

ORCHID SCIENCE LETTERS

Issue 1, September 2011



Participants at the 2011 OSSSU Workshop at Lankester Botanical Garden,

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The Newsletter of Orchid Seed Stores for Sustainable Use (OSSSU)

EDITORIAL

Welcome to the first edition of Orchid Science Letters. As the OSSSU family begins to expand further the time has arrived to begin producing a regular newsletter. We are hoping that this will further facilitate communication between members within the group. We would like to publish short articles about orchid conservation activities around the world and would be happy to receive contributions in either English or Spanish. Clearly the focus will be on the activities of OSSSU members, but we are also interested in publishing information about wider issues in orchid conservation. The key to the success of the project must be sharing of information. We want to know who is doing what, where and when.

We know that there is a lot of good work taking place around the world, but much of it doesn't receive sufficient publicity. As an example of this, many articles are published in scientific journals that are only available through subscriptions. It is our intention to include abstracts of recent papers of interest, and in this issue there are abstracts of papers describing an improved method for tetrazolium testing by our colleagues at UNOESTE in Brazil, and the effects of different media on a range of species written by colleagues at Kew's Seed Conservation and Research Section at Wakehurst Place.

It is our intention to place longer articles in the 'resources' section of the web site, and you will find that we have uploaded protocols for tetrazolium testing in both English and Spanish plus a very interesting report on orchid conservation in St. Eustatius in the Caribbean. We would like to hear about any popular articles you may have published. For example in Samara (www.kew.org/msbp/samara), the International Newsletter of the Millennium Seed Bank Partnership, volume 20, there is a short piece about the Costa Rica workshop in September, 2010. Apologies to Matt Richards. Where were you when the photograph was taken? I have now pasted you into future images of the workshop attendees.

It would be useful if we could include suggestions where students might be able to apply for grants to enable them to participate in orchid conservation projects. It goes without saying perhaps that we need to find ways of both encouraging more students to work with orchids and to find ways where possible of supporting them in their studies. The article about Danielle Ferreira's participation in the work at Atlanta Botanical Garden (ABG) provides a good example of what we are trying to achieve. As a result of Dani meeting Matt Richards at the workshop in San José she was awarded an internship.

From the end of September, there is going to be a change in the management structure of the project and Tim Marks is going to take over from Phil as Project Manager, thereby allowing Phil to focus on the newsletter and the web site. Phil is a past editor of both the Orchid Review and the Orchid Specialist Group's Orchid Conservation News. Over the past few months Phil has found skype to be a very useful way of communicating with some members of the group. We would like to encourage you to use skype if possible. We have found that the most sensible way to use skype is to arrange a time to go on line via email. The listserver has not been a success so far. It will only work if everyone signs up to it and perhaps the newsletter is a better option assuming that it is produced on a regular basis.

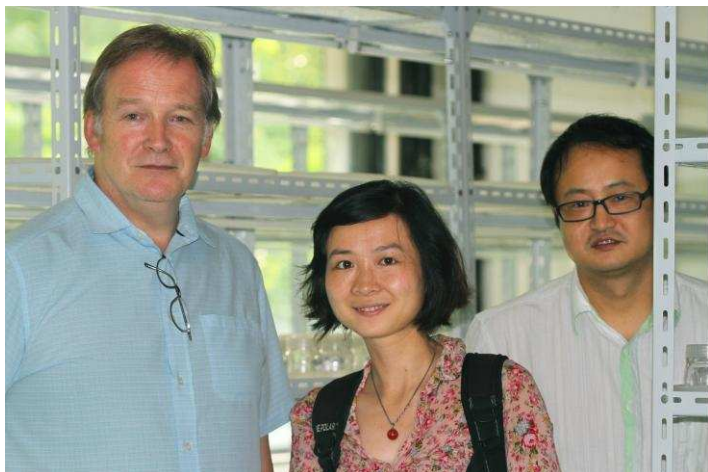
We have received a number of invitations to join LinkedIn, Sonico and Facebook. We are reluctant to use these at present. Are we just old-fashioned? We would value your opinions. In conclusion, we think that the key to the future success of OSSSU2 is good communication combined with the publication of information useful to the participants.

Phil Seaton, Tim Marks and Hugh Pritchard

The Newsletter of Orchid Seed Stores for Sustainable Use (OSSSU)

NEWS

Hugh Pritchard visits Xishuangbanna Tropical Botanical Garden



Hugh was invited to Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Sciences (CAS) during 4-6th August 2011. He visited the seed bank, the ex-situ conservation and reintroduction of endangered species research group and the beautiful garden, where he especially enjoyed the palm collection. His presentation named "Seed Storage of Threatened and Useful Plants" was welcomed by the staff and students.

Prof. Jin CHEN, the garden director, held a symposium between Prof. Hugh and the staff from the conservation biology centre. Orchid seed storage and germination and the germination problem of the hard palm seeds were discussed. This visit laid a firm foundation for orchid conservation and personnel training.

XTBG is located in the southwest of China, covering an area of 900 hectares. Over 10 thousand species of tropical plants are preserved in its 34 living collections. Scientists at the garden are engaged in biodiversity conservation and the sustainable use of plant resources, focusing on forest ecosystem ecology, conservation biology and plant resource development. There is a long history of co-operation between XTBG and Kew.

Lin HUA

Udai Pradhan visits Kanchit Thammasiri in Bangkok



Udai C. Pradhan, Chair, Indian Subcontinent Regional OSG, IUCN/SSC Orchid Specialist Group with his wife visited Kanchit's lab and greenhouse on March 15, 2011 to discuss orchid seed stores and OSSSU work at Mahidol University. Hemlata Pradhan, their daughter and a well-known botanical illustrator showed her work at the exhibition of "Watercolour Paintings of Unique and Exquisite Orchids and other Plants of the Himalayas " at Serindia gallery, Bangkok during March 17- May 1, 2011.

Kanchit Thammasiri

Top left: Prof. Hugh Pritchard, Lin Hua and Prof. Gao Jianguan

Left: Udai Pradhan talking with students at Mahidol University

3rd Orchid Micropropagation Workshop Quito Botanic Garden



Workshop participants

Orchid Micropropagation Quito

Whereas it is the responsibility of the present generation of orchid lovers to preserve as many orchid species as possible for the next generation to enjoy, it is self-evident that the future of orchid conservation lies in the hands of today's young people. The key question is, what can be done to encourage more students to participate in orchid conservation programmes? Nowhere is the task more urgent than in Ecuador, with around 4,300 species of orchids, it is truly a hotspot for orchid biodiversity. An almost incredible one in every four species of flowering plant in Ecuador is an orchid. Many are teetering on the brink of extinction.

The talks

In May this year I was privileged to be invited to participate in the third workshop held at Quito Botanic Garden (JBQ) on the micropropagation of orchids. When I arrived on the first morning the auditorium at JBQ was filled to capacity with students from around Ecuador, eager to participate in this five day workshop. The stage was set by the well-known authority on Ecuadorian orchids, Alex Hirtz, who gave the introductory talk on the topic of 'Why are there so many orchids in Ecuador?' With the discovery of many new orchid species to his credit over a period of many years, Alex has an encyclopedic knowledge of the orchids of his country and a particular interest in their evolution.

There followed a week of lectures in which the underpinning theory was delivered by our colleagues from Quito Botanical Garden, the University of Cuenca, the Universidad Técnica Particular de Loja (UTPL) plus the Ecuadorian Ministry of the Environment. I was there on behalf of OSSSU and as a representative of Kew. The topics ranged widely from the role of CITES in orchid conservation by Teddy Escarabay, safety in the laboratory (although crucial this is never considered by students to be the most exciting topic, and yet José Miguel Romero made his talk one of the most entertaining of the week illustrating important points with cartoons) through to seed viability testing using a new modification to the chemical stain tetrazolium chloride, developed by Nelson Neto and his colleagues in Brazil; construction of a laminar flow hood by Kabir Montesinos and transplanting of orchid seedlings from the flask by Iván Guachizaca.

My own contributions to the workshop (I gave 6 talks) were also in Spanish ... and it must be said ... very bad Spanish! Fortunately, both the students and the other speakers were very kind and happy to help me out. Indeed explaining concepts in a language other than your own has the advantage of making you explain things simply.

Practical activities

Students of course enjoy doing as well as listening (and often more than listening!) and at the heart of the workshop were the practical sessions. Micropropagation is after all a practical activity and it was important to provide the students with as much hands on experience as possible of the various techniques ... there is no substitute for doing. The sessions were led by Eduardo Sánchez, ably assisted by Francisco Merchán and the other lecturers. As we all know, Eduardo is passionate about preserving his country's megabiodiversity. An outstanding lecturer and ambassador for orchid conservation, he is always keen to share his expertise gained thorough more than thirty years growing orchids and raising them from seed in the laboratory.

Students were led through the sequence from beginning to end, from pollination to seed storage; from protocorm to transplanted seedling. Orchid flower structure can be a bit of a puzzle at first and it is important to transfer sufficient pollen to the stigma to obtain good quality seed i.e. where a high proportion of testa contain embryos.

The practicals were conducted as a circus, whereby there were a number of different activities taking place at the same time and students were able to rotate between the different tasks. Leonardo Vaca Granda showed students how to make up culture media. The students were taught various techniques of surface sterilising orchid seeds and sowing using the three laminar flow hoods Phil Seaton demonstrated the packet method, a particularly useful technique where there are only small numbers of seeds available for sowing.

As an added bonus, Paola Cruz Jaramillo demonstrated how to dissect out orchid meristems. She was not used to carrying out such a delicate operation in front of an audience, but despite her shaking hands she gradually revealed the glistening ball of cells that was the



Maxillaria meristem. The students were spellbound. "How did she do that?" "It's so tiny." After Paola's demonstration thirty students clad in pristine white lab coats clustered around binocular microscopes to try out the technique for themselves.

Once the meristem has been obtained the art is to persuade this minute ball of cells to continue dividing and differentiate into a plantlet on an agar medium in a test-tube. As they multiply and

develop within the shoot tip the cells are influenced by various plant hormones and other growth factors. This is the subject of Paolita's research; to tease out the correct balance to induce the ball of cells first to multiply, and then to produce shoots and roots.

Judging by the response of the students the workshop was a great success. A number of the students expressed a strong desire to pursue their studies with orchid-related projects. I came away feeling optimistic about the future prospects for orchid conservation in Ecuador if we can provide the necessary support enabling young people to participate.

Phil Seaton

Phil Seaton would like to express his thanks to the organisers Carolina Jijon and Leonardo Vaca Granda for being invited to participate in the workshop and for the enthusiasm expressed by the students. Sincere thanks to Paola for taking the time to explain her studies.

Saving Florida's Cigar or Cowhorn orchid (*Cyrtopodium punctatum*)



**Danielle pollinating
*Cyrtopodium punctatum***

Loss of habitat due to intensive logging of its large productive stands of bald cypress for timber in the 1940's, combined with harvesting by over-zealous collectors, decimated populations of the cigar or cowhorn orchid (*Cyrtopodium punctatum*) to the extent that by 2007 fewer than 20 individuals were known to occur in Florida's 85,000 acre Fakahatchee Strand Preserve State Park.

Although still encountered in the expansive adjacent Big Cypress National Preserve and Everglades National Park it only occurs sporadically. With no natural seed set or seedling recruitment documented in recent history, the prospects for recovery of the species in the Fakahatchee Strand seemed dismal. As a last ditch attempt to rescue this, one of North America's largest

and showiest native orchids, Atlanta Botanical Garden (ABG) was asked by Florida Division of Recreation and Parks to assist in recovery efforts. Members of ABG's recovery team began cross-pollinating individuals within the park, and in 2009 the first capsule was harvested and sent to the garden's Tissue Culture Lab where staff and dedicated volunteers propagated plants for recovery.

A Masters degree student at the Universidade Federal do Paraná (UFPR – Brazil), Danielle Ferreira visited ABG in the spring of 2011 to participate in this conservation programme. With experience gained through her university studies she was able to participate fully in the daily tasks of the tissue culture lab, where she initiated germination tests for four different

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breeding groups of ***C. punctatum***, the results of cross-pollinations made in 2009 and 2010. The aim was to develop a more efficient protocol for micro-propagating this species, aiming to achieve both high rates of germination and improved growth rates. The experiment involved investigating a range of different culture conditions, including comparisons of 16/8 hours light/dark and complete darkness and two different types of germination media.

Although germination appeared to be marginally better in the dark than in the light, the magic ingredient for both germination and subsequent growth appeared to be the incorporation of banana into the culture medium. The addition of pulped banana is not suitable for all epiphytic orchids, but for ***C. punctatum*** its benefits are clear. Danielle completed the preparation of replating media and transferred thousands of cigar orchid seedlings that had been germinated *in vitro*.

Following the full development of seedlings raised *in vitro*, Danielle assisted with the transfer of hundreds of seedlings from the laboratory to the production greenhouse. Her daily horticultural duties included the care of the seedling collections in the greenhouse. Indeed, in addition to working in the tissue culture lab, Danielle performed many horticultural activities within the Fuqua Orchid Center and greenhouse collections at the ABG. These duties included daily care of orchid collections, watering, spraying, fertilizing and pruning. She also helped to maintain and organize the exhibits landscape of the Fuqua Orchid Center.

While work continued in the lab with fresh batches of seed harvested in 2010 and 2011, there was an opportunity to enhance the natural population of ***C. punctatum*** in the spring of 2011. After four weeks of background preparation for on the ground conservation work, the team traveled to the Fakahatchee Strand Preserve in Copeland, Florida to spend four days in the field collecting fruits pollinated in the previous year. Seed was sown *in situ* at microsites that presented conditions as close as possible to those thought to be required by ***C. punctatum***, with the aim of inducing natural germination. Seed was collected for future *ex situ* production at ABG. Dani cross-pollinated many different individuals to increase genetic diversity within the seed capsules. *In situ* seed packets (deposited earlier) were collected and examined for *in situ* germination. The development of new seedlings from natural seed germination from seed dispersed in 2010 was observed.

Winters in Florida are mild and relatively dry, with a rare cold front bringing freezing conditions. The summers are very hot, wet and sticky, with average temperatures in the low 90's Fahrenheit (low 30's Celsius) and relative humidity in excess of 90%. Rainfall peaks in June/July, when heavy thunderstorms are frequent in the late afternoon. As a result of these extremes the water levels within the Fakahatchee Strand can fluctuate enormously. When Matt Richards, Conservation Coordinator of ABG, had visited the study site in November 2009 he had found himself up to his waist in water but this time, in April 2011, the ground was dry.

The cigar orchid, as you would expect becomes dormant over the dry winter months and loses its leaves. During April/May it begins to produce new roots, flowering before the production of new growths. Seed capsules take 14 months to develop, so that seeds are released in the hot and humid months. During their visits to the site the team from ABG harvests a proportion of the capsules and takes them back to the lab in Atlanta to ripen. Around 10% of the seed is sown or put into dry storage at -20°C. The remainder of the seed is sent back to the Fakahatchee where park rangers play "Johnny Orchid Seed", scattering the seed at suitable sites.



Dennis Giardina with *C. punctatum* attached to tree trunk

The long-term aim of any re-introduction project is to establish a self-sustaining population. Seed enclosed in the classic net packets used for fungal baiting had been deposited the previous year and were collected and examined for signs of in situ germination. This, however, proved to be unnecessary as the researchers were excited to find a clutch of new seedlings from seed dispersed in the previous spring in 2010. In addition a new height was reached in the 3 year project as 88 robust seedlings were introduced back into their natural environments of the Fakahatchee Strand Preserve in 2011. This act alone would more than quadruple the known number of individuals throughout its native haunts within the park. Danielle cross-pollinated a selection of different individuals to increase genetic diversity within future seed collections. Fruits pollinated in the previous year were collected for future ex situ production at ABG, and further seed was sown in situ at appropriate microsites.

Danielle Ferreira & Matt Richards

Initiating and continuing collaborations and sharing experience between institutions both belonging to OSSSU and outside the network are vital for effective conservation both now and in the future. The link established between the Universidade Federal do Paraná (UFPR - Brazil) and Atlanta Botanical Garden (ABG) through the OSSSU network of institutions enabled Dani to participate in the above project. Danielle would like to offer her sincere thanks to the Naples Orchid Society for their gracious support of her internship through their scholarship programme. She would also like to thank Atlanta Botanical Garden for their matching financial contributions, and the Friends of the Fakahatchee Strand for providing additional financial support of this conservation project. This project involved many agencies and partners including FDEP (Florida Department of Environmental Protection), FFWC (Florida Fish and Wildlife Conservation Commission) and Friends of the Fakahatchee Strand. The authors would also like to thank Chuck McCartney for the time and trouble taken in correcting the manuscript.

A full report of Danielle's internship can be found under Resources on the OSSSU web site.

***Brassavola cucullata* Project on St. Eustatius**



**Back row left to right: Raymond Tremblay, Lindsay Galway & Jim Ackerman
Front row: Hannah Madden**

The best National Park intern I ever had was the wonderful Miss Lindsay Galway, a Canadian national who spent six months working alongside me in 2008, and much to my delight chose to do a research project on orchids. Lindsay had just completed her bachelor's degree in ecological determinants of death (faculty of environment). Since leaving Statia she has gone on to complete a master's in Global Public Health and is now doing a PhD in the faculty of health sciences. Did I also mention she is very brainy? Not only did I have the pleasure of working with Lindsay, I also got to work for the first time with world-renowned orchid expert Dr. James Ackerman from the University of Puerto Rico (UPR) and his colleague Dr. Raymond Tremblay, who descended on Statia in June 2008.

Our first day of fieldwork took us to Boven, one of the more challenging trails on the island. The vegetation is thorny, and rocks hidden in the long grass threaten to trip up the unwary hiker. Add to that the blazing heat of the summer months and it becomes quite an adventure. However, we were on a mission and nothing was going to stop us. Hiking to the summit of Boven we passed a former alcohol distillery. The structure remains relatively intact and the stone oven at the rear is still visible. It is easy to imagine the sugar cane juice being heated by the oven, the alcohol being condensed by a copper worm submerged in the liquid. Nearby are the foundations of a plantation-owner's house. Sugar cane was grown in this area and many African slaves spent their lives here, often never leaving the island before they died. It is not unusual to find pieces of clay pipe, pottery, glass bottles and other artefacts. In fact, per square metre, you can find more artefacts on Statia than any other country in the world. The peak of Boven is characterised by gigantic copper coloured boulders which are the remains of numerous layers of volcanic deposits. The red colour is due to oxidisation of the ferro-magnesium mineral in the andesite rock. Upon reaching the



**Raymond Tremblay with
*Brassavola cucullata***

summit at 289 metres, we are rewarded with magnificent views of the island as well as our neighbours: St. Kitts & Nevis, Saba, St. Barths and St. Maarten.

Dripping with sweat, we wandered around the area until we located a large population of ***Brassavola cucullata*** dangling among the huge boulders. Many of these plants had generous numbers of leaves. According to Ackerman one leaf roughly equates to one year of age, therefore we deduced that the plants were healthy, of good age and often located high enough to be out of the reach of the dreaded roaming herbivore, namely goats. On the other side of the peak in a sheer cliff face that is home to many Red-billed Tropicbirds and other seabirds. It is also the preferred habitat of ***Psychilis correllii***. Having a slight fear of falling to my death, I prefer to stay at least a metre from the edge. However, the ever-fearless Lindsay risked her life to pluck a specimen for Jim. At this point, I liked her even more.

The following day we hiked the comparatively cool south western slope of the Quill, a dormant volcano, to look for more ***B. cucullata***. We were not

disappointed. The area is full of Mappoo (***Pisonia subcordata***) trees which the orchids seem to favour, and one does not have to wander far off the trail to find them. Some trees are literally dripping with orchids. Seeing these majestic flowers in their native environment is a sight to behold. Orchids have become the latest craze and not a day goes by without seeing one in a household pot or on TV, but nothing can compare to seeing them in the wild. We are lucky that there is at least one species of orchid in bloom throughout the year on Statia. The casual hiker will walk straight past and not even know it's there, but those that slow down and look around will be rewarded for their observation. Those that hike with a qualified guide will get to see even more. Although due to thicker vegetation the views are less spectacular than on Boven, there is a viewpoint at an area called White Wall. This is a great spot to sit and admire Battery de Windt far below, one of 18 forts and batteries that

were constructed on this tiny island during the slave-trading period. This may sound ridiculous for an area of land only 11 square miles, but back then Statia was home to 20,000 inhabitants and was so highly desired that it changed hands 22 times between the British, French and Dutch.

In the same area of the viewpoint, for those with trained eyes, it is possible to find **B. cucullata**, **Epidendrum ciliare** and the aptly-named Dancing Lady (**Tolumnia urophylla**). The brightly coloured yellow flowers resemble an old fashioned ball gown that gently 'dances' in the breeze. A little further down the trail towards the Botanical Garden we stopped to show Jim our 'orchid tree'. The entire trunk and limbs are literally covered in **E. ciliare** and, despite growing close to the edge of the cliff, it has survived many a hurricane. To see this plant in full bloom is a sight that any orchid lover can only dream of. Suffice to say, photos do not do it justice. Just before we reached the Botanical Garden, much to our surprise, we came across more **B. cucullata** in an area where there are no other orchids. It was a very pleasant end to day two.

Day three, our final day in the field, took us along the rim of the Quill to its highest point, Mazinga peak at 600m above sea level. This trail is full of lush vegetation such as Elephant Ears (**Philodendron giganteum**), Balsam trees (**Clusea major**) and the endangered Copey Vera (**Ternstroemia peduncularis**), which we nicknamed the Japanese Toothpaste Tree due to its similarity to a Japanese cherry blossom and slight aroma of toothpaste. Although **B. cucullata** does not grow here, other orchid species such as **E. ciliare**, **Epidendrum anceps**, **Epidendrum difforme** complex, **Polystachya concreta**, **Ornithidium coccineum** and **Prescottia oligantha** can be found. In fact, it was Jim who discovered **Prescottia oligantha** during his second trip to Statia in 2009. While at Mazinga Peak we were searching the area for **Ornithidium coccineum**, which had recently been discovered by another intern. Suddenly out of the undergrowth I heard the cry: "Prescottia oligantha!". I rushed to Jim's side and there poking out of the moss were a few small inflorescences with tiny pink flowers. Only an expert eye could have spotted them.

Three days was all it took to cement my love of orchids. Thanks to Lindsay, we were now on our way to establishing a long-term monitoring program so that I would have a viable excuse to visit at least two species twice a year. Now in our fourth year, I continue to carry out surveys with a passion and never get tired of seeing **B. cucullata** in bloom. These plants amaze me. How do they know to give off a scent at night to attract pollinators? How is it possible that any creature has a proboscis so long it can reach all the way down the nectar tube, just to be duped by the plant? I still have a lot to learn but thankfully I am (relatively) young and have the rest of my life to continue working in this field. Let's just hope I can still hike when I'm 70!

Hannah Madden

A report written by Lindsay Galway on the St. Eustatius Orchid Conservation Project can be found under Resources on the OSSSU web site

Size Matters

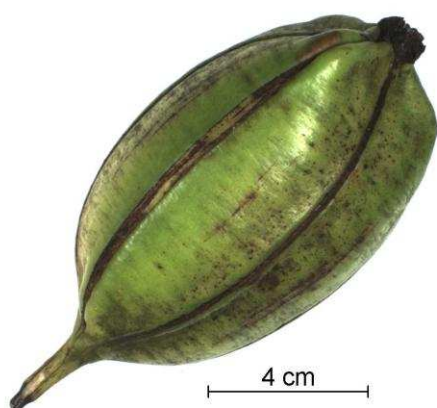
Tim Marks gasped in amazement as he opened the parcel in the dry room at the Kew's Millennium Seed Bank (MSB). He had no idea that orchid seed capsules could be so large. Nestling among 'normal' size capsules of *Aerides odorata* and *Cymbidium finlaysonianum*, substantial seed pods in their own right, were three enormous capsules of *Grammatophylum speciosum* measuring 10 by 6cm. Reputedly the world's largest orchid, *G. speciosum* may also hold the record for producing the orchid world's biggest seed capsules.

Sainiya Samala (Nee) and Suphat Rittirat (Phat), two of Kanchit Thammasiri's students from Mahidol University in Thailand, were visiting for three weeks of training and had brought their own seeds with them. The first question that crossed Tim's mind was, "How many seeds were there in each capsule?" rapidly followed by, "How should we count them?" This was Nee and Phat's first task. After scraping out all of the cream-coloured seeds from one of the capsules, they were weighed. A sub-sample was then taken and weighed in turn. Count the number of seeds in the sub-sample followed by some straightforward maths and you have the answer. Simple! How many seeds in the seed capsule? One million seeds.

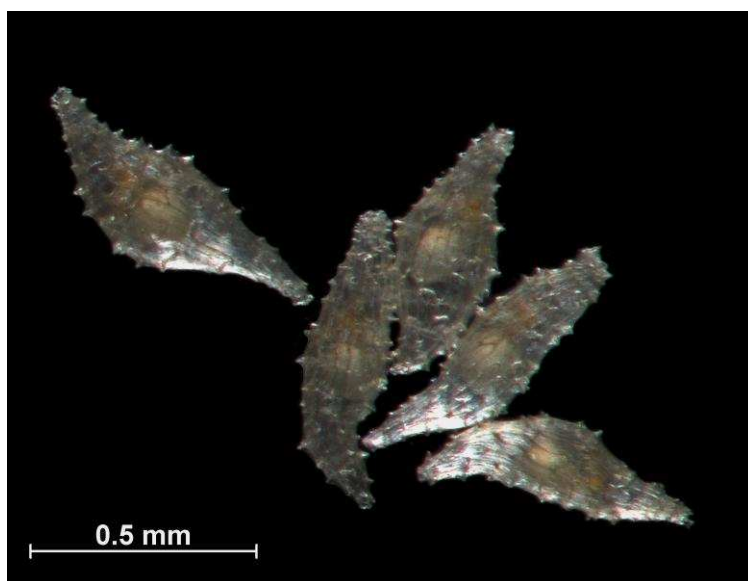
It was found that the second capsule contained almost double the number of seeds, an amazing two million seeds. The next question was, "How big is an individual seed?" This was a much easier task, simply involving using a binocular microscope, a digital camera and some clever software. An individual seed measured 0.2 x 0.07mm.

Next it was the turn of the *Aerides* capsules. This time the seeds were a dark red/brown colour and, although the capsules were much smaller, they still contained 200,000 seeds. On measuring the seeds it was found that they were much smaller than those of the *Grammatophylum*, measuring 0.06 by 0.03mm.

Such seed capsules provide generous quantities of seed for both long-term storage and experimental purposes. The tiny seed capsules of members of the subtribe Pleurothallidinae will contain far fewer seeds of course, and it would be interesting to know how many seeds might be expected in a seed capsule of a *Lepanthes* for example, and how big are the individual seeds. From a seed banking perspective such information would be invaluable. A seed collection of a million seeds sounds (and is) very impressive, and average (non-orchid) seed collection at the MSB is 10,000 to 20,000 seeds. However it could be much more difficult to achieve an adequate collection with a *Lepanthes*, and it would be worth knowing how many seeds you could expect in an average capsule for a range of orchid species producing tiny capsules.



Grammatophylum speciosum capsule and seeds (photos: Wolfgang Stuppy)



Abstracts

Nadarajan J., Wood S., Marks T.R., Seaton P.T. & Pritchard H.W. 2011. Nutritional requirements for in vitro seed germination of 12 terrestrial, lithophytic and epiphytic orchids. Journal of Tropical Forest Science 23(2): 204-212

Although numerous media have been developed to evaluate both orchid seed germination and further seedling development after storage, few studies have attempted to relate nutritional requirements to life history traits. For a range of terrestrial, lithophytic and epiphytic orchid species, comparisons were made of germination on Knudson C, with and without activated charcoal or banana powder, Norstog and Phytamax™ media to represent variations in available nitrogen. Germination varied, with maximum values ranging from just 9% for *Prosthechea cochleata*, *Platanthera* sp. and *Spathoglottis paulinae* to 95% for *Phragmipedium longifolium*. Along with *Paphiopedilum delenatii*, *Paphiopedilum philippinense* and the epiphyte *Guarianthe bowringiana*, *P. longifolium* germinated well on most media. Germination was significantly higher on Norstog than the other media for four of the six epiphytes tested. Germination was maximum on Knudson C medium with activated charcoal for four of the six terrestrial/lithophyte species. The results indicate a greater preference for nitrogen from amino acids rather than ammonium or nitrate salts in seeds of epiphytes compared with some terrestrial orchid species.

Hosomi, S.T., Santos, R.B., Custodio, C.C., Seaton, P.T., Marks, T.R. and Machado-Neto, N.B. 2011. Preconditioning Cattleya seeds to improve the efficacy of the tetrazolium test for viability. Seed Sci. & Technol., 39, 178-189

The tetrazolium test is one of the most reliable techniques used to estimate seed viability. Its utilization with orchid seeds has become important in the evaluation of seed batches for propagation and conservation. The aim of this work was to study the tetrazolium test to verify the efficiency of pre-conditioning, time of exposure and the concentration of tetrazolium solutions in the evaluation of seeds of tropical orchids. Seeds were pre-conditioned in a 10% sucrose solution at room temperature for 24 h followed by immersion in 0.1, 0.25, 0.5 and 1% tetrazolium solutions for 3, 6, 12 and 24 hours at 40°C in a water bath in the dark. The results obtained in the tetrazolium test were compared with germination on Knudson C medium. Evaluation of colour development was performed using a table scanner. Germination was tracked using digital photography. Pre-conditioning in 10% sucrose improved the seed staining, making the differences in seed viability easy to distinguish. Use of tetrazolium solution at concentrations greater than 0.25%, and periods of exposure greater than six hours are recommended when the objective is economy and fast execution, even if the seeds have not been conditioned in 10% sucrose.