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Protocol for Hypovirulence Treatment Of American Chestnut Trees

Scope

This protocol covers the procedures volunteers can use to treat blight on surviving American chestnut trees. It has been prepared by H. F. (Burnie) Burnworth based on information and materials provided by Dr. Donald L. Nuss of the University of Maryland Biotechnology Institute and Mark Double in the Division of Plant and Soil Sciences of West Virginia University.

There are no hazardous materials involved in the hypovirulence treatment described herein.

Background

The American chestnut was once the dominant tree in Appalachian forests. Early in the last century, a bark fungus disease, *Cryphonectria parasitica*, was inadvertently imported into this country, and it eventually killed four billion American chestnut trees.

After several unsuccessful attempts on the part of the federal government and others, to restore the trees, The American Chestnut Foundation (TACF) was formed in 1983 to implement a backcross breeding strategy for developing blight-resistant American chestnuts. (For more details see the TACF Web site: www.acf.org.)

Some American chestnuts, although heavily attacked by the blight, are able to grow and produce nuts. Hypovirulence treatment is a method for prolonging the productive life of these trees and in future, it may even enhance the blight resistance of trees produced by the TACF breeding program.

Chestnut blight produces cankers -- areas of diseased bark, which can completely girdle a tree and cause its death. Control of the blight is

complicated by the fact that there are 64 different vegetative compatibility strains of blight fungus. For hypovirulence to work, a virus must be introduced into one of the types of blight fungus identified in a given canker. This virus infects and weakens the blight fungus on the tree, hopefully enabling the tree's natural defenses to heal the bark and callus over the entire canker.

To introduce a hypovirulent fungal strain into a given blight canker, it is usually necessary that the two fungal strains – with and without the virus – be vegetatively compatible.

The active conversion requires strands of the two fungi to not only grow together but actually to fuse, thus creating an opening for cytoplasmic exchange. This takes place more readily in the bark than in a Petri dish.

The components of the hypovirulent "soup" to be used as inoculum below were prepared by Dr. Nuss from blight samples taken from Barbara Knapp's champion tree (5 vegetative compatibility strains), and by Mark Double from blight samples taken from a survivor in Green Ridge State Forest (2 vegetative compatibility strains). They have been grown individually and then mixed together.

Equipment

- 2 tall stepladders
- 2 cordless drills with ¼ " spade-type (Irwin) bits
- 2 500 ml laboratory wash bottles containing the hypovirulent "soup"
- 2 rolls of 1" masking tape
- 2 yellow tree-marking crayons
- 2 rolls of yellow flagging tape and polka-dot tape
- 2 digital cameras & identification signs

Procedure

1. An advance team identifies cankers for treatment
    a. Mark tree for treatment with polka-dot flagging tape
    b. Select well-defined cankers on strong sprouts
    c. Ignore large cankers threatening to girdle the tree

d. Ensure accessibility for treatment – clear base of tree as needed
e. Mark candidate cankers with yellow tree-marking crayon

2. Two treatment teams apply the hypovirulent “soup”
    a. Find a candidate canker to treat
    b. Photograph it with identification sign
    c. Using a cordless drill, bore ¼” holes, ¼” deep, at the interface of infected and uninfected bark; (The area of infection should be evident – infected bark is brown and uninfected bark is white/green.) Space the holes at intervals of 2” to 3” around the circumference of the canker.
    d. Insert the tip of the wash bottle into each hole and fill with the hypovirulent “soup”
    e. Cover each hole with a square piece of masking tape
    f. Mark the tree with yellow flagging tape when all candidate cankers on it have been treated