Canadian Chestnut Council
Conseil Canadien du Châtaignier

July 2025

Comments addressed to the United States Department of Agriculture (USDA-APHIS) regarding the revised (2024) petition for determination of nonregulated status for blight-tolerant Darling 54 American chestnut (Castanea dentata)

The Canadian Chestnut Council (CCC) is writing to oppose the release of genetically engineered (GE) American chestnut trees and comment on the revised APHIS risk assessments of the GE tree that is now known to be "Darling 54." We ask you to reject the revised petition from the State University of New York College of Environmental Science and Forestry (SUNY ESF) to release (deregulate) this genetically engineered American chestnut tree.

Deregulation of these genetically engineered American chestnut trees would threaten the remaining populations of American chestnut in Canada and pose a concrete risk to our ongoing and promising efforts to restore the American chestnut in Canada. Any continued pursuit of deregulation will put restoration of the American chestnut at risk, in Canada and the United States.

In August 2024, SUNY ESF submitted a revised petition to USDA-APHIS "to provide additional clarifications and corrections about the chestnut event." This revision was triggered by the discovery of a significant error made by the researchers where they mistakenly used the GE event "Darling 54" in their tests instead of the event "Darling 58" which was named in the 2020 petition.

This identity error was a fundamental mistake that has undermined the research conducted on the GE tree thus far, which also forms the basis of their petition. It is also an indication of the unexpected risks that can arise, with unacceptably high stakes for the future of the American chestnut.

The revised petition minimizes this fundamental error and does not clearly respond to the observations of "significant performance limitations" reported by The American Chestnut Foundation (TACF) in December 2023.[i] The TACF reported that the GE tree is not blight tolerant, is significantly shorter and has increased mortality.

Instead of withdrawing their petition as we requested,[ii] SUNY ESF has simply renamed "Darling 58" as "Darling 54" and resubmitted their petition.

However, the mistaken use of Darling 54 is not simply a naming issue. In Darling 54, the OxO gene was inserted into a coding region, causing a deletion (or 1069 base pairs) in a salinity tolerance gene (SAL1), with unknown consequences. The mistaken use of Darling 54 dates back to 2016.

Trials conducted TACF and their research partners also found significant performance limitations. The TACF says its results "indicated striking variability in Darling trees' blight tolerance, significant losses in growth competitiveness, reduction in overall fitness including stunted growth, leaf browning and curling, and increased mortality."[iii] While SUNY ESF maintains that Darling 54 is safe for environmental release, the TACF argues that the multiple performance limitations mean that Darling 54 is not able to function as a restoration tree as planned.

We reiterate our analysis and arguments submitted in 2022 and our request that the USDA-APHIS reject this petition for deregulation. We assess the risks of Darling 54 as follows:

The Canadian Chestnut Council (CCC) is a scientific and charitable organization with the mission to restore the American chestnut to its original ecological (keystone species/mast), economic (chestnuts, timber, chemicals) and cultural (First Nations/historical/hunting) roles in the environment.

Under the Endangered Species Act 2007, the Ontario Ministry of Natural Resources and Forestry followed by the Ontario Ministry of Environment, Parks and Conservation (ESA regulator), the Canadian Chestnut Council has been directed and licensed, first under permits and currently under our Stewardship Agreement, to continue to develop a blight resistant/tolerant American Chestnut based on the surviving indigenous American trees in Ontario and directed not to import or use any external trees, nuts or pollen. The CCC has concentrated its efforts on the pure American Chestnut breeding program and is clearly demonstrating that there is a native resistance to the blight in the Ontario population. Our third generation of blight tolerant selected crosses are approaching maturity to produce the fourth generation of native blight tolerant American Chestnuts.

We disagree with the concept that the "Darling 54" is a restoration tree, and its release will contaminate the remaining population of American Chestnuts and result in possible greater harms. The approval route is taking a horticultural/agricultural approach which end runs or downplays the environmental/ecological issues and concerns of other American Chestnut organizations in North America. "Darling 54" is a single gene therapy to a complex pathogen relationship. The American chestnut utilizes a variety of defense mechanisms to prevent the entry of the fungus involving several genetic traits which are all need to act in unison. The CCC notes several concerns regarding the release of the "Darling 54" as a restoration tree. We anticipate the gene therapy will fail in a relatively short time as demonstrated in many genetically modified agricultural crops as the pathogen evolves. The intervening contamination of wild and research breeding trees with "Darling 54" pollen prior to the failure will certainly damage and set back all efforts to preserve and restore the tree ecologically. The gene therapy of the "Darling 54" may also trigger an evolutionary change in one or more of the 200 plus strains of blight that exist in North America to result in a more virulent strain that could prove in more deadly. The failure of hypovirulence in North America demonstrates the difficulty of dealing with multiple strains of blight. The unique symbiotic relationship of the blight in the "Darling 54" will also act as a source of blight spores which will add to the blight pressure on the remaining wild population, planted gene conservation colonies and on research breeding plots. In Canada, there are approximately 27 Carolinian species of trees identified that harbor chestnut blight and act as carriers for the blight. Any distribution of the "Darling 54", no matter how well regulated, will increase the spread of blight and act as a point source contaminant in the environment.

The Canadian Chestnut Council is also concerned about the lack of regard to the existing restoration efforts /strategies and preservation of the native that will be impacted by the release of "Darling 54". We are making efforts to prevent the extinction of the tree in the northern range and have taken steps to assist its northern migration to areas well outside its historical range to better protect the species. We view the release of the "Darling 54" as major contamination and threat to our efforts

The preservation of the unique Northwest population of the American Chestnut (Stoltz/Husband, 2022) found in Ontario has been a priority of the CCC for many years. In addition to breeding blight tolerant/resistant pure American Chestnuts the council has carried out numerous programs to identify, preserve and enhance the population of native American Chestnuts.

In the Breaking Isolation Program, geographically isolated reproductive trees are provided six to a dozen young but reproductive aged, grafted trees which allow for cross pollination and nut production to be restored. In the process genetic biodiversity within the population and natural recruitment is possible. Best Forestry Management and Practices for the American Chestnut are under development to improve the number of isolated trees that can be incorporated in this program.

In the Gene Conservation Nut Colony Program, a combination of seedlings and grafted trees representing a broad genetic cross section of the Canadian genome are planted in grouping of 200 plus trees to act a gene conservation effort and allow for genetic recombination in the resulting nuts which will be distributed to adjacent woodlands by wildlife. This program is used in areas that lack a wild population of American Chestnut both within and outside the historical range and involve public lands (Conservation Authorities, Land Trusts, Provincial Parks, Nature Conservancy of Canada, Royal Botanical Gardens-Hamilton, First Nations). Colonies are established in blocks or interspersed in reforestation projects so that the best restoration practices can be studied and developed. A vigorous vegetative propagation program gathers scion from all mature wild trees for grafting and planting out in plots to preserve the gene pool. This brings together various chestnut genetics that would normally not occur in the wild due to distance.

In the Survey and Analysis of Remnant American Chestnuts, formal surveys recording the condition, survival, location, ecological and habitat parameters of all known wild, native American Chestnut in the historical range are performed each decade in co-operation with the University of Guelph. The initial survey of 800 trees is currently 1800 plus wild American Chestnut in Ontario. Analysis of the data has provided a variety of papers that provide important insights to the current state of the native trees, persistence of the blight, environmental parameters that influence the survival of the tree in the wild. The Northwest population consists of mature canopy trees with a high number of subcanopy trees and poor natural recruitment due to a closed canopy. Best Forestry Management and Practices are being implemented to release suppressed chestnuts and open light holes for recruitment. These studies also direct our future to better enhance our restoration work and survival of the species.

In DNA Analysis, all native trees and research trees have been tested for purity. The results have shown that the Northwest population is homogenous and uncontaminated by foreign strains. In total only 13 trees have been removed from the survey population due to non-American genetics and the verification of the pure American Chestnut blight resistant breeding program continues. The contaminated trees were all from the Niagara region bordering New York State and indicative of the past work of hobbyists/horticulturalists. In addition, three subpopulations were identified in the historical range in Ontario which require preservation as some are underrepresented in the overall population. Recent

DNA analysis has additionally shown that the Northwest population found in Ontario is genetically unique from the neighboring regions in the United States (Stoltz/Husband, 2022). The closest similar genetic makeup is in Georgia at the extreme southern range which indicates a probable unique migration and development of the northwest population of the species. The D58 being from New York State American Chestnuts would be seen as a contamination to the uniqueness of the Northwest population in Ontario.

The CCC in the past distributed Canadian stock of the American Chestnut to the Maritime provinces for preservation well outside the historical range for safe keeping against the blight. The species has been planted and viable as far north as Sault Ste. Marie and North Bay in Ontario.

The CCC has demonstrated aggressive restoration efforts, and considerable effort and coordination at landscape scales to protect, preserve and restore the American Chestnut in Canada. There are 35000 planted out American Chestnuts in Canada as part of the North American effort to save an endangered species and restore it to its ecological, cultural, and economic roles. The American Chestnut was falsely declared expatriated back in the 1950s in Ontario and has been predicted to be expatriated by 2100 without intervention. The CCC believes that it is reversing that prediction with the addition of more trees by human intervention and natural recruitment. The Blight Resistant Breeding Program is increasing the tolerance to blight and total resistance may not be far off.

If it will take "centuries" for the "Darling 54" to be established and "centuries before conservation impacts are seen" why are the alternative (non-GE) restoration efforts of other American Chestnut organizations such as the CCC not being provided the benefit of this same timeframe?

The CCC finds it highly improbable that any methods for controlling pollination by transgenic chestnuts can be applied to potential restoration programs once the "Darling 54" is released in North America. Commercial growers dealing with chestnut orchards are very different from controlling pollination in a research plot or protecting wild chestnuts from pollen contamination. Hobbyists, horticulturalists, and commercial tree nurseries can not be regulated from the haphazard planting "Darling 54" in areas adjacent to breeding plots or wild native populations. The CCC has consistently battled with residents wanting to import chestnuts from outside sources since the Endangered Species Act 2007 restricts the distribution of pure American Chestnut. The public is ignorant of the work to protect the endangered American Chestnut and the release and importation of the "Darling 54" is a quick fix for their avarice. Regarding Weediness/invasiveness, the CCC views the "Darling 54" tree or pollen as a threat to the native American Chestnut genome of the unique Northwest population. Our population is uncontaminated by non-native chestnut material and unique. As stated prior the "Darling 54" will be a major source of contamination and result in the destruction of the native American Chestnut.
With the current foreign pests and diseases disrupting the remaining Carolinian Eastern Deciduous Forests, we see that the release of the "Darling 54" as a strong opportunistic species that will readily be planted out in reforestation programs as a substitute for the elimination of ash, beech, hickory, oak, etc. We do not see the spread of "Darling 54" as slow or controlled in any manner. It will not be a restoration tree as sold but a commercial/horticultural/timber tree. In fifty years the first timber could be cut as demonstrated previously here in Ontario as the tree is fast growing even in our northern location. Its purported ecological role will be secondary.

The American Chestnut was a Keystone species of the Eastern Deciduous Forest and support a variety of wildlife approaching approximately 275 known species and probably additional lifeforms. Its original demise by the blight resulted in the extinction of at least eleven insects which cascaded in the

decimation of insectivore bird species and decline of larger forest raptors such as the Goshawk. The American Chestnut was the support for a myriad of food chains and intricate food webs. The role of the American Chestnut in the life cycle of the brook trout being one of the most interesting with the high protein leaves feeding the benthic macroinvertebrates that the brook trout feed upon and that the rot resistant wood of the tree provided nearly permanent stream structures for the best trout habitat in a watershed. The CCC questions if sufficient scientific studies have been determined to the possible ecological detrimental impacts "Darling 54" or "Darling 58" may have once in the natural environment. A single bee species study does not cover the multitude of bees and other pollinator species that the tree will host. There is a lack of scientific studies on the insect consumption and survival rate that will feed on the "Darling 54". "Darling 54"'s ecological role as the restoration tree for a keystone species requires additional study before it is released to make certain it will not have unseen irreparable harm on the remaining Eastern Deciduous Forest.

The spread of the "Darling 54" by seed and pollen is very underestimated. In Ontario the isolation distance for pollen is defined as a minimum of one kilometer but literature references up to 18 kilometers. With the increase in wind speed, frequency, and duration due to climate change pollen isolation is questionable. The natural spread of nuts by wildlife, gravity, water, etc may be a few kilometers per century but that is also suspect as many bird species that feed on the smaller American Chestnut are movers of the seed either through dropped nuts or by predation by larger predators. The premise also completely ignores the human factor in the greater distribution of nuts. The assisted migration of nut tree species by First Nation people in the post-glacial period is a prime example. Modern humans will certainly speed the spread of the tree from its out plantings with or without permission.

The assertion that the risk of using "Darling 54" American chestnut in a commercial venture is likely too high for adoption in the foreseeable future is not realistic. Some hobbyists, horticulturalists and commercial tree nurseries growers in Canada are promoting the "Darling 54" as they did with "Darling 58" as the solution since the tree's inception. There are growers and hobbyists are very keen to obtain the "Darling 54" from Ontario tree nurseries that are willing seek importation of the tree if it is released in the United States.

The Canadian Chestnut Council sincerely hopes that serious reconsideration be made prior to any possible approval of the release of the "Darling 54" as a restoration tree or in any other form in the environment. The possible irreparable harm to a continentally shared endangered species is reason enough to take pause.

Submitted on behalf of the Canadian Chestnut Council
Ron Casier
Chair,
Canadian Chestnut Council

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[i] The American Chestnut Foundation. Darling Performance Results. December 2023.
[ii] Canadian Chestnut Council and the Canadian Biotechnology Action Network, *Request for SUNY ESF to withdraw its petition for deregulation of Darling 58,* Letter to Andrew Newhouse, Director. The American Chestnut Project, State University of New York College of Environmental Science and Forestry (SUNY ESF), February 20, 2024
[iii] The American Chestnut Foundation. Darling 58.
https://web.archive.org/web/20230825203949/https://tacf.org/darling-58/