A Storage Method for Pollen Using Freeze Drying

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An easy-to-use and relatively inexpensive pollen storage technique was developed using a freeze dryer and vacuum sealing system. Tree Planters' Notes 40(4):18-19; 1989.

The Erambert Seed Orchard, one of six orchards in the USDA Forest Service, Region 8 Tree Improvement Program, is located in southern Mississippi near the town of Brooklyn. On 330 acres, the orchard contains four geographic variants each of loblolly (*P. taeda* L.), longleaf (*P. palustris* Mill.), shortleaf (*P. echinata* Mill.), and slash (*P. elliottii* Engelm.) pines. Active breeding programs are being conducted with loblolly and slash pines.

It is usually best to use fresh pollen in controlled breeding work. On a practical basis, this is often impossible in complex breeding schemes. The change ability of weather and variation in time of clonal pollen ripening make pollen storage a necessity.

The pollen-processing facilities at the Erambert Seed Orchard have been improved with the purchase and construction of a mechanically refrigerated freeze dryer and vacuum sealing system. We had initially used a dry ice system for freeze drying that was difficult and unreliable. The improved system is simplified, more effective, and relatively inexpensive (about $1,500) and eliminates the problems involved with obtaining, storing, and working with dry ice and the difficulties of sealing bottles with a torch (fig. 1).

Constructing the System

We constructed the new system by converting our dry ice moisture trap to direct mechanical refrigeration, making use of the vacuum pump and the vacuum manifold already at hand. We now have a simple, effective system to freeze dry and vacuum seal pollen for freezer storage. The same principles allow for construction of this type of system with other brands of equipment.

We took our dry ice freezer (Virtis model 10-155), removed the insides of the dry ice trap, and centered the coil from the refrigeration unit (Unicool) in the trap. We covered the top of the trap (which thus becomes the vacuum chamber) with an acrylic top of 10-inch-thick Lucite. We cut about 1 inch from each side so the top would fit between the vacuum tubes on each side of the vacuum chamber.

The Virtis freezer has a stainless steel manifold for attachment of sample bottles. We increased our capacity by adding an additional manifold of heavyduty PVC pipe, 3 inches in diameter, which we drilled for

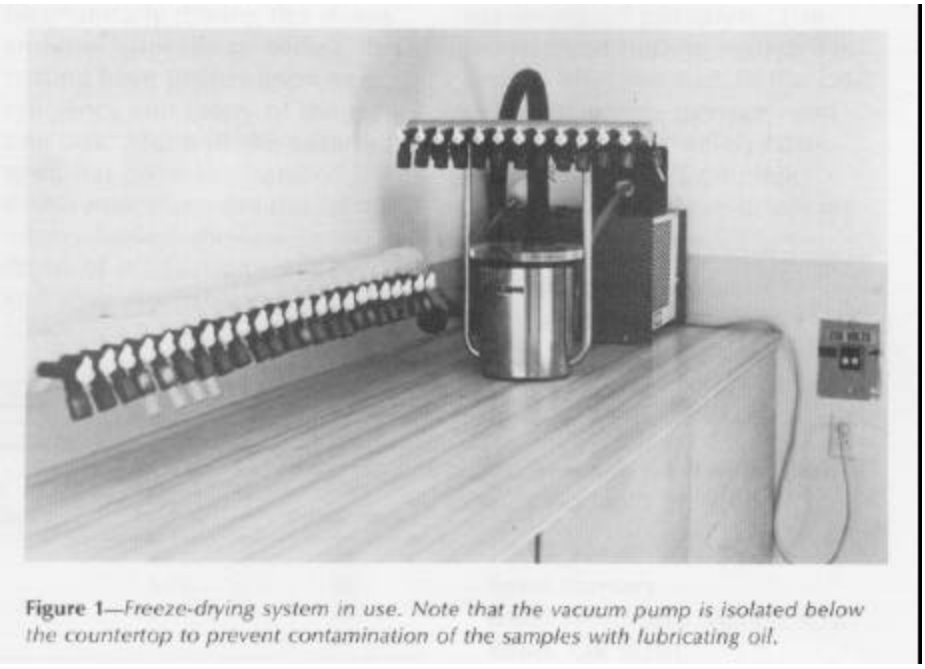


Figure 1—*Freeze-drying system in use. Note that the vacuum pump is isolated below the countertop to prevent contamination of the samples with lubricating oil.*

additional valves. An additional manifold can be as long as necessary if the vacuum system has the capacity to pull the required vacuum (fig. 2).

If a dry ice freezer is not being converted, heavy-duty PVC pipe, 10 to 12 inches long, can be used to construct the manifold for attachment of sample bottles.

Using the Freeze-Drying System

1. Collect catkins, before they start to shed pollen, in brown, kraft bags (lunch bags work well).
2. Place bags in the drying room on a clothesline until the pollen has completely air dried. (A forced-air drying system works much better than just hanging the bags on a clothesline to dry.)
3. Sieve the pollen into 10-ml bottles.
4. Center the top (acrylic) plate on the vacuum chamber so that ice build-up is evenly distant from the inner wall of the chamber.
5. Turn on refrigeration unit and allow it to run for 15 to 20 minutes to maximum low (-50 °C). Turn on vacuum pump and apply vacuum to drying chamber (can be done before or after refrigeration unit is turned on). Only attach sample bottles to drying chamber after refrigeration is turned on.
6. Connect sample bottles to the vacuum manifold by first starting the vacuum pump with all the valves off. Then, connect the first bottle when the vacuum pressure has reached 100 mm or less. Keep watch to ensure that the vacuum is maintained at this pressure or less.
7. After drying the pollen for about 2 hours, screw the bottle in the adapter to seat the split stopper under vacuum. Turn the quick-seal valve off; unscrew the bottle and place a screw cap on it (the screw cap comes with the 10-ml bottle if ordered by part number 178962).

Obtaining Parts for the Freeze Drying System

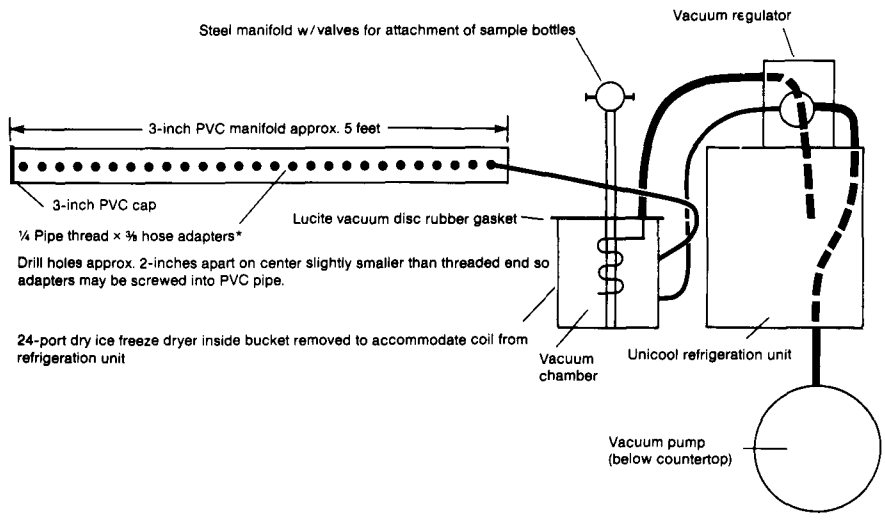
All items can be obtained from

Virtis Co., Inc.
Route 208
Gardiner, NY 12525
Tel: (914) 255-5000
(800) 431-8232

Unicool refrigeration unit (#135756)
Quick-seal valve, 1/2 inch (#171884)
Adapter, 1/2 inch vented (# 177964)
Lucite vacuum disk, 10 inch (#153916)
Bottles, 10-ml, with screw caps (#178962)
Split stopper (#171785)

The 1/4- by 3/8-inch hose adapter can be purchased locally. The vacuum pump used at Erambert Seed Orchard is a Vac Torr 25 (#10282) obtained from

GCA/Precision Scientific 3737
West Cortland Street
Chicago, IL 60647 Tel: (312) 227-2660
(TWX) 910-3122



*Hose adapter may need to be shimmed to accommodate the ½-inch quick-seal valve

Figure 2—*Diagram of freeze-drying system. Drawing is not to scale.*