) where there should have been minus signs (-). The corrected article is at:

http://www.carnivorousplants.org/cpn/articles/CPNv40n3p84_87.pdf

December 2011, vol. 40 no 4, page 131 (Fig. 2): In the small inset photo of seeds, the author erroneously inserted a photo of Byblis 'David' seeds rather than Byblis cf. filifolia ´ liniflora seeds. The corrected figure and further discussion can be found at:

http://icps.proboards.com/index.cgi?board=byblis&action=display&thread=5189

Mycorrhizal formation by various carnivorous plants

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Keywords: physiology: mycorrhizae, roots.

Introduction

Carnivorous plants receive the greatest amount of attention for their modified leaves used for obtaining extra nutrients while they live in low-nutrient soils (Slack 2000). Another way in which plants obtain more nutrients when nutrients in the soil are limiting is the formation of mycorrhizae with fungi (Nemec 1982). The fungi provide a large, highly-absorptive surface area for the plants, able to scavenge rare nutrients efficiently, while the plants provide their fungal partners with carbohydrates. This mycorrhizal habit is found in many forest trees and also in most heaths and in some epiphytes and orchids (Marx 1982; Lambers & Colmer 2005). More and more plants are being discovered to be mycorrhizal (Lambers & Colmer 2005).

With this effect of enhancing the uptake of nutrients from such a relationship, carnivorous plants might be expected to form mycorrhizae, but there are very few reports of such symbioses among carnivores, or even among their fellow wetland plants in general, with one recent report in *Drosera* a rare exception (Fuchs & Haselwandter 2004). Also, Venugopal & Raseshowri (2007) have recently reported the formation of mycorrhizal associations by *Drosera peltata* which actually uses modified underground stems and leaves in place of roots for their connections with mycorrhizal fungi. This contradicts the common belief that there is no association of mycorrhizal fungi with roots of either hygrophylic or xeromorophic carnivorous plants (MacDougal 1899, reviewed by Juniper *et al.* 1989).

In our study, 23 species of greenhouse-grown carnivorous plants from various genera were examined to identify the extent of mycorrhizal colonization and the degree of mycorrhizal development within their root systems.

Materials and Methods

Plants that were used during this study were greenhouse grown in Corydon, Indiana, USA. They were propagated either from seed or from greenhouse-grown vegetative fragments. Plants were not inoculated with mycorrhizal spores. Thus, any mycorrhizae found would have come from the potting materials used, or from other environmental sources in the greenhouse.

Twenty three species were examined in total. A representative sample of different parts of the root (or stolons, for rootless species) from each species was obtained and preserved in 95% ethanol for a minimum of 24 hr. Individual root samples of 1-2 cm were placed on a slide, washed with deionized water and stained using a mixture of 80% (by volume) solution A, and 20% (by volume) solution B. Solution A was 0.3% (by mass) aniline blue in 90% ethanol. Solution B was Lactophenol Blue Solution (FLUKA, Fuchs, Switzerland; Marx 1982; Nemec 1982; Ruzin 1999).

Three 1-2 cm sections of root or stolons were selected randomly from samples of each spe-

Table 1: Mycorrhiza-formation scores (VAM=Vesicular Arbuscular Mycorrhizae)		
Taxon	Root system	Score (0 - 10)
Nepenthaceae		
Nepenthes sanguinea	Weak/medium	4
Lentibulariaceae		
Pinguicula laueana	Weak, and large	6
Pinguicula moranensis	Weak	5
Pinguicula planifolia	Weak, and large	5
Utricularia cornuta	None	0
Utricularia inflata	None	6
Utricularia longifolia	None	2
Utricularia nephrophylla	None	0
Utricularia paulineae	None	3
Utricularia striata	None	0
Sarraceniaceae		
Sarracenia purpurea	Weak/medium	2-3
Sarracenia psittacina	Weak/medium	4
Sarracenia rubra subsp. *gulfensis*	Weak/medium	6
Darlingtonia californica	Weak/medium	7
Droseraceae		
Dionaea muscipula	Weak, but fleshy	4
Drosera adelae	Weak, and large	3
Drosera androsacea	Weak, and large	4
Drosera aliciae	Weak, and large	7
Drosera binata	Weak, and large	6
Drosera patens × *occidentalis*	Weak, and large	3
Drosera scorpioides	Weak, and large	3
Drosera omissa	Weak, and large	5
Drosera prolifera	Weak	3, mostly VAM
Drosera aliciae	Weak, and large	0 (Control)
Drosera patens × *occidentalis*	Weak, and large	0 (Control)

cies and used for staining and analysis. Sections were stained for approximately 30 min, with the exceptions of *Drosera binata* and *Nepenthes sanguinea*, both of these plants having a thick, dark root structure. Their root sections were stained for less than 2 min to allow some detail to be seen after staining and then washed thoroughly, like other samples, with deionized water to remove the excess stain. A cross-section cut from the sample was used in the situation when entire root was too thick or too dark to be viewed under the microscope. After being washed with deionized water for approximately 5 min to remove excess stain the root samples were ex-

amined under a compound microscope. Fungal colonization was measured using the following numerical scale devised by Moberly & Darnowski (unpublished):

0: No staining or staining of bodies clearly identified as structures other than mycorrhizae.
1: 1-3 cells in the field of view with mycorrhizal infection.
2: 4-5 cells in the field of view with some mycorrhizal infection or a similar number or bodies or mass of bodies in a smaller number of cells in the field of view.
3: 6-8 cells in the field of view with some mycorrhizal infection or a similar number or bodies or mass of bodies in a smaller number of cells in the field of view.
4: 9 or more cells in the field of view with some mycorrhizal infection or a similar number or bodies or mass of bodies in a smaller number of cells in the field of view.
5-10: Multiple clusters of cells scoring as in 1-4 above, with the total score for all clusters in the field of view being 5-10.

In using the above ranking scheme, more elaborately detailed or larger mycorrhizal clusters in a given cell raised the score by 1. As negative controls, we used roots from *Drosera aliciae* and *Drosera patens* × *occidentalis* (*i.e., Drosera* sp. "Lake Badgerup") that were grown in aseptic culture media at the lab.

Results

Table 1 illustrates the results of this survey on the presence of mycorrhizal colonization. All but three of the 23 species examined, the exceptions being *Utricularia striata*, *Utricularia cornuta*, and *Utricularia nephrophylla* (all bladderworts and the first and third aquatic plants), formed mycorrhizae when grown in the greenhouse. Wide variation in the shapes and abundance of mycorrhizae was observed in different species. *Drosera aliceae*, *Drosera scorpioides*, *Darlingtonia californica*, and *Sarracenia rubra* subsp. *gulfensis* formed a very large number of fungal colonizations, while other species, such as *Sarracenia purpurea*, *Utricularia longifolia*, and *Utricularia paulineae* formed very few associations.

Discussion

Since the observations of Nitschke (1860) and Burbidge (1897), there has been a general belief that carnivorous plants only have weakly developed roots, *e.g.*, most *Drosera* species, *Dionaea*, *Pinguicula*, *Cephalotus*, *Sarracenia*, and most *Nepenthes*, or no roots at all, *e.g.*, *Utricularia*, *Genlisea*, and *Aldrovanda*. The absence of roots does not necessarily mean, however, that the functions or roots are not needed; in some plants the stem and leaves have replaced their functions (Lambers & Colmer 2005). Therefore, mycorrhizal associations might occur, even in carnivorous plants where true roots have been replaced by modified shoot parts. In particular, this applies to species of *Utricularia*, and as can be seen in Table 1, even some aquatic *Utricularia* showed signs of fungal associations. Given that, it is not surprising that terrestrial and epiphytic species that grow in moist soil and in decomposing organic matter from other plants (Taylor 1989) also often showed signs of mycorrhizal infection.

On the basis of a recent ecophysiological study (Adamec 2005) carnivorous plant roots appear to be physiologically very active per unit biomass, in spite of their relatively weak proportion of total plant biomass. Since the prevailing amount of mineral nutrients in carnivorous plants gained by roots, the activity of which is greatly stimulated by foliar nutrient absorption from prey, the role of roots is crucial also for carnivory (Adamec 2005), making the general presence of mycorrhizae in cultivated carnivorous plants sensible. Further study of wild populations is needed to confirm the presence of mycorrhizae in natural growth conditions.

Ackowledgements: The authors thank the Indiana University Southeast Office of the Dean for Research for Large Grants and Indiana University for an Intercampus Grant and a Research Support Fund grant.

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Mineral nutrition in hydroponically-grown *Pinguicula*

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Keywords: hydroponics, mineral nutrition, *Pinguicula* spp.

Introduction

Hydroponic cultivation consists of growing plants on inert substrates with liquid mineral nutrient solutions. It has recently become popular for home-growing plants in limited spaces and for large-scale greenhouse production of crop plants. It is also a valuable tool for the scientific investigations of plant mineral nutrition. Despite the importance of mineral nutrition in the carnivorous syndrome (Juniper *et al.* 1989; Adamec 1997, 2002; Ellison 2006), there is no recent report on the potential usefulness of hydroponics as an investigation tool for studying mineral nutrition of carnivorous plants. The goal of this study was to evaluate the growth response of carnivorous plants of the genus *Pinguicula* on a peat-based and a rockwool-based hydroponic set-ups under various conditions of mineral deficiencies.

Materials and Methods

Plant material

In total, 21 *Pinguicula* species and hybrids were used in the study. Several Mexican species used in this study were derived from seed stocks originally collected from field sites in Mexico and later grown among hobbyists in Europe: *P. agnata* from El Chico, *P. gigantea* from either Ayautla or Synalta, *P. moranensis* from Zacapoaxtla. *P.* sp. "huahuapan" had an affinity with *P. rectifolia, P.* sp. "la vuelta" with *P. moranensis* and *P.* sp. "pachuca" had no affinity with any known species (for affinity relationships of these undescribed plants with described species, see Cieslak *et al.* 2005). Hybrids used in this study (*P. agnata* × *P.* sp. "huahuapan", *P. gigantea* × *P. moctezumae*, and *P. grandiflora* × *P. vallisneriifolia*) were all prepared by one of the authors (L. L.). Specimens of other species had a commercial origin (Nature et Paysages, France).

Hydroponic growth conditions

All plants were propagated *in vitro* according to Legendre (2011) before being immediately transferred to the hydroponic set-ups during this study. Starting stock plants had a rosette diameter of 0.5 cm unless otherwise stated.

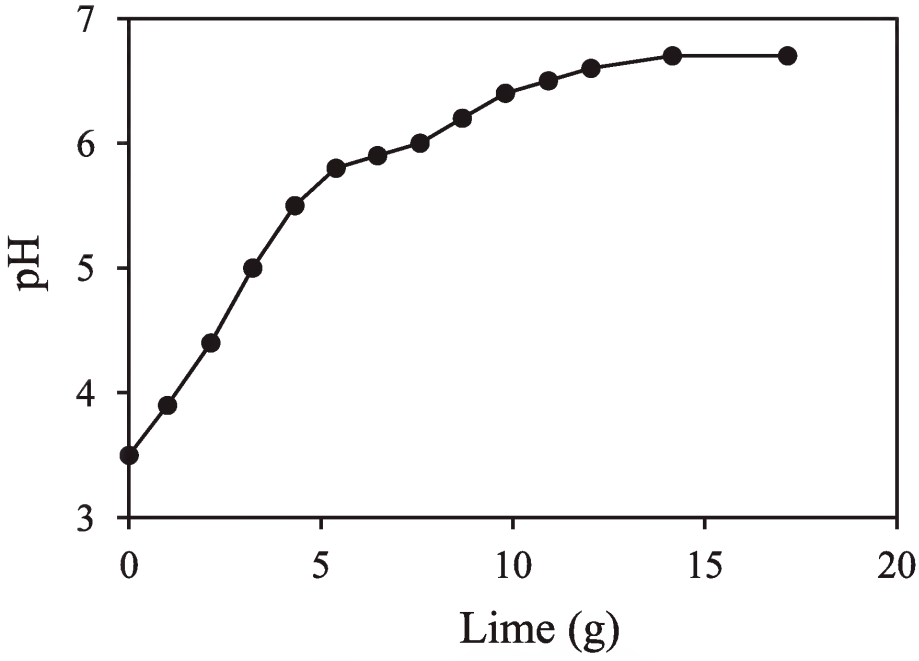

Figure 1: pH-titration curve of 100 g of Lithuanian peat with milled garden lime.

Growth conditions on peat

Plants were grown either on a mixture of peat and sand (2:1) (Lithuanian peat at pH 3.5 and 2 mm quartz sand) or on a similar mixture fertilized as originally published by Baker (1957) as the University of California (UC) basic mix (5 kg dry *Sphagnum* peat, half of the peat volume of 2 mm quartz sand, 11.34 g KNO_3, 11.34 g K_2SO_4, 113.6 g superphosphate 0-20-0, 341 g milled dolomitic lime, 113.6 g milled calcitic limestone). Both growth media were sterilized by autoclaving at 110°C for 2 h. They were laid in closed 70×45×20 cm polystyrene foam containers. Distilled water was added until it reached 2 cm above soil level. After 2 days, excess water was drained away by opening holes at the bottom of the containers. The stock plants were planted in the soil and the containers were placed in a greenhouse under regular top watering with no tray underneath (mist turned on for 15 min every other hour – deionized water was used). Temperature in the naturally lit greenhouse was 35-40°C during the days and 25°C at night. Relative humidity was kept above 90%.

Three to 6 specimens of each of the 13 tested species were planted in a random manner in the same container. Each experiment was replicated three times consecutively. Diameters of plant rosettes were measured after 4 months. Statistical analyses were made by Student t-test.

In order to assess the effect of lime addition on peat pH, a pH-titration curve was constructed by adding small increments of milled lime to a peat-water mixture/suspension. In this experiment, the pH rose markedly from 3.5 to subsequently stabilize at around 6.3-6.7 (see Fig. 1). The UC basic mix used in this study had a pH value of 6.5.

Growth conditions on rockwool

Rockwool slabs (50×44×10 cm) were irrigated with a mineral solution in a tidal table set-up. The mineral nutrient solution was pumped upward into the dish that contained the plants for 15 min at regular intervals 4 times per day. An overflow tube connected this dish

Table 1. Composition of the mineral nutrient solutions after Hoagland and Arnon (1950) modified by Jones (1997) for studying mineral deficiency effects.

Nutrient stock solutions	Concentrations in stocks (g/l)	Volume of stocks used in final solution (ml of stock per litre of final solution)					
		Complete	- N	- P	- K	- Ca	- Mg
1 M $Ca(NO_3)_2.4H_2O$	236	5	0	4	5	0	4
1 M KNO_3	101	5	0	6	0	5	6
1 M KH_2PO_4	136	1	0	0	0	1	1
1 M $MgSO_4.7H_2O$	246	2	2	2	2	2	0
50 mM FeNaEDTA	18.4	1	1	1	1	1	1
50 mM K_2SO_4	8.7	0	5	0	0	0	3
10 mM $CaSO_4.2H_2O$	1.72	0	100	0	0	0	0
50 mM $Ca(HPO_4)$	6.8	5	0	5	0	0	0
Micronutrient stock		1	1	1	1	1	1
H_3BO_3	2.86						
$MnSO_4.H_2O$	1						
$ZnSO_4.7H_2O$	0.22						
$CuSO_4.5H_2O$	0.08						
$(NH_4)_6Mo_7O_{24}$	0.02						

to the nutrient solution storage container to allow the water table to stabilize 1 cm below the base of the plants. When the pump was turned off, the mineral solution flowed back to the storage tank through the pump. The formulas of the mineral nutrient solution used in this study are summarized in Table 1.

Plants (1 cm diameter) were laid on the rockwool and rooting took place in less than a week. Plants were illuminated by fluorescent tubes (industrial white) placed 40 cm above them (10 cm spacing) 16 h each day in an enclosed wooden box. Relative humidity oscillated from 45 to 55% between day and night, respectively.

All species used in an experiment were planted in the same dish (one dish per mineral nutrient solution – minimum of 6 plants per species per dish planted in a random manner). Each experiment was replicated at least 3 times in three independent set-ups. Significance of growth characteristics were estimated with the Student t-test ($p<0.001$).

Mineral content analyses

After 40 d of cultivation on the rockwool set-up, entire plants were collected. Their roots were removed and the leaves dried (at 45°C in a ventilated oven for a week) and powdered for later mineral nutrient analyses. The analyses were conducted on single plants and a minimum of three parallel plants were sampled individually per growth dish for each experimental condition.

The powdered leaf mass was digested and mineralized with concentrated acids and ana-

lysed for N, P, K, Na, Ca, and Mg content as described in Adamec (2002). Results of the nutrient analyses were expressed as % of dry weight (DW) for each nutrient.

Results and Discussion

A comparison of the growth increase of *Pinguicula* plants on fertilized (UC basic mix) and non-fertilized peat-sand revealed that most of the tested plants performed much better on the fertilized mixture (Table 2). The exceptions were *P. primuliflora*, *P. filifolia*, *P. moranensis*, *P.* sp. "la vuelta", and *P. lusitanica* (similar size plants on both media) and *P. sharpii* (died on the UC basic mix). Even though *P. moranensis* and *P.* sp. "la vuelta" did not attain a larger size, they produced more leaves (more active growth) on the fertilized medium. Typically, all plants had short-lived leaves that died (and rotted away) upon contact with the substrate when grown on the non-fertilized medium so that two layers of leaves were never seen stacked above each other. On the contrary, all Mexican *Pinguicula* species (except for *P. sharpii*) developed 2-3 layers of leaves stacked above each other in the fertilized medium (UC basic mix). In a later experiment, temperate European species (*P. vulgaris*, *P. grandiflora*, *P. dertosiensis*) also developed larger rosettes with more active growth on the UC basic mix (data not shown). The use of the UC basic mix generated some unusual phenotypes in some species, such as the appearance of red veins in *P. agnata* and the production of plantlets at the base of *P. lusitanica* and *P. crystallina* (Table 2). These

Table 2. Comparison of the rosette diameter *Pinguicula* species grown on pure peat/sand or on fertilized peat/sand (UC basic mix) after being watered with distilled water during 4 months (starting plant diameter was 0.5 cm). # Statistically-significant differences (t-test) at $p<0.05$ (*ns*, $p>0.05$).

Species name	Plant diameter (mean ± SD) on	
	non-fertilized mixture	fertilized mixture
P. agnata	2.5 ± 0.1	8.2 ± 3.3#
P. crystallina	2.0 ± 1.1	3.7 ± 0.5#
P. emarginata	2.3 ± 0.6	4.0 ± 0.1#
P. filifolia	4.3 ± 0.6	5.3 ± 0.6ns
P. gigantea "ayautla"	2.5 ± 0.2	24 ± 0.1#
P. gigantea "synalta"	9.0 ± 0.1	21 ± 1.0#
P. lusitanica	1.7 ± 0.3	1.7 ± 0.3ns
P. moranensis	5.1 ± 0.1	4.0 ± 0.5ns
P. primuliflora	1.7 ± 0.9	2.2 ± 1.6ns
P. sharpii	3.0 ± 0.2	Dead
P. sp. "huahuapan"	1.5 ± 0.8	2.3 ± 0.6#
P. sp. "la vuelta"	4.0 ± 1.0	4.2 ± 0.8ns
P. sp. "pachuca"	4.0 ± 0.1	5.8 ± 1.3#
P. agnata × *P.* sp. "huahuapan"	0.5 ± 0.1	4.0 ± 1.4#

could be detached and used to multiply the plants. All in all, these results show that *Pinguicula* species can be divided into two subgroups that differ in their growing response to a fertilised substrate. Coincidently, with the exception of *P. sharpii*, this correlates with a major evolutionary event within this genus (Cieslak *et al.* 2005), separating species of the subgenus *Isoloba* from those of the subgenera *Pinguicula* and *Temnoceras*. It also corresponds to a separation of species with opposite growth patterns: tropical growth-form *vs.* temperate growth-form.

Similar observations were made after growing a set of *Pinguicula* species on rockwool with the full-strength Hoagland and Arnon's (1950) hydroponic mineral nutrient solution. Temperate European (*P. vulgaris*, *P. longifolia*, *P. dertosiensis*, *P. nevadensis*, *P. grandiflora*, *P. vallisneriifolia*) as well as most Mexican *Pinguicula* species (*P. moctezumae*, *P. laueana*, *P. moranensis*, *P. rectifolia*, *P. emarginata*, *P. gypsicola*, *P. agnata*, *P. gigantea*) grew very well under these conditions, while the Mexican *P. sharpii*, Caribbean *P. filifolia*, and south-east USA species *P. lutea* died within weeks (data not shown). As shown in Table 3, the plants that fared well grew up to giant sizes after only 3 months of cultivation on the Hoagland and Arnon's (1950) complete nutrient solution (started to bloom shortly after the measurements were made, *ca* 100 days after planting).

Plants grown on a N-deficient solution were markedly smaller. They were pale green to yellow while plants were dark green on the complete solution. Very reproducibly, the leaves of some species became deep reddish-purple on their whole lamina (*P. laueana*, *P.* sp. "la vuelta"), or just along the veins (*P. grandiflora* × *P. vallisneriifolia*), a pigmentation that was at best faint on the complete (N-supplemented) Hoagland and Arnon's (1950) nutrient solution. At the end of the experiment, *P. laueana*, *P. vulgaris*, and *P.* sp. "la vuelta" were starting to enter dormancy on the N-deficient solution. This experiment has been replicated 3 times with similar results.

On a couple of occasions, it was observed that some of the plants on N-deficient nutrient solution (especially those with no reddish pigmentation) spontaneously caught large

Table 3. Effect of nitrogen deficiency on *Pinguicula* growth. Plants were grown in the rockwool hydroponic setup. Measurements were made on 2-4 parallel plants, 92 days after planting 1 cm diameter plantlets and irrigating with Hoagland and Arnon's (1950) full-strength or N-deficient nutrient solution. Differences in growth were significant for all species ($p<0.05$, t-test).

Species name	Rosette diameter (cm ± SD) on	
	complete solution	N-deficient solution
P. laueana	13.6 ± 0.4	3.8 ± 0.5
P. moctezumae	18.5 ± 0.7	7.5 ± 0.7
P. vulgaris	5.4 ± 0.9	1.1 ± 0.1
P. grandiflora × *P. vallisneriifolia*	24.7 ± 0.7	14.3 ± 5.2
P. gigantea × *P. moctezumae*	43.1 ± 1.2	7.5 ± 1.5
P. sp. "huahuapan"	10.5 ± 0.7	6.5 ± 0.5
P. sp. "la vuelta"	15.8 ± 0.5	5.1 ± 0.2
P. sp. "pachuca"	14.0 ± 1.9	7.7 ± 1.5

numbers of insects (10-100 times more root gnats than plants grown on the complete solution). This was very surprising because the trays with both mineral variant solutions were grown side-by-side in the same growth box (closed except during door opening). Unfortunately, insect captures were rare and all attempts to artificially introduce insects as prey failed so that this observation could not be repeated. Though the capture of insects is expected to provide the plants with some external source of nitrogen (and eventually additional minerals not provided by the nutrient solutions), the presence of captured insects on a plant never correlated with an increase in size or a greening of the leaves when compared to plants of the same tray that had not caught any insect. It is likely that the high nutrient concentrations in the nutrient solutions far prevailed over the effects of nutrients originating from the tiny preys.

Between day 17 and 30 after planting, plants of *P.* sp. "la vuelta" and *P.* sp. "pachuca" grown with distilled water (on rockwool or peat) and on N- or K-deficient nutrient solutions increased their rosette diameter by merely 11%, while those grown on the complete solution, Ca-, Mg-, and P-deficient solutions increased by 30%. The differences were significant using Bonferroni test ($p<0.05$). Plants grown on distilled water and on N- or K-deficient nutrient solutions had lighter green leaves (*P.* sp. "pachuca") or red-purple leaves (*P.* sp. "la vuelta") that were always sticky to the touch. Plants grown on the complete solution or on Ca-, Mg-, or P-deficient solutions had dark green leaves and were not sticky (mucilage or glands were not visible to the naked eye, either).

Table 4. Mineral nutrient content of *P.* sp. "la vuelta" and *P.* sp. "pachuca" plants grown under different fertilization regimes. Analyses were made after 40 days of cultivation either on rockwool with variants of the Hoagland and Arnon's (1950) solution (complete: original formula; none: no minerals; -Ca: Ca deficiency; -Mg: Mg deficiency; -K: K deficiency; -N: N deficiency; Dd water: distilled water) or on peat/sand mixture with distilled water. At this stage, the fastest growing plants had not yet doubled in size. Values represent the mean ± SD, n=6 (4 *P.* sp. "la vuelta" and 2 *P.* sp. "pachuca" plants, both species yielding similar results). For the complete solution, n=1.

Substrate	Nutrient solution	Plant mineral content (% DW)					
		N	P	K	Ca	Mg	Na
Peat	Dd water	0.71 ± 0.27	0.044 ± 0.003	0.85 ± 0.37	0.74 ± 0.18	0.34 ± 0.12	0.043 ± 0.012
Rockwool	Dd water	1.14 ± 0.25	0.055 ± 0.013	0.96 ± 0.46	1.06 ± 0.37	0.34 ± 0.11	0.028 ± 0.013
Rockwool	-Ca	2.25 ± 0.24	0.74 ± 0.15	4.56 ± 1.66	0.26 ± 0.18	1.04 ± 0.48	0.066 ± 0.017
Rockwool	-Mg	2.89 ± 0.24	0.50 ± 0.17	4.23 ± 1.28	1.90 ± 0.34	0.13 ± 0.024	0.054 ± 0.018
Rockwool	-K	1.76 ± 0.37	0.058 ± 0.009	1.10 ± 0.40	1.58 ± 0.53	0.52 ± 0.17	0.061 ± 0.047
Rockwool	-N	0.65 ± 0.15	0.051 ± 0.011	1.38 ± 0.40	0.79 ± 0.40	0.60 ± 0.25	0.044 ± 0.016
Rockwool	Complete	3.21	0.73	3.40	1.08	0.41	0.078

Plants grown on the complete solution had much higher tissue nitrogen, potassium, and phosphorus contents than those grown with distilled water (Table 4; the results were similar during distilled water watering on both rockwool and peat). Calcium and magnesium contents were not raised markedly by the use of the complete solution. Nitrogen deficiency appeared to have pleiotropic effects and led to decreased N, K, and P accumulation, while Ca and Mg remained almost unaffected. In agreement with the previous observation that plants grown on K-deficient media had similar phenotypes as those grown on N-deficient media, K-deficient media had the same effects on mineral accumulation in the plants as did N-deficient ones. Accumulations of N, P, and K were therefore found to be linked to each other, and all of these minerals were required for fast growth. On the other hand, Ca and Mg deficiencies did not affect P and N uptake but raised P uptake. The absence of Ca in the solution increased Mg accumulation, and *vice versa*. Thus, the absence of one of these two divalent cations led to a greater accumulation of the other three cations (K, Na, and the other divalent cation) in the plant. Even though tissue Ca content in the plant did not relate to plant growth, high Ca contents have been suggested to enhance plant tolerance to fungal attack (Legendre & Kibellis 2005). On the other hand, high foliar Ca content in carnivorous plants can indicate that the plants were grown under greenhouse conditions at low air humidity, *i.e.*, at high transpiration rates (see Adamec 2002).

In conclusion, the tissue nutrient content, but Ca and Mg, found in two Mexican *Pinguicula* species grown in nutrient-poor substrates (Table 4) was very similar to that reported for circumboreal *P. vulgaris* grown experimentally in slightly enriched peaty substrates (Aldenius *et al.* 1983; Karlsson & Carlsson 1984). Tissue Ca and Mg contents in these Mexican calcicole species remained surprisingly high, even when watered with distilled water (*cf.* Adamec 1997). However, when the two Mexican species were grown in the complete, rather concentrated, nutrient solution (Table 1) their tissue N, P, and K contents roughly tripled and were comparable with those reported for many species of rapidly growing wetland plants (*cf.* Dykyjová 1979). Studies conducted on temperate European *Pinguicula* species (*P. vulgaris, P. alpina, P. villosa*) revealed that they have the capacity to take up mineral nutrients for plant growth both by roots and leaves (Aldenius *et al.* 1983; Karlsson & Carlsson 1984; Hanslin & Karlsson 1996; for a review see Adamec 1997). In this context, this study proved a very great capacity of a large set of *Pinguicula* species (though not all) for nutrient uptake by roots (Table 2, 3) and unveiled a complex network of accumulation interrelationships among them (Table 4).

Acknowledgements: This study was supported partly (to L. A.) by the Research Programme of the Academy of Sciences of the Czech Republic (No. AV0Z60050516).

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An improved mineral nutrient solution for the *in vitro* propagation of *Pinguicula* species

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Keywords: *in vitro* cultivation, *Pinguicula*, tissue culture.

Introduction

Propagating plants *in vitro* (tissue culture) is widely used to rapidly generate large quantities of disease-free plants. It is applied to hundreds of carnivorous plant species including the genus *Pinguicula* whose species are very sensitive to pathogenic microorganisms and, in some cases, may develop poorly under current cultivation practices (Legendre & Kibellis 2005).

Adams *et al.* (1979) and Gonçalves *et al.* (2008) have respectively reported the successful micropropagation of *P. moranensis* and *P. lusitanica* on a diluted version of the mineral nutrient solution originally published by Linsmaier & Skoog (1965). This was based on the nutrient composition described by Murashige & Skoog (1962) with modifications of the vitamin content. Nevertheless, most *Pinguicula* species cannot be grown on this nutrient solution because they will develop leaf tip burns (*P. moctezumae*), reduce in size after each transplanting (*P. filifolia*), quickly form dormant hibernaculae (all temperate European species), or simply do not grow at all (*P. ramosa*, *P. parvifolia*).

This article aims to share some pieces of personal experience and the composition of an improved mineral nutrient solution that alleviates the above-described symptoms and which can be used to successfully propagate most *Pinguicula* species.

Materials and Methods

Plant material

All species used in this study were obtained from a horticultural company (Nature et Paysages, France) except *P. vulgaris*, *P. gigantea*, and *P. macroceras* seeds which had been collected from the Champagne state of France, Ayautla, Mexico, and Douglas Park, California, USA, respectively. *P.* sp. "huahuapan" had affinity with *P. rectifolia* and *P.* sp. "la vuelta", *P.* sp. "santa maria yukuhiti" had affinity with *P. moranensis*. The hybrid *P. moctezumae* × *P. gigantea* was prepared by the author.

Explant sterilisation

Seeds of all species were surface-sterilized before being introduced to tissue culture vessels. For this, they were soaked for 6 min (with regular vigorous shaking) in a 4% calcium hypochlorite solution supplemented with 0.1% Tween-20 (this solution was filtered immediately before use to discard the detergent-induced bleach precipitates) followed by 3 rinses of 10 min each in sterile distilled water. Dead seeds and plant debris floated in the surface foam while live seeds sank to the bottom of the tubes. This allowed their easy separation either by specifically sucking away the live seeds with

a sterile glass Pasteur pipette or by pouring away the sterilizing and rinsing solutions containing the non-seed material.

Growing conditions

Plants were grown in 5 cm diameter glass vessels (3-6 plants per pot) in a growth chamber maintained at constant temperature (23°C) with an air conditioning unit. They were lit with fluorescent tubes (alternating industrial white and warm, reddish, light tubes, each 10 cm apart) placed 40 cm above the plants. Lights were on 16 h per day.

Liquid media were sterilised by autoclaving at 121°C for 20 min. Plant transfers were made in sterile air in laminar flow hoods.

Plants (0.5 cm diameter) were subcultured 3 times on Mix A (see below) before being transferred to Mix A or Mix B for the comparative study. Their diameter was then measured 8 weeks later.

Mineral nutrient solutions

Distilled water was used to prepare all stock and final solutions.

Mix A: Macronutrients 10X stock (stored at 4°C for up to 3 months): NH_4NO_3 (16.5 g/l), KNO_3 (19.0 g/l), $CaCl_2$ (3.322 g/l), $MgSO_4$ (1.807 g/l), KH_2PO_4 (1.70 g/l) (Murashige & Skoog 1962).

Micronutrients 1000X stock (stored at –20°C for several years): KI (0.83 g/l), H_3BO_3 (6.2 g/l), $MnSO_4.H_2O$ (16.9 g/l), $ZnSO_4.7H_2O$ (8.6 g/l), $CuSO_4.5H_2O$ (0.025 g/l), $CoCl_2.6H_2O$ (0.025 g/l), $Na_2MoO_4.2H_2O$ (0.25 g/l). This later substance was dissolved separately in 100 ml water before being added to the final micronutrient mix (Murashige & Skoog 1962).

Fe-EDTA 200X stock (stored at 4°C away from light for up to 3 months): Na_2EDTA (7.45 g/l dissolved in 900 ml of nearly boiling water), $FeSO_4.7H_2O$ (1.86 g/l added with small increments to boiling hot EDTA). The volume was subsequently adjusted to 1 liter with water. A couple of drops of concentrated HCl were added if iron salts did not dissolve. Crystals formed in the refrigerator during storage but could be re-dissolved by a quick boil in a microwave oven (Dalton *et al.* 1983).

Vitamins 1000X stock (stored at –20°C for several years): myo-inositol (100 g/l), Thiamine.HCl (0.4 g/l), glycine (2 g/l) (Linsmaier & Skoog 1965).

Final mix: 25 ml/l 10X macronutrient stock, 1 ml/l 1000X micronutrient stock, 3.78 ml/l 200X iron-EDTA stock, 1 ml/l 1000X vitamin stock, 20 g/l sucrose (food grade), pH 5.9 (adjusted with 1 N KOH), 5 g/l Agargel (Sigma A-3301). This specific grade of Agar gave the best results.

Mix B: Macronutrients 10X stock (stored at 4°C for up to 3 months): NH_4NO_3 (4.125 g/l), KNO_3 (19.0 g/l), $CaCl_2$ (3.322g/l), $MgSO_4$ (1.807 g/l), KH_2PO_4 (1.70g/l).

Micronutrients, Fe-EDTA, and Vitamins stocks were the same as in Mix A.

Final mix: 50 ml/l 10X macronutrient stock, 1 ml/l 1000X micronutrient stock, 3.78 ml/l 200X iron-EDTA stock, 1 ml/l 1000X vitamin stock, 20 g/l sucrose (food grade), pH 5.9 (adjusted with 1 N KOH), 5 g/l Agargel (Sigma A-3301).

Results and Discussion

In a first series of experiments, the growth of *P. moranensis* and *P. agnata* were compared when grown on serial dilutions of Linsmaier & Skoog's (1965) nutrient solution (undiluted and 2-, 4-,

8-, and 16-fold dilutions). The fastest growth was obtained with 4- to 8-fold diluted nutrient solutions in agreement with the formulation used by Adams *et al.* (1979) and Gonçalves *et al.* (2008). A comparison of the growth of the same two species on 4-fold diluted nutrient solutions, the pH of which were either adjusted with NaOH or with KOH, revealed that sodium ions (Na^+) were toxic to these species and the use of KOH yielded faster growth (data not shown). Similarly, the use of the iron-EDTA formulation developed by Dalton *et al.* (1983) was found to significantly improve the performance of these two species (prevents iron, and other metal ions, from precipitating during autoclaving). Because repeated transplanting led to regularly more stunted specimens, the nutrient solution was modified to dilute the macronutrients 4 times while maintaining a constant concentration of the other nutrients (micronutrients, iron-EDTA, and vitamins). Several studies have indeed demonstrated that micronutrients can be growth-limiting if their concentrations are half of optimum values that are, more or less, common to most land plants (Jones 1997). All of these basic adjustments of composition of the nutrient solution have led to the design of Mix A of the present study.

Nevertheless, other species (most noticeably *P. filifolia*, for example) did not grow optimally on Mix A and displayed reduced growth after each subculture. For this reason, a new formulation (called Mix B) was designed and assayed in a comparative study after an initial round of propagation on Mix A. Mix B differed from Mix A by having half the ammonium nitrate and twice the other macronutrients (1/8 the ammonium nitrate and half of the other macronutrients than in Murashige & Skoog, 1962). As shown in Table 1, Mix B allowed most of the tested *Pinguicula* species to develop to larger sizes. Species, like *P. filifolia* and all European temperate species, responded very positively to the change in nutrient solution, while others (from Mexico) were unaffected. Typically, those species that exhibited size reduction during subculturing on Mix A were the ones that fared better on Mix B. All of the species listed in Table 1 were subsequently subcultured 12 times on Mix B with no significant reduction in growth rate or size.

Table 1. Comparative growth of some *Pinguicula* species on tissue culture mineral nutrient solutions Mix A and Mix B.

Species name	Final plant diameter (cm; mean ± SD)*	
	Mix A	Mix B
P. filifolia #	4.0 ± 1.0	8.0 ± 1.0
P. grandiflora #	1.5 ± 0.6	3.7 ± 0.6
P. longifolia subsp *longifolia* #	2.3 ± 0.6	4.7 ± 0.6
P. moctezumae #	3.0 ± 0.9	5.5 ± 0.6
P. vulgaris #	1.2 ± 0.6	3.0 ± 0.1
P. macroceras #	1.7 ± 0.5	2.7 ± 0.6
P. sp. "huahuapan" ns	4.0 ± 1.0	4.3 ± 0.6
P. sp. "la vuelta" #	2.7 ± 0.6	3.7 ± 1.5
P. sp. "santa maria yukuhiti" #	1.5 ± 0.6	2.5 ± 0.6
P. moctezumae × *P. gigantea* ns	2.7 ± 0.9	3.0 ± 0.8

* 8-week old plants – starting plant diameter: 0.5cm; # Significant difference in growth between the two lots of plants (Student t-test, $p<0.05$). ns: non-significant ($p>0.05$). n=3-6.

A set of species that did not develop (or grew very slowly) on Mix A (*P. parvifolia*, *P. heterophylla*, *P. oblongiloba*, and *P. ramosa*) was directly cultured on Mix B. All species developed surprisingly well on Mix B.

Two interesting observations were made during the comparative study of the nutrient solutions, Mix A and B. First of all, long-leaf species such as *P. moctezumae* and its hybrids developed dead leaf tips on Mix A, a phenomenon that also occurs occasionally when plants are grown *ex vitro* (on soil). This disorder was absent from plants grown on Mix B. Additionally, temperate European species did not form winter buds (and entered dormancy) as quickly on Mix B as on Mix A. So, not only did they develop faster on Mix B, but they remained longer in active growth on this mix, growing larger plants in a shorter time. This suggests that both phenomena may be caused by a deficiency of some minerals.

Unlike in Adams *et al.* (1979), the exogenous addition of hormones to the tissue culture medium was not an absolute requirement to induce the multiplication of *Pinguicula* species *in vitro* (also observed by Gonçalves *et al.*, 2008). All species multiplied spontaneously when left long enough on their growth media.

One drawback of *in vitro* technology is that it leads to an inevitable drift in the plants genetic make-up. Though tissue cultured plants are multiplied vegetatively, and are therefore, supposed to be genetically equal, somaclonal variants spontaneously arise (even when no hormones are added exogenously). These variants can be unintentionally selected because of the massive number of plants that are generated in comparison to the small number of specimens that are selected when subculturing fresh media for the next round of propagation, especially in the case of species that grow slowly. I have indeed noticed that I was systematically transferring the best-looking (best-growing) specimens. This led, for example, to the selection, after two years of micropropagation, of a clone of *P. ramosa* that was growing much faster and was making plants twice as large as the mother plant from which it originated.

Acknowledgement: The author wishes to thank Lubomir Adamec for his careful review and suggestions about this manuscript.

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Fishing spiders in the hanging stomachs of Borneo

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Keywords: Field studies: Borneo, spiders, ants, *Nepenthes gracilis*, *Misumenops nepenthicola*, *Oecophylla smaragdina*.

Desperate for a drink, the famous naturalist Alfred Wallace drank the liquid from a group of pitcher plants while exploring Malaysia. Although the fluid was full of dead insects and looked "uninviting", he wrote in 1890 that he and his friends "found it very palatable, though rather warm, and we all quenched our thirst from these natural jugs". They must have been almost delirious with dehydration to have quaffed a few pitchers, as one local name for these plants translates to "the place where rats pee", probably because of the urine-like smell from decomposing insects. While I have drunk the fluid from unopened pitchers of *Nepenthes gracilis* (slightly sweet and slimy), when it smells like a rat urinal, and comes from a vessel often called a "hanging stomach", it does not sound like a drink that will improve with age.

In many ways the pitchers are like the stomachs of animals. Although not bubbling cauldrons of flesh-dissolving digestive fluid, they do contain bacteria and antioxidants that break the insects down. One animal that often ends up with pitcher-plant food in its own stomach is the crab spider *Misumenops nepenthicola*. It spends almost its entire life living within the pitcher and captures insects lured to the plant's nectar, as well as aquatic larvae living in the pitcher-plant fluid.

Misumenops not only lives with a number of Asian species of *Nepenthes* and steals their food,

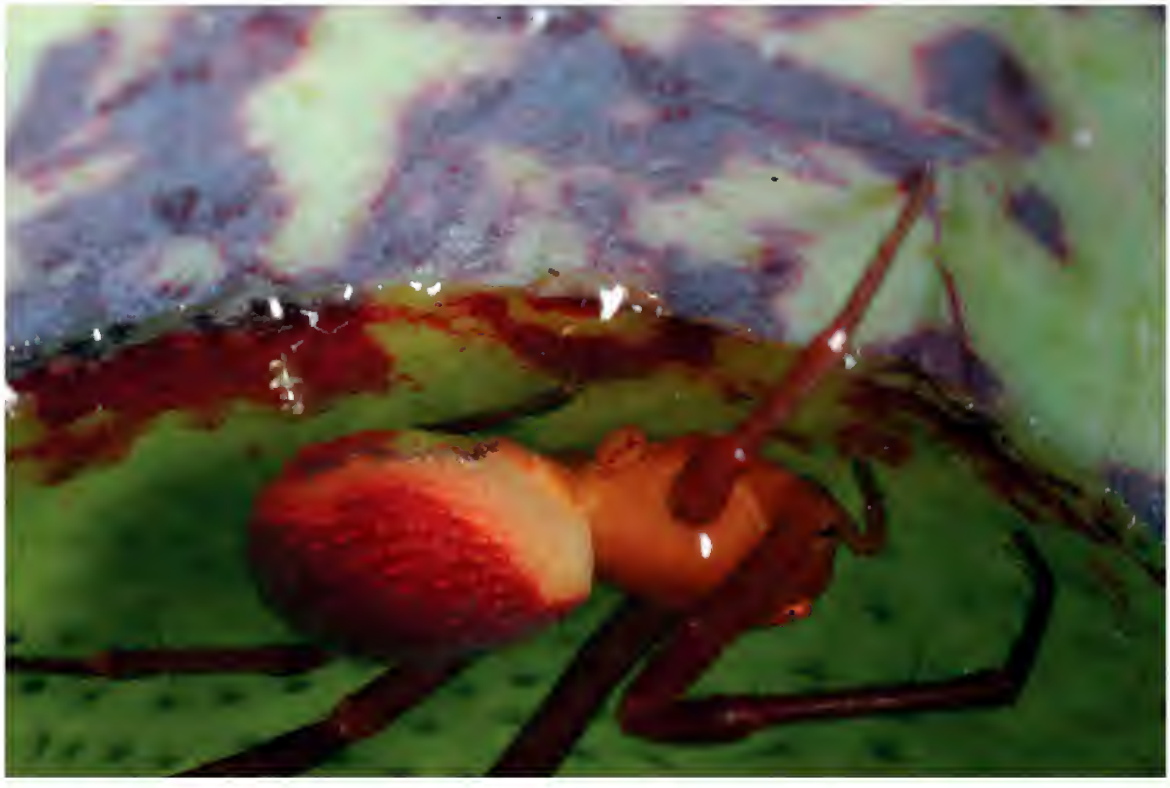

Figure 1: *Misumenops* with air bubble.

Figure 2: *Misumenops* dragging ant.

Figure 3: *Misumenops* and larva.

but it even uses the plant's insect trap to defend itself. When I first started peering into the pitchers of *Nepenthes gracilis* in Bako National Park in Sarawak on the island of Borneo, I was sure I sometimes caught a flash of red. This turned out to be the spider leaping from the walls or lip of the jug into the fluid. And predators a lot smaller than me have the same effect. The footsteps of large nectar-loving ants, three to four times the size of the spider, are enough to make the spider take the plunge. Fortunately for the spider, no underwater wrestling ensues, as these ants are somehow able to avoid the pitfalls of a slippery lip. *Misumenops* does not end up permanently in the pitcher because it leaves draglines of silk hanging from the lip of the pitcher down to the fluid and even below its surface. It uses these to move very quickly around the slippery walls of the pitcher and when it emerges from the fluid, it just grabs a silken line to help it get to the dry pitcher wall.

If the pitcher had only recently opened for business and was not full of drowned victims being broken down into plant nutrients, I could see the spider clearly beneath the fluid. When the pitcher was a murky mass of bodies (or "necromass"), the spider would scuttle crab-like beneath the corpses in their watery grave and later emerge, zombie-like, with its body cloaked in the partially digested bodies of ants and other insects.

How does this spider manage to breathe underwater? Unlike some aquatic spiders, which trap a bubble of air over their entire body, adult and large juvenile *Misumenops* trap only a small air bubble over a pit on the anterior ventral abdomen next to the book lungs, which are used for breathing (see Fig. 1). The pit is covered in water-repellent hairs that help push the air bubble against the sides of the pit and keep it in place. This elaborate mechanism is to overcome a property of the pitcher-plant fluid that encourages drowning rather than breathing. Pitcher plants produce a surfactant or wetting

Figure 4: *Misumenops* underwater with larvae.

agent (hence the liquid's slimy feel when I drank it) that reduces the surface tension on the fluid's surface. This facilitates the smooth transition from air to liquid for plummeting insects. Obviously, pitcher plants want their food *inside* their stomachs, not skating around on the surface breathing tiny sighs of relief! However, for a plummeting spider, low surface tension also makes it more difficult to trap an air bubble; hence the hairy pit.

With an air bubble attached, the spider can stay underwater for about 40 minutes. If it needs to come up for a breather it can just raise its hairy pit above the surface and take down another bubble of air. *Misumenops* does not have a water-repellent cuticle, like some aquatic spiders, and so it emerges from the fluid looking like the proverbial drowned rat. Although the fluid only digests the dead, the spider carefully grooms itself dry, before deciding its next move.

Just how does *Misumenops* steal sweet-toothed insects from the jug? For insects like flies and beetles, *Misumenops* is a rather malevolent lifeguard as it saves some of them that have just fallen in, from drowning, by hauling them out of the fluid. The kiss of death rather than the kiss of life follows the rescue and the spider then sucks its victim dry. Many other species of crab spiders also exploit the attractiveness of nectar by living on flowers and ambushing pollinating insects.

The most common victim of *Nepenthes gracilis* in Bako National Park are green weaver ants (*Oecophylla smaragdina*) and *Misumenops* has a very clever way of catching them. The ants are far too dangerous for the spider to catch on its own, so it lets the plant do most of the work. At times, the spider will detect the splash of a green weaver ant as it falls in. After a minute or two the ant sinks and drowns in the fluid. Out of sight but not out of mind, the spider waits about ten minutes before submerging and retrieving the freshly-dead and harmless ant (see Fig. 2).

Misumenops also goes fishing for aquatic larvae that are living in the pitcher fluid (see Fig. 3). It will stick its long front legs into the fluid and grab larvae as they come to the surface to breathe, or it will drop into the fluid and catch them underwater (see Fig. 4). It will even crawl into the necromass,

throw a little spider tantrum by thrashing around, and flush out larvae which are hiding there. As the larvae swim away from the disturbed necromass they are captured by the spider.

Female *Misumenops* build an egg sac just above the pitcher's fluid line, and when the baby spiders emerge they disperse around the dry walls inside the pitcher. Just like their parents, the babies also leap into the fluid when disturbed. The sight of 50-odd spiderlings making tiny splashes as they submerge reminds me of an arachnid version of "Titanic"! Because the spiderlings are so small, they can trap an air bubble over their entire abdomen, and will stay submerged for as long as the adults. Ironically, while the lower surface tension makes it more difficult for the adults to trap a bubble of air, the babies actually benefit from the surfactant, as it allows their small bodies to slip underwater more easily. When I saw the babies jump into regular water, a number got stuck on the surface "skin". Eventually the spiderlings leave their nursery to find their own pitchers.

What does the carnivorous plant get out of having a free-loading carnivore living in its insect trap? Well, probably nothing, except the spider at least uses the pitcher as a gigantic toilet and returns some of what it stole from the plant. This makes me feel even less like following Wallace's "line"; but if I ever had to, it would be one cocktail I would neither shake nor stir.

Carnivorous plant cultivar names registered in 2011

Dionaea 'Fondue' G.Bily, Carniv.Pl.Newslett.40:95 (2011)	20 Oct.
Dionaea 'JA1' J.A.Gonzalez Dominguez, Carniv.Pl.Newslett.40:140 (2011)	30 Dec.
Drosera 'Ambrosia' B.Barnes, Carniv.Pl.Newslett.40:25 (2011)	3 Apr.
Drosera 'Dreamsicle' B.Barnes, Carniv.Pl.Newslett.40:25 (2011)	3 Apr.
Drosera 'Golden Dew' S.Fretwell, G.Bourke & S.Spence, Vic.Carniv.Pl.Soc.101:5 (2011)	20 Oct.
Drosera 'Leo Bourke' G.Bourke, Carniflora Australis 7(3):4 (2010)	29 Jun.
Drosera 'Woolly Beast' B.Barnes, Carniv.Pl.Newslett.40:26 (2011)	3 Apr.
Drosera 'Woolly Red' B.Barnes, Carniv.Pl.Newslett.40:26 (2011)	3 Apr.
Nepenthes 'Fat Boy' G.Bourke, Carniflora Australis 7(3):5 (2010)	29 Jun.
Nepenthes 'Red Rocket' G.Bourke, Carniflora Australis 7(3):5 (2010)	29 Jun.
Sarracenia 'Blood Sweat & Tears' P.Wilson, Carniv.Pl.Soc.J.(UK) 33:52 (2011)	7 Nov.
Sarracenia 'Deep Throat' P.D'Amato, Carniv.Pl.Newslett.40:138 (2011)	30 Dec.
Sarracenia 'Godzuki' P.D'Amato, Carniv.Pl.Newslett.40:96 (2011)	20 Oct.
Sarracenia 'Gorey' J.Lechtman, Carniv.Pl.Newslett.40:136 (2011)	30 Dec.
Sarracenia 'Leo Song' P.D'Amato, Carniv.Pl.Newslett.40:137 (2011)	30 Dec.
Sarracenia 'Red and White' P.D'Amato, Savage Garden:82 (1998)	30 Dec.
Sarracenia 'Seurat' J.Lechtman, Carniv.Pl.Newslett.40:136 (2011)	30 Dec.
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Utricularia 'Merrie Heart' P.D'Amato, Carniv.Pl.Newslett.40:97 (2011)	20 Oct.

Drosera cistiflora L.

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Keywords: Cultivation: *Drosera cistiflora*

Drosera cistiflora is one of those species that everyone is familiar with, but unfortunately is grown in very few collections, because of the unavailability of plant material, and also in part to the rather unfair reputation it has of being difficult. Admittedly, it's not as straightforward as many of the commonly grown species, but is worth the extra little effort.

It is a native of the Cape region of South Africa, and inhabits seasonally wet areas, with the wet season being the cool damp winter months. It is the growth pattern of this (and indeed all species) that one should consider when trying to understand the best way to succeed with cultivation. Firstly, as it grows in the winter months in the wild, so it will in the confines of a greenhouse, giving a welcome breath of life at an usually dull and lifeless time. While the *Sarracenia* are cold and static, this plant bursts out of the soil anytime from late September through early January, with an erect green shoot that opens revealing the first sticky carnivorous leaves. These initial leaves reflex downwards and produce a small rosette, but subsequent growth takes on a different form, with a vertical stem reaching skywards, interrupted at regular intervals by 3 cm leaves held at somewhere between 30° and 45° (see Fig. 1). The stem itself varies in height from a few centimeters, up to over 30 cm (some populations considerably more) decorated with their glistening leaves. The main claim to fame of this species is, however, the flowers (see Front Cover), considered by many to be

Figure 1: *Drosera cistiflora* plants in cultivation.

Figure 2: The white flowered *Drosera cistiflora* plants from Darling.

the largest in the genus. From my own experience these are up to 5 cm in diameter, and when seen for the first time cannot fail to impress even the most hardened of botanical philistines.

I currently have three types which have flowered for me. My first plants which are now some 13 years old are completely reliable, and every year produce 3 cm diameter pink flowers with white petal bases darkening to a green center. The plants themselves are rather wiry, and unusually continue to produce a growing stem from the last leaf axil below the flower which forms another 2 or 3 leaves. This characteristic has led to this plant being given its own specific rank–*D. variegata* Debbert. These plants originate from Gifberg.

A couple of years later I had success with a white-flowered form from Darling, which like the previous has become a regular performer. This is an altogether shorter stockier form to 20 cm in height, and without the wiry appearance of the pink form. The flowers are larger at about 4 cm in diameter, and although I call it white, there is an element of very pale yellow in the mix, which contrasts perfectly with a dark green center. (see Fig. 2).

A few years ago, I was treated to one of those rare moments when you are literally stopped in your tracks. A supposedly red form of this plant, also from Darling, had been in bud for some time and on this particular morning was the first thing to catch my eye as I walked in to the greenhouse. I had heard that the color of these plants could be intense, but in nearly 30 years of growing carnivorous plants, I have yet to see a flower which could rival what this plant had produced. At a little over 5 cm in diameter, this is the largest *D. cistiflora* flower I have seen, and sported a color I can best describe as pillar box red with a dark green centre (see Fig. 3). All of the images I took at the time show the flower as being somewhat orange-red. The plants when they flowered were approximately

25 cm in height, with pink tentacled leaves. Unfortunately, this flowering has yet to be repeated, and I have noticed that the plants are smaller than they were then. Perhaps a lack of food, or maybe I allowed them to dry out too much? I shall experiment with feeding them to see if the flowering is as a result of available food stored in the roots.

Figure 3: Stunning red *Drosera cistiflora* flowers!

After flowering, the plants gradually yellow and die back as the sun becomes stronger and the days become longer and drier, eventually losing their visible parts and retreating underground to survive the hot dry summer months as thick, fleshy roots. Here they remain while the ground above them bakes solid, only re-emerging with the returning rains and cooler temperatures to begin their growth period once again.

In cultivation, they should be kept in a little water whilst in growth, and this should be allowed to be taken up by the plants before replenishing, taking care not to allow them to dry. Once they begin to die back, reduce the watering gradually so as to allow them to dry slowly until they disappear completely. Over the summer months, they should be given an occasional quantity of water to their bases, not enough to soak them, but sufficient to prevent the roots desiccating. For this reason, it is wise to grow them in large pots with room for their roots to spread around, in a compost of 8 parts silver sand to 2 parts peat moss.

The best way to propagate this species is by seed, and it was thought that as they are winter growers, the seed should be sown in the autumn. However, as I stated earlier let's consider their wild growth cycle. The plants flower and set seed at the end of their growth period before the onset of warm spring weather and as their soil is drying. The seed then sits in its dormant state through the fierce summer heat, only germinating after the first rains and when the temperature is a little more conducive to growth. Therefore, I now sow seeds of this and other winter growing species in the spring, at the time they would naturally be shed by the parent plant, and allow them the hot dry desiccating summer they need before autumn germination. Once they are soaked through in September or October, many tiny green shoots can be seen in a matter of only a few weeks.

The first year is the most critical for these plants, as they take a while to develop the substantial roots required to sustain them through the summer, and you can easily destroy them at this stage if they dry too much.

I have also had success with smoking the seeds in a barbeque with smoldering peat over night. This replicates the bush fires that are a vital part of so many ecosystems in South Africa. Simply sow the seeds in pots and wet thoroughly, light the peat (this can be the tricky part), and when smoking well, position the tray well above. Cover and leave. This can be done in the autumn, and is another way of unlocking the inherent dormancy the seeds have.

Although as I said earlier, there is more care required, the extra effort is well worth the kind of reward that these incredible plants can repay.

New cultivars

Keywords: cultivar: *Dionaea* 'Scarlatine' (English: *D.* 'Scarlet Fever'), *Sarracenia purpurea* 'Phoenix', *Sarracenia* 'Zjahnine'

Dionaea 'Scarlatine' (English: *D.* 'Scarlet Fever')

Submitted: 28 September 2011

The parentage of *Dionaea* 'Scarlatine' is *D.* 'Dentate Traps' × *D.* 'Royal Red'. This *Dionaea muscipula* cultivar may be reproduced vegetatively by rhizome or leaf cuttings in order to preserve the characteristics of the mother plant, as follows:

Trap margin with short irregular dentition of a dentate sawtooth type, having a yellow-green color displaying red sprinkles or spots (see Fig. 1). The petiole with the same background coloration as the trap likewise shows red sputtering.

Dionaea 'Scarlatine' was bred in April 2008 and named in April 2011 by the author. The epithet refers to the spots visible on the plant that resemble the rash caused by the childhood disease scarlet fever.

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Figure 1: *Dionaea* 'Scarlatine' plant and trap. Photo by Lucien Blacher.

Submitted: 29 October 2011

Sarracenia purpurea 'Phoenix' has a rather interesting history. In the early 1990s, Jim Bockowski and I frequently took trips to the area locally called the New Jersey Pine Barrens or Pinelands. On one of these trips, we stopped by one of his favorite spots near an old railroad bed that had a great diversity of carnivorous plants including several species of *Utricularia* and *Drosera* and many *S. purpurea*. One of these plants stood out.

The young plant grew on the south side of the railroad bed partly submerged in water and baking in full sun. The colors of this plant were striking: dark maroon/red throughout but the hood margin was lime green (see Fig. 2). Several years later I was treated with an additional bonus – the plant's flowers were orange/yellow – which made the plant even more unique (see Fig. 2).

In the late 1990s, the plant was transferred to Jim Bockowski's care. He verified the orange flower colors (he thought I was crazy when I first mentioned it to him) and produced seed through self-fertilization. Shortly thereafter, the parent plant perished due to a fungus attack. Offspring of this plant that exhibit the diagnostic characteristics are called *S. purpurea* 'Phoenix' referring to a mythical bird with a colorful plumage and a tail of gold and scarlet (or purple, blue, and green according to some legends) and which has an ability to be reborn from its own ashes. The registrant believes the name fits with the plant's history and dark maroon/red leaf color and orange/yellow flowers.

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Figure 2: Pitcher and flower of *Sarracenia purpurea* 'Phoenix'. Photo by Christoph Belanger.

Submitted: 16 December 2011

This exceptional *Sarracenia* hybrid is the result of the pollination of *Sarracenia oreophila* by *Sarracenia leucophylla*. The pollination was conducted by fellow New Zealand grower, Don Gray, in 2003. The seed was subsequently germinated by me in 2004 and from the resultant seedlings I selected this clone in 2008 for its distinctive properties.

Following the growth pattern of the *S. oreophila* parent, only one flush of pitchers is produced early in the spring following flowering. In form the pitchers are a blend of both the *S. oreophila* and *S. leucophylla* parents being upright with a large undulate hood, a notable spout, and attain a height of 70 cm. Ensiform non-carnivorous leaves intermediate between those of the parents are produced midsummer and endure throughout the winter. Flowers are simple yellow with pink petals.

The noteworthy characteristic of this hybrid is its extreme vivid coloration which evolves rapidly once each pitcher opens. Initially the pitchers are pleasant yellowish green with intricate red venation over the upper third of the leaf (see Fig. 3). Some white areolae are discernible on the hood within its vein network. Within three days red begins to suffuse within the nectar roll, the interior of the mouth, and the interior and exterior of the hood (see Fig. 4). Over the course of the next month this red suffusion becomes more intensive, darkening notably, and white areolae become more apparent on the exterior of the hood (see Fig. 5). By the end of the second month the pitcher's venation, the nectar roll, and the interior of the pitcher have become a very dark purple (see Back Cover). At this stage the white areolae present on the hood have become entirely visible against the dark suffused background (see Fig. 6). Throughout the attainment of this ultimate coloration the veins of the pitcher tube itself remain distinct against the yellowish green of the tube, the exterior of the column, and mid-line of the rear of the hood. A very limited number of small areolae may be found on the tube exterior below the mouth on some pitchers. The lower two-thirds of the pitcher remain yellowish green almost devoid of veining. These features are established at a point mid growth season and well before pitcher desiccation at the onset of dormancy. The photos are of a specimen grown permanently outdoors.

The name 'Zjahnine' is a phonetic re-spelling of the person's name, Janine, and is in honor of a valued friend.

Vegetative propagation is necessary to maintain the unique features of this hybrid.

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Figure 3: *Sarracenia* 'Zjahnine' opening pitcher. Photos by Julian Brook.

Figure 4: *Sarracenia* 'Zjahnine' pitcher three days after opening.

Figure 5: *Sarracenia* 'Zjahnine' pitcher one month after opening.

Figure 6: *Sarracenia* 'Zjahnine' mature pitcher lid.

A CASE OF BIRD CAPTURE BY A CULTIVATED SPECIMEN OF THE HYBRID *NEPENTHES* × *MIXTA*

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Keywords: Cultivation: *Nepenthes* × *mixta*, prey capture, bird

As a nurseryman, I'm used to being asked what is the biggest prey item caught by my plants. Of course, as a general rule the answer is insects, but there are some species, especially *Nepenthes*, which can consume larger animals such as lizards, frogs, small rodents, and even rats—as has been well documented. Indeed there have been several reports of cultivated *Nepenthes* specimens catching mice, although it is hardly common in comparison to wild plants where a much broader spectrum of fauna is to be found.

Exceptional prey capture is not only limited to *Nepenthes* though. A number of years ago I found several small lizards had been caught by the Venus Flytrap, *Dionaea muscipula*, at the previous nursery location in Surrey (Hewitt-Cooper 1999), and in more recent times I rescued a Robin, *Erithacus rubecula*, from a *Roridula dentata*, which although missing a few feathers and somewhat sticky footed, flew away hopefully not too traumatized.

I recently made a discovery which I believe to be a first for a plant in the UK. A specimen of *Nepenthes* × *mixta* (*N. northiana* × *maxima*) hangs during the summer months in my tropical garden, from the branches of an old cider apple tree, where it grows quite happily despite a lack

Figure 1: *Nepenthes* × *mixta* plant *in situ*.

Figure 2: Tail feathers protruding from pitcher.

Figure 3: Decomposition of the lower section of the pitcher.

Figure 4: Five days later, and the pitcher has virtually died.

of attention on my part (see Fig. 1). It grows in a 20-liter container pot which holds sufficient moisture to prevent it drying out too much, and is a specimen of the common clone known as *N.* "Miranda", which was left over from one of the flower show displays I had staged. It was placed outside as an experiment, which has clearly been successful as it is enjoying its third summer in this position.

Outside, these and indeed many other carnivorous plant species are much more efficient at catching prey, and the pitchers are frequently over half full of flies and wasps—far more than the plant would catch in a greenhouse environment, proving their effectiveness.

In July 2011, I found in one trap the body of a small Blue Tit (*Cyanistes caeruleus*), which was wedged head down in the trap with the tail feathers protruding from the pitcher mouth (see Fig. 2). The bird had been in this position, I estimate, for 2-3 days, was clearly dead, and the base of the trap had started decaying due to the presence of such an unorthodox and large capture, as can be seen clearly (see Fig. 3).

The trap was of the upper type, with the characteristic infundibular shape of most *Nepenthes*, and measured 160 mm from the lowest point at the base to the top of the peristome at the lid attachment. The opening of the mouth was 51 mm high by 24 mm at its widest point.

A prey item of this size in what is not a particularly large trap by *Nepenthes* standards, would decompose long before the plant could gain any nutritional benefit, and five days later the pitcher had died back considerably (see Fig. 4).

Naturally of course, *Nepenthes* do not actively attract birds, as indeed they do not on their own entice the other documented prey mentioned earlier, but the abundance of insect life in and around these plants is a convenient food source for other insect eating organisms.

In this case, the unfortunate bird, I believe, landed on the base of the peristome, leaned in to retrieve an insect probably floating on the surface of the pitcher fluid within, and either became wedged or toppled forward too far and fell below the water line.

I would be interested to hear of other unusual prey items discovered by growers, both by *Nepenthes* and other carnivorous plant species.

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Utricularia humboldtii Schomb.

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Keywords: ecology: *Utricularia humboldtii*, cultivation.

Of all of the 230 or so species of *Utricularia*, the sections with the most beautiful flowers are undoubtedly Orchioides and Iperua. *Utricularia humboldtii* falls in to the latter, along with *U. reniformis*, *U. nelumbifolia*, *U. nephrophylla*, and *U. geminiloba*, and indeed Iperua translates as "wonderful flower" in the Arawak Indian language.

This plant has the accolade of having the largest flowers in the genus, and also possibly the largest traps at nearly 1 cm across (although the Australian ephemeral *U. arnhemica* is also a contender).

Described by Robert Schomburgk in 1840, *Utricularia humboldtii* is a native of Venezuela, Guyana, and Brazil where, according to Taylor (1989), it is an epiphyte in bromeliad leaf axils or on mossy trees, and also as a sub-aquatic terrestrial in wet soil in open savannah or forest clearings. As an epiphyte it is sometimes found in the leaf axils of bromeliads, most notably *Brocchinia tatei* which can attain gigantic proportions (see Fig. 1). As a terrestrial it is seen in wet peaty soils, generally in highland savannah (see Fig. 2) alongside other *Utricularia* species amongst grasses where its' characteristic bright green, slightly waxy, coriaceous paddle-shaped leaves can be up to 60 cm in height, and with a leaf blade up to 10 cm high and 5 cm across, held atop red wiry stems (see Fig. 3).

Figure 1: The author with *Brocchinia tatei* Mount Roraima, Venezuela.

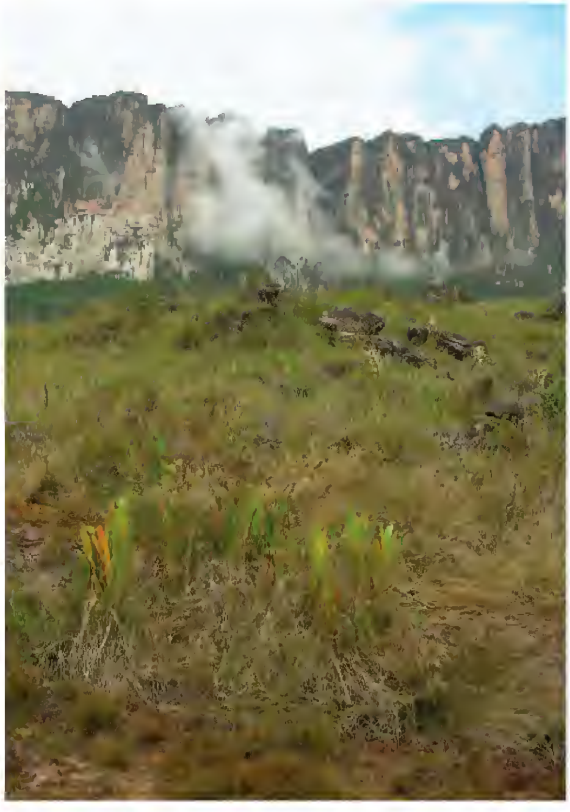

Figure 2: Typical highland habitat with *Brocchinia hechtioides*.

Figure 3: *Utricularia humboldtii* leaf blade.

Figure 4: Flowering *Utricularia humboldtii* with Kukenam Tepui in the background.

Two distinct types of trap are produced, many small ones to 2 mm, and the occasional giant trap to 10 mm across as mentioned above.

The flowers are this species most notable characteristic. They are up to 7.5 cm in width and 5 cm high, dark violet with two vertical bright yellow parallel stripes on the upper corolla lobe. The two calyx lobes on the rear of the flower are a contrasting darker purple (see Fig. 4). Up to 8 flowers are commonly produced on each flower scape, which can be up to 100 cm in height, circular in cross section, and has a strong wiry quality. Generally, a couple of flowers open simultaneously, starting with the lowest and ending with the highest.

An interesting phenomenon occurs when this plant is found growing in the axils of bromeliads. Pale green stolons are produced which rise out of the water, arch over, and descend back in to another water source. It was long assumed that this is a method by which the plant can colonise other host bromeliads, but it tends not to travel very far. Certainly in cultivation the stolons tend to only reach a few cm and so would have little chance of stretching far enough to reach a new host. It has been suggested that as the host plant grows new leaves from its center, the bladderwort would be gradually pushed out to the dead, and hence dry, outer leaves where it would perish. Therefore, the plant could be replanting itself via stolons back into the centre of the bromeliad to ensure its' survival.

In cultivation this is a fairly forgiving species, and I grow mine in a small pond basket half filled with peat and topped with live sphagnum moss, and stood in 2-5 cm of rain water. The pot will simply act as a base from which the plant will grow and spread, escaping in to the surrounding water and wandering freely around and between the other pots on the bench in full sun.

I have grown it for a number of years, and it has flatly refused to flower for me. However, my patience recently paid off and I was finally privileged to see this incredible plant flower (see Fig. 5). Although it may be coincidence, the in-

florescence was produced after I inadvertently allowed the water level in the bed to drop dramatically, and I believe it was a panic response on the part of the plant to produce seed prior to dying. Whatever the reason, 4 stunning flowers were produced which lasted approximately 3 weeks. The peculiar characteristic green embryo containing "live" seeds weren't produced, so one assumes either manual pollination is necessary, or the plant is self-sterile.

I was hoping to experiment by allowing the plant to "dry out" this year to see if flowering could be forcibly stimulated, but unfortunately in the cold conditions of the previous winter, the plant appears to have died when the temperature dropped below 7°C.

A winter minimum of 10°C seems to be safe, at which temperature it retains a few evergreen leaves.

As with most *Utricularia* species, this plant is best propagated from division as it seems fairly vigorous, and further experimentation with allowing the plant to dry a little, may see this traditional shy flowering plant bloom more frequently.

Figure 5: *Utricularia humboldtii* flowers in cultivation.

Reference

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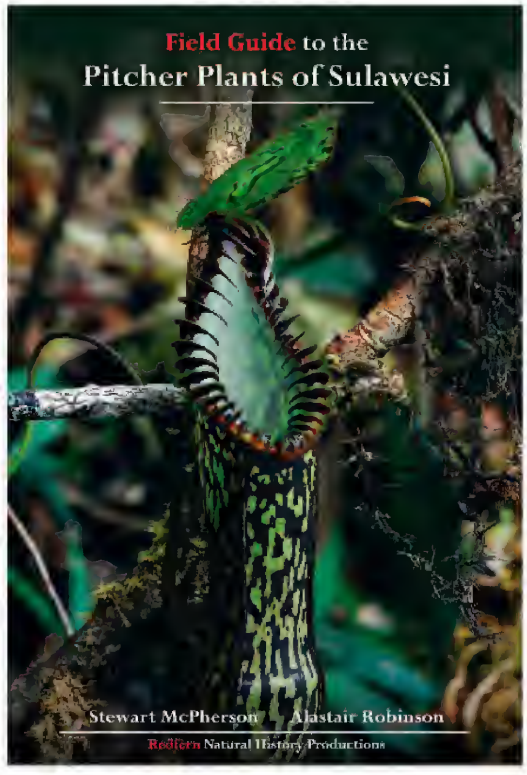
New Nepenthes Volume One. This 596 page work comprises 491 images and provides an update on the genus *Nepenthes*, covering all new species and discoveries made since the publication of the first complete monograph in 2009. Highlights include; descriptions of eight new *Nepenthes* species, emended descriptions of *N. lamii* and *N. thorelii*, an account of a newly discovered population of *N. pitopangii*, new understanding of the diversity of *N. philippinensis* and related taxa, expedition reports, a comparison of all highland *Nepenthes* of Palawan, complete descriptions and a comparison of all recently described Indochinese taxa… and much, much more.

Field Guide to the Pitcher Plants of the Philippines. A lavishly ilustrated and colourful overview of the twenty seven species of Philippine *Nepenthes*. Several of the species that feature in this work have been discovered only in the last two years and are documented here in unique detail (*N. cecilae*, *N. palawanensis* and *N. pulchra*).

Field Guide to the Pitcher Plants of Sulawesi. A beautiful guide to all *Nepenthes* of Sulawesi, including never-before published images of *N. hamata*, *N. eymae*, *N. nigra*, *N. undulatifolia* and a unique variant of *N. mirabilis*. This work also includes the first ever listing of all *Nepenthes* hybrids known from Sulawesi and photos of three hybrid plants.

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