



Inoculating Chestnuts with the Blight Fungus

What is inoculation?

Inoculation is the process whereby fungal mycelium of the chestnut blight is introduced into the bark of a chestnut tree in order to test resistance of the tree to the fungus.

Why Inoculate?

- Inoculation is the first step in determining the resistance of chestnut trees to blight
- B3-F1 trees range in resistance from highly susceptible to moderately resistant. Inoculate B3-F1 orchards in order to determine which trees are moderately resistant and should be used to produce B3-F2 nuts.



Before Inoculating:

- Tree must be in fifth year of growth or later
- Diameter of tree at the highest inoculation point must be at least 1 1/2 inch, though larger is preferred
- Remove tree shelters to allow trunks to harden off
- Measure and mark trees to be inoculated in advance
- Inoculate in early June, the best growing time for the fungus



Cork borer

Supplies:

- Inoculum: SG 2-3 and EP155. Order from Fred Hebard
- 1 quart 190-proof grain alcohol (Everclear)
- 1 pint 70% ethanol alcohol (3 parts 190-proof, 1 part water)
- Test tubes for alcohol
- Small bottles to carry alcohol
- 10 rolls 1/2 inch masking tape
- Several caddies to carry supplies
- 6 rags per team
- 6 cigarette lighters per team
- 2 #1 cork borers, about 1/8in preferably, or 3/16th in, per team
- Cork borer sharpener. Make sure it will sharpen your particular cork borers.
- 3 spatulas per team – 8” long with 1” long sharp end and 1 “ long blunt end



Cork borer sharpener

Caddy:

- Label caddy with inoculum that is being used
- Tape test tube securely to handle of caddy
- 2 cigarette lighters
- Spatula
- 2 petri dishes of either SG1 or Ep155 inoculum, well labeled.
- Sharpie marker
- 70% ethanol
- Rag



Organization:

- Organize into groups of 4 – 6 people: One **plugger**, one to two **inoculators**, one to two **tapers**, and one **recorder**.
- **Plugger**: sanitizes cork borer by flaming with 190 proof alcohol. Prepares rag with 70% ethanol to sanitize area of trunk to be inoculated. Uses cork borer to cut holes in bark. Take caution with fire. Don't put cork borer/spatula too far in alcohol. Flame is blue and hard to see.
- **Inoculator**: Uses sterilized cork borers to cut disks of agar and mycelium of the fungus. Make sure not to cut plugs in agar without fungus. It is important to cut inoculum only from the margin of a culture that **HAS NOT REACHED** the edge of the plate. Avoid areas with contamination.
- **Tapers** use ½ inch masking tape to tape over inoculation holes
- **Recorders** record which trees have been inoculated with which inoculum, how many holes made, and any errors made in the process. Need list of trees in orchard. Make sure to record date, orchard name, and list of team members.

Procedure:

1. **Plugger** selects appropriate trees (dbh above 1 1/8") and four inoculation sites on trunk. Avoid crotches, cankers, and the green stripes that run up and down the stem. Holes should be 12" – 18" apart. Wipe hole sites with ethanol rag.
2. **Plugger** pushes cork borer into bark at inoculation site. Push and twist one half turn until you feel hardwood. Plunge out the bark plugs after each tree with the plunger. You can make several holes between flaming if you are careful not to let the cork borer touch anything. Be sure to sterilize the cork borer plunger between uses as well.
3. **Plugger** makes sure that inoculators know where holes are and which holes to fill.



4. Inoculator uses SG-1 (less virulent strain) in two upper holes. Locate all four holes. Flame both ends of spatula and take care not to touch them to anything. Slip one end of spatula into the pre-cut agar plug and gently lift out of petri dish. Place the inoculum plug into the top hole, mycelium in toward the cambium of the tree. Smear inoculum into hole by squishing with spatula. Wipe spatula off on pants or rag and use the other end of the spatula to pick up the second plug and place in hole directly below the upper hole. If you put the plug in the wrong hole, use a permanent marker to mark the hole with the inoculum name. The recorder should take note with explicit detail of error. Flame both end of the spatula after dipping in the alcohol and move on the next tree. Keep petri dishes shaded so mycelium won't heat up and die.



- 5.** The second **inoculator** follows the same process as the first, just using the more virulent strain, EP-155, and putting the fungal plugs into the lower two holes.
- 6. Tapers** make sure the proper hole was inoculated with the proper mycelium. Immediately after **inoculators** fill holes, **tapers** cover them with a piece of masking tape about 3-4" long. One **taper** follows the same **inoculator** all the way through to ensure proper procedure has been followed. The taper is the critical quality-control person. Inoculum in un-taped holes generally does not infect the tree, and, of course, if a hole is not filled with inoculum, no infection occurs.



7. Recorders record information about inoculation. Needs list of trees in orchard.

Records:

- Orchard name
- Date
- List of team members
- Which trees were inoculated
- Number of holes inoculated,
- Any errors made

Summary:

- Organize into groups of 4 – 6: one **plugger**, one to two **inoculators**, one to two **tapers**, and one **recorder**.
- Prepare two caddies per group: one for each **inoculator**. Label each caddy with inoculum. Each caddy should include: test tube taped to handle to hold ethanol, 2 cigarette lighters, spatula, extra cork borer, petri dish with inoculum, sharpie, and a rag. **Tapers** should have two rolls of ½” tape and **plugger** should have bottle of 70% ethanol rag, and test tube full of 190 proof alcohol.
- **Plugger** picks qualifying trees and selects four inoculation sites, at least 12” apart from each other, on the stem. **Plugger** wipes the areas with 70% ethanol, dips cork borer in 190 proof alcohol and flames, and cuts the four inoculation holes in the tree. Alcohol and flame cork borer between each tree.
- **1st inoculator** dips spatula in 190 proof alcohol and flames, then inserts SG-1 plug into two top holes, mycelium facing the cambium, wiping spatula each time and using sanitized side of spatula each time.
- **2nd inoculator** follows same procedure for 1st inoculator, except puts EP-155 into bottom two holes.
- **Taper** tapes over each hole.
- **Recorder** records all information for each tree, including any mistakes.

Suppliers:

- Fisher Scientific: www.fishersci.com, 1-800-776-7000
 - Cork borer: set of 3 ranging from 4.8 – 7.8 mm: \$13.55
 - Cork borer sharpener: \$36.40
 - Semimicro spatula with one tapered end, one rounded end: \$10.31
- Presque Isle Wine Cellars, North East, PA 800-488-7493
 - Cork borer 3/16 inch, \$7.25
 - Cork borer sharpener
- Ward’s Natural Science: www.wardsci.com, 1-800-962-2660
 - Cork borer 4.8mm or 7.8mm: \$5.10
- Lake Charles Manufacturing, www.testtubesonline.com, 1-866-739-4600
 - 50 16x150mm glass test tubes: \$7.04
 - 50 16mm test tube caps: \$3.40
- Superior Scientific, <http://lab-suppliesonline.com>, 1-216-701-8806
 - Cork borer sharpener: \$18.27