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THE AMERICAN CHESTNUT FOUNDATION®

TACF Fact Sheet

Transgenic American Chestnut:
'Darling 58'

Researchers at the State University of New York College of Environmental Science and Forestry (ESF) have developed 'Darling 58' American chestnut (*Castanea dentata*) trees with enhanced blight tolerance. ESF is a valued TACF partner in our work to use breeding, biotechnology, and biocontrol to rescue and restore the American chestnut. These trees are not meant to replace the surviving remnant American chestnut population, but to help rescue it by allowing introduction of the blight tolerance trait to produce a viable and diverse restoration population. These may be the first bioengineered organisms with the goal of ecological restoration, and they represent a unique application for this technology to be used for conservation and cultural benefits.

Background

The American chestnut was an ecologically, economically, and culturally important tree species within its range in the eastern United States. In the late 1800s, the introduction of the chestnut blight fungus (*Cryphonectria parasitica*) from Asia decimated more than three billion American chestnuts. Tolerance to this pathogen in Darling 58 American chestnuts was enhanced by adding a gene for oxalate oxidase (OxO), which breaks down a toxin produced by the blight fungus.

Cankers after intentional inoculation with the blight fungus: Wild-type stem at left shows large, sunken, girdling canker. Darling 58 stem at right shows smaller swollen canker, indicating living tissue is responding and the stem will survive.

What is Oxalate Oxidase (OxO)?

Oxalate oxidase is a common enzyme that has been studied for over 100 years and is found in grains, many crops and food products, wild plants and microbes. There are even functionally similar genes in Chinese chestnuts, which may partially contribute to the blight tolerance observed in these trees. Darling 58 chestnuts with OxO can still get blight cankers, but they are typically superficial and don't kill the tree (see figure). Although it is from wheat, OxO is not related to gluten and does not match any known allergens.

Are Darling 58 Chestnut Trees Safe?

Over decades of study, several experiments have been performed on OxO-expressing American chestnuts and results consistently confirm a lack of novel risks or unintentional consequences. To date, these experiments on insects, aquatic life, other plants, soil microbes, and nut nutrition show that trees with OxO are at least as safe as their non-transgenic relatives. Many of these results have been described in a series of articles in the *Chestnut* journal and can be found on our website's biotechnology page: https://www.acf.org/science-strategies/biotechnology/

Examples of ecological interactions that have been studied with transgenic chestnuts: a bee visiting a catkin (left), and a small seedling germinating through a decomposing chestnut leaf (right).

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Can I Plant Darling 58 American Chestnut?
ESF has submitted a petition to the United States Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS) to request that Darling 58 be granted "nonregulated" status. Nonregulated status, along with approval from two other regulatory agencies in the U.S., would mean that Darling 58s and their offspring can be distributed and planted like wild-type or traditionally bred chestnut trees. They would be made available for not-for-profit distribution to the public and to groups taking on restoration programs. (Currently, all transgenic chestnuts are planted only under USDA-APHIS permits.)

Who Started and Funded This Research?
The entire transgenic chestnut research program was initiated by public chestnut enthusiasts who became founding members of the New York Chapter of TACF. The vast majority of ESF's research funding has come from public, government, philanthropic, and other non-corporate sources.

What Would Restoration Look Like Using Transgenic Chestnut?
TACF and ESF are working on crossing Darling 58 with a diverse set of surviving American chestnuts over multiple generations, to create a resilient population suitable for potential large-scale restoration efforts. This is part of a broader restoration effort including complementary approaches such as backcross breeding and biocontrol treatments, as well as managing other threats like Phytophthora root rot.

ESF and TACF are continually working to optimize existing procedures and explore new techniques to ensure the best likelihood of restoration. Regardless of the methods used, meaningful restoration will require patience and dedication, because American chestnuts, compared to other hardwood tree species within their natural range, are relatively slow to spread to new areas. Therefore, efforts toward outcrossing with wild chestnuts and the resulting increase in genetic diversity will rely on the public to restore this keystone species to our forests.

**This text was adapted with permission from the Executive Summary of the Petition for Determination of Nonregulated Status for Blight-Tolerant Darling 58 American Chestnut, by Dr. William Powell and several collaborators with the ESF chestnut research team. For more information about transgenic chestnut, or to access the full petition, please visit: www.esf.edu/chestnut*

ESF graduate student climbs a ladder to place pollination bags over immature female flowers, like the ones shown in the foreground.