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Flower to Seed to Plantlets to Speculations

As this issue is begun, we jump back several months to Sunday-14 December, 2014. The unregistered hybrid Pl#280606-15. Rlc. (Pennsylvania Spring × Aqui-Finn) Clone #1) was in flower (Fig.1). The registration forms were sent off to the United Kingdom, to the official registrar of hybrids in the Royal Horticultural Society which has charge of such registrations worldwide. The forms and fees were sent for two hybrids.

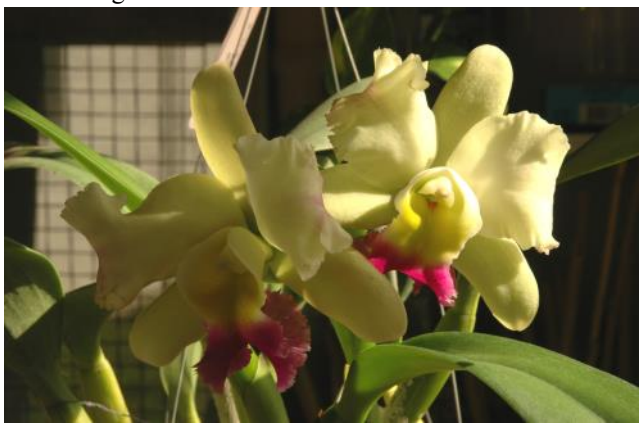

Fig. 1. Pl#280606-15. Rlc. Wilma Ferry, Clone #1 = (Pennsylvania Spring × Aqui-Finn).
DSC_6583 Sun-14Dec-14.

The official name, Rlc. Wilma Ferry, was registered on 08 January, 2015+ (RHS registration confirmation Ref. P. 24764), at the same time and on the same sheet of paper as another hybrid, Den. Nora Olsen (Fig. 2).

Fig. 2. Dendrobium Nora Olsen = (Den. Walter Oumae × Den. Somsak).
DSC_5504. Digital photo: Tues-27Aug-13.

As personal and local problems continued into the fall of 2015, additions to the library seemed to be out of the question for some time, and your editor had serious reservations regarding how long it might be before seed capsules would continue to be carried on a cross done in late January, 2015. The literature references tended to hover around 180 days for pollination-to-dehiscing, and by 01 Nov-15, 283 days had passed. The two seed capsules looked healthy and were large-sized, but the length of time and the continued energy drain on the plants began to give some concern to this worker. Consequently, it was decided to open one capsule and possibly both to see if something unusual might be going on within. Rather than simply sacrifice one or both capsules due to mere curiosity, the flasking hood was thoroughly sprayed with

alcohol and elaborate preparations were undertaken to see if this worker - after much literature searching and equipment sterilization - might sow the orchid seed (if any) successfully. Previous attempts had resulted in mold killing everything, and his last successful orchid seeding had been in the early-mid 1980's.

After much preparation and constant cross-checking with Wilma (who had never done this sort of thing), we harvested first capsule Sat-05Dec-15 (318 days after doing the pollination, and the second one (the backcross) Thur-10Dec-15.

Preparation of flasks

Prior to actually harvesting the capsules, a little pressure cooking needed to be done. A liter of distilled water was mixed with the powdered agar (34 grams of agar powder to 1 liter of water). This was poured into a stainless steel container which was heated sufficiently to dissolve the agar powder. What are currently used are square shaped pint bottles with screw-caps from the Grainger Company in McAllen and elsewhere. Heat to well below boiling; just enough to dissolve the agar thoroughly. This solution was poured into each of several pint bottles to a premeasured level.

Fig. 3. Pouring agar solution in bottles prior to pressure cooking
Photo: DSC_7473 Thur-03 Dec-15.

To arrive at the correct level of the agar solution in the bottle, one is stood upright and filled with just enough water so the bottle's neck is not touched when the bottle is laid horizontal on a level surface. Now, with the bottle standing upright, the water level is marked on the bottle, using a sharp, permanent felt pen. This is the level you will want when pouring in the uncooked agar-mixed solution (Figs. 3,4). Be certain your pressure cooker will hold bottles standing up, or nearly so, during cooking.

Fig. 4. Pouring agar solution in bottles prior to cooking, using a pre-measured upright bottle as a guide.
Photo: DSC_7474 Thur-03 Dec-15.

Fill bottles to the level of the one marked. Leave all bottles on the level surface and compare. They should all be filled very closely to the same level. Screw each cap on lightly (<u>not</u> tightly!), and cover each capped bottle with a square of foil big enough to overlap the capped bottle to 4-5 cm below the

cap (Figs. 5,6). With the bottle caps screwed on loosely, put on the aluminum foil and snug a rubber band around the foil below the cap. Fill the pressure cooker, put on the lid, and place the pressure weight on top of the pressure-release peg, and cook the bottles.

Thompson (1977) states that 5 minutes at 5 psi is sufficient, but the pressure

Fig. 5 rapped and rubber banded, flasks are ready to be laid down to harden
Photo: DSC_7483 Sun-11Aug-13.

Fig. 6. Cover the capped bottles and place upright in pressure cooker.
Photo: DSC_7476 Sun-11Aug-13.

release weight for your editor's pressure cooker lets the cooker's internal pressure go up to 15 pounds. Your editor gave the flasks 15 pounds pressure for 15 minutes. This was supposedly far too long at a much higher pressure, but flasks thus cooked have subsequently been stored for months with no fungi sprouting

within, and the agar plate within seems to have undergone no significant deterioration in quality as a growth medium. The figures in this article (3, 4, 5, 6) should illustrate this portion of the protocol reasonably well.

After cooking and releasing the cooker's internal pressure, stand the flasks upright on the towel's firm surface (use gloves: the bottles will be hot!) Tighten the bottle caps, but not overly tight. Screwing them on tightly may glue them on and cause great difficulty later getting them unscrewed! Form the foil around each flask and hold it in place with a rubber band (Fig. 5). Now gently lay each hot flask on its side, taking care to not allow the agar to run onto the interior of the cap. Allow the agar to cool and harden into a jelly mass.

At this point, the preparation for seed sowing has been completed, but no seed has been sown. If no seed is available, the sealed flasks may remain, lying flat, on the shelf for some time. Storage on a lighted shelf for a couple of weeks has the advantage of confirming that no mold spores have remained within the flask. Other sterilized implements should remain wrapped and in a safe, clean space, but still should be sprayed with alcohol when opened within the flasking case.

Fig. 7. Cross #280606-15 × 190206-12) Seed capsule opened and seeds in a pile.
Photo: DSC_7495 Sat-05Dec-15.

With all of the preceding preparations completed, the capsules are finally harvested and the pile of seeds may be visible as just so much white or cream colored talcum powder (Fig. 7). Four mother flasks (already discussed earlier in this issue) had already been sown from this particular pile, yet enough seed remains for 30 to forty more mother flasks! The extra seed has been wrapped and stored in a container, and kept dry, and free from fungal degradation. Seeds from the cross and the back-cross were wrapped separately and might remain viable for several years.

Elementary Culture

In nature, "survival of the fittest" is to have the natural capability to survive within whatever the natural environment throws at an organism. Simple short-term minds often misconstrue "fittest" for "fight-est". This was so in Darwin's day and it's true today, but the "fittest" is actually the adaptive capability of the organism naturally "fitted" (biologically, genetically equipped) to survive within whatever the natural, local conditions are for it and its seed. The old saying of "healthy mommies make healthy babies" applies to *all* organisms from orchid plants to humans, and it's a sad commentary on so-called "human intelligence" to see the blasphemy many present-day reproductive humans do to themselves as creators of members of a generation to come!

When it comes to producing orchid plants from seed, it is incumbent on the grower to provide the proper culture for an orchid plant to have the strength to bear a seed capsule, and that seed capsule may take months to mature. Over the years, this grower has often heard the complaint that "it flowered, and then it just died". Orchid plants will try their best to reproduce, both vegetatively and by seed, and with poor *plant* care, a plant may put all its resources into flowering and then just not have the strength left to survive as a plant; so powerful is the natural drive to reproduce one way or another!

The advice to the orchidist is obvious: if you intend to grow orchids from seed, begin by growing healthy plants! Don't even begin to do a pollination on a plant that's diseased or otherwise in poor condition! Start with a healthy plant and then don't wait so long after the plant has opened the flower that you risk deterioration of both the pollen and columnar parts of the plant.

Contamination

The most obvious enemy for orchid seeds in a sterile container is fungus! The particular species of fungus isn't important. It's enough to know that fungi grow a lot faster than orchid seed! Orchid seed may take a couple of weeks to start greening within a flask, but the agar plate can be completely covered over with fungus growth within twenty four hours, and within a few seconds of breaking open an orchid seed capsule the seeds will be contaminated by airborne fungus spores! The only solution is to either (a) keep the seed capsule in a sterile environment when it is cut open; or (b) sterilize both the dry seed and whatever fungus spores are with the dry seed with an agent that kills the fungus spores but does not kill the seeds.

There is a protocol for method (a), but the following remarks it will be assumed the orchidist is working with already exposed orchid seed and protocol (b) will be covered in detail in this article.

Keep in mind that cleanliness is important if the flask's internal environment is to remain absolutely clear of fungus!

Seed Sterilization

The sterilization Substance

Calcium hypochlorite (bleaching powder), is an effective seed sterilization agent. It's also a cheap and easy chemical to obtain. Buy the smallest possible package of what's called "shock" at your local swimming pool supply store.

Weigh out 10 grams and mix it thoroughly with 100 ml of water. It won't dissolve completely, but will form an off-white suspension. Add a drop or two of dish-washing detergent, shake well, and then let this mixture set a while. It will separate into a clear green liquid on top, with the bottom portion an opaque white liquid. Siphon off the top clear green liquid (the Brits call this Fairy Liquid. I know not why). This clear green portion is what you will want to use. Save the excess in a stoppered or screwed-tight bottle.

For the following, you will want a few test tubes and or small clean teacups or beakers available. Add a small quantity of seed (about a quarter to a half of a level teaspoon) to about 5 to 10 ml of the bleach solution and shake it well for about 3 minutes. If necessary (and if you have the arm strength!) you can keep this up for as long as 15 minutes, but three to five minutes is usually sufficient to kill fungi and bacteria, but not injure the plant cells. You now have seed that's sufficiently sterilized, but the bleach still remains.

Use a funnel and some fast-running filter paper and strain off the liquid. Turn the funnel over (the seed's in it) and pour distilled water through it. The distilled water should have had a drop or two of a wetting agent (surfactant) added; just enough to wet the seeds. Drain the seed and distilled water into a beaker that's been alcohol rinsed and allowed to dry inverted. Rinse the seeds again, using distilled water. Rinsing the seeds a third time with distilled water should yield seeds that are clean and in distilled water. Some may settle to the bottom, but many - being so light - may remain suspended in the water.

After giving the seeds a little time to settle, and the concentrated bottom portion may be collected for sowing, or the lighter portion (also with seeds) may be collected if it looks like the flask could use a little more water in the interior.

Fig. 8. The orchid seeds are in distilled water. The water has had 1 to 2 drops of soft soap as a surfactant. RJF is adjusting mixing tools
DSC_4588 Photo Credit: Wilma Ferry Mon – 14May-12.

Needles:

I have used 10cc glass Ideal brand syringes for several years. A #14 stainless steel needle turns and clips into the syringe. It has a needle 7.5 cm long which allows one to load the syringe and literally squirt orchid seed far back into an old square quart glass milk bottle which is what, years ago, I used for sowing orchid seed flasks. Along the way, there was marking tape put around the needle box. My practice has been to use, and then rinse syringe and needle separately, and then pressure-cook the wrapped, disassembled pair, and keep them sterile-wrapped until it's time to use again. If they've been stored for any great length of time, I re-sterilize the needle-syringe unit. When it comes to items like needles and syringes, they should be stored with great care lest these tools fall into hands and be treated as toys or weapons! For purposes of growing orchids from seed, knowing the size of these needles can prove useful. If one should try, for example, to use the tiny needles used for diabetic injections, the result is usually a jammed needle! Orchid seed is microscopic, but it can stick together and make small clumps of sufficient size to inspire the expression of some uncomplimentary verbal opinions about a clogged needle!

Sowing Orchid Seed:

At his point, you should have ready the number of prepared flasks in which you wish to sow orchid seed. They should still be wearing their aluminum foil covers. The flasks should be laid down, each with the internally held agar plate on the bottom. At this point the rubber band guards may have been cut off and each bottle's cap loosened, but not removed. Now you can proceed individually or collectively. Do not cough or sneeze or you may pollute any opened flask!

With flasks open, load your syringe and shoot into the flask about 3 to 5 ccs of the seed/water solution. Continue until all flasks are inoculated. Lay the needle aside, carefully tighten the lid on a flask, and proceed to do the same on the next one until all caps are tightened. Return to the first flask, secure its aluminum foil cover and fasten a new rubber band holding unit in place.

Carefully transport each flask to its place on the lighted shelf. As you lift and transport the flask try gently to spread seed-bearing liquid over the plate's surface, but -in any case - do not let the internal seed-bearing liquid slop onto or even touch the flask's tightened lid! With the flasks secure, clean all implements with alcohol and put them back in secure places. Especially do this with needles and syringes!

The flasks have been seed-inoculated. Now they're lying stationary on a shelf, and the grow-light above is on a timer to afford the flasks 16 hours of light and 8 hours of darkness. Now you wait! Your room temperature may be between 68 to higher; even up to the high 70's , The room temperature is not critical. What *is* critical is that the flasks' interiors don't get too cold or become over-heated into the 90°F. range.

You continue waiting! How long you will need to wait depends. If you've been sloppy anywhere along the line, you'll see a great crop of mold within the flask

within a couple of days. If that happens, you shrug and go back and try to figure out what you did that was wrong, and you take steps to correct your errors! Don't lose your temper and give up: this is just ordinary bona fide practical science that's been done by ordinary people for (by now) for seventy five years or so. You just have to do the procedure correctly!

If you've done everything correctly, you should see greening within the flask from a week to as long as 5– 6 weeks depending on the species (or hybrid) the local temperatures, and other factors. If you don't see mold forming, promptly, be patient: the chances are you'll see greening within the flask soon.

Fig. 14. Green protocorms visible within flask of Rlc (♀Wilma Ferry × ♂Sierra Blanca cross. DSC_7679 Mon-02Feb-16.

Now we review your editor's plant records:

On 08 January, 2015, two orchid hybrids were registered. One was registered as Den Nora Olsen and the other as Rlc. (Blc.) Wilma Ferry. Following are entries from this second hybrid.

"Thur-22Jan-15/01:00 hours: the cross and back-cross were made as follows:

♀ Pl#280606-15. (Clone#1). Rlc. Wilma Ferry × ♂ Pl#190206-12. C. Sierra Blanca 'Mount Whitney' AM/AOS; and ♀ Pl#190206-12. C. Sierra Blanca 'Mount Whitney AM/AOS × ♂ Pl#280606-15. (Clone#1) Rlc. Wilma Ferry.

Sun-14Feb-15: both plants photographed bearing seed capsules forming (DSC_6683: Rlc. Wilma Ferry on left). Allowing approximately 180 days for maturation, these seed capsules should be mature around 21Jul-15.

Sat-05Dec-15: capsule from Wilma Ferry as capsule-carrying plant: Capsule not dried & dehisced; excised from plant. One suture partly begun but not opened. The capsule showed much chaff, and seeds with nuclei were scant. Four mother flasks

were inoculated this date from a capsule of which Pl#280606-15. Wilma Ferry Clone #1 was listed as the mother plant (date of pollination 22Jan-15), and Pl#190206-12. C. Sierra Blanca 'Mount Whitney' AM/AOS was the pollen parent.

Thur-10Dec-15/10:15 hours: capsule from the Sierra Blanca plant: Seed capsules not dehisced, but severed from plant as with the Wilma Ferry capsule. Seeds with nuclei were profuse from the Sierra Blanca capsule. Three flasks were sown.

Three ♀Clone#1 Wilma Ferry × ♂Sierra Mount Whitney and three ♀Sierra × ♂Clone#1 Wilma Ferry flasks (six mother flasks in total) have been sown, sealed, and placed under lights. The seed remaining from each capsule has been collected, wrapped, and stored in a desiccant jar. A small beaker of additional seed from the ♀Sierra capsule also was placed (open) in an open small beaker which was placed (open) in the desiccant jar. Both are stored for possible future sowing as dry seed.

Outlook: From the amount of chaff observed in the Wilma Ferry capsule, it's possible the capsule had opened and the nuclei dried out and died. It's also possible the Wilma Ferry plant is pollen-fertile only. It is also possible the Wilma Ferry pollen only caused the Sierra plant to self-pollinate and produce seed of basically Sierra × self. I will have to wait and see what results!

Sat-16Jan-16: Pin-point sized protocorms observed in all three flasks of the back-cross! Two of the ♀Clone#1 Wilma Ferry cross had mold and the third had a large patch of mold that does not appear to be spreading in the back end of the flask. However, the front area of the flask is showing the same pinpoint-sized protocorms, so we have seed sprouting from both the cross and back-cross!

Time from pollination to capsule harvest: 322 days; time from sowing to greening: 37 days.

Mon-02 Feb-16: The fungal patch in the lone flask of the Wilma Ferry as the capsule carrying plant has not seemed to be enlarging, but it has grown to 4.2 cm diameter, at the far end (away from the flask entrance), and so a tool was fabricated out of sheet aluminum about 24 cm long by 2.0 cm wide (diameter of flask mouth: 3.9 cm) and following outside spraying of tools and flask exterior, I reached into the flask, cut the fungus about in half, and scooped up the first part and carried it out of the flask (hopefully without spilling any of it en route out. I did the same with the second part, sterilizing and rinsing the tool between trips. It *looks* like all of it was removed, but we wait and see what happens.

While the flask was open, a portion of agar and protocorms was transferred to a fresh flask in case the original flask had any mold reappear. Additionally, as the agar looked somewhat dry in both the original mother flask and the additional one, 6 ccs of distilled water were gently syringed into each flask. Both flasks were resealed and restored to positions under the timed lights.

The mold patch was examined under the worker's 10-power jeweler's magnifying headpiece and appeared to be a dormant patch of a Zygomycota fungus. The flask was resealed, but the damage had been done: within 24 hours, the flask with the mold patch and the two others with protocorms attempted to be taken were all covered with mold! What might have worked might have been to take the protocorms for transplanting out *before* attempting to remove the fungal growth from the back area of the flask. Past experience (from years ago) says it's better to leave such a contaminated flask along and if plants survive to actually transplanting size (usually well over 3-4cm height) they can be washed in a (mild) fungicide solution and will survive. However, the solution that usually works best is to start all over again with dry seed that's been saved, and re-do the cross!

Conclusions

We have followed this procedure in detail from pollination on Thur-22Jan-15 to greening of protocorms within the flask on Sat-16Jan-16; just a few days short of one year (359 days), At this point, the next step is to raise the plants until they are about 0.5cm high or simply appear to have become overcrowded within the flask, and then transfer them into a replate flask, again under sterile conditions. In replated flasks individual plantlets will be spaced about 1.5 to 2.0 cm apart (allowing for about 18 to 20 plantlets per replate flask). These plantlets will be grown in the replated flask until they are close to touching the top of the flask, until they appear to be crowding their quarters or the orchidist decides they are to be deflasked and cultured in the "outside" world. Once outside the flask, the results are seen when plants first flower.

How long might be the time period from pollination of the flower to seeing the first flowers from seedlings? Experience says that period could be between 3½ to 5 years, depending on the species. *Phalaenopsis* plantlets have been known to be "forced" into first flowers in as little as 2½ years, while seedlings from cattleyas have been known to take from 5 to 7 years to show their first flowers. Growing orchids from seed is not necessarily difficult, but it can provide some excellent lessons in patience!

Expectations

Every never-before-done orchid hybrid is in many respects a gamble when it comes to what will be produced when it comes to flower size, color, and other variables. Some general predictions can be made, within what is known about certain genetic traits being dominant or recessive, but more often than not, it boils down to a wait-and-see game.

Expectations and Hopes: Suppose you've come across an unnamed hybrid with promising-looking flowers; a hybrid probably done by reputable individuals who have passed on. It might be worth crossing that flower with one you know, and know its ancestors. You do the cross with expectations for something pretty, but in the back of your mind, it would be special blessing if it turned out to produce one of those one-in-a-thousand classically *beautiful* orchid flowers!

There's still another aspect to the process of growing orchid plants from seed. During the years from pollination to seeing the first flowers, there can be a certain joy in speculating what might come, but there is that unique feeling of knowing that what is being produced has never been done before. There's a happiness that the teamwork of two to several individuals: in being able to share something worthwhile with others, and in the happiness that goes with that long-teamwork!

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