PRESERVATION METHOD—POLLEN

NLGRP POLLEN CARYA CRYOPRESERVATION 1

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File Name
NLGRP_POLLEN_CARYA_CRYOPRESERVATION_1

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Abstract
Catkins flowers are harvested when they have plump, yellow-green anthers, some of which are splitting but not yet dehiscing. Catkins are spread in a single layer on paper in a still, dry room. Turn the air conditioner thermostat down to 15°C overnight and set the dehumidifier to its lowest setting. In the morning, raise the temperature to about 21°C. Gently lift and shake the dried catkins to release the remainder of the pollen and put through a sieve before shipping to NLGRP overnight. Once at NLGRP, begin the moisture equilibration process using a vacuum desiccator containing a calcium nitrate saturated salt solution. Let pollen sit in desiccator at room temperature overnight. Package pollen into 2mL vials and plunge into vapor phase of LN.

Study Reason
Preservation

Introduction
This is the standard method of cryopreserving *Carya* pollen germplasm at NLGRP.

Source of Plant Material
In mid-spring, pollen is collected from the catkin flowers of field grown *Carya* trees at sites of the National Collection of Genetic Resources for Pecans and Hickories in College Station and Brownwood, Texas.

Plant Material Description
Carya catkin flowers are collected and dried in College Station, Texas or Brownwood, Texas, in mid-spring. Catkins at the ideal stage of development for collection are 7.6 to 15.2 cm long, with plump, yellow-green anthers, some of which are splitting. On an individual tree, catkins on low limbs near the interior of the tree usually shed first. Catkins at the top of the tree are the last to shed. Once catkins are mature, pollen dehiscence may occur within a short period, especially if conditions are dry and windy. Pollen shed is delayed by rainy conditions or high relative humidity (>85%). Routine monitoring of target trees is therefore very important for successful collection. Pollen shed often begins in mid-morning after relative humidity decreases, so the best time to monitor and collect catkins is in the early morning before the pollen shed begins. When lightly tapped, mature catkins will release a cloud of yellow pollen. At this stage (or slightly before), catkins should be carefully pulled

from the tree and placed in a large paper bag labeled with the cultivar, location, and collection date.

Plant Material Cleanliness
The material should be free of insects or other contaminants. Upon opening a new shipment, material is carefully inspected for insects. Otherwise, the material is not tested for pathogens.

Plant Material Preparation/Pretreatment
Catkins are spread in a single layer on unprinted newsprint paper in a still, dry room. Turn the air conditioner thermostat down to 15°C (60°F) overnight and set the dehumidifier to its lowest setting to run continuously. In the morning, raise the temperature to about 21°C (70°F). The catkins will not wilt under these cool, dry conditions, and pollen should be shed within 24 h of collection. Gently lift and shake the dried catkins to release the remainder of the pollen, then discard them. Pollen can be separated from the plant debris by sieving through 100 mesh screens (#100 =150 µm) onto a single layer of newsprint. The dry pollen will pour like fine flour. It should be placed into a screw-cap 50 mL conical centrifuge tube. 30 mL of pollen per accession is the desired amount to maximize the amount of pollen in long-term storage. Properly label the tube with the cultivar, location, and collection date, and place it in a refrigerator until shipped overnight to NLGRP in a Styrofoam cooler with an ice pack. The pollen arrives at NLGRP ready to begin the moisture equilibration process immediately. It is important to process the pollen very quickly.

Moisture Equilibration
At NLGRP, the pollen moisture content (MC) must be reduced to approximately 6-10% MC on a fresh weight basis (FWB). To begin this moisture equilibration process, have a room temperature (~25°C) Pyrex vacuum desiccator (250 mm diameter) prepared with a saturated salt solution of calcium nitrate, which has critical relative humidity of approximately 47%. The saturated salt solution is made by adding calcium nitrate salt to a large Petri dish (140 x 20 mm), with enough water to make a salt slurry; fill the dish but leave a few millimeters of space at the top to make handling easier. Place the uncovered Petri dish containing the saturated salt solution in the bottom of the desiccation chamber. Check daily to ensure that undissolved salts are present in the slurry. Pour pollen into an appropriately sized Petri dish, making sure the pollen is evenly distributed with a depth of no more than a few millimeters. Place uncovered Petri dishes containing pollen into the prepared desiccator and ensure the lid of the desiccator is sealed shut. Let sit at room temperature overnight.

To determine moisture contents, a tiny amount of pollen (~15 mg at most) must be sacrificed for taking weights. The following process will be done twice, the first time with fresh pollen as soon as it arrives at the lab, the second time it will be done with the pollen after it has been moisture equilibrated overnight.

For each accession, three weigh boats are prepared and weighed for tare weights. Weigh boats are made using a small square of aluminum foil and an unsharpened pencil. Wrap the foil around the base of the unsharpened pencil and then slide the foil off to create what looks like a tiny bucket. Weigh the empty weigh boat to get the tare weight. Place a small amount of fresh pollen into the weigh boat, then gently close the weigh boat using forceps and weigh it again for the tare+FW (fresh weight) amount. The fresh weight (FW) is the tare weight subtracted from the tare+FW amount. These samples are then placed in a 90°C oven for 2 days. After 2 days, the oven-dried pollen in the weigh boats is weighed again for the tare+DW (dry weight) amount. The dry weight (DW) is the tare weight subtracted from the tare+DW amount. The following equation is used to calculate the moisture content on a fresh weight basis: MC=((FW-DW)/FW), and on a dry weight basis: MC=((FW-DW)/DW).

Moisture content is determined for the pollen before and after moisture adjustment. Only the moisture content after equilibration is recorded in the eCryocard and GRIN-Global.

Packaging
Package the moisture equilibrated pollen into 2 mL cryovials containing approximately 2 mL pollen each. The goal is to fill 15 vials per accession, placing 5 vials on a cane, achieving 3 canes in total. Canes are inserted into labeled plastic sleeves.

Cryopreservation Method
Remove one Petri dish of pollen from the desiccation chamber at a time and quickly package pollen into 2 mL cryovials. Plunge vials into the vapor phase of liquid nitrogen for a very fast cooling rate.

Storage Conditions
The loaded canes in sleeves are transferred to cryoboxes and stored in the vapor phase of liquid nitrogen. Viability of cryopreserved pollen is assessed and samples with greater than 5% viable pollen are placed into cryotanks designated for long-term storage.

Comments
N/A

References
Connor KF, Towill LE. 1993. Pollen-handling protocol and hydration/dehydration characteristics of pollen for application to long-term storage. Euphytica 68:77–84

Appendices
N/A