CARNIVOROUS PLANT NEWSLETTER

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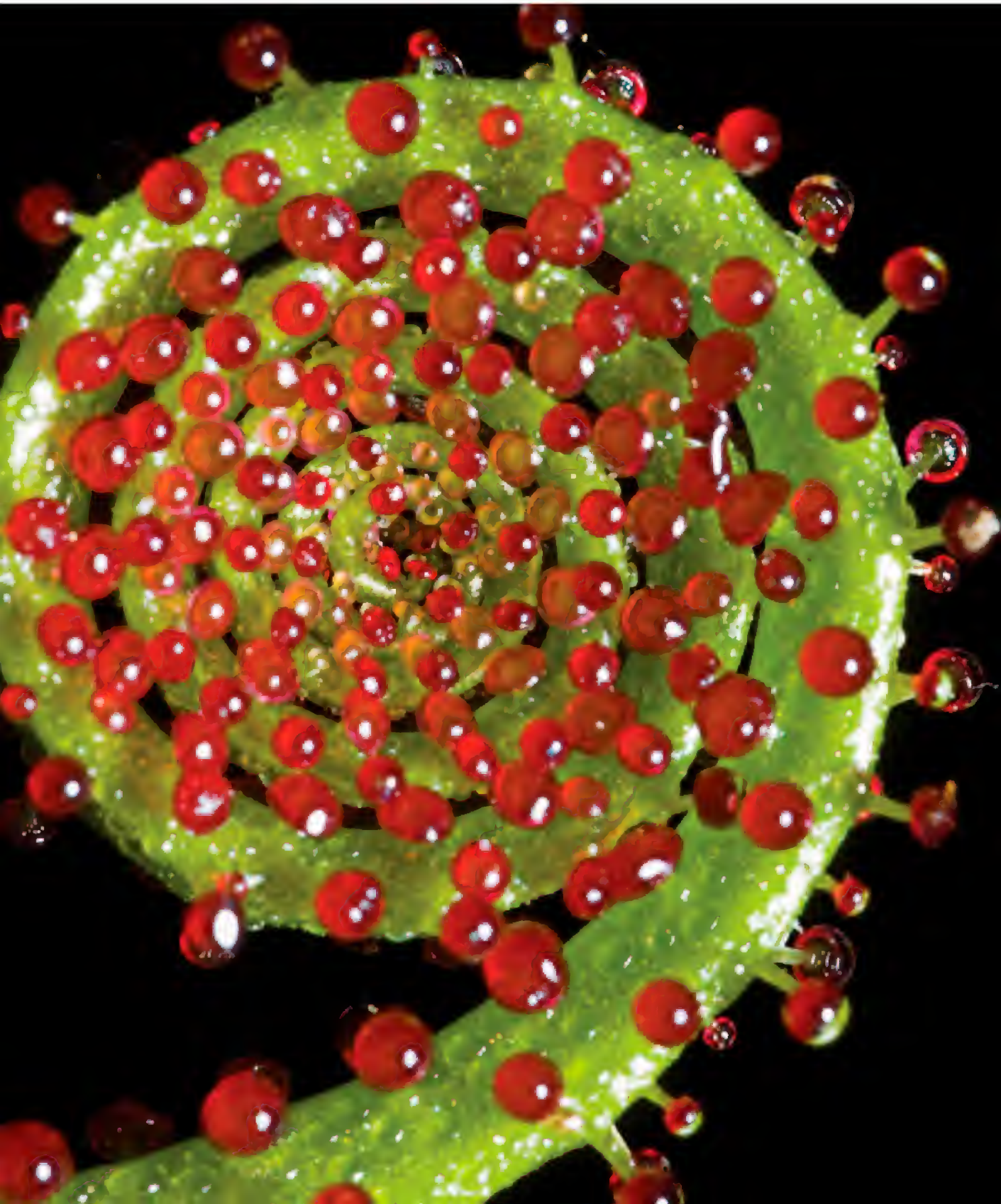

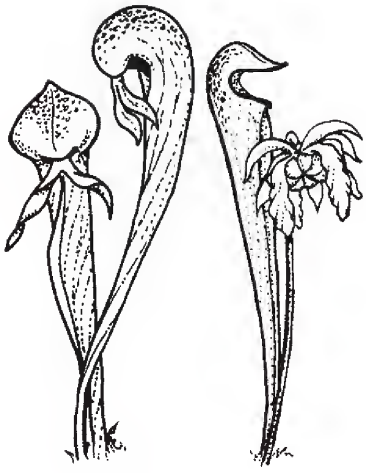

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Front Cover: Unfurling *Drosophyllum lusitanicum* leaf spiral. Photo by Barry Rice. Article on page 143.

Back Cover: A comparison of *Roridula gorgonias* (left) and *Roridula dentata* (right). Photo by Nigel Hewitt-Cooper. Article on page 146.

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Call for proposals
10th ICPS Conference 2014

After this summer's successful and exciting 9th ICPS Conference in Seekonk, USA, it is now time to begin thinking about the next ICPS Conference scheduled for 2014. According to the principle of fair global distribution and the set schedule, the next venue should preferably be located in Asia/Australia/New Zealand.

If you or your society feels this should be your turn, please email a proposal by **31 March 2013** to marcel@carnivorousplants.org.

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Drosera bicolor Lowrie & Carlquist

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Keywords: taxonomy: *Drosera bicolor.*

Drosera bicolor Lowrie & Carlquist is endemic to southwestern Australia (Lowrie & Carlquist 1992). It is an erect tuberous sundew with alternate leaves and a basal rosette, placed in *Drosera* subgenus *Ergaleium* section *Ergaleium*. Prior to its formal description this species was informally known as '*D. peltata* 'Hammersley". Since its description, debate has continued as to whether it is a distinct taxon or is better treated in the broader concept of *Drosera peltata* Thunb (*e.g.* Lowrie & Carlquist 1992; Schlauer 2012). This paper presents a more detailed description of this taxon and discusses its taxonomic status.

Drosera bicolor grows in the sandplains of the Upper Phillips River about 35 km northwest from Ravensthorpe (Lowrie 1998; FloraBase 2012). It grows in gaps between shrubs in a low, open shrubland, and appears to be most common in small shallow depressions fringed by various species of restiads (Restionaceae) (Fig. 1(a)). It often grows in the company of *D. zonaria* Planch. The growing medium is deep pale brown fine-grained quartz sand that develops a brittle surface crust. So far this species is only known from its type location, but it is likely to occur more widely throughout this area in the same habitat.

The general habit of *D. bicolor* has a flat basal rosette of leaves from which an erect stem with alternate crescentic petiolate leaves, with a peltate attachment of the petiole, and flowers arranged in a one-sided scorpioid cyme (Fig. 1(a)). This growth habitat is typical of members of the *D. peltata* complex (Gibson *et al.* 2012). *Drosera bicolor* has a calyx with sepals that bear glandular hairs on the margin and outer surface (Fig. 1(e)), and ovoid seeds with a shallowly pitted reticulate surface texture that are up to 0.3 mm long (Fig. 1(h)) which may explain why *D. bicolor* was initially considered to represent a strange form of *D. peltata.*

However, *Drosera bicolor* was described as a separate species due to the following characters: the red patch of pigment at the base of each otherwise white petal (Fig. 1(e)), the style segments arranged in a flat rosette (Fig. 1(f,g)), cauline leaves that increase in size towards the stem apex (Fig. 1(c,d)), and the lengthening and changing orientation of the petioles (from stem-appressed to spreading) of successive cauline leaves (Lowrie & Carlquist 1992; Lowrie 1998). These characters are not found in any members of the *D. peltata* complex, including the superficially similar *D. yilgarnensis* R.P. Gibson & B.J. Conn (previously '*D. peltata* 'Western Australian Form" *sensu* Lowrie (1987; pp. 88-91)).

Close examination of *D. bicolor* plants has revealed some other unique characters to the cauline leaves that pertain to the gradual change in leaf size and shape, and in the point of attachment of the petiole to the leaf blade in successively produced leaves. Mature plants of *D. bicolor* produce an erect stem between 5 and 20 cm tall with between about 9 and 15 cauline leaves. Stem internode length varies between successive leaves, and is between about 2 and 4 mm long in the lower part of the stem; between about 5 and 10 mm between the middle leaves, and up to 15 mm long between the uppermost leaves.

Figure 1: Photomosaic of *Drosera bicolor:* (a) mature plants in bud in the wild; (b) developing rosette of a plant in cultivation; (c) cultivated plant with small lower stem leaves; (d) cultivated plant with maturing stems with noticeably larger leaves towards the stem apex; (e) open flower with distinctive bicolored petals; (f) ovary and styles from above; (g) oblique view of the flower structure showing the short stamens; and (h) SEM of a *D. bicolor* seed (R. Gibson 028, NSW), scale bar is 0.1 mm long. All photos but (h) by R. Gibson; photo (h) by P. Littlefield.

The lowermost cauline leaves are broadly obovate in shape, to 2 mm wide by 1.5 mm long with a flat upper margin. Petioles are about 1 to 1.5 mm long, attached to the midpoint of the leaf upper margin (Fig. 1(c)). Successive leaves increase marginally in size, so they are about 2 mm wide by 2 mm long, and the upper leaf margin becomes emarginate so that the middle leaves are crescentic in shape. Petioles are between about 2 and 4 mm long and are attached on the mid-point of the upper leaf margin (Fig. 1(d)). Leaves in the upper third of the stem are up to 2.5 mm wide by 2 mm long with a deeply emarginate upper leaf margin. Petioles are 4 to 7 mm long and now have a peltate attachment to the back of the lamina (Fig. 1(d)). The upper leaves also have horn-like extensions of the upper corners of the lamina, or auricules; that in this species can be up to 3.5 mm long (Lowrie 1998). In addition to the unique style architecture this species also has remarkably short filaments so that the stamens are shorter than the gynoecium (Figs. 1(e-g)).

The curious gradual variation in the size of the cauline lamina and the length of the cauline leaf petiole in *D. bicolor* is only found in three other Australian sundews: remarkably only one of these is another tuberous sundew: *D. menziesii* R.Br. ex DC. subsp. *basifolia* N.G.Marchant & Lowrie (Lowrie 1998). The other two species, *D. banksii* R.Br. ex DC. and *D. subtilis* N.G.Marchant (Lowrie 1998), are annual species from northern tropical Australia, the former has been placed in *Drosera* subgenus *Lasiocephala* (Rivadavia *et al.* 2003). The shared leaf characters between these two distantly-related pairs of species suggest that this may be an example of convergent evolution. There may be a number of factors favoring this unusual change in cauline leaves, these hypotheses include: (1) the close-pressed leaves at the base of the stem form a barrier to some walking (or crawling) herbivores that may otherwise eat the new stem growth; (2) the lower leaves capture small ground-dwelling and flying prey, such as springtails (*Collembola*) and fungus gnats (*Diptera*), while the upper cauline leaves are dedicated to capture (larger) flying insects; (3) these sundews may initially grow faster than sympatric herbs but then start to be shaded by them, thus leaf shape size variation reflects the most efficient allocation of resources to plant parts; and (4) the initial stem growth is acting as a small target to reduce the chance of browsing kangaroos and wallabies in taxa that may lack other means of defense (such as bad-tasting compounds). When a plant is browsed new stem growth can develop from the remaining stem portion but this secondary growth may not flower, or produce fewer flowers than an undamaged plant and thus produce less seed in that growing season. It would be interesting to run some experiments and make further field observations to test these hypotheses, and develop other ideas that may explain this marvelous leaf pattern.

The overall plant structure of a basal rosette with a central stem with alternate leaves that include some that are crescentic and some that are peltate and flowers borne in a terminal inflorescence with hairy sepals is reminiscent of plants in the *D. peltata* complex. However the disc-like styles, stamens shorter than the gynoecium, petals with a red basal blotch, and cauline leaves with a gradual change in size, shape, petiole length, and petiole attachment are not found in any members of the *D. peltata* complex. Therefore, based on this investigation *D. bicolor* is considered to be a distinctive species that is not part of the *D. peltata* complex.

Acknowledgements: Thanks to Mr. Phill Mann for his assistance with fieldwork and many excellent discussions about this species. Thanks also to Mr. Patrick Littlefield (UNE) for his assistance in producing the SEM image used in this paper. The author gratefully acknowledges funding from a Noel C. W. Beadle Scholarship (UNE) that helped fund the use of the SEM microscope. I also wish to thank anonymous reviewers for their helpful comments that improved this paper.

References

FloraBase 2012. *Drosera bicolor*. http://florabase.dec.wa.gov.au/browse/profile/13386 [viewed 28 May 2012].

Gibson, R., Conn, B.J., and Bruhl, J.J. 2012. Morphological evaluation of the *Drosera peltata* complex (Droseraceae). Australian Systematic Botany 25: 49-80.

Lowrie, A. 1987. Carnivorous Plants of Australia: Vol. 1. University of Western Australia Press, Nedlands.

Lowrie, A. 1989. Carnivorous Plants of Australia: Vol. 2. University of Western Australia Press, Nedlands.

Lowrie, A. 1998. Carnivorous Plants of Australia: Vol. 3. University of Western Australia Press, Nedlands.

Lowrie, A. and Carlquist, S. 1992. Eight new taxa of *Drosera* from Australia. Phytologia 73: 98-116.

Rivadavia, F., Kondo, K., Kato, M., and Hasebe, M. 2003. Phylogeny of the sundews, *Drosera* (Droseraceae), based on chloroplast *rbcL* and nuclear 18S ribosomal DNA Sequences. American Journal of Botany 90(1): 123-130.

Schlauer, J. 2012. Carnivorous Plant Names Database. http://www.carnivorousplants.org/cpdb/SearchCPdb.php [viewed 21 Sept. 2012]

The digestive fluid of *Drosera indica* contains a cysteine endopeptidase ("droserain") similar to dionain from *Dionaea muscipula*

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Keywords: chemistry, *Drosera indica*, cysteine endopeptidase, droserain, *Dionaea muscipula*, dionain, aspartic endopeptidase.

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Introduction

Carnivorous plants are known to secrete various endopeptidases extracellularly to digest prey proteins. Previously we purified two isoforms of nepenthesin to homogeneity and elucidated their enzymatic and structural characteristics (Athauda *et al.* 1998, 2002, 2004; Takahashi *et al.* 2003, 2005). In a continuation of these studies, we have been attempting to characterize these and other endopeptidases secreted by carnivorous plants to digest prey proteins (Takahashi *et al.* 2009). Recently, we found and partially characterized a cysteine endopeptidase in the digestive fluid of *Dionaea muscipula* and named it "dionain" (Takahashi *et al.* 2011). In the present report, we describe the occurrence of a similar cysteine endopeptidase in the digestive fluid of *Drosera indica* and propose the name "droserain" to this enzyme. In this connection, we also propose the names of aspartic endopeptidases from the digestive fluids of relevant carnivorous plants.

Materials and Methods

The crude digestive fluid of wild specimens of *Drosera indica* was obtained in the Watarase retarding basin area, Tochigi. The digestive fluid was collected by soaking thirty leaves successively (1 leaf for 1 min at a time) in 10 ml of distilled water in a test tube to wash out the digestive fluid through up-and-down strokes. The diluted digestive fluid thus obtained was stored frozen until use. Benzyloxycarbonyl-Phe-Arg 4-methyl-7-coumarylamide (Z-Phe-Arg-MCA), a cysteine endopeptidase substrate, *trans*-epoxysuccinyl-L-leucylamido(4-guanidino)butane (E-64) and pepstatin A were obtained from Peptide Institute, Osaka. Other reagents used were of analytical grade.

To measure the activity toward Z-Phe-Arg-MCA, the reaction mixture contained 20 µL of the diluted fluid, 5 µl of 2 mM Z-Phe-Arg-MCA in dimethyl sulfoxide, 75 µl of 100 mM buffer at various pH values, and ±10 mM dithiothreitol (DTT). The mixture was incubated at 37°C and the increase in fluorescence at 460 nm with excitation at 370 nm was measured at 5-min intervals for 60 min, and the activity was determined from the slope of the digestion curve. To measure the effects of other agents, a small volume of each reagent solution (*e.g.*, 1 µl of 1 M DTT and 1 µl of 1 mM E-64) was

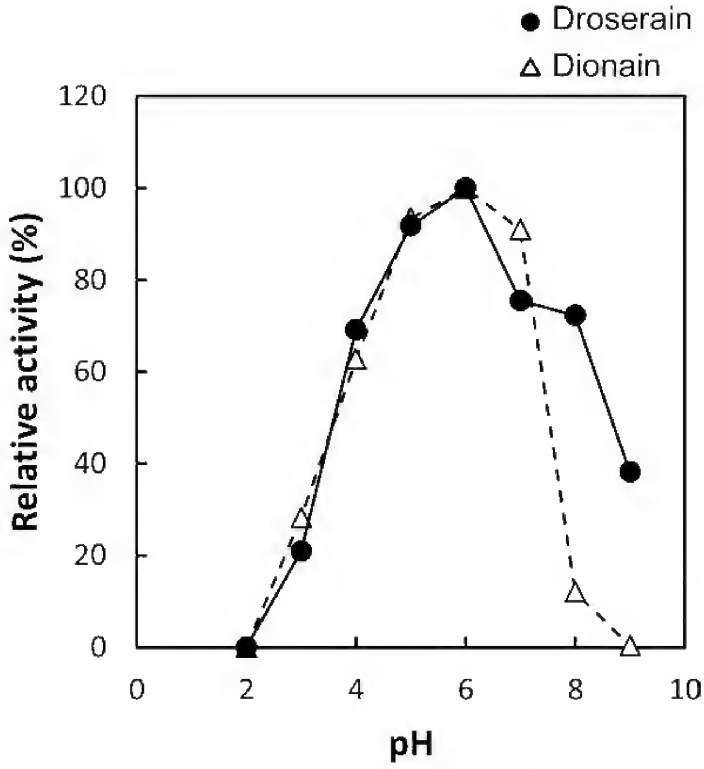

Figure 1: pH dependence of the activities toward Z-Phe-Arg-MCA of the digestive fluids of *Drosera indica* (droserain) and *Dionaea muscipula* (dionain) in the presence of 10 mM DTT. The activity at pH 6.0 was taken as 100%. The buffers (100 mM) used were KCl-HCl, pH 2.0, sodium citrate, pH 3.0-6.0, potassium phosphate, pH 6.0-8.0, and Tris-HCL, pH 8.0 and 9.0.

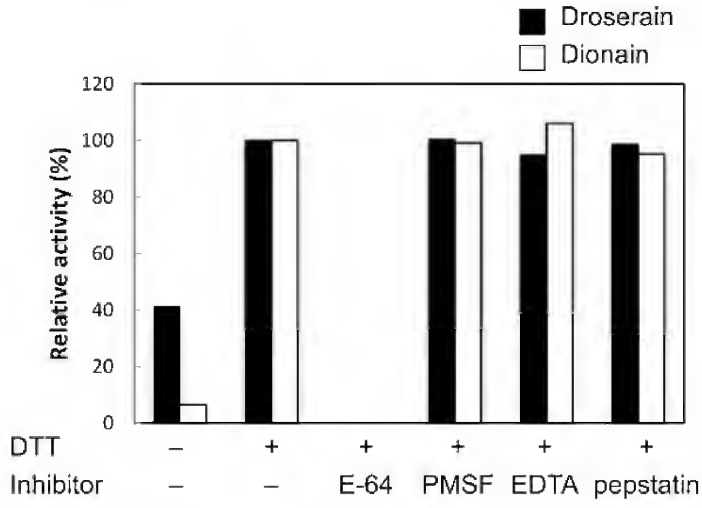

Figure 2: Effects of inhibitors on the activities toward Z-Phe-Arg-MCA of the digestive fluids of *Drosera indica* (droserain) and *Dionaea muscipula* (dionain) in the presence of 10 mM DTT at pH 6.0. The final concentrations of the inhibitors were E-64: 10 µM, PMSF: 1mM, EDTA: 5 mM, and pepstatin A: 1 µM.

added, and the mixture was preincubated at 37°C for 5 min before addition of the substrate. For comparison, the activity of dionain in the digestive fluid of *Dionaea muscipula* was also measured under the same conditions using 2 µl of the crude digestive fluid in a total volume of 100 µl made up with distilled water.

Results and Discussion

Figure 1 shows the pH dependence of endopeptidase activity toward Z-Phe-Arg-MCA in the presence of 10 mM DTT. The maximal activity was observed at pH 6.0, and about 20% and 40% of the maximal activity was observed even at pH 3.0 and 9.0, respectively. This indicates that the enzyme is capable of acting in a wide range of pH extending from acidic to weakly alkaline region. In the absence of DTT, about 40% of the activity in the presence of 10 mM DTT was observed (Fig. 2). A similar pH dependence of activity was observed with dionain in the presence of 10 mM DTT except that the present enzyme showed notable activity at pH 8-9 unlike dionain. The shoulder of activity at pH 8-9 may indicate the presence of the second cysteine endopeptidase.

Furthermore, the enzyme was completely inhibited by 10 µM E-64 (a cysteine peptidase inhibitor) whereas it was not inhibited by phenylmethanesulfonyl fluoride (a serine peptidase inhibitor), EDTA (a metallopeptidase inhibitor), and pepstatin A (aspartic peptidase inhibitor) as shown in Figure 2. The inhibitory profiles of these inhibitors were essentially the same with dionain (Fig. 2). These results show the occurrence of a cysteine endo-

peptidase similar to dionain in the digestive fluid of *Drosera indica*. We propose the name "droserain" for this enzyme. In this connection, we also propose the names of aspartic endopeptidases from the digestive fluids of *Dionaea muscipula* (Venus Flytrap), *Drosera* (sundew) sp., and *Cephalotus follicularis* as "dionaeasin", "droserasin", and "cephalotusin", respectively, as previously suggested (Takahashi 2003).

In the present study, 20 µl of the diluted digestive fluid was used for the assay of droserain, whereas 2 µl of the crude digestive fluid was used for the assay of dionain, and the activity observed for the latter was 4.2 times higher than that for the former. Therefore, one leaf of *D. indica* was calculated to contain approximately 8 µl equivalent of the digestive fluid of *D. muscipula*. This value appears to be fairly reasonable. The present enzyme is thought to work in concert with the aspartic endopeptidase "droserasin" in the digestive fluid of *Drosera* sp. like dionain and dionaeasin in *Dionaea muscipula*. This resemblance is reasonable since *Dionaea* and *Drosera* sp. are closely located in the phylogenetic tree. It remains to be clarified what kind of reducing agent is present in the digestive fluids of these carnivorous plants to activate the cysteine endopeptidases.

References

Athauda, S.B.P., Inoue, H., Iwamatsu, A., and Takahashi, K. 1998. Acid proteinase from *Nepenthes distillatoria* (Badura). Adv. Exp. Med. Biol. 436: 453-458.

Athauda, S.B.P., Inoue, H., Iwamatsu, A., and Takahashi, K. 2002. Purification and enzymatic characterization of an aspartic proteinase (Nepenthesin) from the insectivorous plant *Nepenthes distillatoria*. Proc. 4th Interntl. Carnivor. Plant Conf. Tokyo, 117-124.

Athauda, S.B.P., Matsumoto, K., Rajapakshe, S., Kuribayashi, M., Kojima, M., Kubomura-Yoshida, N., Iwamatsu, A., Shibata, C., Inoue, H., and Takahashi, K. 2004. Enzymic and structural characterization of nepenthesin, a unique member of a novel subfamily of aspartic proteinases. Biochem. J. 380: 295-306.

Takahashi, K., Athauda, S.B.P., Matsumoto, K., Rajapakshe, S., Kuribayashi, M., Kaga, T., Muramatsu, M., Niwa, H., Shimaya, K., Kubota, K., Nishii, W., Kojima, M., Inoue, H., Kubomura, N., Iwamatsu, A., and Shibata, C. 2003. Unique characteristics of carnivorous plant aspartic proteinases with special reference to nepenthesin. Program and Abstracts, Interntl. Conf. Asp. Proteases and Inhibitors, Kyoto, 20.

Takahashi, K., Athauda, S.B.P., Matsumoto, K., Rajapakshe, S., Kuribayashi, M., Kojima, M., Kubomura-Yoshida, N., Iwamatsu, A., Shibata, C., and Inoue, H. 2005. Nepenthesin, a unique member of a novel subfamily of aspartic proteinases: enzymatic and structural characteristics. Curr. Protein Pept. Sci. 6: 513-525.

Takahashi, K., Matsumoto, K., Nishii, W., Murakami, M., Kubota, K., Shibata, C., and Athauda, S.B.P. 2009. Comparative studies on the acid proteinase activities in the digestive fluids of *Nepenthes*, *Cephalotus*, *Dionaea*, and *Drosera*. Carniv. Pl. Newslett. 38: 75-82.

Takahashi, K., Suzuki, T., Nishii, W., Kubota, K., Shibata, C., Isobe, T., and Dohmae, N. 2011. A cysteine endopeptidase ("dionain") is involved in the digestive fluid of *Dionaea muscipula* (Venus's fly-trap). Biosci. Biotechnol. Biochem. 75: 346-348.

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In vitro rooting of *Nepenthes truncata* Macf.

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Keywords: *in vitro* rooting, *Nepenthes truncata*, IBA.

Rooting is an important stage in propagation of *in vitro*-grown delicate plants such as *Nepenthes truncata*. With well-developed root systems, plants are hardy enough to withstand changes in the environment during transfer from laboratory to nursery, thereby ensuring high survival rates.

In order to establish well-developed roots of *N. truncata* cuttings, we designed a series of experiments to explore a more suitable alternative to agar-based media and determine the appropriate plant growth regulator (PGR), PGR concentration, and method of application. Root length, root color, shoot length, shoot color, and survival rates were assessed. Statistical analyses were done using ANOVA (α=0.05, CropStat ver. 7.2.3). All culture media used full Murashige and Skoog media adjusted to 5.75±0.05 pH prior to sterilization; plantlet cultures were kept under 25±2°C, with light intensity of 3000 lux.

Effect of Plantlet Size and PGR on *In Vitro* Root Development

To determine the effect of plantlet size and PGR on root development of *in vitro Nepenthes truncata,* small (<1 cm height, leaf width ≤2 mm) and medium (between 1 cm to 3 cm height, leaf width >2 mm). Clone 4 plantlets were grown in (0.6% w/v) agar media supplemented with different Plant Growth Regulator (PGR) concentrations: 1 mL·L^{-1} Hormex®, 3 mL·L^{-1} Hormex®, 1 mg·L^{-1} IBA and 3 mg·L^{-1} IBA. Plantlet survival and rooting parameters were noted for 4 months. It was a completely randomized 2 × 4 factorial experiment consisting of 8 replicates with 5 plantlets per replicate.

Rooting parameters and plantlet survival were significantly affected by the PGRs used and these were constantly highest in plantlets supplemented with 3 mg·L^{-1} IBA. Plantlets supplemented with Hormex®, a commercially-available PGR containing vitamin B1, NAA, and IBA, produced callus while those supplemented solely with IBA grew root hairs (Fig. 1). Callus production impeded plantlet survival as callus creates an incomplete and weak connection between roots and shoots. In contrast, the presence of root hairs in plantlets supplemented with IBA aided in water absorption and translocation (Dhillon *et al.* 2011).

Figure 1: Roots in medium plantlets supplemented with and 3 mg·L^{-1} Indole-3-butyricacid (a), 1 mL·L^{-1} Hormex® (b), and 3 mL·L^{-1} Hormex® (c).

Table 1. Mean ± s.e. root count, root length rating, root color rating, and number of survivors of *Nepenthes truncata* clones under different rooting treatments for three months.

Note: Each value is the mean of 8 replicates with 5 plantlets per replicate. Values were sqrt(x+0.5) transformed prior to analysis.

Treatments	Root count	Root length	Root color	Survivor
Size				
Small	1.03±0.42	0.92±0.35	0.89±0.24	1.72±0.70
Medium	1.06±0.63	1.02±0.42	0.93±0.38	2.00±0.67
PGR				
Control (Co)	0.88±0.10	0.85±0.15	0.80±0.04	1.85±0.79
1 mL·L^{-1} Hormex® (P1)	0.13±0.24	0.99±0.21	0.96±0.24	1.72±0.67
3 mL·L^{-1} Hormex® (P2)	0.74±0.80	0.76±0.53	0.72±0.41	1.63±0.13
1 mg·L^{-1} IBA (P3)	0.79±0.43	0.78±0.36	0.78±0.25	1.81±0.72
3 mg·L^{-1} IBA (P4)	1.67±0.58	1.46±0.41	1.28±0.37	2.29±0.68
Size × PGR				
Small				
Co	0.71±0.00	0.71±0.00	0.71±0.00	1.58±0.27
P1	1.28±0.38	1.06±0.20	1.04±0.16	1.62±0.07
P2	0.71±0.19	0.71±0.13	0.71±0.08	1.47±0.22
P3	0.71±0.21	0.71±0.15	0.71±0.12	1.70±0.26
P4	1.73±0.03	1.45±0.06	1.27±0.02	2.23±0.28
Medium				
Co	1.06±0.06	0.10±0.06	0.90±0.03	2.11±0.23
P1	0.97±0.22	0.93±0.20	0.87±0.13	1.82±0.00
P2	0.78±0.00	0.82±0.00	0.74±0.00	1.80±0.27
P3	0.87±0.13	0.85±0.08	0.86±0.08	1.92±0.24
P4	1.61±0.00	1.51±0.00	1.29±0.00	2.35±0.30
P-Values				
Size	0.76	0.16	0.44	0.04
PGR	0.00	0.00	0.00	0.03
Size × PGR	0.02	0.00	0.00	0.04

Although plantlet size did not significantly affect root count, root length, and root color (Table 1), survival was significantly higher ($p = 0.04$) in medium plantlets. In addition, unlike medium plantlets, small plantlets grown in PGR-free media did not root.

Effect of Rooting Media on Root and Shoot Development and Survival of *In Vitro* Plantlets

Five pooled clone lines (Clones 3, 4, 7, 11, and 18) under a unifactorial completely randomized experiment were used to determine the effect of rooting media on root development of *in vitro N. truncata* plantlets. Pooled clone lines comprised 23 replicates with 5 plantlets per replicate. Replicates 1 to 6, 7 to 10, 11 to 17, 18 to 20, and 21 to 23 were assigned to Clones 4, 3, 11, 7, and 18, respectively. Medium-sized (1 to 3 cm) plantlets were transferred to: (1) 10 mL (0.6% w/v) agar media and 10 g of (2) vermiculite and (3) Silvosa medium (2:1:1 ratio of coco coir:osmunda fiber:charcoaled rice hull). PGR and activated charcoal were not incorporated in the media. Plantlet survival, shooting, and rooting parameters were noted for 3 months.

Table 2. Mean ± s.e. number of survivors, shoot count, shoot length, shoot color, root count, root length, and root color and P-values of *Nepenthes truncata* grown in different media for 3 months.

Note: Each value is the mean of 23 replicates with 5 plantlets per replicate. Values were sqrt(x+0.5) transformed prior to analysis.

Survivors	Agar	Vermiculite	Silvosa	P-value	LSD (0.05)
	1.27±0.45	1.85±0.51	1.21±0.64	0.00	0.30
Shoot					
Count	2.96±1.76	1.90±0.56	1.20±0.64	0.00	0.68
Length	1.37±0.36	1.43±0.16	1.42±0.30	0.58	0.13
Color	1.44±0.39	1.57±0.00	1.54±0.28	1.21	0.13
Root					
Count	0.79±0.38	1.07±0.24	0.84±0.33	0.01	0.17
Length	1.54±0.00	1.57±0.28	1.50±0.18	0.21	0.08
Color	1.44±0.00	1.47±0.26	1.40±0.00	0.24	0.08

Vermiculite was apparently the best rooting medium as it significantly produced the greatest number of survivors and the most roots (Table 2). Root length was also highest in vermiculite. In a study comparing plantlet rooting in vermiculite and gerlite (a solid medium), vermiculite promoted rooting better and it promoted penetration and aeration of the roots more than the latter (Jay-Allemand *et al.* 1992).

Shoot count was significantly highest among plantlets grown in agar. Shoot production was ten times faster in agar than in vermiculite and Silvosa medium (Figs. 2 & 3).

Because vermiculite is only good for rooting and agar for shoot formation, it would be better to grow plantlets in agar until they produced ample number of shoots prior to transfer in vermiculite. Transferring previously rooted plantlets to agar might degrade root quality since nutrient reserves of plantlets will then be utilized for shoot production.

Effect of Cutting Source and IBA Concentration on Root and Shoot Development of *In Vitro* Plantlets

The effect of 3 IBA concentrations (3 mg·L^{-1} IBA, 6 mg·L^{-1} IBA, and 9 mg·L^{-1} IBA) on root development of tip and base *in vitro* plantlets was also tested in Clone 4 where 8 replicates with 5 plantlets per replicate were used. It was a completely randomized 3 × 2 factorial experiment. Plantlets were irrigated with 18 mL full-strength MS and grown in 10 g vermiculite. Shooting and rooting parameters were observed for 4 months.

Root and shoot parameters were not significantly different between plantlets from different cutting locations (tip and base) supplemented with different IBA concentrations (Table 3). The root and shoot parameters were not significantly affected by the application of 3 different IBA concentrations. Loca-

Figure 2: *Nepenthes truncata* plantlets placed in agar (a), vermiculite (b), and Silvosa medium (c) for 4 months.

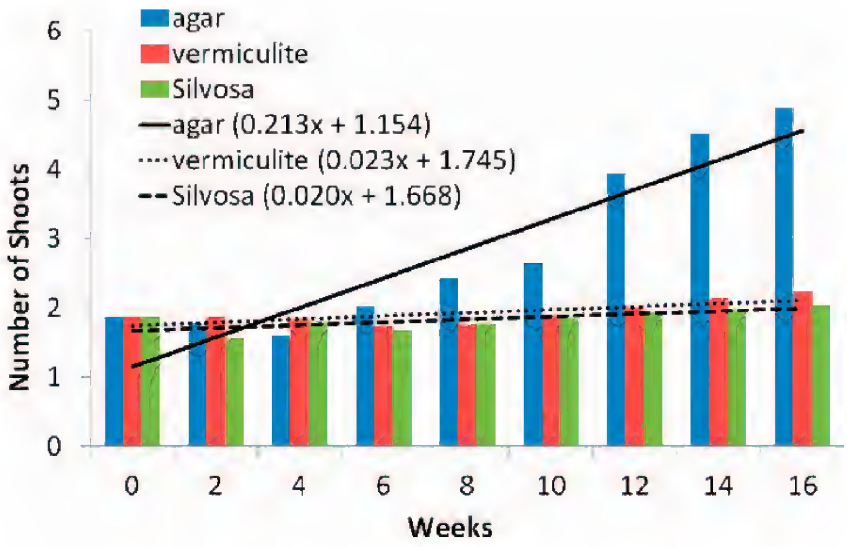

Figure 3: Shoot count of plantlets grown in different rooting media for 4 months.

tion of plantlet cuttings also did not influence root and shoot development. In the 2 cutting locations, the supplemented auxin was likely equally absorbed, and there was also no disparity in mineral composition (Schwambach *et al.* 2005).

Effect of IBA Application on Root and Shoot Development of *In Vitro* Plantlets

Two methods of IBA application were also compared in Clone 18: (a) incorporating IBA in 10 g vermiculite and (b) dipping cuttings for 10 minutes in IBA ranging from 10 mg·L^{-1} to 100 mg·L^{-1} prior to transferring in IBA-free 10 g vermiculite (Table 4). It was a completely randomized unifactorial experiment consisting of 4 replicates with 5 explants per replicate. Shooting and rooting parameters were observed for one month.

Although in most cases, shoot and root development of plantlets dipped for 10 minutes in differ-

Table 3. Mean ± s.e. number of survivors, shoot count, shoot length, shoot color, root count, root length, and root color of tip and base and P-values of *Nepenthes truncata* plantlets supplemented with different indole-3-butyric acid (IBA) concentrations for 3 months.

Note: Each value is the mean of 8 replicates with 5 plantlets per replicate. Values were sqrt(x+0.5) transformed prior to analysis.

Location	IBA concentration (Conc.) mg·L^{-1})	Shoot			Root		
		Count	Length	Color	Count	Length	Color
Tip	3	2.04±0.21	1.67±0.10	1.59±0.022	1.93±0.52	1.56±0.29	1.51±0.24
	6	1.95±0.16	1.65±0.05	1.59±0.047	2.02±0.75	1.65±0.38	1.46±0.43
	9	2.00±0.16	1.60±0.10	1.56±0.02	1.67±0.70	1.31±0.37	1.25±0.42
Base	3	1.94±0.13	1.65±0.10	1.58±0.02	1.81±0.57	1.56±0.31	1.51±0.25
	6	1.89±0.11	1.66±0.06	1.59±0.00	1.91±0.40	1.56±0.27	1.46±0.22
	9	1.95±0.18	1.61±0.06	1.58±0.00	2.00±0.84	1.66±0.31	1.34±0.32
P-value	Conc.	0.40	0.50	0.14	0.53	0.19	0.55
	Location	0.56	0.78	0.06	0.59	0.07	0.73
	Conc. × Loc.	0.37	0.27	0.34	0.99	0.74	0.16

Table 4. Mean ± s.e. root count, root length, root color, shoot count, shoot length, and shoot color of *Nepenthes truncata* plantlets dipped at different IBA concentrations for 10 minutes and grown in IBA-free media for one month.

Note: Each value is the mean of 4 replicates with 5 plantlets per replicate. Values were sqrt(x+0.5) transformed prior to analysis. IM= incorporated in the media.

IBA concentration ($mg \cdot L^{-1}$)	Shoot			Root		
	Count	Length	Color	Count	Length	Color
3 (IM)	1.87±0.09	1.64±0.04	1.58±0.00	1.02±0.22	0.85±0.09	0.90±0.14
10	1.75±0.06	1.63±0.03	1.54±0.04	0.97±0.13	0.95±0.11	0.92±0.10
20	1.90±0.09	1.76±0.13	1.58±0.00	1.11±0.20	0.96±0.12	0.92±0.10
30	1.86±0.14	1.59±0.06	1.53±0.07	0.93±0.14	0.86±0.13	0.81±0.07
40	1.86±0.13	1.61±0.03	1.58±0.03	1.63±0.34	1.33±0.21	1.21±0.18
50	1.85±0.04	1.67±0.07	1.62±0.09	0.84±0.14	0.81±0.10	0.80±0.09
60	1.90±0.09	1.68±0.06	1.51±0.05	1.07±0.19	0.86±0.07	0.86±0.07
70	1.75±0.10	1.59±0.05	1.52±0.04	0.91±0.21	0.79±0.08	0.79±0.08
80	1.80±0.04	1.64±0.04	1.56±0.02	0.99±0.21	0.83±0.08	0.81±0.07
90	1.86±0.04	1.71±0.05	1.52±0.05	1.11±0.22	0.96±0.17	0.91±0.11
100	1.80±0.08	1.65±0.11	1.47±0.07	0.93±0.23	0.82±0.11	0.85±0.15
p-value	0.583	0.676	0.727	0.237	0.089	0.223
LSD (0.05)	0.179	0.162	0.139	0.513	0.318	0.292

ent IBA concentrations were similar to plantlets grown in media supplemented with 3 $mg \cdot L^{-1}$ IBA, the latter is time-efficient and less laborious.

Conclusions

Our study shows that vermiculite can be used as a more suitable alternative to agar-based media for rooting *in vitro* grown *Nepenthes truncata*. Plantlet size, cutting location, and method of application did not significantly affect *in vitro* rooting. Dipping cuttings for 10 minutes in IBA concentrations ranging from 10 $mg \cdot L^{-1}$ to 100 $mg \cdot L^{-1}$ before transferring in an IBA-free medium is just as efficient as incorporating 3 $mg \cdot L^{-1}$ IBA in the medium.

References

Dhillon, R.S., Hooda, M.S., Pundeer, J.S., Ahlawat, K.S., and Chopra, I. 2011. Effects of auxins and thiamine on the efficacy of techniques of clonal propagation in *Jatropha curcas* L. Biomass and Bioenergy 35(4): 1502-1510.

Jay-Allemand, C., Capelli, P., and Cornu, D. 1992. Root development of in vitro hybrid walnut microcuttings in a vermiculite-containing gelrite medium. Scientia Horticulturae 51(3-4): 335-342.

Ofori, D.A., Newton, A.C., Leakey, R.R.B., and Grace, J. 1996. Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: effects of auxin concentration, leaf area and rooting medium. Forest Ecology and Management 84(1-3): 39-48.

Schwambach, J., Fadanelli, C., and Fett-Neto, A.G. 2005. Mineral nutrition and adventitious rooting in microcuttings of *Eucalyptus globulus*. Tree Physiol 25(4): 487-494.

Wounding and chemical treatment effects on *Drosera capensis* bud formation on leaf cuttings

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Introduction

Drosera capensis L. (Droseraceae) is one of the most commonly grown carnivorous plants prized for its ease of culture and rapid growth. In cultivation, seed is preferred for propagating this species; however, in the case of cultivar propagation, asexual propagation must be used. *D. capensis* readily propagates from both leaf and root cuttings. Leaf cuttings can be used to asexually propagate *D. capensis* (Brittnacher 2011). Wounding and exogenous hormone applications are commonly used in herbaceous and woody plant asexual propagation to enhance adventitious root and shoot formation. The purpose of this study was to investigate the effect of wounding and exogenous hormone application (auxin and cytokinin) on adventitious shoot formation on leaf cuttings of *D. capensis*.

Materials and Methods

The experiment was replicated in three separate flats using a 1:1 peat and milled sphagnum soil mixture. Leaf cuttings were taken from established plants maintained in the N.C. State University conservatory. Leaves used for cuttings were the youngest, fully unfurled leaves. The five treatments in this experiment consisted of an untreated control, two separate wounding treatments, a liquid auxin dip and a liquid cytokinin dip. The first wounding treatment entailed making a slight cut down the middle of the midvein on the adaxial side of the leaf using a single edge razor blade (razor wounding). The second wounding treatment involved poking the adaxial side of the leaf about ten times with a needle in a uniform pattern (needle wounding). For both hormone treatments, leaves were dipped in a solution for 10 seconds and then laid flat on the propagation media. For the auxin and cytokinin treatments, a 100 ppm solution of the potassium salt of indole-3 butyric acid (K-IBA) and 200 ppm solution of N6 benzyladenine (BA) were used, respectively. For the control and all treatments, leaf cuttings were firmly placed on the media, abaxial side down, and placed under a misting regime (6-second duration at 8-minute intervals) in a greenhouse maintained at an average of 75°F. Thirty leaf cuttings (ten per replication) were used for each treatment, divided evenly among three flats. Cuttings were checked weekly, beginning a week after the experiment was initiated. Once plantlets began to form, the cuttings were checked weekly at 3-4 day intervals. Evaluations were based on days until plantlet formation, number of plantlets per leaf, number of plantlets that produced roots, and number of leaves per plantlet.

Results and Discussion

There were no obvious differences between treatments for percentage of leaves that produced at least one adventitious shoot (Fig. 1), except for the K-IBA treatment, which dramatically inhib-

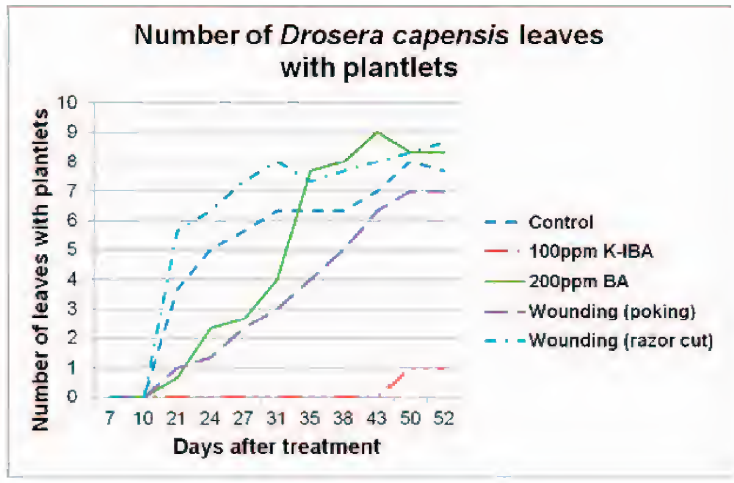

Figure 1: Average number of *D. capensis* leaves that produced at least one adventitious plantlet (out of 10 leaves total per replication). Data represent the mean of 3 replications.

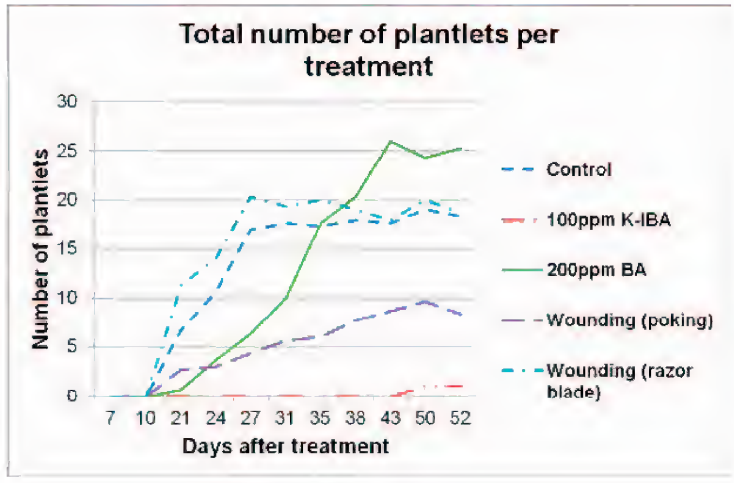

Figure 2: Total number of adventitious plantlets produced on leaves of *D. capensis* (out of 10 leaves total per replication). Data represent the mean of 3 replications.

ited the number of leaves forming plantlets and total plantlet formation. The midvein wounding treatment produced more plantlets per leaf on average than any other treatment up to about week 4, but was only slightly superior to the control treatment response. However, the BA treatment ultimately resulted in more plantlets per leaf at week 6 (Fig. 2) compared to all other treatments. Based on the data from this experiment, wounding by cutting the midvein produces the most plantlets in the least amount of time when compared to the other treatments performed in this experiment, but the BA treatment ultimately promoted the greatest number of plantlets by the end of the experiment. The midrib wounding treatment may be more time and cost effective than is the BA hormone dip treatment.

In order to provide the best yield in a shorter amount of time, removing the plantlets from the leaf cutting and planting them out should be done between three and five weeks after the initial cuttings are taken. This will help increase the number of plantlets that survive versus if the plantlets are left on the leaf cutting. After five weeks, the plantlets began to die as they competed with each other, with an exception in the case of the cytokinin treatment.

During the first three weeks of the treatment, bud proliferation was rapid for all the treatments except the K-IBA treatment. By the end of the third week, many of the buds began to grow their first true leaves and by the end of the fifth week we observed that many of the buds did not become plantlets. This is probably due to the excess of algae and fungal growth on the media surrounding the cuttings.

Conclusion

Result of this experiment show that wounding leaves of *D. capensis* Broadleaf form by cutting down the midvein led to a greater proliferation of plantlets than any of the other treatments in five weeks. Based on the data, wounding the midvein of the leaf produced a budding response for leaf cuttings of *D. capensis* Broadleaf form, but more research is needed to confirm this conclusively. After five weeks this experiment showed that a BA hormone dip greatly increases the number of plantlets produced. In future experiments it would be good for researchers to test wounding only the midrib versus the entire leaf surface. It would also be suggested to research the effects of different types of propagation media to determine whether the media was a limiting factor for this experiment or if this propagation method would produce the same response in other species of *Drosera*.

References

Brittnacher, J. 2011. Propagation-Drosera Leaf Cuttings. International Carnivorous Plant Society. http://www.carnivorousplants.org/howto/Propagation/DroseraLeafCuttings.php

D'Amato, P. 1998. The Sundews. The Savage Garden: Cultivating Carnivorous Plants. Berkeley, CA: Ten Speed Press. 119-129.

Drosophyllum lusitanicum L.

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Keywords: cultivation, *Drosophyllum lusitanicum.*

Regarded by many as the "odd man out" amongst carnivorous plants, *Drosophyllum lusitanicum* is one of those species not readily grown in cultivation, but which is surprisingly easy when a few simple rules are adhered to.

It produces long linear leaves to approximately 20 cm in length, which taper at their apex to a point. The long-lived leaves are held erect at first, and gradually lower to horizontal, finally resting at ground level where they die back, giving the plant a dome-like appearance. The base of the plant is skirted in the remains of the old leaves. The outer facing surface of the leaves is covered in numerous red stalked, mushroom-shaped glands which are topped with a droplet of mucilage (Fig. 1 & Front Cover). The red coloration of these glands is a stark contrast to the vibrant green of the leaves. There are also numerous sessile glands on the leaf surface from which digestive enzymes are released. This plant is unique in being one of only two plants to have leaves which unfurl facing outwards from the center of the growth point, rather than facing inwards as a fern does. This phenomenon is known as outward circinate vernation, a characteristic shared only with a couple of *Byblis* species.

The leaves are arranged in such a way as to resemble the growth point of a pine (*Pinus*), giving rise to one of its common names, the Portuguese Dewy Pine. Mature plants produce a strong honey aroma which is used as an attractant, and which on plants outside, is extremely effective as the leaves can be almost black with the carcasses of dead flies.

When an insect lands on a leaf, the mucilage detaches from the stalked glands, adhering to the

Figure 1: Mature *Drosophyllum lusitanicum* (left) and unfurling leaf (right).

Figure 2: Captured prey (left) and open flowers (right).

animal's exoskeleton. As it struggles to escape it makes contact with numerous glands, each depositing another droplet of glue until the insect's exoskeleton is coated and it suffocates. Digestive enzymes are then released from the sessile glands which break down the soft element of the animal, leaving only the chitinous remains (Fig. 2).

In July and August flowering commences, with the flowers produced atop a thick glandular stem to 30 cm in height, and circular in cross section. The calyx lobes are also glandular. The stunning bright yellow 5-petalled flowers are approximately 2.5 cm in diameter, and open for 3-4 days (Fig. 2).

Once pollinated, the corolla is shed and the seeds develop in a conically triangular-shaped, translucent green seed pod which grows to about 1.25 cm in length, and is held upright (Fig. 3). When mature in October, the capsule splits longitudinally along the 5 seams to expose 15-20 pyriform seeds, each about 3 mm in length (Fig. 3).

When a single growth point flowers, it does not leaf again, and is taken over by side shoots.

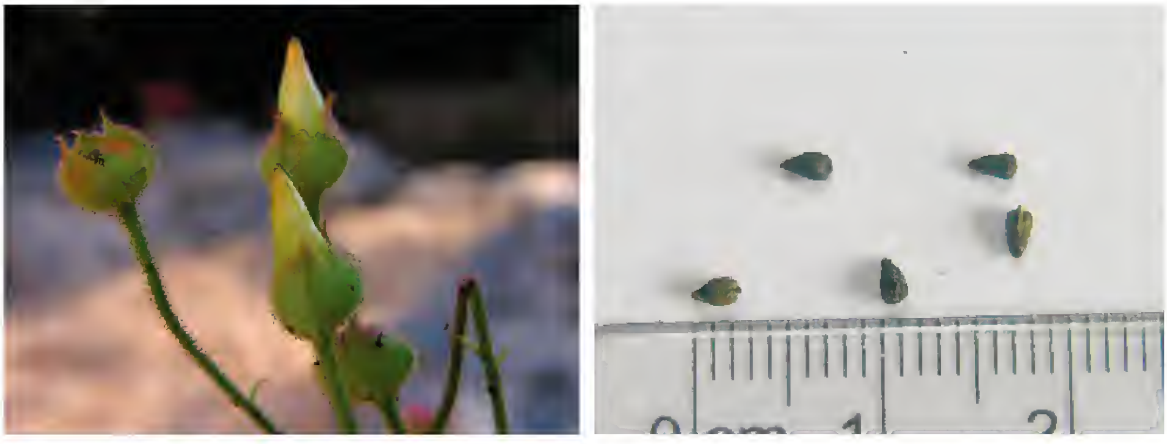

Figure 3: Conical seed heads (left) and seeds (right).

The natural habitat of this plant is southern Spain, western Portugal, and northern Morocco, and it is somewhat different to many other carnivorous plants, with a preference of dry stony hillsides with comparatively little rainfall.

The plant is long-lived and over time forms a thick stem, and must have a substantial root system to penetrate deep into the substrate in search of moisture, which I feel is a key to long-term cultivation.

I first grew this plant in the mid-1980's, the original plant living for about 3 years, and indeed over the years have grown *Drosophyllum* many times, but found that each would live only 2 years before flowering and dying. Considering the substantial bulk of wild plants, one can surmise that they live for considerably longer, so there must be a method by which the longevity can be extended.

My early attempts at cultivation were using the so-called Slack method, whereby a small clay pot is set in a larger pot of the same type (Slack 1986), which I felt was too restrictive for the plant as the roots can only enter the larger container via the small drainage hole. With this in mind I started germinating seeds in those small fiber pots, which I then set in a large (20 cm) clay pot, the thinking being that the roots would not be as restricted, but still found that the plants would live only 2 years.

Whilst considering the wild conditions of the natural habitat, and seeing habitat photographs (McPherson 2008), I came to the conclusion that the roots are still too restricted so I exchanged the large clay pot for a 10 liter black plastic container pot with a diameter of 26 cm, and a depth of 22 cm. Also considering the arid, stony soil conditions, I chose to use cornish grit as a medium, with the addition of a handful of coarse lumps of peat moss to help maintain a small amount of moisture.

Still using the fiber pots for germination, I transferred a young 7.5 cm-tall seedling in to the container pot in the summer of 2010, allowing the plant to establish over the cooler winter months in a greenhouse with a minimum temperature of 7°C. Once the danger of frost was over, the pot was lifted outside to a full sun position for the summer, where it received virtually no water (except during an exceptionally dry spell), apart from the natural rainfall. By July the plant was some 35 cm tall, with many leaves, and certainly benefiting from the abundance of insects to be found outside, with the leaves turning almost black with carcasses. It's interesting to note that the aroma produced by the plant can be detected from up to 3 m away.

I move the plant back to a frost-free location in the autumn, though I suspect a light frost will do little damage.

Propagation of this plant is by seed, which germinates easily, and has a long viability (especially if refrigerated). I have germinated 5-year-old seeds with no problems, and have heard of older seed remaining viable. I sow them in the small fiber pots mentioned earlier, which are about 6 cm in diameter in a 50:50 mix of peat moss and silver sand, and cover them to a depth of about 5 mm. I sow a single seed in each pot, and stand them in a tray of rain water in full sun. Germination should take 4-6 weeks. Keep the young plants in this position until they are about 6 cm tall, at which time they can be transplanted into a large container pot as described above. I now prefer plastic pots as they do not dry as rapidly as clay, and therefore the frequency of watering is minimal, especially in a damp location such as Somerset.

Outdoor cultivation of this plant appears to work well during the summer and certainly in a position of full sun when the plant will become less lax and more compact than those grown under glass, more closely resembling wild plants.

Seed kits of this fascinating plant are sometimes available from www.hccarnivorousplants.co.uk

References

Slack, Adrian. 1986. Insect-eating plants and how to grow them. Alphabooks Ltd, Sherborne, Dorset, England. 172 p.

McPherson, Stewart. 2008. Glistening Carnivores: The Sticky-Leaved Insect-Eating Plants. Redfern Natural History Productions, Poole, Dorset, England. 392 p.

The Roridulaceae

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Keywords: Cultivation, *Roridula gorgonias*, *Roridula dentata*, *Pameridea.*

The family Roridulaceae comprises of a single genus, *Roridula*, which is represented by two species: *R. gorgonias* and *R. dentata* (see Back Cover). Both are endemic to the Cape region of South Africa, with *R. gorgonias* having its narrow range in the coastal mountains of the South West Cape, and *R. dentata* found in the somewhat more arid area to the north. Both are woody perennial herbaceous shrubs and can attain large proportions with both species capable of reaching 2 m in height. In habitat both species grow in sandy soils, with *R. gorgonias* enjoying wetter conditions and being often found growing alongside *Drosera* and *Utricularia* species.

Much has been written about the symbiotic relationship these plants have with the capsid bugs *Pameridea* (Fig. 1), and each plant species has its own *Pameridea* species–*P. roridulae* on *Roridula gorgonias* and *P. marlothii* on *Roridula dentata*.

Because the plants do not produce their own digestive enzymes, they rely on their incumbent army of hemipteran warriors to consume the vast numbers of animals caught on the resinous leaves. The *Pameridea* are able to negotiate the sticky forest with the aid of an oil secreted on to their exoskeletons, enabling them to literally run along the leaves and stems unhindered. Once an animal is caught by the plant, the bugs move in, and using needle-like stylets suck the unfortunate victim dry. Insects are the predominant prey item, but as the plants become larger, animals as substantial as birds are also caught. I have had to remove an adult Robin (*Erithacus rubecula*) from the sticky branches of *Roridula dentata* which was held fast and had lost many feathers. After feeding, the *Pameridea* excrete liquid faeces on to the leaves which are then absorbed by the plant. This foliar feed provides the *Roridula* with the nutrients it requires. It is in effect a complete symbiosis.

The *Pameridea* species live their complete life cycle on the plant. They can often be seen mating and the females can be observed injecting the eggs in to the bark of the branches during the summer months.

Roridula gorgonias produces long woody stems which branch only after flowering and gradually become multi-headed, each topped with a fairly tight cluster of bronzy green lanceolate leaves 7.5-10 cm in length (Fig. 2). Each cluster is held erect at first and then slowly fan outwards before dying off and pointing downwards, with the dead growth clinging to the stem so they retain a sticky skirt beneath the green leaves. As the stem lengthens this dead material is shed, exposing the bare brown branches.

Bright pink flowers are produced during the spring and throughout the summer months, each having 5 fine, acutely ovate petals and a cluster of 5 sulphur yellow stamens. These flowers are approximately 2 cm in diameter, and seem to be attractive to the *Pameridea* bugs. Indeed, it has been suggested that they aid in the pollination of these plants, although hover flies are also sometimes seen on cultivated plants (Fig. 2). The flowers remain open for several days, finally closing when pollination occurs. Approximately 4 months later, the dark brown 2-3 mm angular seeds are produced (Fig. 4). These have a distinctly verrucose testa, and typically 4-5 are produced per capsule. When dehiscence occurs, the fruit remains erect (Fig. 5), and as such the seeds can be easily removed by hand with the aid of tweezers. This prompts the question, why are the seeds not readily shed from the mother plant? Could it be that the seeds are retained on the plant until fire burns through the habitat, in much the same way as is seen with some *Protea* species?

Figure 1: *Pameridea* on *Roridula dentata*.

Roridula dentata is a rather more untidy plant (Fig. 3) which branches freely from a young age without the necessity of flowering. The leaves are similar in shape to *R. gorgonias* but are pinnate, having 7-8 dentations on either side and are to 7.5 cm in length. They are a light apple green color and grow from a bright red soft stem which gradually becomes woody and brown with age (Fig. 3). Unlike *R. gorgonias* the leaves are produced singly along the stem from an open rosette, but age and die back in the same way, remaining attached for some time before falling from the plant. An interesting observation with this species is the pungent smell it emits during hot weather, best described as a mix of sweet corn and sewer gas!

As the plant develops and branches it can become large and rambling, eventually attaining over 2 m in height, and having an equal width (see Back Cover). Pale pink flowers with 5 broadly ovate, acutely tipped petals to 2 cm in diameter are produced during the late winter and early spring, and again these seem to attract the attention of the *Pameridea* who may aid pollination.

Four to five seeds are produced per capsule some 4 months later (Fig. 4). These are pale brown in color, cigar shaped, bear a distinct longitudinal ridge along one plane, and have an alveolate (honeycombed) surface. They are larger in size than *R. gorgonias* at about 4-5 mm in length and 1.5-2 mm in width. Dehiscence is achieved by the seed capsule splitting longitudinally along 3 planes, and the seeds fall to the ground as the fruit is pendulous (Fig. 5). Interestingly, very few seeds are to be found caught on the resinous leaves, suggesting that the seeds are either thrown clear of the mother plant (which is doubtful as seeds are usually found at the base), or they have a coating which in some way does not adhere to the leaves.

Figure 2: *Roridula gorgonias* growth point (left) and hover fly on the flower (right).

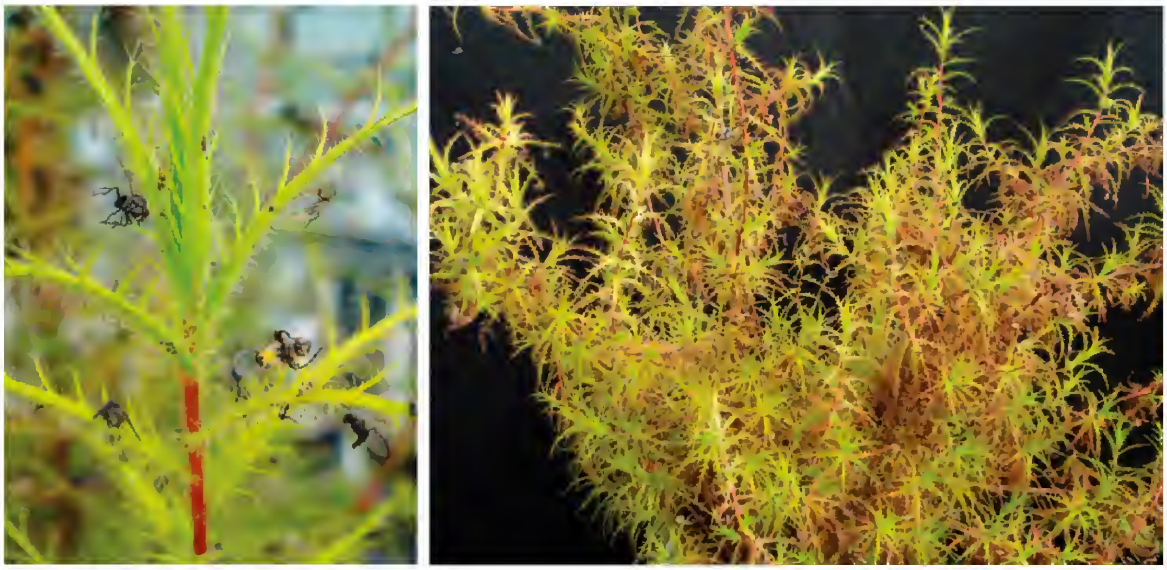

Figure 3: *Roridula dentata* growth point (left) and plant (right).

There has been little written about the cultivation of the Roridulaceae and they remain fairly rare in collections–certainly this is the case for *R. dentata* which requires rather more care to maintain successfully. The biggest obstacle to overcome is that of seed germination, which must be treated first. Both species require the same method of germination and perhaps the biggest clue lies with the fact that *R. gorgonias* retains its seeds as mentioned above. As has been published previously, many South African species require fire to germinate, or rather the smoke and chemicals contained therein. The easiest method of smoking seeds uses the method described by Lowrie (1996) and Reiner (2003), using a barbeque to smolder dry peat. This will replicate the effects of a bush fire and encourage growth.

Once treated keep the seeds in a sunny position, ideally in a greenhouse and keep wet. Germination can take several weeks, and eventually the testa will split and a root will emerge. The seedling will grow and shed the testa allowing the leaves to develop. *R. dentata* often takes a further few weeks to shed its' seed coat and it can appear to be struggling to dispose of it. Don't be tempted to remove it as you are likely to snap the developing shoot inside and kill the plant. In August 2007, I tried this method of smoking the seeds with 7 seeds of each species, and within 6 weeks all of the *R. gorgonias* had produced upright shoots, and all of the *R. dentata* had split and were starting to produce their first root. Hardly a scientific experiment due to the low number of seeds sown, but interesting just the same.

Figure 4: Seed comparison: *Roridula dentata* (left) and *R. gorgonias* (right).

Seedlings of *R. gorgonias* develop more slowly than *R. dentata*, which is faster in all respects, but because of the difficulty of this species it remains far rarer.

Figure 5: *Roridula gorgonias* dehiscence (left) and *Roridula dentata* dehiscence (right).

Cultivation

Roridula gorgonias is the easier of the species; we'll look at the successful cultivation of this plant first.

Light: Keep in full sun. I suspect that low light levels will produce etiolated, sickly plants which would not survive for long.

Water: Stand in 1 cm of rain water during the spring/summer months, and just damp in winter. Do not allow them to dry completely as they will rapidly decline. I lost a 60 cm specimen by allowing this to happen. When it was re-watered it gradually rotted and died over a period of several weeks–a sad end to a plant I had nurtured for 6 years.

Compost: I grow mine in a mix of peat moss and silver sand in a ratio of 1:2. This allows for free drainage during the winter so the plant does not remain too wet. The addition of 1 part coarse perlite will also aid in this, but bear in mind they will need careful and regular scrutiny to avoid drying too much. A good sized container allows healthy root run, and for a plant 30-60 cm high a 5-liter 25-cm pot is ideal.

Temperature: During the summer months the temperature in the greenhouse can rise to over 38°C with no negative effects. A winter minimum of 7°C is adequate. Growth ceases over the winter months and the population of *Pameridea* crashes, due probably to a combination of low temperature and lack of food. A few banded crickets (*Gryllodes sigillatus*), of the variety sold as reptile food, placed on the leaves will provide sustenance, but beware not to position too many as they are likely to rot during the cooler, damper months. However, the population will return in the spring when the temperature increases and the eggs laid the previous year will hatch.

General: As long as the conditions above are maintained this is a fairly easy and rewarding plant to grow. Allow good space around the plants as the branches tend to rot if they are cramped together and are touching. Good air movement is also an important factor to consider, especially during the winter months when greenhouse doors and windows are closed. A circulation fan is recommended to discourage fungal spores in the air from settling on any dead material and causing molds to flourish.

Roridula dentata requires similar conditions, but with some extra care-especially during the winter.

Light: As for *R. gorgonias*, it requires full sun to grow well.

Water: Stand in ½ cm of rain water during the spring and summer months when in full growth, allowing the tray to dry before replenishing. During the winter, the compost must be just damp, allowing a little water at a time to the base to ensure the plant does not desiccate. Conversely, too much water will rot the plant in a matter of days.

Compost: This species prefers dryer conditions than *R. gorgonias* and therefore likes a mix of peat, silver sand, and perlite in a ratio of 1:2:1. A large container is advisable to allow good root run, and will also prevent the compost drying too quickly. I have a 120 cm plant in a 25-liter pot. A 5-liter 25-cm pot will suffice for smaller individuals.

Temperature: As for *R. gorgonias*.

General: Most failures of this species occur during the winter months when they are extremely susceptible to rotting. Even in ideal conditions large plants will lose individual growth points to rot. They simply turn a grey/green color and wither. Regular inspection is required as rot can spread rapidly and affect whole branches, so be sure to remove any affected growth immediately, cutting back to healthy growth to allow a good margin. Even during the summer months this can occur during damp and overcast periods so vigilance is necessary all year. Good air movement is essential and air circulation fans I feel are imperative during the winter.

As mentioned above too much water will cause the plants to rot. I lost a 150 cm specimen when a small leak developed in the greenhouse roof. The gradual dripping fell directly on to the soil surface and saturated the pot. Within only a few days the whole plant had rotted.

Cuttings seem possible with this species. They must be fresh red stems, as the older woody stems never appear to root. Nevertheless, it is a slow process and roots seem to take a number of months to develop. Keep the cuttings small, ideally 7.5-10 cm, and push them in to a peat and sand mix to a depth of approximately 2.5 cm. Stand them in 2 cm of water until they have resumed growth, which will possibly be before roots develop, and keep in full sun. Once they are clearly growing, gradually reduce the amount of water to encourage the roots to form.

Because of the precise conditions *R. dentata* requires, I would only recommend it to the experienced grower who has succeeded well with *R. gorgonias*, and who has the correct growing facilities. However, to succeed with these incredible plants is ample reward for the effort required as they are surely among the most unusual subjects of the plant kingdom.

References

Lowrie, A. 1996. An easy method to smoke treat carnivorous plant seed. Bull. Austral. Carniv. Plant Soc. 15(2): 3-5.

Obermeyer, A. 1970. Droseraceae. In: L. Codd, B. De Winter, J. Killick, and H. Rycroft (eds.) Flora of Southern Africa, Vol. 13, Government Printer, Pretoria: 187-201.

Reiner, M. 2003. Die Gattung *Roridula* und ihre Freunde. 13 p. http://www.drosophyllum.com/Dateien/Roridula.pdf

Drosera slackii Cheek

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Keywords: Cultivation: *Drosera slackii.*

South Africa is renowned among carnivorous plant growers for its *Drosera*, with stunning species such as *D. cistiflora* and *D. pauciflora*, and also a number of rosetted species. For some, these species hold little appeal, but as with many plants I feel a closer look is required to appreciate the finer beauty of these plants. *Drosera slackii*, named after Adrian Slack, was discovered as recently as 1979, and named in 1987, being placed in section *Drosera* within the genus. lt ranks as one of the most impressive rosetted *Drosera*, and can be confused with no other (Fig. 1).

Found around the Cape region of South Africa, the deep red rosettes can reach 7.5 cm in diameter, with narrowly cuneate leaves with a distinct bulge on the petiole (Fig. 1). There is a conspicuous bright red multi lobed stipule at each leaf base, and the rear of the leaf has a number of coarse red hairs, predominately on the petiole section, with a few extending on to the rear of the lamina.

The upper surface of the lamina is covered in the characteristic insect catching glandular tentacles, with a single line of non-sticky, longer hairs to 8 mm in length along the apex. Each of these is topped with a cylindrical, non-sticky gland, the purpose of which is to hold in place a large struggling insect whilst the sticky glandular hairs gradually curl over and make contact. These so-called "snap tentacles" are found in number of rosetted species, and are capable of extremely rapid movement, often bending through 180° in as little as half a second. Like all rosetted *Drosera*, the leaves themselves are able to roll over completely to ensure as many hairs as possible are in contact with the insect prey.

After a few years, *D. slackii* becomes pedestal forming, with the live rosette sitting on top of a column of previous year's dead growth, in the same way as the well known *D. roraimae* of Venezuela (Fig. 2). I have a few plants which have remained in the same pot for 6 years and are now approximately 7.5 cm high. To produce this effect it seems important to regularly flush through the compost from above with rain water. I have found that after 3-4 years of being in the same compost, the rosettes of many species start to lack vigour and reduce in size. This I believe is due to a build up of minerals in the upper layer of the compost, brought about by the constant upward imbibement of water. No matter how pure the water used, there will always be an element, however small, of mineral salts which will accumulate in the upper surface, and must be detrimental to growth. For the past 4 years I have regularly flushed the *Drosera* plants from above, usually every 3-4 weeks in the

Figure 1: Deep crimson rosettes of *Drosera slackii* (left) and closeup of lamina (right).

Figure 2: Old pedestal forming rosettes.

growing season, with the result that the plants have thrived, with none of the reduction in rosette size previously seen. In the wild of course, the natural rainfall will have the same effect.

It would be interesting for this theory to be tested—a task I shall leave to someone somewhat more technically minded than myself.

A few years ago, I discovered another interesting phenomenon with this species. A hundred or so plants were on a bench in the nursery, and as I passed them I bent down to retrieve something off of the floor. As I stooped down past the level of the plants, I was struck by a strong floral perfume, which on closer investigation was being produced by the rosettes themselves, rather than the flowers

Figure 3: Glandular scape (left) and closeup of flower (right).

Figure 4: Hover fly pollinating the flowers (left) and tiny fusiform *Drosera slackii* seeds (right).

which have no fragrance. One can only assume that this acts as an attractant for insect prey, but I have found no reference to it elsewhere.

In early summer, tall wiry glandular stems (Fig. 3) to approximately 50 cm are produced with 20-25 bright purple-pink flowers to 1.25 cm in diameter, each with 6 bifid stigma tips, and each opening for a single day (Fig. 3). I have seen the flowers pollinated by hover flies in the nursery (Fig. 4).

Seed set on this species seems to be poor, with comparatively few produced when compared to other South African plants. The seeds are fusiform, blunt at one end, and 0.75 mm in length (Fig. 4).

I keep my plants in full sun, in a compost of silver sand and moss peat to a ratio of 2:1, and standing in 2-5 cm of rain water in the summer months, reducing down to keep just damp over the winter with a minimum temperature of 7°C. At these temperatures the plants remain in growth all year, though the rate of growth decreases considerably over the winter.

I have subjected this species to sub-zero temperatures, down to as low as −6°C, the result of which was the destruction of the rosettes, but re-growth from the long wiry roots occurred the following spring with new shoots emerging in April.

Individual plants will, in time, divide and can produce a dense colony. These can be divided and potted separately. Root cuttings work well, and in early spring adult plants can simply be removed from their pots, the lower 50% of the roots removed, and these laid out on the surface of some compost and lightly covered with the same. Re-pot the adult plants. Keep the compost with cuttings standing in rain water and in full sun, and tiny rosettes will begin to develop within 6 weeks.

Seed also works, and can simply be surface sown in the spring and treated as the root cuttings above. Germination occurs in 2-4 weeks.

This species is available from www.hccarnivorousplants.co.uk

Germination of 22-year-old *Drosophyllum lusitanicum* and *Byblis gigantea* seeds

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While cleaning out an old refrigerator in the lunchroom at my office, I discovered a paper bag containing packets of seeds of *Drosophyllum lusitanicum*, *Byblis gigantea*, and several *Drosera* species from the ICPS Seed Bank that had been there for 22 years! As you can imagine, the seed storage conditions in an office lunchroom refrigerator are less than ideal. The paper bag and seed packets were stained with all sorts of unknown spilled food stuff.

In late March, I scratched the seed coat of 100 of the 22-year-old *Drosophyllum* seeds on fine sandpaper until I saw the white endosperm. These scarified seeds were then soaked in tap water for 24 hours. I put the 100 seeds on the surface of moist vermiculite to see if they would germinate. To my great surprise, the first seeds began to germinate in 13 days. At the end of two months, 76 of the 100 seeds had germinated (Fig. 1).

I sprinkled the old *Byblis gigantea* seeds on the surface of a wet mixture of equal parts Canadian milled sphagnum peat and silica sand in a glazed ceramic pot. Two days later, I put a handful of dry grass on top and burned it. The seeds began to germinate within 35 days. I did not count the number of small seeds or estimate germinate rate. I was just surprised that the 22-year-old seeds germinated.

None of the old *Drosera* seeds germinated.

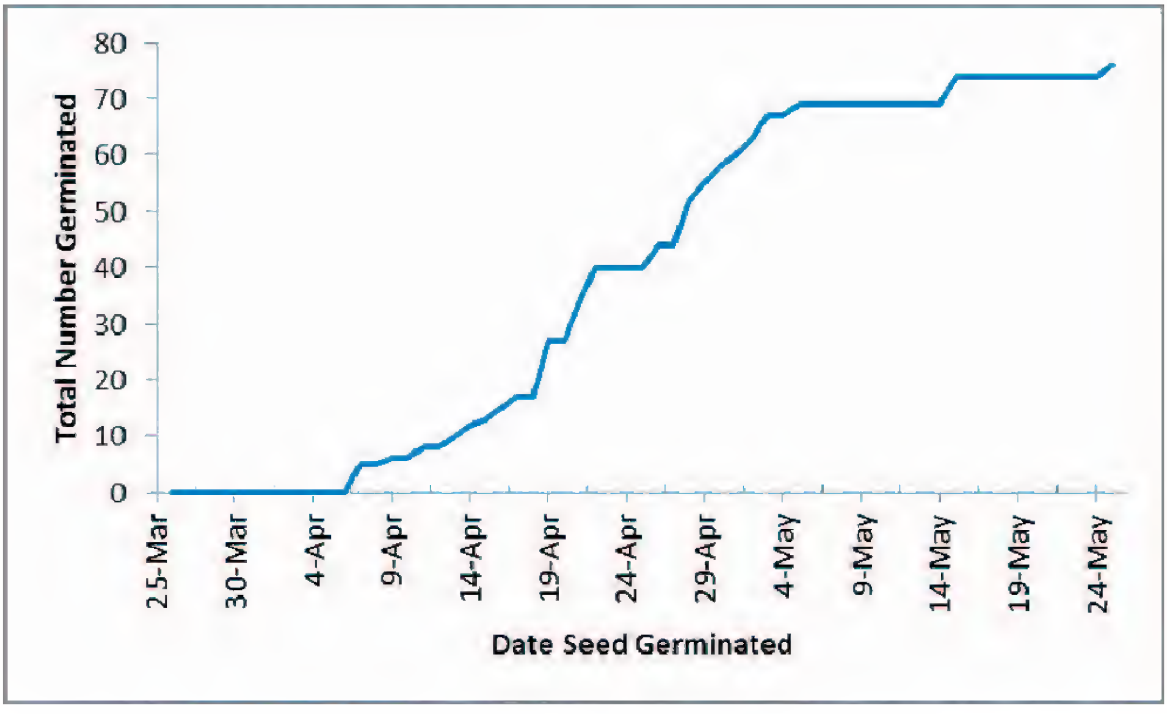

Figure 1: Germination of 22-year-old *Drosophyllum lusitanicum* seeds.

Apology

Photographic credit error in: Cross, A. 2012. *Aldrovanda* - The Waterwheel Plant. Redfern Natural History Productions, UK.

In August, I was pleased to release my first book, '*Aldrovanda* - The Waterwheel Plant'. I am extremely thankful to the numerous researchers and carnivorous plant hobbyists from all over the world who were willing to contribute to this work by sharing their knowledge and photographs with me, all of whom are gratefully acknowledged in the book.

Unfortunately, several images taken by Dr. Bartosz Płachno of the Department of Plant Cytology and Embryology, Jagiellonian University, Poland, were accidentally included among a large number of files sent to me, and are incorrectly attributed to Dr. Lubomír Adamec in the text. These include the two images of Figure 23, and the single image of Figure 24.

The error will be corrected in any further reprints or volumes of the book, and I offer my apologies to Dr. Plachno for the accidental inclusion and incorrect credit of his images.

–Adam Cross • School of Plant Biology • The University of Western Australia • Botanic Gardens & Parks Authority • Fraser Avenue • West Perth WA 6005 • Australia

Mail delays?

Members occasionally complain that it takes a long time to actually receive the hard-copy printed CPN in the mail. In some locations it seems to take a month to receive the CPN. Sometimes members let us know that they have not received their CPN at all.

We apologize for problems with the domestic and international postal services. We have made many attempts to improve delivery. We recently changed from a clear to opaque mailer to reduce theft. Since the cost of postage is a major part of the CPN production budget, we are limited in what we can accomplish.

CPN is published and mailed on March 1, June 1, September 1, and December 1. One option for rapid gratification is that the PDF of each issue is immediately posted on our website http://icps.clubexpress.com/