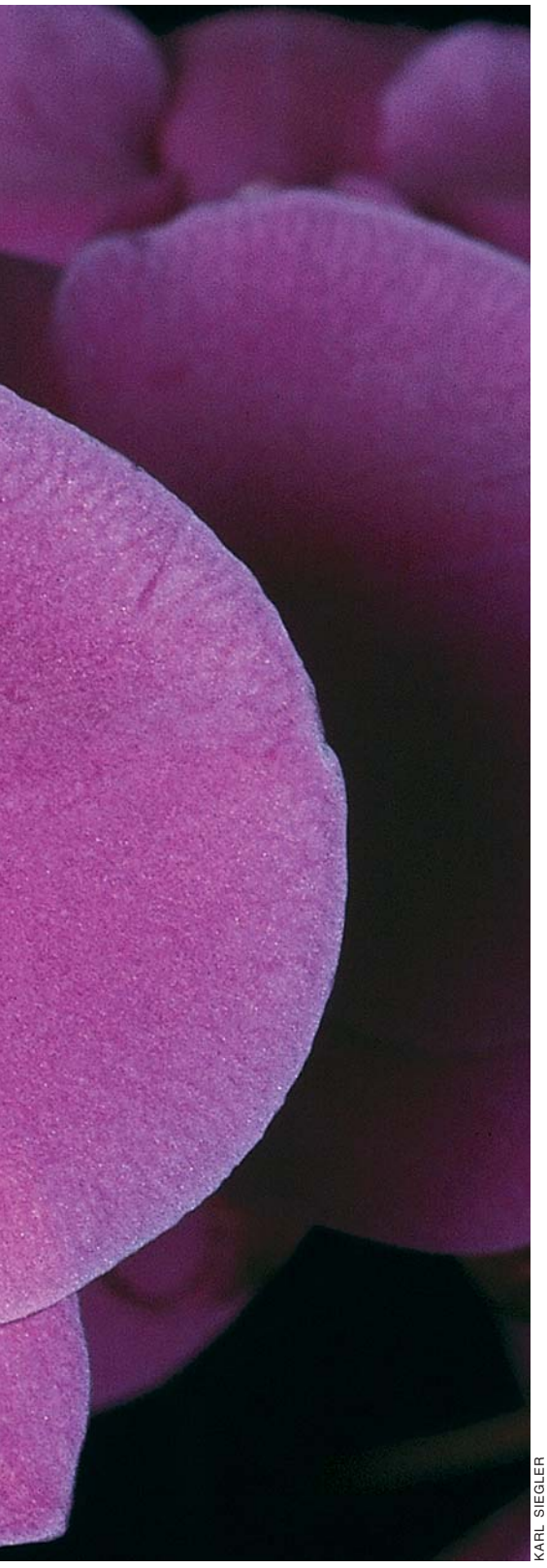
Sowing Orchid Seed

An Easy Approach to New Rewards

TEXT AND TECHNIQUE PHOTOGRAPHS BY FRED J. BERGMAN



KARL SIEGLER

LEFT The author made the cross *Phalaenopsis* Fred's Purple Gem (Abendrot × Polita Dupuy) that he grew from seed and took to AOS judging, where the clone 'Pat', won an AM/AOS. Grower: Fred Bergman.

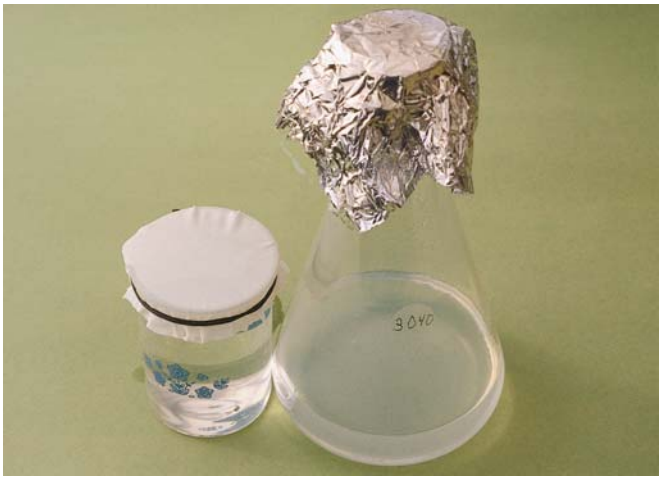
IF YOU ARE LOOKING FOR A NEW orchid-growing challenge, you can follow the usual path of cultivating new genera or you can try something different. Try making your own crosses and sowing the seed. I find blooming one of my own crosses provides tremendous satisfaction. But, could anything be more rewarding? For me, the answer is yes. When one of my crosses, *Phalaenopsis* Fred's Purple Gem 'Pat ' (Abendrot × Polita Dupuy) garnered an AM/AOS, I had an additional incentive for making crosses and sowing the seed.

A number of books and articles that describe making crosses are available, and much is written about seed sowing, too. Most procedures are designed for commercial seed sowing and tissue-culture laboratories, and as such they are overkill for an individual sowing only a few crosses. This article describes a simplified procedure that requires no experience, little special equipment and no special facilities. Give seed sowing a try. If the steps described here are followed carefully, you have an excellent chance of succeeding. Only a minimum of special equipment and few special supplies are required (see page 528).

GETTING SEED Obtain seed by making a cross. Articles that tell you how to make a cross are readily available. Seldom mentioned, but important, is the correct harvesting of seed. This simple seed-sowing method is designed for use with mature seed, which is harvested after the capsule turns yellow or splits (check for splitting daily when capsule starts turning yellow). When a seed capsule is ready, it should be removed from the plant, opened and placed on a piece of paper in a protected, warm, dry place. A day later the capsule will be dry enough to shake out the seed. Fold the collected seed in unglazed paper to form a closed packet and store the seed with a packet of drying agent. Suitable drying agent packets, usually silica, can be found packed with electronic equipment and some medication. Look for a small paper packet enclosed with the products. Heat the packet for one minute in a microwave oven and let it cool before placing it in a bottle with the seed. The bottle containing the seed and the drying agent should be tightly capped and stored in a refrigerator's vegetable crisper. I have sown seed after being stored this way for four years and obtained good germination.

WORK AREA The work area can be anywhere there is a smooth surface that will not be damaged by a bleach solution. I sow in the service building connecting my greenhouses. The space is used to pot plants, prepare potting mix and to carry out other operations associated growing plants. The air should be reasonably still. In the home, a kitchen counter is an option.

THE PROCESS Make a solution of calcium hypochlorite for disinfecting your seed. Do not substitute other disinfectants, especially if you are sowing phalaenopsis or other sensitive seed. Calcium hypochlorite is readily available at most hardware- or pool-supply stores. The label will state that the active ingredient is calcium hypochlorite and give the percent available chlorine (AC). Multiply the AC number by 0.114 to find the volume of pure water in fluid ounces (30 ml/oz) required when you use ½ level teaspoon of hypochlorite. Add the powder to the required amount of water and stir thoroughly. The solution will contain approximately 0.5 percent AC. Any concentration between 0.5 and 0.1 percent is usually effective but using the higher concentration gives a margin of safety. If you want to try lower concentrations, figure that each additional ounce of pure water will lower the hypochlorite concentration 0.06 percent. For example, if you add an extra ounce of water to the 0.5 percent solution you will have a



solution containing 0.44 percent AC. After you mix the water and calcium hypochlorite, let the solution stand undisturbed for about one hour. A precipitate will form and settle, after which the clear solution at the top should be poured into a screw-capped glass bottle. Tightly capped and stored in a refrigerator, the solution will remain usable for at least six months.

You will also need a saturated copper sulfate solution. The solution is stable so it can be prepared ahead of time. Copper sulfate crystals are available at most garden stores. A saturated solution is prepared by adding a few ounces of blue copper sulfate crystals to a small amount of water in a container until undissolved crystals remain in the solution.

Rubber stoppers can also be prepared ahead by tightly packing absorbent cotton in the stopper hole until the hole is filled to a minimum depth of ¼ inch (.6 cm).

CONTAINERS Fill a small, wide-mouthed glass jar (around 2 ounces or 60 ml) to within ¼ inch (6 mm) of the top with pure water. Cover the jar with a piece of thin rubber sheeting (obtained from a rubber glove) and tie the sheeting tightly over the top of the jar using string. The container of pure water, after sterilization, will be used to rinse and distribute the seed following the calcium hypochlorite treatment.

Pressure cookers come with racks to hold their contents above the water. A missing rack should be replaced, using a piece of heavy hardware cloth or rust-resistant perforated metal. The rack should elevate the cooker contents about 2 inches (5 cm) above the bottom of the vessel. Add water to the pressure cooker to a level just below the rack.

MEDIA Preparation and sterilization of medium should be done the day before sowing. Dehydrated media is dissolved using a double boiler. As a substitute for a double boiler use two containers, one fitting inside a larger one with space between. Add tap water to the larger container and 4 cups (.95 L) of pure water to the smaller container. Add an entire .25 gallon (1 L) package of dehydrated media to the smaller container while stirring. Using the entire package of dehydrated medium avoids the need for weighing. It's a waste of medium but you can throw out a lot of medium for the cost of a precision balance. Even if you are going to sow only one cross, consider instead sowing at least two flasks so you will have a backup in case one flask is contaminated. A 1 liter package of medium will produce enough solution to fill six 500 ml flasks about 1 inch (2.5 cm), the recommended depth. If you must use smaller flasks to fit in a smaller pressure cooker, also fill them to a depth of about 1 inch (2.5 cm).

Now turn the heat on under the double boiler and bring the water to a slow boil. Continue heating for 20 to 30 minutes and occasionally stir with a wooden spoon. At the end of 30 minutes the medium should be clear and ready for pouring into the flasks.

Using a funnel, distribute the medium equally in the flasks while it is still hot. Always use a funnel and exercise care not to spill any on the inside of the flask neck. If some is spilled, remove the spill from the neck using a damp cloth. Wiping up any spilled medium should be as complete as possible to prevent creating a path for organisms to grow past the rubber stopper. Remember, the seedlings will remain in the flask for as long as a year.

There are a number of prepared dehydrated media available. I have evaluated most of them using phalaenopsis seed and found one of the best to be Hill's mother flask medium. Good results were obtained using Knudson's C or Vacin & Went when sowing other tropical orchids. Your cross may be a *Paphiopedilum* or *Phragmipedium*, two genera that require a different germination medium. A simple medium that has produced good results for me was described by

Supplies

THE following common household supplies can be used to germinate seed:

pressure cooker (the larger the better)
absorbent cotton
1 or more Erlenmeyer flasks to fit in cooker
2 tightly woven cotton hand towels
one-hole rubber stopper to fit each flask
set of measuring spoons and cup
1 bottle of orchid medium
funnel
one 5–10 ml syringe with detachable needle
nesting pots or a double boiler
large-bore needle for syringe
distilled or deionized (pure) water
calcium hypochlorite
several small glass containers
small glass bottle with screw cap
heavy aluminum foil
copper sulfate
1 shallow large glass bowl or cake pan
disposable gloves
bottle of household bleach
eye dropper
wooden spoon

Rose and Lauer (1977). The medium consists of Knudson's C with 100 to 200 ml of the water replaced by coconut milk.

I do not use charcoal in media because it requires shaking the flasks to prevent settling while the agar gels. Shaking the flasks requires removing them from the pressure cooker. An early flask removal increases the risk of contaminating the outside of the flask and the subsequent sowing.

Cut 5-inch (12.5-cm) squares of aluminum foil, place one of the cotton-packed rubber stoppers loosely in the neck of each flask and cover the stoppers loosely with the aluminum foil. Both the stopper and aluminum foil should be placed loosely on the flask to allow steam to enter and exit the flask during sterilization. Place the flasks, while still hot, along with the rubber-covered pure water bottle in the pressure cooker. Close the pressure cooker and begin heating. If the pressure cooker has adjustable pressure settings, set the pressure for at least 15 psi (105 pascal). As soon as the pressure control valve starts emitting steam, start timing the sterilization. Cut back the heat until the cooker is just steaming and continue the sterilization procedure for 30 minutes. Turn off the heat but leave the pressure cooker closed. Do not open the pressure cooker until you are ready to sow seed. Keeping the pressure cooker closed keeps the outside of the flasks clean and free of organisms until you are ready to sow.

SOWING THE SEED After 24 hours, the medium will have set enough to sow your seed. Use a measuring cup and add 1cup (.25 L) of bleach and 9 cups (2.1 L) of tap water to a shallow glass or plastic pan. Place two cotton towels in the bleach solution. Remove the seed and the calcium hypochlorite solution from the refrigerator and bring them to the work area. Take the syringe needle and place a small plug of absorbent cotton in the bottom of the needle hub and attach the needle to the syringe fitting. Remove the plunger from the syringe and place a very small amount of seed (about the size of a pinhead) in the barrel of the syringe, reinsert the plunger and depress the plunger to the bottom of the syringe. Pour some calcium hypochlorite solution (about 20 ml) into a small container, add one drop of dish detergent and stir. Insert the syringe needle into the calcium hypochlorite-detergent solution, and draw 3 or 4 ml into the syringe. (The step within these parentheses is not mandatory but improves your chance of obtaining a contamination-free sowing. Insert the syringe needle into a spare rubber stopper to seal the needle end and pull out on the plunger as hard as you can. This creates a vacuum in the syringe that helps the hypochlorite solution penetrate the seed coat. Release the plunger, shake the syringe and repeat the process for a total of three times. Finally, remove the rubber stopper from the needle). The syringe containing the seed should be shaken occasionally for a total of 15 minutes. Between shakes, put on a pair of rubber gloves, wring out the towels and spread one on top of the other on a work surface. You will be working between the two towels.

Remove the rubber-covered pure water container from the pressure cooker and place it under the top towel. Put the lid back on the pressure cooker. At the end of the 15-minute shaking period, hold the syringe needle over a waste container and depress the plunger until the syringe is empty.

OPPOSITE LEFT Ready for sterilization, a rubber-covered water container next to a flask loosely covered with aluminum foil.
OPPOSITE RIGHT Sowing seed under a towel dampened with dilute bleach.
ABOVE LEFT A flask positioned for removing the mold without contaminating the plants.
TOP Seedlings in flasks ready to be removed and planted in seedling flats.
ABOVE The author's flasks in a controlled environment of 86–90 F (30–32 C) with a 16-hour day.

Submerge the syringe in the shallow pan of Clorox-water for two minutes and thoroughly wet the outside. Move the syringe to a position under the top towel. Insert the syringe needle through the rubber cover of the pure water container and draw pure sterile water into the syringe. You can look

under the edge of the towel to help in performing the following operations. Shake once and discharge the water into the waste container; repeat the rinse a second time. Next, fill the syringe with 1 to 2 ml of pure sterile water from the pure water container and place a flask taken from the pressure cooker under the top towel. Keep everything under the top towel as much as possible while you remove the aluminum foil from the flask. Remove the needle from the syringe and lay the needle aside. Give the syringe a good shake, quickly lift the stopper from the flask, and inject part or all of the syringe contents into the flask. Replace the stopper and remove the flask from under the towel covering. Finally, use the eye dropper to place only one drop of copper sulfate solution on the cotton plug in the stopper and a drop in the crack between the flask neck and the rubber stopper. Cover the rubber stopper and the neck of the flask tightly with the aluminum foil and label your flask. Now sit back; you are done flasking.

ONE MORE STEP After flasking, one more operation may be required. After sowing, examine your flasks daily. If there is contamination, it usually becomes visible in three to seven days. If you want to try to save a contaminated flask, it is essential to act while a flask contains only small growths on the surface. As soon as you spot any contamination, make a 1:9 bleach solution in the shallow container, as was done for seed sowing, and place the two towels in the solution. Take a long-handled iced-tea spoon (checked earlier to make sure it will fit through the neck of the flask) and place the spoon in the bleach solution with the towels. Wring out the two towels; place them on a work surface, as was done for seed sowing, and place the contaminated flask between the towels. Working between the towels, remove the aluminum foil and rubber stopper and place the stopper, bottom side down, on the lower towel. Hold the flask at a 45-degree angle and move the towel edge up until you can just see the growth on the surface of the media. Keep the towel over the neck of the flask and insert the iced-tea spoon into the flask under the towel. Scoop out the contamination keeping back from any growth at least ½ inch (1.2 cm). If you touch or come too close to a growth, or need to take more than one scooping, you should remove the spoon, dump its contents, and redip the spoon in the bleach solution. Generally, failure in using the spoon salvage technique is caused by trying to save too much of the sowing. Be wasteful and remove a large portion of the medium surface. Finally, replace the stopper, re-treat the space between the stopper and the flask neck with a drop of copper sulfate solution and replace the aluminum foil. If contamination redevelops, it is recommended that you discard the sowing. A second attempt at saving a flask is seldom successful. Of course, you can always try again for the practice and get lucky.

This procedure is designed to replace the more complicated procedures and is intended for sowing only a few crosses. It incorporates experience gained from sowing 75 to 100 crosses a year for an accumulated sowing of several thousand crosses. The procedure was used successfully to demonstrate seed sowing for the Orchid Society of Greater Kansas City and for a seed-sowing study group. When the procedure was shown at the Society meeting, a clear plastic sheet was substituted for the cotton towels to allow the membership to observe the process. You may wish to use clear plastic, but it is slightly more difficult to disinfect. This requires wiping the plastic down with a 1:9 bleach solution, much harder than simply dipping. If you plan to sow a number of crosses over an extended period, consider building a seed-sowing chamber. Plans for such enclosures can be found in many articles and books including Arditti (1982) and Hicks (2004).

For further information on seed sowing, I conditionally recommend three books, specifically those by Arditti (1982), Hicks (2004) and Thompson (1977). Again, the only seed disinfectant that should be considered is calcium hypochlorite. Follow the directions given here, not the frequently quoted 10 g/140 ml or 7 g/100 ml of water. The concentration usually specified in literature is neither saturated, as it has been described, nor at the concentration specified by Wilson that is usually cited (1915). The cause of errors found in most literature that describes the use of calcium hypochlorite was detailed in *Orchids*, Bergman (1996). Among other problems, calcium hypochlorite currently available

contains approximately 75 percent AC, and prepared at 7 g/100 ml produces a solution containing 5.25 percent AC. For comparison, the 1:9 dilution of bleach (sodium hypochlorite) normally recommended produces a solution containing 0.525 percent AC. The calcium hypochlorite prepared following standard directions is 10 times stronger than a 1:9 dilution of household bleach. Some genera, such as *Cattleya*, can tolerate this strength, but for sensitive seed like Phalaenopsis it is sudden death.

CONTAMINATION Over the years, a number of contamination sources have been located. One of the most significant sources has been identified as the failure of the hypochlorite solution to penetrate the seed coat. Incomplete penetration of the seed coat is lessened by vacuum treating the seed-hypochlorite mixture as described by Hicks (2004). To create a vacuum, I use a Hozon siphon injector to pull a vacuum on the containers holding the seed-hypochlorite mixture, releasing the vacuum, and shaking the mixture vigorously. The vacuum treatment is repeated three times. Seed sown this year using vacuum eliminated contamination in all but one of 72 flasks, and the contaminated flask was saved.

Using this simplified procedure avoids the need to reflask. If you prefer reflasking, consider obtaining a laminar airflow hood. Reflasking without one is possible but the contamination rate is generally very high. By following the simplified procedure, sowing thin and providing good growing conditions

after the removal from the flask you will not need to reflask.

I prefer seed from a capsule that has been left on the plant until the capsule splits. This avoids sowing a flask that does not germinate and it does not require reflasking. Because seed from a mature capsule can be stored, you have additional chances to re-sow the cross if there is contamination. In addition, if you bloom the cross and it proves to be exceptional you can make another sowing years later

Many articles suggest using containers other than flasks for sowing seed. I prefer flasks because they have a large planting area in relation to a small neck opening. Containers with large openings increase the risk of a contaminated sowing.

Some literature suggests that a microwave oven can be used to sterilize flasks. Before trying a microwave, it is suggested you read the sterilization section of any good microbiology book. You will find that you cannot sterilize using a microwave oven. Sterilization requires killing everything including spores and that requires temperatures above the boiling point of water. Because a container in a microwave oven is open, the maximum temperature reached will be that of boiling water. If you live at a higher elevation, a microwave oven may reach only 90 F (32 C) or less, well below the required 250 F (121 C) produced by steam at 15 psig. To be certain sterilization is complete, one needs saturated steam at 15 psig for enough time to permit heat to thoroughly penetrate the medium. You must use an autoclave or pressure cooker for complete sterilization.

GROWING CONDITIONS My flasks are kept in a temperature-controlled chamber at 86 to 90 F (30–32 C). Light is from cool-white fluorescent bulbs using a 16-hour day. After one year in the chamber, the seedlings are removed from the flask and planted into flats containing 4 inches (10 cm) of mix. The mix is prepared as described by Davidson (1965) using fine-grade bark. Optimum growth is obtained after removal from the flask by supplying increased heat and humidity. Maintaining high humidity is essential, but higher temperatures are optional. The higher temperatures serve only to speed up the rate of growth. The flats are suspended over water heated to 90 F (32 C) and covered with glass plates to create high humidity. Plants stay in the high temperature-humidity system for one year and are then moved to individual containers. Phalaenopsis seedlings occasionally bloom in the flats, and frequently one year after being moved to individual pots.

Any seed-sowing procedure can be modified to meet individual requirements. Study the procedure until you under-stand the underlying principal and then make the alteration. I find it helpful to visualize working in the rain, with every operation designed to prevent rainwater from entering the flask.

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OPPOSITE The phalaenopsis plants shown here, which were removed from flask four months earlier, are in flats in a heated chamber (glass top removed). ABOVE LEFT This photograph, taken July 12, 2004, shows plants flowering in 2-inch (5-cm) pots. Left: cross made May 21, 2002 and sown on July 25, 2003; Center: cross made June 22, 2002 and sown on May 18, 2003; Right: cross made June 21, 2002, and sown on March 24, 2003. ABOVE Red phalaenopsis sometimes grow more slowly. This first-bloom seedling was registered as *Phalaenopsis* Fred's Cardinal (Brother Purple Spotted × Brother Purple). The cross was made on March 19, 2001, sown on December 9, 2001 and photographed on July 16, 2004.

Fred Bergman has been growing orchids for 56 years, now retired after emplyment as a chemist for 40 years. He planted his first orchid seed in 1950, finally obtaining a contamination-free flask in 1954. 9401 East 103rd Street, Kansas City, Missouri 64134 (e-mail fjbergman@mfire.com).